Deciphering the genetic architecture of native resistance and tolerance to western corn rootworm larval feeding

by

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DEDICATION

I would like to dedicate this dissertation to my family and friends who have been instrumental in shaping me as an individual and encouraging me to pursue my passion for science and agriculture. My grandparents for instilling in me an appreciation for agriculture, and teaching me the value of farmers to society. I'd like to particularly thank my parents for instilling in me a level of determination that has dictated my life and for their love and support throughout my education. My wife, Rene, for being such an important light in my life and for all of the insightful discussions that I always love having with her, and for being so patient through all the early mornings, late nights, and midday frustrations. I'd also like to thank my major professor, Nick Lauter, for being an exceptional supervisor, mentor, and friend that really got me interested in the field of crop genetics research, and directed my career on a research-based path, for which I am sincerely appreciative.

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ABSTRACT

Plants can exploit complex suites of biochemical, morphological, and physiological mechanisms to defend against herbivory. This research expands that body of knowledge by investigating mechanisms of defense in maize (*Zea mays*) against one of its most economically important pests, the western corn rootworm (*Diabrotica virgifera virgifera*, WCR). Natural variation for resistance and tolerance to WCR larval herbivory has been previously reported; however, characterization of the underlying genetic architecture has remained elusive. The results from three separate studies are presented that confirm heritable variation exists for WCR resistance that is both experimentally tractable and reproducible. The findings highlight that both genetic and environmental components contribute to the observed variation and interactions exist between rootworm population dynamics and root phenology. Using F_2, BC_1, and DH populations capturing natural variation for three native resistance traits, we demonstrate that discrete regions on chromosomes 2, 3, 5, and 7 are consistently associated with a resistance phenotype. QTL co-localized across analysis populations that were evaluated in different locations and years. Among 21 QTL fixed in the DH population, between 46% and 56% of the variation was explained for three resistance traits. The alleles were found to act robustly by reducing node-injury and increasing root biomass, which was confirmed in hybrid testcrosses. In a separate study, we identified particular physiological and genetic mechanisms of response to WCR root herbivory and revealed evidence of genetic overcompensation. A QTL on c3 (bin 3.05) was localized to a 2.8 cM region and was associated with increased growth rate under high herbivory. The *sps2* gene involved in regulating source-sink transition fell precisely within the QTL interval, and is a possible candidate in the herbivory stress response. These results advance our current understanding of host-plant defense and also provide a route for applied maize improvement by providing a genetic framework for native resistance that can be exploited to reduce larval feeding damage by WCRs.

CHAPTER 1: GENERAL INTRODUCTION

Abbreviations

WCR: Western corn rootworm

NCR: Northern corn rootworm

SCR: Southern corn rootworm

ECB: European corn borer

MCB: Mexican corn borer

QTL: Quantitative trait loci

RIL: Recombinant inbred line

AI: Artificial infestation

SS: Stiff-stalk

NSS: Nonstiff-stalk

Project Goals and Objectives in the Context of Agricultural Challenges

In the past 150 years, the human population has skyrocketed to over 7 billion individuals, vastly more than any other time in human history. This trend will likely persist for the next several decades, driving up the demand for food and basic human resources (Godfray, Beddington, et al., 2010). Based on trend levels achieved in the first years of the 21st century, projections anticipate the world will need to produce 70-100% more food by 2050 in order to support our growing population (Royal Society of London, 2009, Tilman, Balzer, et al., 2011, World Bank, 2007). Much of the world's population resides in developing countries, which in coming years will have greater access to livestock and other agricultural commodities, increasing the demands on Earth's natural resources. At the same time, the effects of global warming are expected to worsen (Godfray, Beddington, et al., 2010). This puts tremendous pressure on food producers to maximize yields in a world where arable land is diminishing. Adapting to such changes will be dependent on making genetic improvements to food products, as well as deploying other integrated and sustainable agriculture practices.

Maize (*Zea mays* L. spp. *mays)*, as one of the world's most productive crops, will be at the forefront of our food demand (USDA-ERS, 2014). Nearly 900 million metric tons were produced during the 2011/2012 growing seasons, which combined with the outputs from

soybeans, wheat, and rice, accounts for about 75% of human sustenance (Lobell, Schlenker, et al., 2011, WAO Board, 2014). Therefore, securing yields and productivity in maize is an important step in meeting global crop production demands. One way of making sustainable agricultural improvements is by finding new solutions to problems that can lead to yield losses or reduce plant fitness. In addition to abiotic factors like soil temperature and precipitation, biotic factors such as pathogens, insects, and weeds can have a huge impact on plant health. Annually, insect pests destroy approximately 14% of potential U.S. crop yields, translating to nearly $16 billion (Losey and Vaughan, 2006). Additionally, an estimated $10 billion in pesticides are applied each year to limit such losses (Pimentel, 2005). Furthermore, the more volatile spatial and temporal fluctuations in temperature, precipitation, and CO_2 that accompany climate change are expected to increase the negative impact of pests on agricultural systems (Estay, Lima, et al., 2009, Gregory, Johnson, et al., 2009, Ziska, Blumenthal, et al., 2011).

The overarching objective of this dissertation was to characterize the genetic mechanisms of host-plant resistance to one of the most substantial insect pests of maize, the western corn rootworm (WCR, Diabrotica virgifera virgifera LeConte). Understanding how natural genetic variation determines differences in the host-plant's ability to resist or tolerate WCR herbivory will be a major milestone in securing future maize productivity. Revealing genetic variation is particularly useful because it represents a resolvable target that can be placed into a broader genomic context. Once alleles are identified and their *modus operandi* confirmed, they can be used in further mechanistic studies to interrogate the genetic pathway and examine they how they orchestrate defense against herbivory. Secondarily, positional knowledge of the important genetic factors can be used for allele mining to reveal additional variation that may be associated with stronger levels of resistance. As a byproduct, examining the role of genotype on phenotype, can translate into refining of heritability estimates, which can subsequently be used in models to predict performance and the effect that climate change may have on herbivory.

The specific goals of this dissertation research were to 1) investigate differences in rootworm population dynamics and plant phenology between two experimental approaches used to apply rootworm pressure and assess their ability to capture genetic variation in native resistance screens, 2) characterize the morphological and physiological responses to high

WCR larval feeding pressure and the genetic loci driving the response, 3) use germplasm known to harbor native resistance alleles to characterize their genomic positions and effects, 4) develop maize isolines capturing extensive variation in root architecture and resistance to WCR larval injury, and 5) increase knowledge about the genetic basis of insect resistance in plants.

Overview of the Pest System

The WCR is one of the most destructive pests of maize worldwide, resulting in billions of dollars in revenue losses from yield reductions and management expenses over the last several years (Flint-Garcia, Dashiell, et al., 2009, Gray, Sappington, et al., 2009, Metcalf, 1986). The WCR was first reported in the U.S. in the 1860s, existing on isolated patches of grasses in the western half of the Great Plains (Branson and Ortman, 1967, Branson and Ortman, 1970, LeConte, 1868). However, the introduction of modern agricultural practices, including dry-land irrigation systems, expanded the territory of cultivated corn and provided an ideal host for the WCR. This allowed for a rapid expansion of the pest across most of the corn-producing areas in the U.S. in less than 40 years (Chiang, 1973, Gray, Sappington, et al., 2009). An even more dramatic and invasive expansion in Europe has occurred more recently. Since it was first reported in 1992, the WCR has reached over 20 European countries, with Eastern Europe being severely affected (Bača, 1994, Gray, Sappington, et al., 2009, Kiss, Edwards, et al., 2005). Remarkably, evidence suggests at least three independent intercontinental invasion events have taken place in Europe, exemplifying the formidability of WCR (Ciosi, Miller, et al., 2008, Miller, Estoup, et al., 2005). The beetle is expected to continue expanding its territory, with several high-risk zones already established throughout Eurasia, further intensifying the need for developing new methods to combat WCR on an international scale (Aragon, Baselga, et al., 2010, Kuhlmann and Van der Burgt, 1998). The demonstrated ability of WCR to adapt to new environments and hosts cannot be understated. Controlling this pest will require that we develop and utilize new sources of native and transgenic resistance, and that we improve implementation of integrated pest management plans appropriate for solidifying these gains.

Anticipated Effects of Climate Change on Agricultural Pests, Pathogens, and their Host-Plant Interactions

Changes in climate over the coming decades are expected to exacerbate the WCR problem on several fronts. Plants that are stressed from exposure to long periods of drought have fewer resources to devote to insect and pathogen defense, while at the same time their succulent tissues are likely to be even more attractive to insects when water is scarce. The peak mortality of WCR occurs during egg overwintering, so as winter temperatures rise, a greater number of eggs are likely to survive to eclosion (Levine, Oloumi-Sadeghi, et al., 1992). It is well established that modern agricultural systems are highly sensitive to fluctuations in climate, with some regions experiencing extreme negative effects, while other regions may actually benefit (Fuhrer, 2006). When the effects of plant pests and pathogens are included in the cost-benefit equation, the impacts of climate change become more severe. An increasing number of examples have shown that increases in CO_2, temperature, and/or rainfall may intensify the pressure from plant pests and pathogens on agricultural cropping systems (Gregory, Johnson, et al., 2009). For instance, one study found that soybean (*Glycine max* L.) responded to elevated CO_2 levels by down-regulating genes involved in the cysteine protease inhibitor pathway, which are vital deterrents of coleopteran insects. This resulted in increased foliar damage from the adult WCR and Japanese beetles (*Popilla japonica*) (Zavala, Casteel, et al., 2008). Extreme fluctuations in climate can result in rapid increases in pest populations, which can pose a major challenge for developing and implementing a management plan. In addition, climate can influence the geographical distribution and appearance of pests and pathogens, introducing invasive species into new areas or altering the onset of peak incidence (Gregory, Johnson, et al., 2009). In general, the effects of a changing climate and more extreme weather on plant pathogens and pests are expected to be less predictable and more consequential, elevating the need to develop more robust methods to guard against these biotic stressors.

Lifecycle and Herbivory Patterns of the WCR

Understanding the life history and herbivory patterns of this pest is an important step in evaluating resistance and developing experiments that most accurately assess resistance. The WCR is a chrysomelid beetle and possesses several distinct life stages including egg, larvae,

pupa, and adult forms. The larvae typically cause the most severe damage to corn by feeding on root tissue resulting in reduced water and nutrient uptake (Kahler, Olness, et al., 1985, Riedell, 1990) as well as plant lodging (Spike and Tollefson, 1989). Eggs are laid in the soil in late summer and overwinter there. Egg hatching is initiated the following spring once cumulative soil temperatures reach a critical level, although a small percentage of eggs will undergo an extended diapause (Levine, Oloumi-Sadeghi, et al., 1992). The larvae carry chemoreceptors that detect carbon dioxide and other volatiles released from developing maize seedlings which act as attractants and trigger an herbivory response (Hibbard and Bjostad, 1988, Strnad, Bergman, et al., 1986). Feeding scars left by the larvae can also serve as entry sites for pathogens (Spencer, Hibbard, et al., 2009). Most of this damage is inflicted between the V4 and V11 stages of plant development (Hibbard, Schweikert, et al., 2008). After WCR pupate, they emerge from the soil as adult beetles and begin feeding on aboveground plant organs including both foliar and reproductive organs of corn, causing additional crop losses (Branson and Krysan, 1981, Moeser and Vidal, 2005). Collectively, the stress induced on the plant can manifest in significant yield reductions (Godfrey, Meinke, et al., 1993, Spike and Tollefson, 1991, Urías-López and Meinke, 2001). The most relevant measure of resistance is made through direct evaluation of the root system during the critical developmental phase when larval feeding occurs.

The WCR has traditionally preferred maize as a host-plant; however its adaptiveness and invasiveness have allowed it to circumvent several management strategies. This has resulted in heritable changes in WCR populations that are under strong positive selection. Several of these variant WCRs have been identified including those resistant to crop rotation by altering their host-plant behavior (Levine, Spencer, et al., 2002), and insecticides (Ball and Weekman, 1962, Meinke, Siegfried, et al., 1998, Wright, Scharf, et al., 2000). Surprisingly, rotation-resistant WCRs were found to be as destructive on *Miscanthus*, a crop which is becoming increasingly cultivated for biofuels, as they were on maize (Meinke, Sappington, et al., 2009). Because crop rotation was the major means of controlling this pest, the advent of the rotation-resistant variant resulted in significant economic impacts and sparked a renewed interest in seeking alternative management tactics (Levine, Spencer, et al., 2002).

A more recent addition to the repertoire of WCR management options is the use of transgenic corn expressing cryptochrome protein endotoxins, such as Cry3Bb1 from the bacterium *Bacillus thuringiensis* Berliner (Vaughn, Cavato, et al., 2005). The use of transgenes to control WCR was quite effective initially, however, resistance to the endotoxin has been reported in laboratory selection experiments after only a few generations (Meihls, Higdon, et al., 2008, Oswald, French, et al., 2011), and more recently in field populations (Gassmann, Petzold-Maxwell, et al., 2011). However, a new mode of action has been developed using an RNA interference approach that appears promising, although field validation has yet to be reported (Baum, Bogaert, et al., 2007, Rangasamy and Siegfried, 2012). The use of refuge plants can help to delay the evolution of resistance by WCR populations, but the high frequency of resistance alleles in some populations threatens future corn crops even when refuge strategies are fully implemented (Tabashnik, Gassmann, et al., 2008).

Evolution of insecticide resistance has also been documented in over 400 different species, providing evidence that mechanisms of adaptation are not uncommon and can be evolutionarily favored (Tabashnik, 2008). Gassmann, Onstad, et al. (2009) highlights the main challenges that limit the adaptation of herbivores in natural and artificial systems and points out that host-plant resistance is often one of the strongest forces operating against insects in both types of ecosystems. Given the history of insecticide resistance and the selection pressures existing in modern agricultural systems, native resistance has emerged as a powerful resource for resistance management strategies**.**

History of Maize Breeding for Resistance to the WCR

Several breeding programs have been implemented to develop WCR native-resistant varieties, but to date, no varieties have been awarded the label of resistant (Ivezić, Raspudić, et al., 2009). Intensive screening of U.S. germplasm began in the 1930s and 1940s with an emphasis on identifying tolerant varieties (Bigger, 1941). Later, Painter (1951)introduced a concept of insect defense based on 3 distinct mechanisms: tolerance, antixenosis (nonpreference), and antibiosis. Smith (1989) clarified that these more appropriately represent resistance categories/classifications which are governed by underlying chemical or morphological mechanisms. Owens, Peters, et al. (1974) showed that germplasm tolerant to

WCR injury had more extensive root systems, as well as an ability to more strongly regenerate root tissue during the growing season. Subsequent work identified several inbred lines and hybrids tolerant to WCR root damage (Jenison, Shank, et al., 1981, Riedell and Evenson, 1993).

Since the 1970s, when methods to more directly quantify root injury were developed, additional tolerant germplasm has been identified with characteristically larger root systems (Hibbard, Darrah, et al., 1999, Kahler, Telkamp, et al., 1985). Tolerance via changes in root architecture has been described as the primary defense mechanism and has been shown to preserve yield after larval injury (Ivezić, Raspudić, et al., 2009, Riedell and Evenson, 1993). In 2007, the variety CRW3(S1)C6 was released after repeated open-pollinated selections showed reduced root damage from WCR larval feeding (Hibbard, Willmot, et al., 2007). Extensive evaluation using a number of traits on this germplasm, and 6 other previously reported native-resistant varieties, confirmed earlier reports and also identified two possible germplasm sources of antibiosis, SUM2162 and SUM2068 (El Khishen, Bohn, et al., 2009). Bernklau, Hibbard, et al. (2010) evaluated both of these lines in soil bioassays and established that antixenosis was the mechanism of resistance in SUM2162 and that an unidentified characteristic of the root epidermis acted as a deterrent to WCR larvae. It remains unclear at this time if SUM2162 employs multiple resistance mechanisms or if the antibiosis reported in El Khishen, Bohn, et al. (2009) was actually a consequence of nonpreference. These recent contributions to our knowledge of WCR native resistance suggest that all three host-plant resistance mechanisms are heritably operating in maize, however, no causative genetic factors have been reported to account for any of these mechanisms.

Overview of Previous Genetic Analyses of Insect Resistance in Maize

To gain a better understanding of how host-plant resistance mechanisms are orchestrated in maize, it is useful to review the 35 years of genetic research on resistance to aboveground pests of corn. One of the first studies of quantitative resistance to insects used a set of translocation stocks crossed onto Oh43 and W123 for evaluation of European corn borer (ECB, *Ostrinia nubilalis)* damage, which showed that three separate chromosomes harbored genetic resistance factors (Onukogu, Guthrie, et al., 1978). ECB resistance was later analyzed using 300 (B73 x B52)F_3 lines and a set of 87 molecular markers; seven QTL affecting stalk tunneling were

identified on chromosomes 1, 2, 3, 7, and 10, explaining a total of 38% of the phenotypic variance for resistance (Schon, Lee, et al., 1993). Although the chromosomes identified in initial ECB studies are coincidently located with those from the molecular marker study reported 15 years earlier, the gains in genetic resolution afforded by the advent of molecular markers are significant. A summary of insect-resistance QTL reported in the past 20 years is shown in **Fig. 1**. While these results come almost exclusively from Lepidopteran pest systems, their examination is informative in terms of the type of genetic architecture, genetic resolution, and genetic consistency of effects measured across years, locations and populations.

The next major ECB study identified 9 QTL for stalk tunneling using a recombinant inbred line (RIL) population derived from B73 x B52, marking the development of isoline use in resistance studies (Cardinal, Lee, et al., 2001). These QTL were localized to chromosomes 2, 3, 5, 7, and 9, showing only a 50% overlap with earlier work on the same biparental contrast. The majority of the resistance was contributed by B52. Next, analyzed F_2 families from the cross B73Ht x Mo47 for ECB leaf feeding and stalk tunneling resistance were used to identify QTL for the two separate stages of larval development, termed ECB1 (leaf feeding at whorl stage) and ECB2 (stalk tunneling) (Jampatong, McMullen, et al., 2002). For ECB1 they identified nine QTL in bins 1.01, 1.06, 1.11, 2.09, 4.01, 4.06, 5.05, 6.02, and 8.06. For ECB2 they identified seven QTL in bins 2.01, 5.05, 5.08, 6.00, 6.07, 8.03, and 9.02. The lack of overlap in QTL detected between these distinct stages may indicate that different mechanisms exist in different maize organs, and/or that developmental changes drive different resistance mechanisms. Krakowsky, Lee, et al. (2004) used a RIL population derived from B73 x De811 to reveal several QTL for resistance to stalk tunneling by the ECB. Analyzing data across multiple traits, they identified QTL on chromosome 1, 2, 5, 6, 7, and 9 that explained over 40% of the phenotypic variance. All three of these studies report a general reduction in insect resistance associated with the B73 allele. This inbred has been widely incorporated into U.S. commercial hybrids, which might explain why the WCR has been so invasive on non-transgenic commercially grown corn (Lee and Tracy, 2009, Mikel and Dudley, 2006). Another commonality among these studies and those reported earlier is that the majority of genetic variance is additive in nature, suggesting the accuracy of predicting breeding values for such traits might be quite good. This may also lead to

a more robust estimation of hybrid performance and general combining ability. Importantly, these studies repeatedly highlight the challenges of identifying robust QTL across environments and years, and thus demonstrate the need to assess environmental variance as part of native resistance studies.

Most commercially grown corn is hybrid corn. Therefore, if resistance depends on any genetic factors with a recessive mode of gene action, performance in the inbred may not be a reflection of performance in the hybrid (Flint-Garcia, Dashiell, et al., 2009, Hallauer, 1990). The extent to which inbred performance can predict hybrid performance is largely dependent on the trait being analyzed. Papst, Bohn, et al. (2004) identified QTL for ECB stalk damage in independent trials using a set of $F_{2:3}$ lines and their testcross progenies. They found fewer QTLs in the $F_{2:3}$ lines compared to their testcross progeny, (4 vs. 6), although, three of the QTLs were localized to a similar region in both analysis groups. Other studies have revealed inconsistencies between inbred line *per se* and hybrid performance (Groh, Gonzalez-de-Leon, et al., 1998, Kreps, Gumber, et al., 1998, Thome, Smith, et al., 1992). In addition to dominance, these discrepancies could be due to: segregating alleles between the testcross lines and their tester, inconsistent power to detect QTL between the two populations, low to moderate heritabilities for the trait, or differences in environment for line *per se* performance and testcross performance (Papst, Bohn, et al., 2004). This emphasizes the need to evaluate both inbreds and their hybrids, and to pursue selection within heterotic groups to maintain a heterotic component. Recent advances in genomic selection have indicated that in livestock, purebred performance can be used to accurately predict crossbred performance without including breed as a parameter in the model (Ibanz-Escriche, Fernando, et al., 2009). With high-density marker genotyping applied to a large and diverse sample, this could also prove true for host-plant resistance to insects.

Two studies to date have identified QTL in maize for resistance to the Mediterranean corn borer (MCB, *Sesamia nonagrioides*) (Ordas, Malvar, et al., 2010, Ordas, Malvar, et al., 2009). The 2009 study used the intermated B73 x Mo17 (IBM) RIL population (Lee, Sharopova, et al., 2002) of maize to reveal one QTL for kernel damage in bin 8.05 and two QTL for stalk tunneling in bins 1.06 and 9.04. These stalk tunneling QTL explained 8.5% of the genetic variance, and were positionally coincident with other QTL that had previously been identified for ECB

resistance. The 2010 study deployed a European flint RIL population to identify QTL for *S. nonagrioides* resistance as well as several other agronomic traits. They detected QTL for stalk tunneling in bins 1.02, 3.05, and 8.05 which accounted for 7.5% of the genetic variance in the population when cross-validation was performed. Interestingly, two of these QTL co-localized with QTL for plant architecture and yield-related traits. The QTL identified in bin 8.05 appears be shared between these studies, although it was detected in different traits. The QTL detected in both studies were in the same or adjacent bins to earlier reports for resistance to *O. nubilalis*. (Cardinal, Lee, et al., 2001, Jampatong, McMullen, et al., 2002, Krakowsky, Brinkman, et al., 2002, Krakowsky, Lee, et al., 2004, Ordas, Malvar, et al., 2009, Papst, Bohn, et al., 2004, Schon, Lee, et al., 1993). Resistance has also been successfully mapped in the Southwestern corn borer, the fall armyworm, the sugar cane borer, and the maize weevil and found to be largely additive in nature (Bohn, Schulz, et al., 2000, Bohn, Khairallah, et al., 1997, Brooks, Willcox, et al., 2005, Garcia-Lara, Khairallah, et al., 2009). **Fig. 1** provides a graphical depiction of all QTL identified for insect resistance from the studies cited, projected onto the IBM2 genetic map (Lee, Sharopova, et al., 2002). Since these studies used different mapping populations, the markers flanking the confidence interval for the QTL were identified on the IBM2 map to obtain the IBM2 map interval. In cases where no markers were given, the bin, chromosome arm, or entire chromosome were used to project the equivalent interval in the IBM2 map. The size of the QTL on the IBM2 map is entirely contingent on the map resolution and availability of data for a reported QTL.

There is extensive variation in the reported genomic sizes and the magnitude of phenotypic variance explained among the QTL (**Fig. 1**). Not surprisingly, as these studies were conducted over 2 decades and for different traits and experimental conditions. However, a comparative perspective such as this does provide course estimates for regions of interest and highlights some of those regions in the maize genome that are likely to harbor insect resistance alleles. Regions on chromosomes 1, 2, 5, 6, 7, 8, 9, and 10 all show overlap in at least five of the studies. QTL were detected in bins 2.08, 2.09, 6.07, and 10.04 for 7 of the 11 studies surveyed. With the exception of the 6.07 QTL, all four of these binned regions housed QTL for resistance to multiple insect pests. These regions represent likely candidates for housing resistance genes

involved in broad-spectrum defense pathways. Most of the loci consist of very large stretches of genomic DNA, and considerable research still needs to be done to further validate these studies and delineate the boundaries of individual QTL. Nevertheless, these studies do provide a solid foundation on which future scientific inquiries can build. Since there are currently no reports of genomic characterization of resistance to the WCR, insights in this regard have the potential to not only reveal specific resistance mechanisms to an important agricultural pest, but also reveal general defenses that are exploited in response to herbivory or other types of non-specific defense mechanisms.

Dissertation Organization

The dissertation is organized into five main chapters with chapters 2-4 constituting the original research projects. Chapter 1 introduces the project goals and objectives, places the research in the context of current and future agricultural challenges, provides details about the pest system, and reviews the literature on resistance breeding as well as genetic investigations of native resistance traits in maize.

Chapter 2 is an analysis of methods paper that examines treatment differences between two approaches used to apply rootworm pressure in experimental studies and identifies factors that contribute to variation in native resistance screening. Aaron Gassmann provided the emergence cages and training on adult and larval WCR sampling. N. Lauter played an important role in germplasm development and handling, provision of the trap nursery and WCR eggs, and in experimental implementation. D. Hessel designed and managed the experiments, collected phenotype data, and performed the analysis and writing.

Chapter 3 reports on efforts to identify native resistance germplasm and the positional and functional interrogation of genetic variation controlling resistance in a series of biparental populations. M. Blanco orchestrated the screening of GEM materials and provided data on the original GEM materials. L. Pollak, L. Lewis and N. Lauter collaborated on screening the top 50 entries with resistance potential. N. Lauter produced initial F_1 seed from resistance sources and worked together with D. Hessel to develop and analyze F_2, BC_1 and DH populations. B. Hibbard managed the native resistance phenotyping at the two Missouri locations and provided Mir604 seed as a check. A. Gassmann provided expertise on experimental design and collaboration of

field space at the Crawfordsville location. D. Hessel and N. Lauter led the design and execution of the experiments, which were supported by several technical personnel in each of five nursery seasons. D. Hessel led the analysis and writing.

Chapter 4 examines the physiological and genetic responses to high rootworm pressure using a high resolution hybrid mapping population by examining line performance in damaged and undamaged states. M.P. Scott, A. Moran Lauter and N. Lauter produced and curated the sets of reciprocal hybrids developed from crosses between B101 and IBMRILs. D. Hessel and N. Lauter led the design and execution of the experiments, which were supported by several technical personnel in two nursery seasons. D. Hessel led the analysis and writing.

Chapter 5 provides general conclusions and extended interpretations from this work as they pertain to host-plant defense and crop improvement. As the primary author of this dissertation, D. Hessel performed the initial analysis and interpretation of each result, and composed the figures, tables and narrative for their communication in this document.

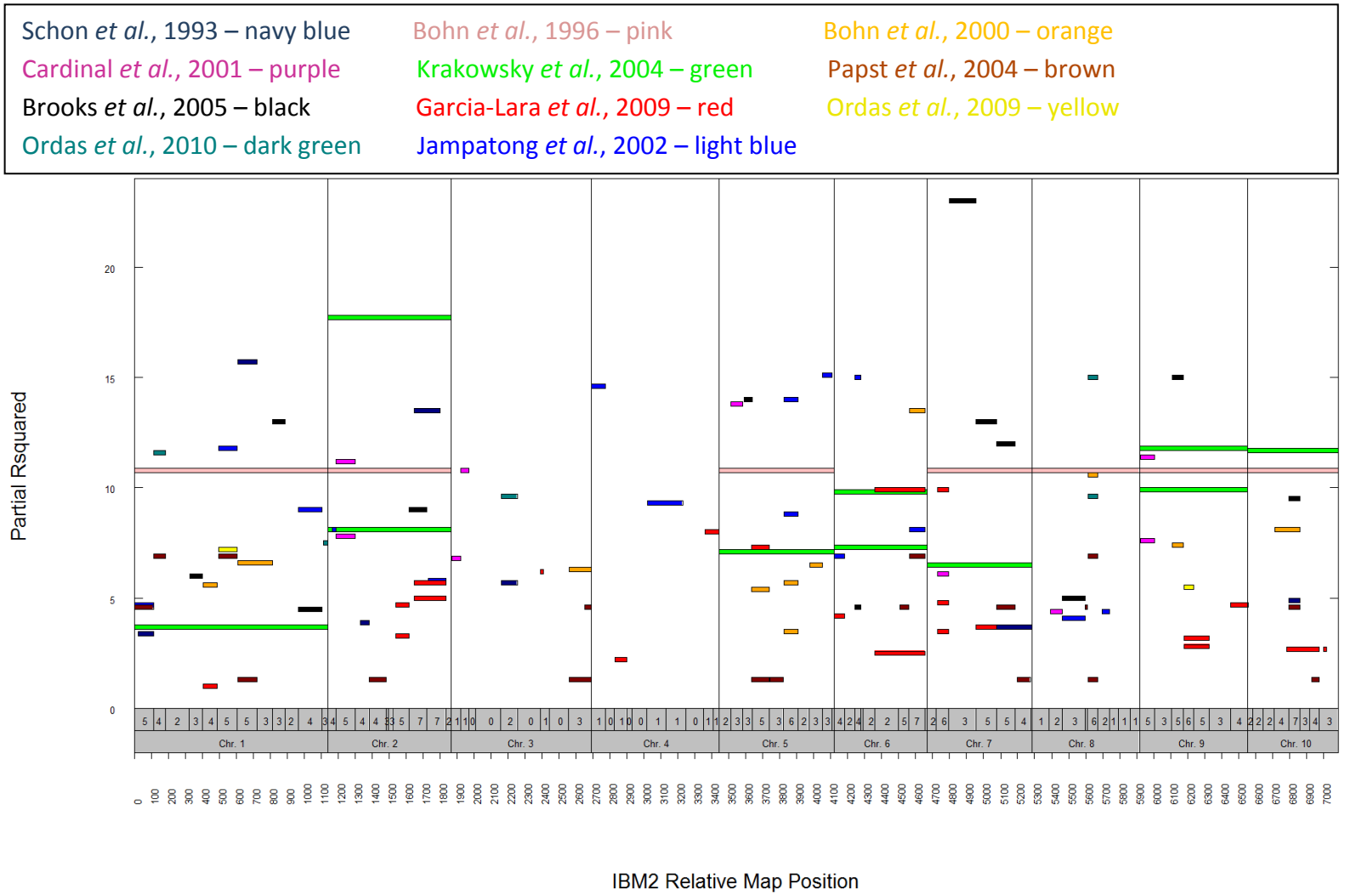

Figure 1. Summary of effect strengths and genetic positions for insect resistance QTL identified and reported in the current literature for maize. The intermated B73 x Mo17 recombinant inbred line genetic map (IBM2) was used to anchor QTL using flanking markers and positional information as available for each study included in this display. Thus, the limited resolution of a QTL support interval may result, in part, from our conservative approach to extrapolation from one map to another.

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CHAPTER 2. EXPERIMENTAL COMPARISON BETWEEN TRAP CROP AND ARTIFICIAL INFESTATION TREATMENTS FOR SCREENING NATIVE RESISTANCE TO CORN ROOTWORM LARVAL FEEDING

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Abstract

Overview: We conducted a detailed comparison of two methods of applying western corn rootworm (WCR, *Diabrotica virgifera virgifera*) pressure in empirical studies and native resistance screens. The two treatments explored were a trap crop (Trap) designed to naturally capture large numbers of WCR, and artificial infestation (AI) of a calibrated number of WCR eggs. The treatments were compared for WCR larval abundance and adult emergence, as well as for several host traits measured using a broad array of corn genotypes: root node-injury, root size and regrowth, standability, and yield-related components. The study was conducted in two years and included 4 different analysis experimental units (maize populations) to assess how the treatment influences rootworm dynamics and subsequent native resistance evaluation.

Results: Overall, AI resulted in higher larval and adult WCR densities with spatial distributions that were more uniform as compared to the Trap treatment. We recovered an average of 49.0 ± 7.5 larvae and 9.0 ± 1.0 adults per genotype in AI, but only 19.0 ± 3.7 larvae and 3.7 ± 1.0 adults in the Trap treatment. Moreover, the correlation between larval abundance and adult emergence was stronger in the AI treatment. We observed that ~20% of WCR larvae survived to adulthood, and that this was widely variable. In several cases, we hypothesize that this variation may be caused by density-dependent mortality of WCR at early life stages. A significant interaction between WCR and northern corn rootworm emergence was detected but only in the Trap treatment. Larval feeding damage was found to be more strongly correlated with root architecture under AI. We also detected a strong treatment by population effect for root size and regrowth, and revealed that node-injury is a more robust trait than size and regrowth, both across treatments and experimental units. Treatment by year variation was negligible for the root

traits but played a significant role in adult emergence. Moderate heritabilities were detected for all three root traits, but considerably more genotype by treatment variation was found for root size and regrowth and is likely attributable to differences in soil composition rather than larval feeding. We also showed that the accuracy of using lodging to predict node-injury was improved in the AI treatment. The elevated tolerance response observed in the AI treatment was found to have a significant effect on yield.

***Conclusions*:** Our results show that artificial infestation results in more uniform larval pressure and is more accurate for predicting levels of node-injury in native resistance assessments. Our data suggest that density-dependent mortality can be detected with AI concentrations of 750 eggs/plant and that this infestation level is also sufficient to detect genetic differences in the degree of native resistance. Under lower larval pressures, interspecific interactions among *Diabrotica* species are more likely to occur and this may have an effect on native resistance assessments. We hypothesize that the observed reductions in environmental variance associated with AI allow for more accurate assessment of native resistance by directly measuring the effects of larval feeding. Several considerations and recommendations are provided for improving models of native resistance and explaining sources of variation including the effects of treatment, year, and population type on the ability to capture heritable variation.

Keywords: Trap crop, artificial infestation, western corn rootworm, native resistance, tolerance, density-dependent mortality.

Introduction

The western corn rootworm (*Diabrotica virgifera virgifera* LeConte) is perhaps the most economically significant pest of maize encountered in modern history. Economic damage caused by the WCR complex has been estimated to be upwards of $1 billion annual in the U. S. alone, and the damage on the global scale far exceeds this number (Gray et al. 2009, Kaster and Gray 2005, Metcalf 1986). Larval feeding by WCRs can result in severe root node-injury, lodging, and decreased plant growth, which can manifest in yield reductions (Godfrey et al. 1993, Gray and Steffey 1998, Urías-López and Meinke 2001). On top of this, exorbitant management and control expenses are typically accrued, adding economic insult to injury (Meinke et al. 2009). The relationship between WCR and cultivated maize has a dynamic history that reflects both the complexity of the problem and the challenges encountered in managing it. For instance, over the

last several decades, WCR populations resistant to insecticides (Ball and Weekman 1962, Meinke et al. 1998, Parimi et al. 2003), crop rotation (Levine et al. 2002), and more recently, Bt-transgenes have been reported (Gassmann et al. 2011, Meihls et al. 2008). Given these challenges, there has been renewed interested and urgency in screening for native resistance and conducting research to better explain the pest-plant relationship.

Some of the biggest challenges in screening for native resistance are the amounts of environmental variation that can be seen in root phenology and WCR population dynamics, which can blur the relationships between cause and effect. Early screens of germplasm conducted for native resistance, insecticide efficacy, and for establishing economic injury levels consistently encountered problems in deciphering results due to a general lack of uniformity in WCR dynamics (Ortman et al. 1974). Given this, the need arose to more precisely measure rootworm pressure in empirical studies. Two main approaches that were developed, and for which are still commonly used, are trap-cropping and artificial infestation of WCR eggs. The use of a "trap" for the WCR arose out of the observation that late-planted corn tended to attract adult beetles from the earlier planted areas, and these adults would oviposit more frequently in the trap crop area (Darnell et al. 2000, Hill and Mayo 1974). This would result in elevated levels of node-injury for corn plants planted the following season inside the trap area. What was originally developed as a management tactic for farmers, later turned out to be a useful tool for researchers studying the WCR. It was later identified that trapping can be enhanced by intermixing maize with a Cucurbitaceous species, since WCR adults, while polyphagous, are strongly attracted to cucurbit vegetative and floral tissues (Branson and Sutter 1989).

Breakthroughs were eventually made in WCR rearing and colony maintenance (Howe and George 1966, Jackson 1986, Jackson and Davis 1978), and techniques to artificially infest WCR eggs to supply larval feeding pressure began to emerge. Initial attempts were performed using egg-soil mixtures (Chiang et al. 1972, Ortman and Fitzgerald 1964), but later progressed to agar suspensions that could be stored for longer periods and resulted in more uniform egg concentrations (Palmer et al. 1977). Benefits of using artificial infestation include more uniformity in the timing of egg hatch and larval development, and more evenly spaced egg distributions. Because there is less variation than natural populations, fewer locations needed to be screened, although the need for replication still exists (Branson and Sutter 1989). For trap cropping, on the other hand, the major benefit is realized in the number of experimental entries

that can be screened under high rootworm pressure without the added labor costs associated with artificial infestations. The procedures used to process and inject eggs into the soil can take time and be cumbersome, and are difficult to achieve on a whole-field scale. However, equipment has been developed to help in the process, allowing for larger-scale infestations (Branson and Sutter 1989, Chiang et al. 1975). Although both Trap and AI treatments have been successfully deployed as a means of applying rootworm pressure, the differences in rootworm dynamics and their interactions with maize phenology have largely gone ignored, making results from germplasm screens more difficult to compare and validate.

Trap crops can be initiated either by trapping adults exclusively from naturally occurring populations, or via boosting a founder population with artificial infestation of eggs. Regardless of how they are initiated, the assumption follows that later flowering plants will recruit more gravid females, since adults feeding on maize primarily graze on the soft tissues (leaves, silks, immature kernels, and pollen), and have a clear preference for younger tissue (Moeser and Hibbard 2005, Moeser and Vidal 2005). Because WCR oviposition tends to take place where feeding occurs, there will be increased oviposition in the later-maturing material (Branson and Krysan 1981).

Eggs deposited in the soil in late summer will overwinter and hatch the following spring once cumulative soil temperatures reach a critical level contingent on a developmental threshold of 11ºC, although a small percentage of eggs will undergo an extended diapause (Levine et al. 2002, Meinke et al., 2009, Schaafsma et al. 1991, Wilde 1971). In larval stages, the WCR can travel only a few centimeters in the soil. The larvae use chemoreceptors that detect carbon dioxide and other volatiles released from developing maize seedlings which act as attractants and trigger an herbivory response (Hibbard and Bjostad 1988, Strnad et al. 1986). Larvae may be able to travel as much as 100 cm from egg hatch to adult emergence, but adults can travel much larger distances by flying up to several kilometers a day, so the ability of a trap crop to maintain high insect pressure relies on the assumption that WCR beetles preferentially establish in fields close to where they emerged (Coats et al. 1986, Grant and Seevers 1989, Hibbard et al. 2004, Short and Luedtke 1970, Spencer et al. 2009, Spencer et al. 2003, Suttle et al. 1967, Toepfer et al. 2006).

Natural populations of WCR can be highly variable across space and time within a given field (Darnell et al. 1999, Meinke et al. 2009, Park and Tollefson 2005). Environmental factors

like temperature, soil moisture, surface residue, soil depth, and total egg density can all play a role in larval abundance and distributions of emerging adults (Godfrey et al. 1995, Onstad et al. 2006, Toepfer and Kuhlmann 2005). In fields continuously grown with corn, such as a trap crop, total adult densities tend to be greater than rotated fields (Godfrey and Turpin 1983, Pierce and Gray 2007). Onstad et al. (2006) reported that natural populations of WCR tend to be more clumped than artificial infestations, and because mechanical infestations have more uniform egg depth there tends to be less variation in egg hatch than natural populations. However, after egg hatch the patchy distribution of eggs can be mitigated as larvae disburse from higher to lower densities (Hibbard et al. 2003, Hibbard et al. 2004, Hibbard et al. 2005). Only a small fraction of the eggs laid will survive to adulthood, which has been estimated to be between 5 and 15% in field experiments (Gray and Tollefson 1988, Onstad et al., 2006, Pierce and Gray 2007). In addition to environmental factors, maize phenology plays a critical role in WCR development and distribution at larval and adult stages (Bergman and Turpin 1986, Campbell and Meinke 2006, Darnell, Meinke and Young 2000, Naranjo 1991, Pierce and Gray 2007).

This introduction had covered only some of the variables that influence WCR populations in the context of experimental field studies. Reducing field variability in order to reveal underlying causative effects has been a major focus in WCR studies, however, it is still not understood how differences in treatment affect the ability to screen native resistance. Advancing this understanding can lead to more accurate estimates of genetic effects and may reveal sources of resistance that would otherwise have been missed. Here, we examine how inherent differences in trap crops and artificial infestations manifest in terms of WCR population dynamics and host-plant root phenology. We hypothesize that using AI results in more uniform WCR densities and because of this more of the variation captured is due to genetic differences in native resistance. In addition to providing details on treatment differences in the context of native resistance screens, we also report on considerations that should be made when analyzing and comparing results from such studies.

Materials and Methods

Treatments and Experimental Units

This study consisted of 4 experimental units (EUs) assigned to two treatments. The two treatments were 1) a trap crop field that had been maintained for 4 years prior to planting (Trap), and 2) a field grown under typical Iowa soil conditions and manually injected with ca.750 WCR eggs/plant (AI). The EUs for the experiment are displayed in **Table 1**. EU1 consisted of a set of 13 pure-breeding lines including 10 with previously demonstrated native resistance in prior screenings, and two expired Plant Variety Protection lines susceptible to larval feeding, LH51 (Holden Foundation) and PHZ51 (Pioneer), but have good agronomic performance (Mikel 2006, Mikel 2011). The 10 resistant lines included two inbreds, AGR9 (AgReliant Genetics) and NGSDCRW1(S2)C4 , as well as 7 doubled haploid lines developed by AgReliant Genetics from AGR9 x NGSDCRW1(S2)C4 F_1 plants (Jim Uphaus, personal communication). NGSDCRW1(S2)C4 originated from a synthetic population of 57 diverse germplasm stocks of primarily yellow dent background that subsequently underwent three cycles of S_2-family selection for reduced root damage (Kahler et al. 1985). An additional synthetic line, CRW8-1, was included because of previously reported resistance and its use in native resistance screens. It was originally developed from BS19 (GP 72)/BS20 (GP 73) rootworm synthetic populations (Prischmann et al. 2007, Russell et al. 1976). Both CRW8-1 and NGSDCRW1(S2)C4 have subsequently been used as resistance checks and reported to have some level of resistance to rootworm larval feeding (Hibbard, Darrah, et al. 1999, Hibbard et al. 2007, Prischmann et al. 2009, Prischmann et al. 2007). An additional expired PVP line, PHG84 was included in the study because of its agronomic performance and good combining ability.

EU2 consisted of a set of hybrids including 3 resistant and 1 susceptible generated using PHZ51 as a common tester parent (**Table 1**). These hybrids have consistently been used as checks in rootworm resistance screens (Jim Uphaus, personal communication). An additional set of hybrids derived from crossing selected germplasm in EU1 to PHG84 was used to assess treatment differences between these lines *per se* and a common hybrid tester. EU3 comprised 16 randomly selected hybrids from a set of 202 derived from the cross of selected members from the intermated B73 x Mo17 recombinant inbred lines (IBMRILs) with a common inbred parent, B101 (Hallauer and Wright 1995, Hessel et al. 2012, Lee et al. 2002). EU4 constituted 80 plants from a backcross population segregating for WCR larval feeding resistance. This population was

derived in 2007 from the following cross: B86 x (FS8B(S):S0316-053-1 x B86). The FS8 source was originally developed by E. S. Horner and later included as a donor in the GEM project (Horner, 1990, Pollak, 2003). Each FS8B(S):S0316-053-1 plant was crossed to the inbred B86 (Reg. No.GP-77) for two generations to generate several BC_1 families (Russell et al. 1976).

Field Design and Rootworm Infestations

The experimental field trials took place during the summer of 2010 and 2011. In 2010, plots were planted on 25 May at the Iowa State University Bruner Research Farm (41° 60′ 35″ N, 93° 44′ 11″ W). This site served as the Trap treatment which was established in 2006 by manual injection of ca. 750 WCR eggs/plant followed by continuous late season corn-on-corn cultivation. Each EU was randomly assigned to a particular block in the field and within each block accessions were randomly assigned to plot locations. Each plot had 25 kernels evenly distributed across 4.572 m, and 0.914 m of alley separating plots in adjacent ranges. An Almaco 4-row cone planter was used for planting, with adjacent rows 0.762 m apart. The AI treatment was planted at the Ag Engineering and Agronomy Research Farm (42° 0′ 60″ N, 93° 46′ 11″ W) on 24 May 2010. In 2011, both the trap crop and the AI fields were located at the Bruner Farm in an attempt to minimize soil composition differences between the treatments. Planting was performed on 18 May 2011 using the same planter as in 2010.

For both years, eggs supplied from the USDA-ARS North Central Agricultural Research Laboratory (NCARL) at Brookings, SD were washed and suspended in 0.15% agar solution according to Palmer, Windels and Chiang (1977) before infestation at a concentration of 750 eggs/plant. These eggs came from a diapausing *D. v. virgifera* lab strain which has been maintained at the NCARL since 1987 (Hibbard, Barry, et al. 1999). This strain has been observed to have similar levels of damage to wild populations and loss of genetic variation has been minimal compared to the non-diapausing strain because genetic diversity can be maintained by adding new wild-caught beetles to the colony (Hibbard, Barry et al. 1999, Kim et al. 2007). Infestations took place on 3 June and 2 June in 2010 and 2011, respectively, which corresponds with the V3-V4 maize growth stage for the corn plants. For each plant to be infested, a vertical hole with a 12 cm depth is made by pressing a dowel with a 1 cm diameter into the soil ~8 cm from the base of the plant. A 5 mL pipet is then used to dispense 2.5 mL of egg-agar solution, calibrated to a concentration of 300 ± 20 eggs/mL, into the base of each hole. Finally, the upper portion of the hole is covered over with soil such that desiccation stresses are minimized for the

eggs. EU1 and EU2 were replicated in 2011 evaluations, but because of resource limitations and experimental requirements, EU3 and EU4 were only evaluated in 2010.

Measuring Rootworm Abundance

Larval abundance was assessed using a modified Berlese funnel method similar to that described by Crossley Jr and Blair (1991), with the key components shown in **Fig. 1A**. This method of soil extraction is also described in detail in Hibbard et al. (2003) and Coleman et al. (1996). Here we describe a new, simple, efficient and effective funnel system we developed using standard parts that can be purchased and replaced at general hardware stores. Seven 8 cm diameter holes were drilled into a 60 cm x 60 cm wooden board, with six in the shape of a hexagon surrounding one central hole. This hole size allowed a 10.16 cm to 7.62 cm duct reducer to fit within the hole so that the 7.62 cm opening protruded below the level of the board. A 10.15 cm duct connector fit snuggly into the 10.20 cm opening of the duct reducer. Under each duct reducer a disposable 266.16 mL plastic cup was placed containing 88.72 mL of water/alcohol/ethylene glycol so that larvae would not survive or dry out. The resulting assembly held seven individual soil samples per Berlese funnel station (**Fig. 1B**). Eight full stations were set up in the Iowa State University Plant Pathology & Microbiology greenhouse with a capacity to process 56 soil samples per batch. Greenhouse lights (465 watts, 120 volts) were suspended 20 cm of the top of the duct connector to provide full and equal coverage of each group of seven soil samples. Duct connector pieces were labeled, and transported out to the field in plastic totes. Selected plants were cut at the stalk and the duct connector was placed over the stalk and pressed into the soil. Each sample, held intact by the metal cylinder was dug out from the soil and prepared such that the exposed soil ends were flush and the side of the cylinder was clean. Each sample was then placed into a mesh harvest bag and transported back to the greenhouse for assembly onto the duct reducer.

Adult rootworm abundance was measured using emergence cages. For each genotype to be tested, emergence cages were placed over randomly selected plants (excluding end row plants) with two plants per genotype per treatment as soon as plants were mature enough to clear the threshold of the emergence trap. The emergence cages used in 2010 were 38.1 cm wide by 76.2 cm long and were modified from the design of Fisher (1980), whereby plants were allowed to grow throughout the season by supplying a space in the center for vertical expansion and using a twist-tie to attach a mesh sleeve to the stalk. In 2011, a different type of emergence cage was

used. In this case, the bottoms were cut out from heavy duty injection-molded nursery pots (30.2 cm by 27.9 cm). Patches of shiny tulle fabric were cut into rectangles 91.4 cm by 61.0 cm and sewn to make a sleeve. Individual sleeves were placed over the lip of the nursery pot and taped into place. Pots were placed over selected plants and twisted into the soil to a depth of ca. 10.2 cm. Twist-ties were used to seal the upper sleeve opening to the plant stalk. Due to limited availability of emergence cages, we focused on EU1 and EU2 for monitoring rootworm abundance. Abundance of WCR, NCR, and southern corn rootworm (SCR, Diabrotica undecimpunctata howardi Barber) were recorded at 6 time points starting 6 July 2010 and 29 June 2011 and ending 23 August 2010 and 30 July 2011 for the two years of study.

Plant Phenotype Data Collection

Lodging scores were collected for each of the genotypes on a per row basis as the percentage of plants in a row leaning (> 30º from vertical) or goosenecked. Lodging and goosenecking were assessed between 17 Aug. and 28 Aug. in both years. Three root traits were also collected: size, compensatory growth, and node-injury. This required excision of intact roots from the soil at a point when maximal larval damage typically takes place at around 500-600 degree day units (DDU) (Hibbard et al. 2008). This process started on 8 July 2010 and 12 July 2011 for the two years of the study. DDUs were calculated by subtracting the developmental threshold of 11.1 ºC from the average soil temperature per day (Levine et al. 1992, Meinke et al. 2009, Wilde 1971). Plants were excised from the soil using digging shovels and transported in batches to a centralized facility where 68.14 L totes were stationed and filled with water. Plants were placed in totes and allowed to soak for 24 h before washing off remaining soil with a high-pressure garden hose attached to a gas-powered pump and water trailer. Larval feeding damage was scored using the 0-3 Node-Injury Scale (Oleson et al. 2005). Each root was rated blindly, and all roots in both years were scored by the same individual to minimize experimenter error and bias. The root size and compensatory growth were rated on the Eiben 1-6 scale (Branson and Sutter 1989, Rogers et al. 1975). On this scale a “1” represents the smallest roots and regrowth, and a “6” represents the largest roots with the most extensive compensatory growth. For some cases, particularly those where no compensatory growth was observed, plants received a score of “0” if either the size of the root system was markedly smaller than a “1” on the root size scale, or compensatory growth was absent. For each plot, a total of 8 plants were dug and rated for the root traits.

For EU1 and EU2, information on several ear morphology and grain-filling traits were also collected to analyze differences in yield between treatments. For this pursuit, 5 ears per plot were harvested and dried to ca. 12% moisture. Prior to shelling, the number of kernel rows (KRN), kernels per row (KPR), and the number of kernel rows not filled (KNF) were counted on each of the five ears per plot. All five ears were compositely weighed to get a total ear weight (EW) for the plot, shelled, and the cobs (CW) and kernels (GW) were weighed. For each of these traits, averages across the plot were calculated prior to performing statistical analysis.

Statistics Analysis

All statistical tests were performed using either R or JMP® Pro 10.0.0 statistical software (R Development Core Team 2008, SAS Institute Inc. 2012). For linear models, the standard least squares analysis function was used for testing mean separations. Each of the following effects was included in the model and tested for significance on phenotype: Date (fixed), Experimental Unit (fixed), Treatment (fixed), Rep (random), Block (random), and Genotype (random). REML was used for estimating variance components for random effects. For each model explaining a phenotypic trait, the alternative hypothesis (Ha: $\mu Trap \neq \mu AI$) was tested against the null hypothesis that there was no difference between treatments (Ho: $\mu Trap - \mu AI$). Cases wherein one-sided t-tests were performed are explicitly indicated in the text. Unless otherwise noted, all tests of significance used a P-value threshold for rejecting Ho of 0.05.

Results

Treatment Differences in Western Corn Rootworm Larval Abundance

The abundance of WCR larvae in soil samples was a function of time, plant genotype, and treatment. Under AI treatment conditions, greater than 50% of the recovered larvae were detected on the first and second sampling dates (23 June and 27 June, **Fig. 2**). For the Trap and non-infested (used as an internal control within AI) treatment groups, the 50% recovery point was not achieved until 3 July 2011. An average of 27.4 ± 5.02 larvae per sample were recovered on 23 June 2011, significantly higher than any other date x treatment combination ($F_{2, 41} = 7.33$, $P = 0.002$). For all three treatment groups, 75% of the larvae were recovered by 7 July 2011. Three of the six sampling dates that included non-infested plants were indistinguishable from the mean larval abundance in the Trap treatment. The mean larval abundance per sample was higher in AI than Trap on every sampling date except 3 July 2011. **Fig. 2** also shows that for the first 3

sampling dates, the number of larvae recovered from non-infested plants remained low (3.18 ± 2.12), but as the summer progressed, a greater number of larvae were recovered (7.7 ± 2.15). This is in contrast to the other treatments which had a mean larval abundance twice as high in the first 4 sampling dates than in the last 4 (10.8 ± 2.80 vs. 5.6 ± 2.41 for AI, and 5.5 ± 2.49 vs. 2.4 ± 2.21 for Trap). Aside from the 30 June sampling date, there was not a significant difference in mean larval abundance between sampling dates for the non-infested and Trap groups ($\alpha = 0.05$, $t_{272} = 1.97$).

Another way of looking at larval abundance data is to assess the mean number of larvae recovered by treatment across all sampling dates. **Fig. 3** shows that the AI treatment had significantly more larvae recovered per genotype than either the Trap or non-infested groups (AI vs. Trap: $t_{288} = -4.12$, $P < 0.001$; AI vs. Control: $t_{288} = -2.54$, $P = 0.006$), and that the mean larval recovery in the control was indistinguishable from the Trap ($t_{288} = -1.06$, $P = 0.144$). Not surprisingly, given that in 2011 attempts were made to minimize soil composition differences by cultivating both treatments in adjacent field blocks rather than at separate locations. Therefore, wild WCRs are likely to have been deposited to some extent outside of the designated Trap field the previous year.

Significant differences in larval abundance for individual genotypes across AI and trap treatments were detected (**Fig. 4**). Among the 24 genotypes sampled for larval abundance, only 6 had significant differences between treatments, all of which had greater abundance in AI. Within EU1, 4 genotypes had significantly greater numbers of larvae recovered in the AI treatment at a p-value threshold of 0.05. Although EU2 did not have any significant treatment differences by genotype at this threshold, there were two hybrids that were significant at $P = 0.10$. The genotype x treatment interaction effect was not significant ($F_{22,213} = 0.827$, $P = 0.690$). Only 1 genotype, (AGR_9 x NGSDCRW-1)DH-2, in AI could be statistically separated from the other genotype x treatment combinations ($t_{191} = 2.21$, $P = 0.029$). There was not a significant difference in mean larval abundance between EU1 and EU2 for either AI ($t_{105} = 0.220$, $P = 0.826$) or Trap ($t_{105} = 0.965$, $P = 0.337$) treatments, and in both treatments there was a positive correlation between the isolines in EU1 and their hybrids in EU2 (Trap: 83%, n = 18, $P_{(1\text{-tailed})} < 0.001$, AI: 57%, n = 18, $P_{(1\text{-tailed})} = 0.007$). The genotypic effect on larval abundance was also slightly more significant and predictive in the Trap ($R^2 = 0.33$, $P = 0.023$) than in the AI treatment ($R^2 = 0.16$, $P = 0.782$).

Treatment Differences in Rootworm Adult Emergence

In contrast to larval abundance, there was no significant difference in mean WCR emergence between the Trap and AI treatments (**Fig. 5**). Mean WCR emergence per sample was not significant between treatments in either year of the study. However, there were more WCR beetles detected per cage in 2011 than in 2010 ($t_{146} = 1.93$, $P = 0.028$). Thus, when making comparisons between experimental rootworm treatments, it is necessary to have both treatments present in a given year. Among the 3 rootworm species collected in 2010 and 2011, *D. virgifera* was by far the most abundant for both treatments. An average of 8.37 ± 1.46 WCR adults per sample were collected across all cages and both years compared to only 0.912 ± 0.23 NCR and 0.128 ± 0.05 SCR. This trend was true for both the mean adult abundance and the total abundance collected over the course of each summer. Therefore, both treatments were effective in enriching the abundance of WCRs relative to other rootworm species. This suggests that over a wider spatial area, there exists a few feral NCR and SCR randomly distributed, rather than small localized patches of NCR and SCR at relative high density.

NCR emergence was not significantly different across the two years (1.183 ± 0.195 in 2010 vs. 0.727 ± 0.161 in 2011); however, the treatment effect was different. NCR emergence was significantly higher in AI than in the Trap in 2010 for both EU1 and EU2, while the reverse was true in 2011 (**Fig. 5**). Neither genotype nor genotype x treatment effects had a significant impact on NCR emergence. Interestingly, there was a positive interaction between NCR and WCR emergence that was only detected in the Trap treatment ($F_{1,73} = 9.16$, $P = 0.0034$), explaining 11% of the variation from one species to the other. SCR abundance was significantly higher in the Trap than in the AI treatment, but there was no difference in SCR emergence between 2010 and 2011, or between genotypes. This provides further evidence that these two species tend to be randomly distributed across a natural field environment. It also suggests that at least in the case of the NCR, there may be interactions at the root interface between these rootworm species.

Just as larval abundance followed a variable distribution across time, so too did adult emergence. In both years, peak emergence occurred between 21 July and 30 July, and the emergence curve in AI occurred slightly right-shifted relative to emergence in the Trap (**Fig. 6**). In both years, over 80% of emergence occurred after 14 July. The WCR emergence pattern in 2011 fit well with the temporal abundance of larvae recovered in 2011 (**Figs. 2 and 6**). Given that 75% of recovered larvae had been collected by 7 July, a typical pupation period of two

weeks would correspond with a peak in adult emergence around 21 July, which is precisely where peaks started to occur, first for natural populations and then artificially-infested populations.

The treatment effect magnitude and direction on total WCR beetle emergence was largely dependent on genotype. Within EU1, variation existed in total WCR adult emergence across genotypes, years, and treatments (**Fig. 7**). For both years, 4 of the 7 genotypes had greater emergence under artificial infestation than in the Trap, but which genotypes this was true for was different for the two years. For example, in 2010 there were 54 total WCR adults collected for PHZ51, and only 5 were observed in the Trap nursery, compared to 11 and 16 in 2011 for AI and Trap, respectively. For both treatments, there was more total emergence in 2011 than in 2010, and the treatment x year effect was largest for AI ($t_1 = 1.98$, $P = 0.048$). The correlation in total emergence between AI and Trap was stronger in 2011 than in 2010 ($r = 0.523$ vs. 0.058), and there was a greater consistency in emergence between years for AI than for Trap ($r = 0.329$ vs. 0.160). The greater correspondence observed between treatments in 2011 shows the improvement that can be achieved when both treatments present in the same geo-spatial area.

Fitting a model with genotype, year, and treatment main effects and their interactions explained 13% of the variance in WCR adult emergence ($F_{59, 717} = 1.81$, $P = 0.001$). Among these terms, genotype and treatment x year had a significant effect, with genotype being the most significant (**Table 2**). A moderately significant genotype x treatment x year 3-way interaction effect was also detected. However, there was not a difference in mean adult emergence between treatments or years. The four significant treatment effects that were detected by genotype, all were hybrids belonging to EU2 (**Fig. 8**). In two cases, AI had greater mean emergence, and in two cases, the greater emergence was observed in the Trap. There was significantly more WCR emergence in EU2 than in EU1 ($t_{144} = 3.84$, $P = 0.0002$), but the treatment effect was not different between the two EUs. Furthermore, differences between genotypes explained more of the phenotypic variation observed in EU2 (10%) than in EU1 (< 1%).

Larval abundance was more predictive of adult emergence under AI treatment conditions. This was true for both experimental units assessed (**Fig. 9**). The strongest correlation was observed for EU1 grown under artificial infestation ($r = 0.63$). This relationship was considerably stronger than the correspondence for EU1 in the Trap ($r = 0.17$). The greater prediction accuracy captured in the AI treatment is likely due to the controlled infestations,

whereby uniform egg depth results in more synchronous development, allowing for more accurate abundance estimates. Another interesting trend observed between developmental stages was the directionality function of each EU. At higher levels of larval abundance, fewer adults emerged in EU2, whereas for EU1, higher larval numbers tended to correspond with greater numbers of emerged adults. This could be due to density-dependent mortality among the greater numbers of competing larvae on hybrid roots vs. their isogenic parents. Further support for this comes from the survivorship ratios from larva to adulthood (**Table 3**). Comparing the mean in total larval abundance with the mean in total emergence revealed an average survivorship of about 20% in both treatments. Evidence for a density-dependent effect was detected, with the highest larval density resulting in the lowest survivorship (EU2 AI), and the lowest density the highest survivorship (EU1 Trap). This confirms that densities in the AI treatment reached a level where density-dependent mortality could be observed.

Treatment Differences in Root Phenology

Analysis of root phenotypes for the two experimental units that were evaluated in both 2010 and 2011 (EU1 and EU2) reveals that AI tends to result in more severe node-injury, larger sized roots, and more extensive root regrowth than the Trap (**Fig. 10**). This was true across both years and for both EU1 and EU2, albeit not all treatment comparisons were significantly different. For EU1, there was a significant treatment effect for NI in both years, and root size in 2011. EU2 however, had significant treatment effects for RS in 2010, and RR for both years. Node-injury was higher in 2011 than in 2010 and was inversely proportional to the root size, indicating a trade-off between these two traits. A model that included treatment, year, and treatment x year interactions explained 15% of the variance observed for NI in EU1, but only 4% of the variance in EU2. For RS and RR, less variation was attributable to these effects for both EUs (2.3% for RR and 8.1% for RS). RR was the most robust trait to variations in treatment, year, and treatment x year, which accounted for only 2.3% of the variance in EU1 and 4.3% in EU2.

Fig. 11 shows the difference in treatment means between the AI and Trap treatments for all 4 experimental units. Clearly, EU3 and EU4 responded differently than EU1 and EU2. They accrued less node-injury and greater differences between treatments for RS and RR. Among the possible treatment by EU comparisons, only two were insignificant, NI for EU3, and RS for EU1. Root size and regrowth appear to be particularly vulnerable to this treatment variation. The two experimental possessing the greatest genetic variation in root architecture (EU3 and EU4)

resulted in the greatest differences between trap and AI treatments. There actually was not a difference in node-injury between the treatments (**Fig. 11**), so the difference in RS and RR was not due to larval feeding. Changes in root architecture due to node-injury would manifest more like **Fig. 10**, whereby, as node-injury goes down, RS and RR go up, and vice versa. Rather, what most likely accounts for the difference is the fact that the treatments were grown in separate fields in the year in which EU3 and EU4 were evaluated and the differences in soil composition and resource availability between these fields have a substantive effect on root architecture. This also provides evidence that the node-injury scale for assessing larval feeding by the corn rootworm is more robust to differences between fields than is RS and RR (**Fig. 10 and 11**).

Node-Injury was moderately predictive of the root architecture traits and was more so in the AI treatment (**Table 4**). The R^2 between NI and RR was particularly low for EU3 and EU4 in the Trap treatment, suggesting that factors other than rootworm feeding are influencing root architecture. Moreover, root architecture appears to be more treatment-dependent than line-dependent. **Table 4** also shows that NI is more predictive of RR than RS in most cases. The strongest relationship existed between NI and RR for EU1 and EU2, where approximately 40% of the variation in RR was due to differences in node-injury.

Variation accounted for by genetic effects and genotype x year, genotype x treatment interactions accounts for a major portion of the phenotypic variation observed for NI and root architecture. Using a model that includes treatment and year fixed effects, and fitting genotype and the relevant interaction terms as random effects explained between 19% and 77% of the phenotypic variance depending on the EU and NR trait (**Table 5**). Variation between genotypes in EU1 accounted for most of the variation observed in RS and RR, and was the second largest source of variation for NI. For EU4 however, genotype alone did not explain any measurable portion of the total variation, but a large portion was accounted for by genotype x treatment interactions. The interaction between genotype and treatment had a measureable effect on RS for all EUs except EU1, and was largest for EU2, accounting for 34% of the total variation. Treatment x year variation was generally very small, about 1% of the total variation for the two EUs evaluated over two years. This indicates that comparisons across years for the three root traits analyzed can be reasonably achieved if such comparisons involve the same treatment. It also means that including a fixed treatment effect into current models of native resistance will result in more accurate comparisons for native resistance. Genotype x year variation had the

largest effect on EU2, and was particularly important for RS and RR. NI was relatively more robust to variation across years, although the 3-way interaction between genotype, treatment, and year was the largest source of explainable variation.

For EU3 there was a significant difference between AI and Trap treatments for RS and RR but not for NI (**Fig. 11**). Genotype and G x T interactions explained between 2% and 12% of the total variation (**Table 5**). The R-squared for the full model including fixed and random effects was highest among all EUs for RR (0.770) in EU3 but lowest for NI (0.190) in EU3. However, the REML residual variance was the largest among all EUs and traits indicating that other sources of variation are unaccounted for in explaining phenotypic variation in EU3 specifically. Although the treatment effect was not significant for NI, the genotypic effect was only significant in the AI treatment and accounted for 28% of phenotypic variation ($F_{15,94} = 2.02$, $P = 0.024$). Two lines, IBMRI MO055 x B101 and IBMRI MO263 x B101, had extensively more node-injury than the other 14 hybrids. These two lines had regression estimates of greater than 0.35 on the node-injury scale, indicating they are particularly susceptible to larval feeding by the WCR. For RS, the treatment effect was very significant ($F_{1,189} = 326.52$, $P < 0.001$), with plants having roots on average 47% smaller in the Trap relative to AI. The trap treatment also had 69% less regrowth than was observed in the AI treatment ($F_{1,189} = 495.23$, $P < 0.001$). For both RS and RR, the treatment effect was the most significant model parameter and explained the greatest proportion of the phenotypic variation: 77% and 82% for the two traits respectively. Interestingly, the genotypic effect on these traits was only significant in the Trap treatment (RS: $F_{15,94} = 2.06$, $P = 0.021$; RR: $F_{15,94} = 2.53$, $P = 0.004$). This again provides more evidence for the idea of resource availability as being a major determinant in root architecture and suggests that genotypic differences between lines become more evident under nutrient-limiting conditions, as would be expected in the corn-on-corn Trap treatment. Additionally, the larger genotypic effect in the AI treatment suggests that for screening node-injury resistance, this treatment is preferred.

For EU4, BC_1 plants grown in the AI treatment had more severe node-injury, but they also had larger sized roots and more root regrowth, consistent with the observation in the other experimental units (**Fig. 11**). A model that fit treatment, BC_1 family (genotype), and BC_1 family x treatment effects explained 26% of the phenotypic variation in NI ($P < 0.001$). Interestingly, as a random effect, genotype did not account for any of the measurable variance, but when treated as a fixed effect, it explained 31% of the variance in NI for the AI treatment ($F_{11,115} = 4.16$, $P <$

0.0001), and 25% of the variance for the Trap treatment ($F_{7,756} = 36.23$, $P < 0.0001$). Thus, differences between genotypic levels, and therefore heritability estimates, were more easily resolved under AI treatment conditions. Heritability estimates for RR was lower in the AI treatment, accounting for 22% and 27% of the variation in regrowth in the two treatments. For RS, a large G x T interaction was detected (**Table 5**) that resulted in a significant genotype effect only in the Trap treatment that explained 18% of the phenotypic variance ($F_{7,756} = 24.52$, $P < 0.001$). This phenomenon of increased genetic variation for NI, and reduced variation for RS and RR that occurred in the AI treatment, provides evidence that controlled infestations provide an advantage in applying uniform rootworm pressure and assigning differences in node-injury to genetic variation. Root architecture, on the other hand, is less controlled by the infestations and more by resource availability.

Associations Among Traits and Accuracy of Trait Predictions

There was a significant correlation detected between the three root traits and the abundance of both adult and larval WCRs with respect to EU1 and EU2. For the AI treatment, a correlation of 0.1683 ($P = 0.206$) existed between larval abundance and NI, whereas the same correlation in the Trap was 0.3904 ($P = 0.048$). For EU2, both treatments had a correlation between NI and mean larval abundance of 0.33, but in EU1 there was a stronger correlation in the Trap treatment ($r = 0.484$, $P = 0.012$) than AI ($r = 0.002$, $P = 0.992$) (**Appendix Table 1**). For a given genotype, greater numbers of recovered larvae tended to correspond with reduced root architecture and more severe node-injury. There was a less consistent relationship between mean adult emergence per genotype and the three root traits due in part to the variation in emergence by year. However, there was a correlation of 0.604 ($P = 0.022$) between NI and total adult emergence by genotype. This provides further evidence that more severe root injury is a result of greater numbers of feeding larvae, rather than more intense feeding done by a smaller number of larvae.

Plant standability (% lodged or goosenecked per row) was typically positively correlated with NI and negatively correlated with RS and RR (**Appendix Table 1**). The strongest associations between NI and lodging approached a level of $r = 0.75$ and occurred for EU1 Trap, EU2 AI, and EU4 Trap and was lowest for EU3 Trap. Looking across all EUs, there was a stronger correlation in the AI treatment ($r = 0.720$, $P < 0.0001$) than in the Trap ($r = 0.424$, $P = 0.0008$) (**Fig. 12**). Thus, the use of lodging is a justifiable way of assessing node-injury and is more accurately assessed in the AI treatment. This can save both time and resources in native

resistance screens, allowing a greater number of genotypes to be evaluated. The other two root traits were also more closely associated with standability in the AI treatment. RS and standability had a -0.81 correlation, while RR and standability had a correlation of -0.76, compared to -0.425 and 0.018 in the Trap for RS and RR, respectively. There was also a positive association between larval abundance and the extent of lodging and goosenecking (**Appendix Table 1**).

Several ear morphology and grain-filling traits were also collected for EU1 and EU2 in both trap and AI treatments to get an estimate of how these traits vary between the treatments. Among the traits listed in **Appendix Table 2**, ear weight, grain weight, cob weight, and ear length had significant differences between treatments. Among these, all except ear length were increased in the AI treatment relative to the Trap. Several genotypic differences between treatments were also observed, but these only occurred for the ear morphology traits and not for the grain weight traits. The fact that yield indicators were greater in the AI treatment provides further support for the idea of greater resource availability, either as a by-product of having larger root systems/compensatory growth, or as a direct effect of resource acquisition.

Discussion

Variation in Larval Densities in Relation to WCR Population Dynamics

When larval abundance was assessed in Trap and AI treatments, we found considerably more larvae present in the AI treatment. This held true for both the mean larval abundance across all genotypes as well as for individual genotype tests between treatments (**Fig. 3 and 4**). The abundance pattern under artificial infestation followed a left-skewed pattern whereby most larvae were recovered in the first two sampling dates and decreased over time (**Fig. 2**). Conversely, the abundance profile in the Trap was more temporally uniform, with lower densities. This confirms that larval populations in the AI treatment are in closer developmental agreement and progressed through life stages at about the same time, largely because of the uniformity in soil depth that occurs from in artifical infestations (Onstad et al. 2006). This has implications on screening for root damage, which is usually assessed at the time of peak larval abundance (Branson and Sutter 1989). The abundance pattern is consistent with what is known about WCR population dynamics. The developmental threshold for the WCR is 11 ºC, so egg hatching had already started at the point when infestations took place, but development can vary depending on soil depth, temperature, and moisture (Fisher et al. 1991, Levine, Oloumi-Sadeghi and Ellis 1992). Egg hatch occurs over a time span lasting approximately 30 days in the US Corn Belt beginning

around the 1st week of June and reaching 50% hatch at about 400 degree days (Levine, Oloumi-Sadeghi and Ellis 1992, Musick and Fairchild 1971). For instance, Hibbard et al (2003) reported a sharp decrease in larval abundance between 16 June and 3 July. This is in agreement with the pattern of larval abundance observed in this experiment which peaked between 23 June and 27 June. The tighter hatching interval under AI conditions suggests this treatment is better at applying uniform pressure and for accurately predicting the peak in larval abundance in screening for node-injury damage.

We detected similar patterns in larval abundance between trap and non-infested plants. Hence, their did not appear to be an ovipositional preference among gravid females from the previous year, even though the Trap treatment was planted several weeks later. Given that WCR adults can survive about 60 days and travel up to 6-17 m/day it is not surprising that there would be some egg laying outside the designated Trap boundary (Branson and Johnson 1973, Hill 1975, Spencer et al. 2009). The use of aritificial infestation adds more larvae to the natural population, which would result in the greater larval densities recovered. This is further supported by the observation that the larvae recovered in the Trap treatment were of variable size and instar stage, and natural WCR poplations tend to be charatistically more variable, with clumped distributions (Branson 1986, Branson and Sutter 1989).

There was a stronger association between genotype and larval abundance within the Trap treatment. One explanation for this is that at lower densities of larvae, the differences between genotypes can be more easily resolved. A more probable explanation is that in the AI treatment, the number of larvae injected into each plant is consistent. Thus, there should be fewer differences between the genotypes, especially if antibiosis is not the main defense mechanism controlling the herbivory response, which is usually not the predominat mechanism (Gray et al. 2009, Riedell and Evenson 1993). Of the reported cases of resistance among the germplasm used in this study, NGSDCRW1(S2)C4 was shown to be tolerant to WCR larval feeding, and only one genotype, CRW8-1 was reported to have reduced larval feeding (Hibbard, Darrah and Barry 1999, Kahler et al. 1985, Prischmann, Dashiell and Hibbard 2009, Prischmann, Dashiell, Schneider and Hibbard 2007, Russell et al. 1976). So the antibiosis explanation seems less likely. In the Trap, the larvae can persist for longer periods in the soil, lending to a greater opportunity for interplant larvae mobility, which if manifested in a preferrential way, would result in differences between genotypes (Onstad et al. 2006). Larvae have been documented to travel as

much as 100 cm in the soil before pupation, so larval preferences can result in differences in local abundance (Hibbard et al. 2003, Short and Luedtke 1970, Suttle, Musick and Fairchil 1967). The fact that the G x T interaction was not significant provides evidence that relative larval densities were fairly consistent between the two treatments. Additionally, antixenosis has been reported as a mechanism of resistance to the WCR (Bernklau et al. 2010). Furthermore, we have confirmed the role of host-plant genotype in regulating WCR larval densities, a finding consistent with other reports in (Bernklau, Hibbard and Bjostad 2010, El Khishen et al. 2009)

Adult Population Dynamics in AI and Trap Treatments

Although larval numbers were significantly higher for the AI treatment, this difference did not manifest in terms of mean adult emergence (**Fig. 5**). This suggests that a greater proportion of larvae survived to adulthood in the Trap than in AI, possibly because due to a density-dependent effect or some other treatment-specific condition. We further confirmed this phenomon, and showed that fewer adults emerged at particularly high larval densities in the AI treatment. Density-dependent mortality has been reported by several others to be an important mechanism behind larva-to-adult surviviorship (Hibbard et al. 2010, Onstad et al. 2006). Combining data from multiple years and across published reports, Hibbard et al. (2010) concluded that density-dependent mortality begins at around 800 eggs per 30.5 cm, which would be consistent with the ~750 eggs/plant used in this study. We also observed similar survivorship ratios from egg and larvae to adulthood, albiet lower in both cases (**Table 3**). One explanation for the lower survivorship reported here could be due to the method of assessing larval abundance, which only captured a fraction of the larvae feeding on any given plant.

Other lines of evidence were also detected that point to density-dependent mortality contributing to rootworm population dynamics. Fewer numbers of larvae were subsisting on plants grown in the Trap treatment, and there was less root node-injury, so these plants would be a good food source for other root-feeding insects seeking to minimize competition. The fact that a positive interaction was detected between NCR and WCR adult emergence only in Trap treatment provides evidence that feral NCR larvae were able to take advantage of this opportunity. Under the same egg densities, NCRs usually have lower survivorship than WCRs, so given that we detected the interaction at the adult stage may indicate an even stronger interaction at the root interface where larval feeding occurs (Onstad et al. 2006). This interspecific interaction is supported by the findings that NCR survivorship is more affected by

interspecific competition whereas for the WCR, intraspecific competition is more important (Woodson 1994). Roots supporting larger WCR larval numbers as was seen in the AI treatment would be a less ideal food source for newly hatched NCR larvae, and therefore movement to plants supporting lower population densities would favor their survivorship.

Because of the varition that exists across emergence cages, total emergence was a less accurate measure of rootworm pressure than mean rootworm emergence. By averaging across samples for a genotype, treatment, or year, individual sample variances have less influence on the total emergence variation and thus more power is achieved. Even so, the correlation between emergence in 2010 and 2011 was twice as high for the AI treatment than for the Trap, indicating that when total emergence is desired, the AI treatment should be the preferred method. This is futher justified by finding insignificant treatment and year effects on mean emergence and a highly significant genotype contribution. The type of germplasm being screened for native resistance is also an important indicator of total emergence in our study. Among the four significant genotype x treatment comparisons, all were members of EU2, suggesting that hybrids respond differently to rootworm pressure than isoline populations (**Fig. 8**). This would be supported by the findings from Flint-Garcia et al. (2009), that WCR larval damage ratings among a set of inbreds were not correlated with their hybrid values when crossed to the inbred B73. Our results would indicate that the differences between line *per se* and hybrid node-injury may be due more to larval preference than than to actual differences in host-plant resistance, which has implications for screening native resistance and developing new sources of germplasm with putative resistance mechanisms.

Theoretically, adult emergence should be reflective of the larval abundance, albiet many factors can influence mortality between larval feeding and adult emergence, and also the accuracy of capturing the representative numbers of individuals per sampling. Our data shows that larval abundance is more predictive of adult emergence under AI treatment conditions, and that the relationship was particularly strong for EU1 (**Fig. 9**). This is likely due to the reduction in AI of the environmental/treatment variance inherently biased in naturally-infested rootworm fields. We observed multiple lines of evidence for density-dependent mortality, and identified an interaction between WCR and NCR emergence. Our data also suggests that differences in WCR dynamics exists between isoline and hybrid populations, whereby at higher larval numbers, fewer adults emerged for hybrids; whereas, for isolines, higher larval numbers translated into

higher adult emergence. This again appears to be a sign of density-dependent mortality among the greater numbers of competing larvae on hybrid roots versus their isogenic parents. Information of this nature will be helpful in building upon current models of WCR poulation dyanmics and can be used to make improvements in the efficiency and accuracy of resistance screening.

Treatment Effects Node-Injury, Environment Effects Root Morphology

There was a clear and evident difference in root phenology between plants cultivated in the AI treatment and those in the Trap. Plants in the AI treatment had more severe node-injury, larger sized roots, and more extensive root regrowth than their counterparts in the Trap and this was consistent across all EUs (**Fig. 11**). However, the magnitude of the treatment effect was dependent on experimental unit. For the isolines in EU1, variation attributed to year, treatment, and there interactions explained three times the variation explained by these factors in EU2. EU3 and EU4 responded in a different way to the treatments than did EU1 and EU2, having less severe node-injury and greater differences between RS and RR. One possible hypothesis to explain this phenomenon is that the treatment differences on root achetecture are exasperated on the larger-sized roots of hybrids making up EU3 and EU4. However, one line of evidence that points against this hypothesis is the greater difference between AI and Trap in 2010 for EU2, which also is comprised of a set of hybrid genotypes. Thus, it appears that the differences in root archectecture that were observed in 2010 were largely due to the inherrant differences between the two fields included in the 2010 evaluations, rather than a true treatment difference. Athough it still should be noted that significantly more regrowth occurred in AI in 2011, so this trait is at least partially influenced by the treatment itself, whereas root size is more environmentally controlled. This is further supported by the fact that only a moderate difference in NI was detected between treatments in EU4, and no treatment difference detected for EU3, so it wasn't node-injury accounting for the root archetecture changes, but rather the differences between the two fields. This also indicates that node-injury is less vulnerable to changes across different environments and across years.

NI was more predictive of RS and RR in the AI treatment and the correlation was stronger for RR (**Table 4**). This is expected, given that regrowth is a measure of how much secondary root growth is present following nodal damage, but serves as a further line of evidence that the AI treatment is better at capturing this physiological connection. This is futher supported by the

WCR abundance data, which showed significantly more larval abundance in the AI treatment, resulting in more severe node-injury. Selecting on root archetecture has been successfully achieved as a means of generating tolerant germplasm (Branson 1986, Gray et al. 2009, Jenison et al. 1981, Owens et al. 1974). This data indicates the accuracy of the selection may be improved under AI conditions. This may be especially important given that selection on these trait can help to preserve the yield potential of injured plants (Spike and Tollefson 1989). In the Trap, factors other than rootworm feeding are influencing root archectecture to a greater extent than in AI. Additionally, the moderate correlation between RR/RS and NI even under controlled infestations indicates that both traits should be collected if extrapolations between root damage and root archetecture are to be explored.

Not only were the root architecture traits more predictive of node-injury in the AI treatment, but they were consistently elevated under AI pressures. This has implications for resistance screening, and should be a consideration when deciphering resistance mechanisms. For instance, if tolerance is a direct consequence of larval feeding, then screening in the AI treatment may be better able to capture the genetic variation. However, if tolerance is more a result of resource allocation/ultilization, then the varying larval densities in trap crops may have less influence on root architecture. The consistent treatment effect on root phenology also means that genotypic relationships should be relatively stable across both treatments, even if mean trait values are significantly different.

The elevated tolerance response that was detected in the AI treatment did have an effect on yeild related traits, namely ear weight, grain weight, and cob weight. These yeild components were significantly higher in the AI treatment even though more larvae were recovered and more severe node-injury was observed. Differences in yeild were not a result of soil composition or nutrient avialability, given that these traits were collected in 2011 (the year in which both treatments were cultivated in the same corn-on-corn location). Rather, it appears that the elevated root growth was a tolerance response to increased larval feeding pressure, which helped to preserve the yeild potential. This relationship between tolerance and yield is not always observed (Gray and Steffey 1998), so perhaps it is the stronger pressures applied in the AI treatment that results in a more profound tolerance response. Additonal research in this area will help to better understand environmnetal factors that influence tolerance and its effect on yeild.

The relative effect of the interaction between genotype (G), year (Y), and treatment (T) on root phenology is highly dependent on what type of germplasm is being screened, consistent with the findings of Flint-Garcia et al. (2009). Isolines in our study had more variation controlled by genetic effects while the hybrid populations had more variation controlled by interactions and residual effects. We postulate that the larger sized-roots of hybrids are a product of multiple sources of biotic and abiotic inputs, and that smaller-sized roots have had fewer inputs, and thus a greater amount of their variation is controlled by genotypic effects. Our models also show that NI is more robust to interactions between genotype and environment, and is particularly robust to variation across years. T x Y interactions explained only a negeligable portion of the total variation for all three traits (**Table 5**).

Another point of evidence that provides validity to the root phenotypes used in this study is their correlation with WCR larval abundance. Genotypes that had more larvae recovered also generally had reduced root size and regrowth, and more severe node-injury, providing additional support that node-injury reflects the abundance of feeding larvae rather than feeding intensity. A strong correlation was detected between the three root traits and plant standability, with lodging and goosenecking postively correlated with NI, and negetively correlated the root architecture traits. The association with node-injury was stronger in the AI treatment than in the Trap (**Fig. 12**). Plant standability, therefore, can be an accurate alternative to the laborous process of digging and collecting root phenotypes for screening of native resistance to the WCR, and appears to be particularly accurate under controlled infestations. Root lodging has been reported to be an efficient and effective method of evaluating rootworm pressure in the past, although because of its vulnerability to environmental variation, the method should be repeated at mulitiple locations (Branson and Sutter 1989, Rogers et al. 1977). Because of the controlled nature of artificial infestations, some of this environmental variance is likely reduced, allowing for a more accurate assessment of node-injury damage. Although, phenotyping lodging alone can result in missing of genotypes that possess antibiosis resistance mechanisms. This is because lodging is only an indirect measure of tolerance to larval feeding, and should be a consideration in resistance screens (Gray et al. 2009).

This study has yielded a detailed experimental comparison between Trap and AI treatments for screening native resistance to corn rootworm larval feeding. Both methods have been effectively used to apply rootworm pressure in experimental settings, but we demonstrate that

artifical infestation has the advantage of controlling environmental variance inherantly associated with trap-cropping. This can translate into improvements in resistance screening and allow for more accurate predictions between traits. Nevertheless, trap treatments do save time associated with preparing and mannually injecting eggs, and may be a better predicate to natural WCR populations. Here, we have offered several suggestions and considerations when conducting field resistance screens to the WCR. This information is of benefit to other researchers working in this field and should help to improve models of native resistance and validation of results across studies.

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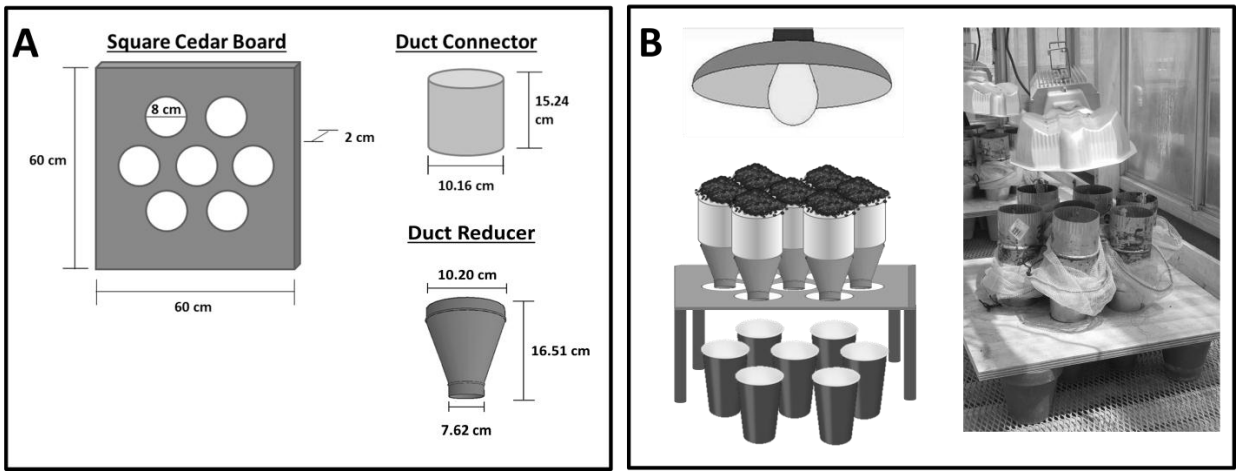

Figure 1. Equipment used to collect soil samples for measuring larval abundance.
A) Diagrammatic view and dimensions of the 3 main components of Berlese funnels used in the experiment. B) Fully assembled unit shown as a diagram (left) and a photo of the actual funnel system (right).

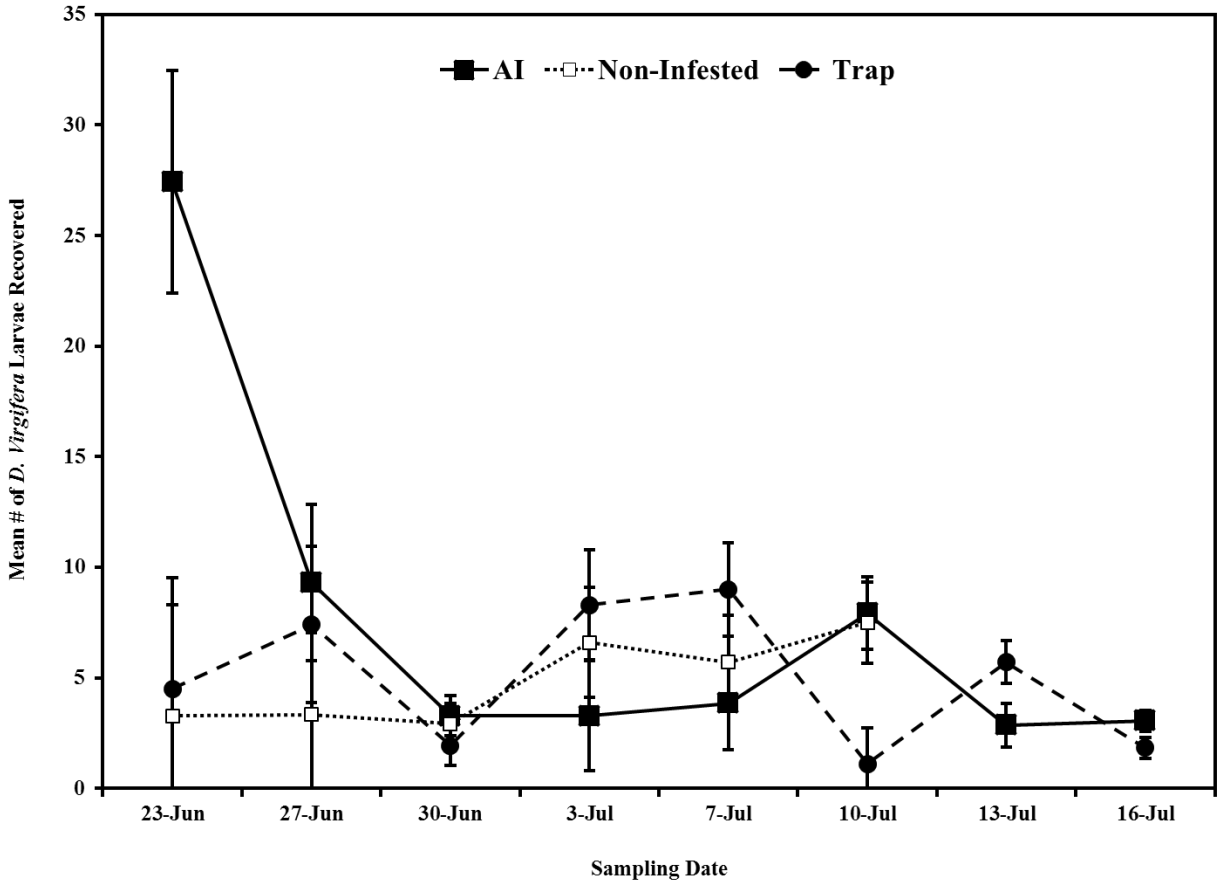

Figure 2. Mean number of larvae recovered from Berlese samples across 8 sampling dates in 2011 for artificial infestation, trap crop, and non-infested treatments. Error bars show the standard error of the mean for each date by treatment combination. Seven samples were collected for each treatment group on each sampling date.

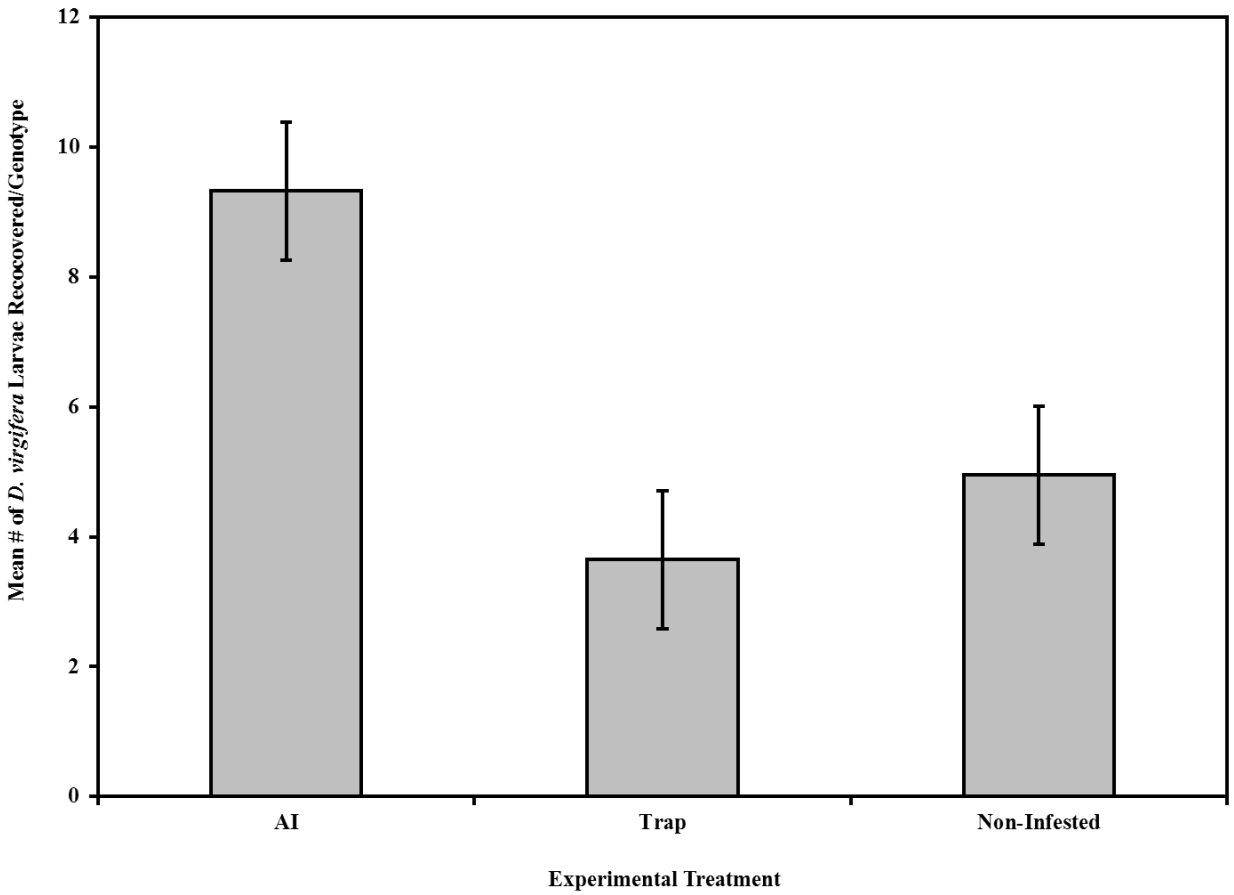

Figure 3. Mean larval recovery per genotype among artificial infestation, trap crop, and non-infested treatments. Each treatment mean is calculated from among 24 genotypes sampled between three and eight times in each treatment group during the summer of 2011. Each set of genotypes sampled on a given date in one treatment were the same set sampled in the other treatment groups on that date (Figure 2). Error bars show the standard error of the mean.

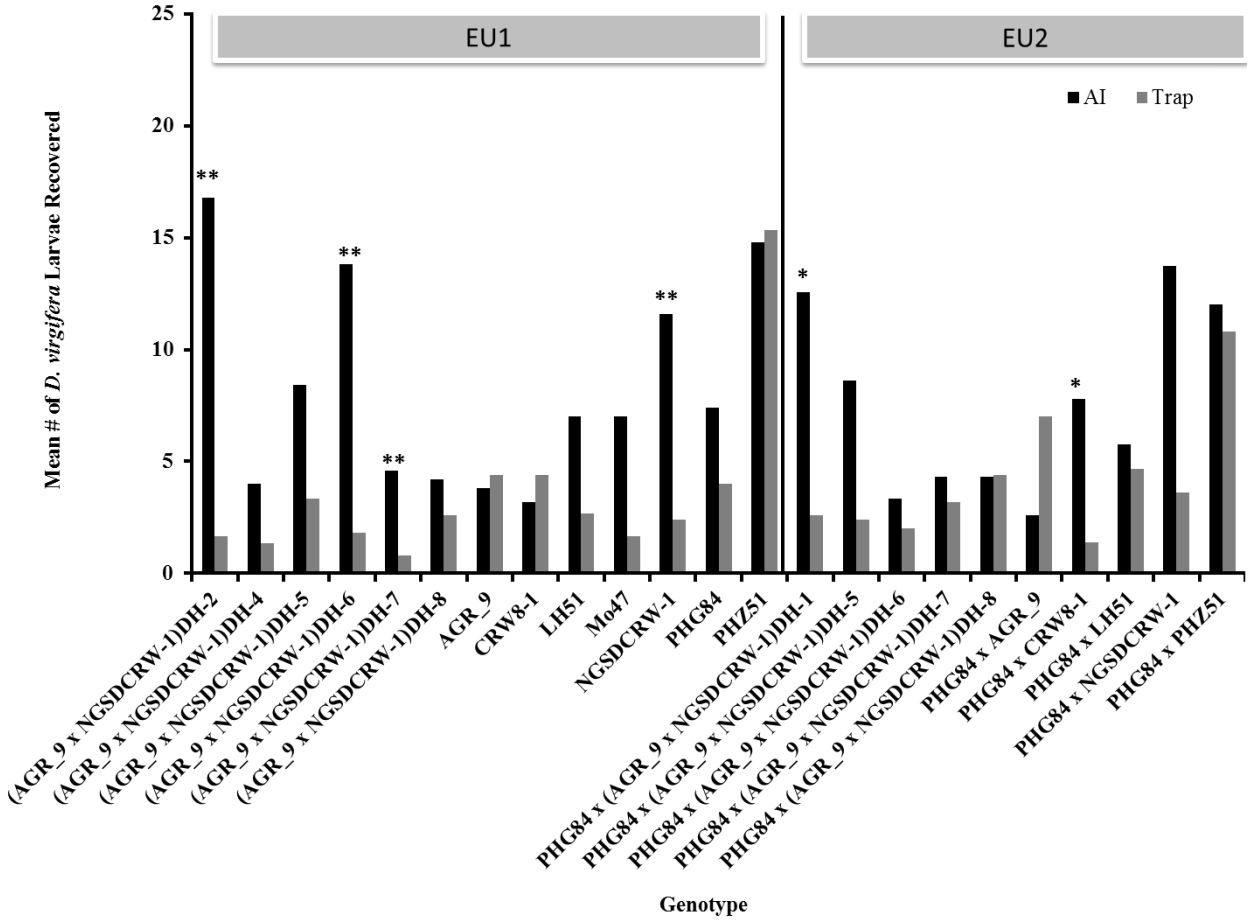

Figure 4. Mean number of western corn rootworm larvae recovered per treatment from 13 isoline genotypes (EU1) and 10 hybrid genotypes (EU2) in 2011. Each genotype was sampled at least three times in each treatment. Asterisks denote significant differences in paired t-tests between Trap and artificial infestation treatments for a given genotype. EU = experimental unit. * $P \leq 0.10$, ** $P \leq 0.05$

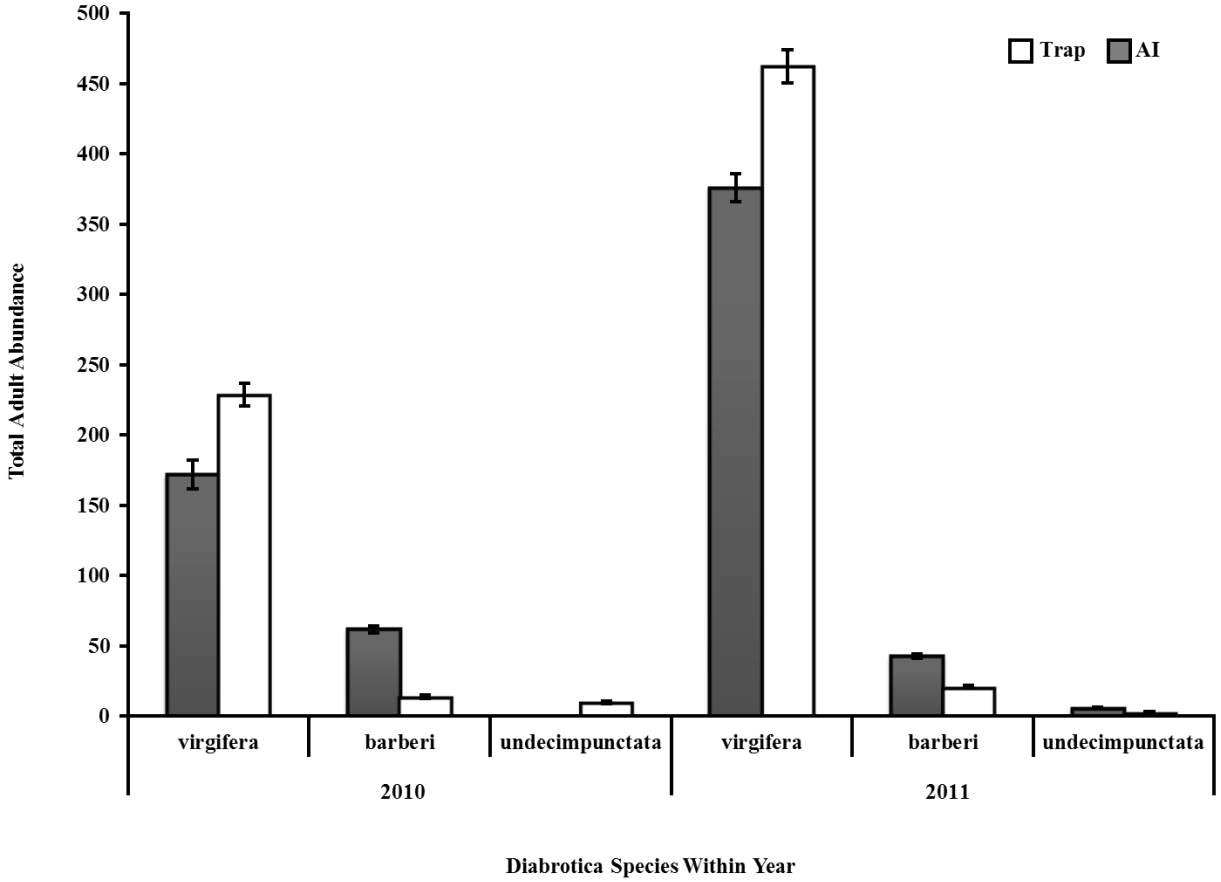

Figure 5. Mean adult abundance per sample of three *Diabrotica* species for both artificial infestation and trap crop treatments replicated across two summers. Each sample is defined as a single cage per time point. Error bars show the standard error of the mean.

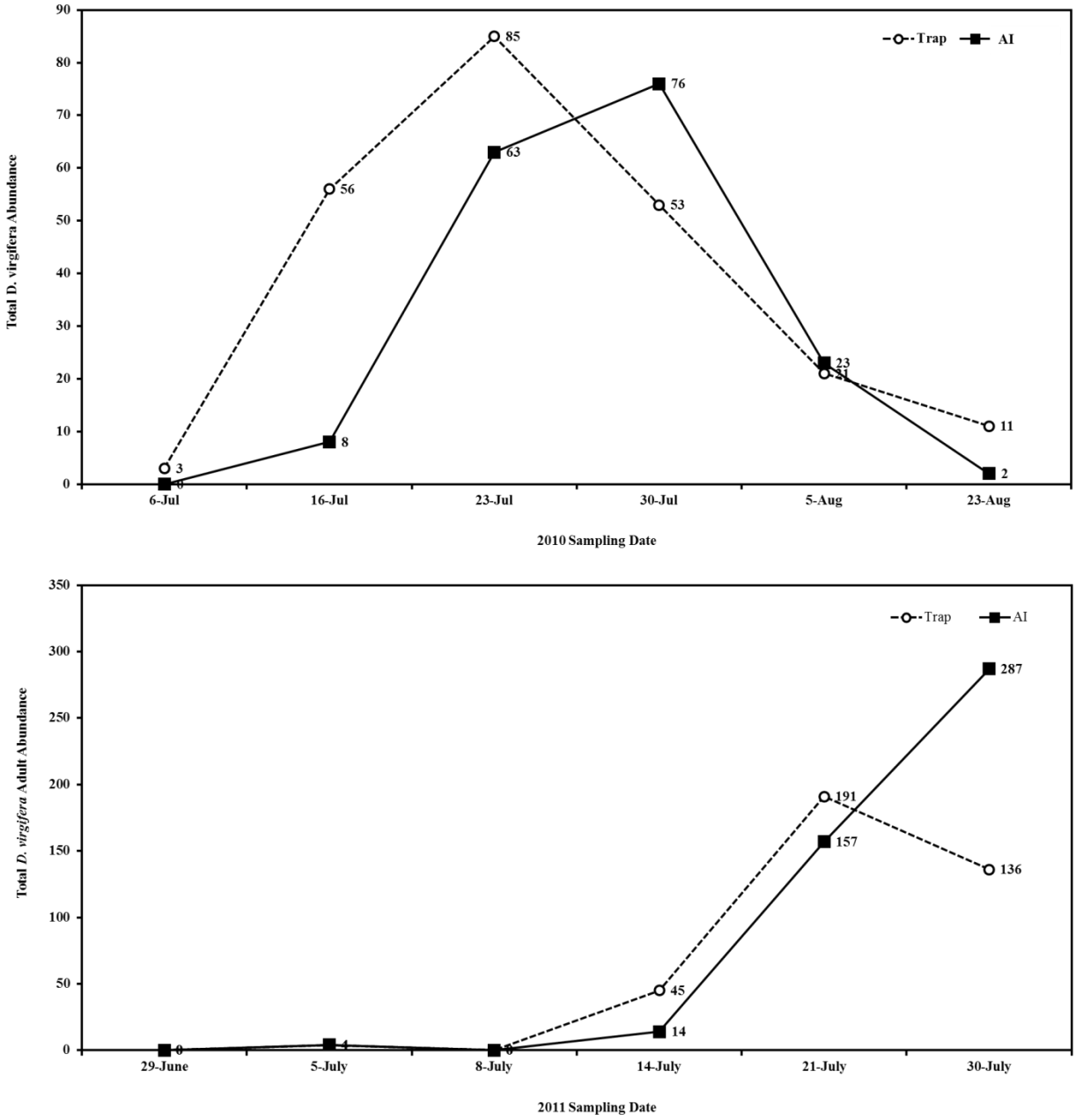

Figure 6. Total *D. virgifera virgifera* adult beetle abundance collected on 6 sampling dates in 2010 and 2011. The number of emergence cages per treatment in 2010 was 32, and in 2011 the number was 44. Because of differences in planting and infestation dates between 2010 and 2011 different dates were chosen for sampling and sampling started one week earlier in 2011.

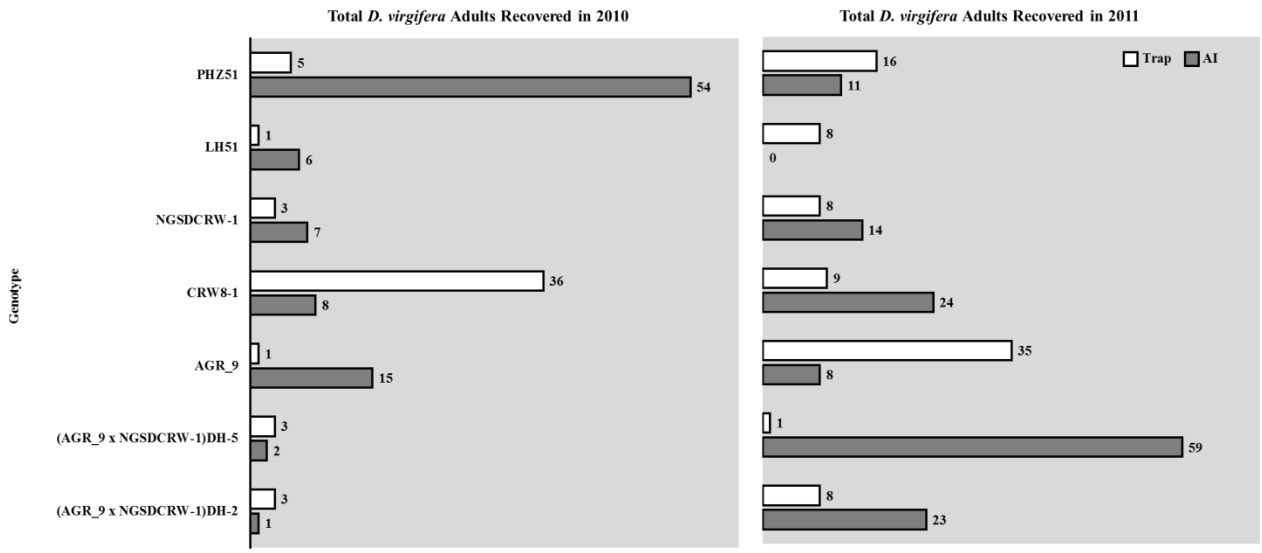

Figure 7. Total *D. virgifera* adults collected from the seven genotypes from EU1 grown in trap crop and artificial infestation treatments over the course of two summers (left: 2010, right: 2011). Each genotype had two cages per treatment, and each cage was sampled 6 times each summer.

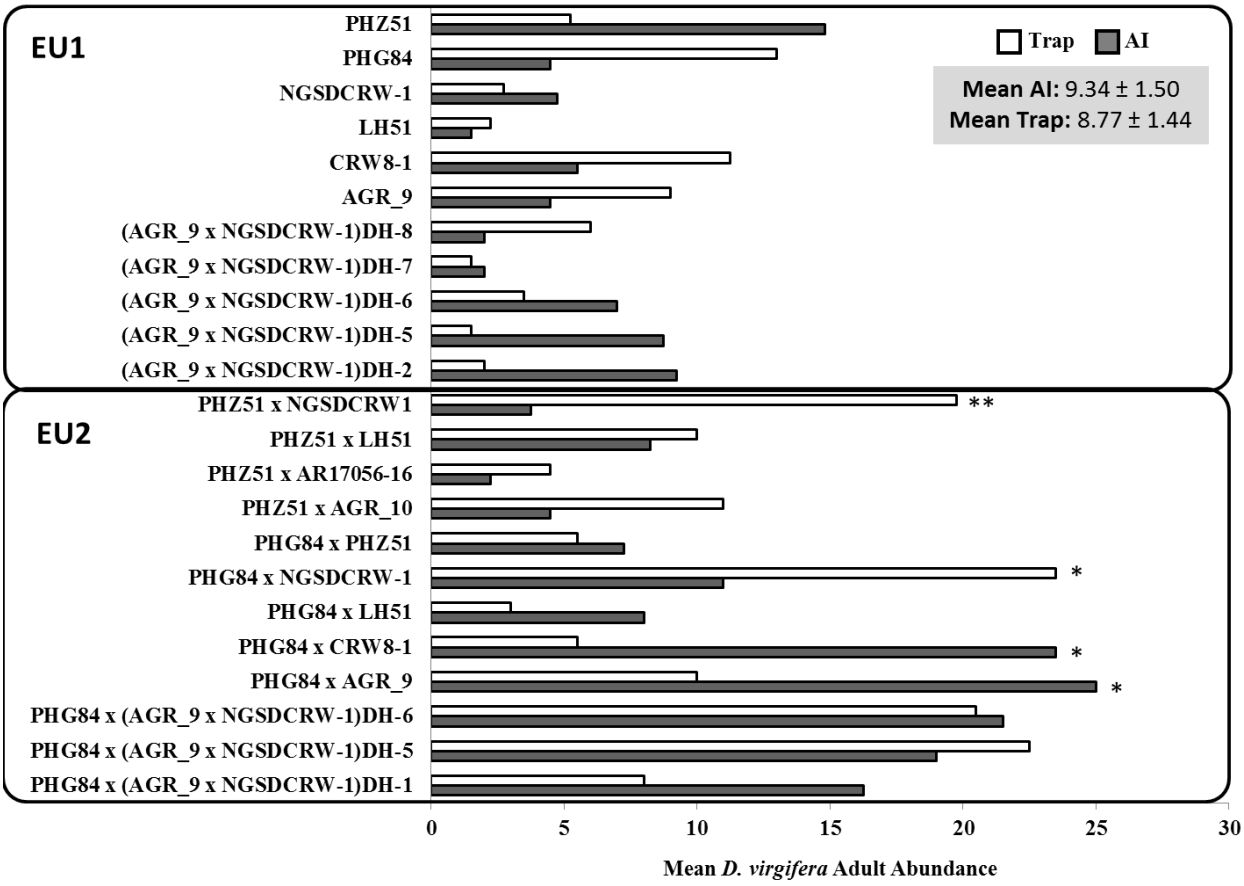

Figure 8. Mean number of adult *D. virgifera* beetles emerged per genotype in trap crop and artificial infestation treatments. Depending on whether or not the genotype was included in both 2010 and 2011, there were either 2 or 4 cages per genotype-treatment. EU = experimental unit. * $P \leq 0.05$, ** $P \leq 0.01$

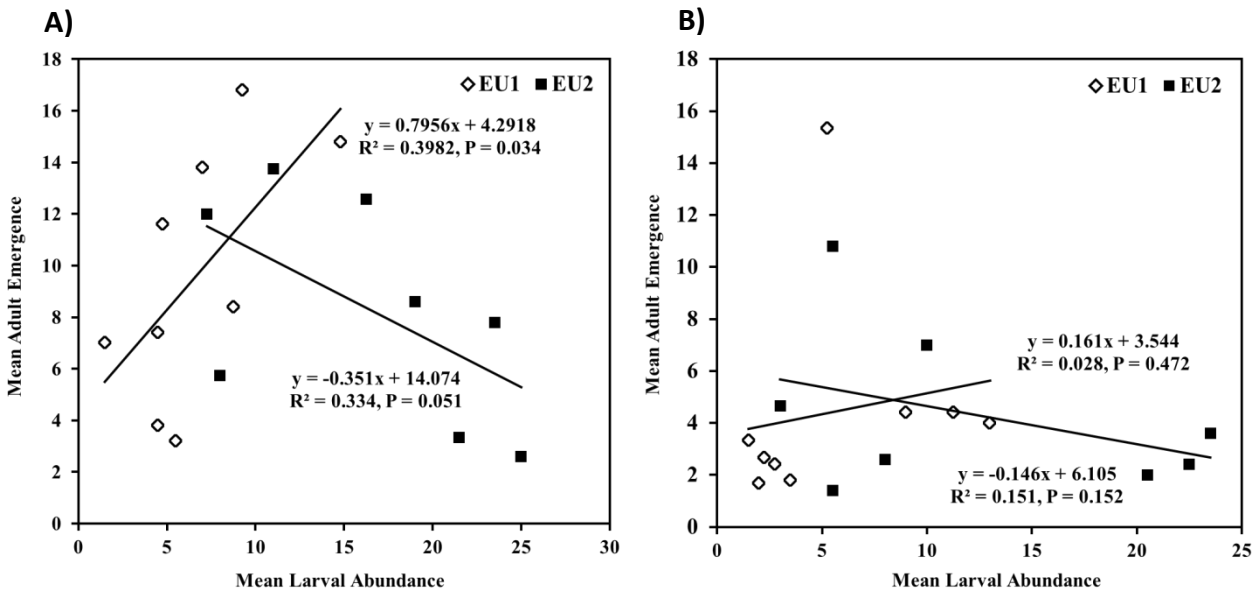

Figure 9. Linear relationship between mean larval abundance and mean adult emergence for isolines in EU1 and for hybrids in EU2 grown under artificial infestation (A) and trap crop (B) treatments. Each mean was calculated by summing the total beetles per cage and taking the average among cages for a particular genotype. Depending on whether the genotype was included in both 2010 and 2011, there were either 2 or 4 cages per genotype-treatment. EU = experimental unit.

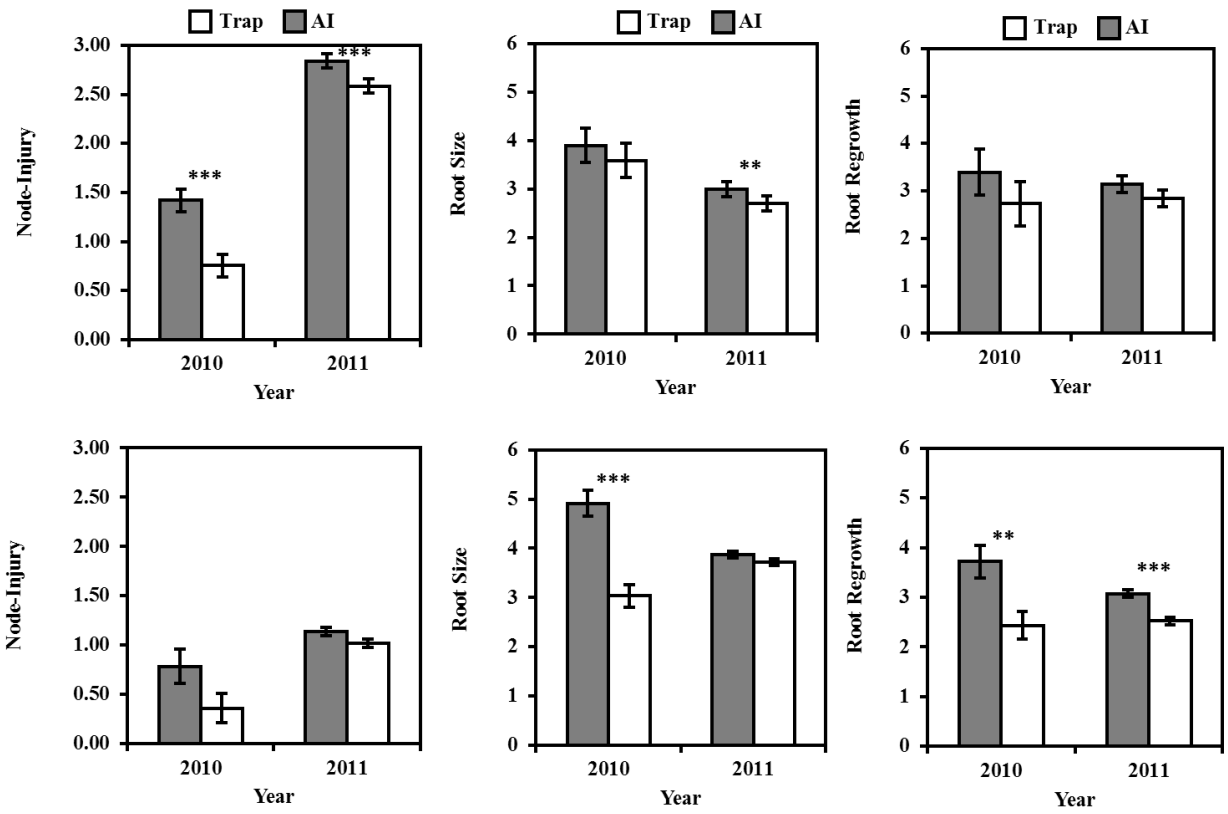

Figure 10. Average root phenotypes for EU1 (top panel) and EU2 (bottom panel) per year and treatment for node-injury (left), root size (center), and root regrowth (right). Each average is calculated from the mean genotype ratings for each treatment within year. This was an average of among 12 isolines in 2010 and 14 in 2011. Error bars show the standard error of the mean. Asterisks denote significant differences between AI and Trap treatments.* $P \leq 0.005$, ** $P \leq 0.0005$.

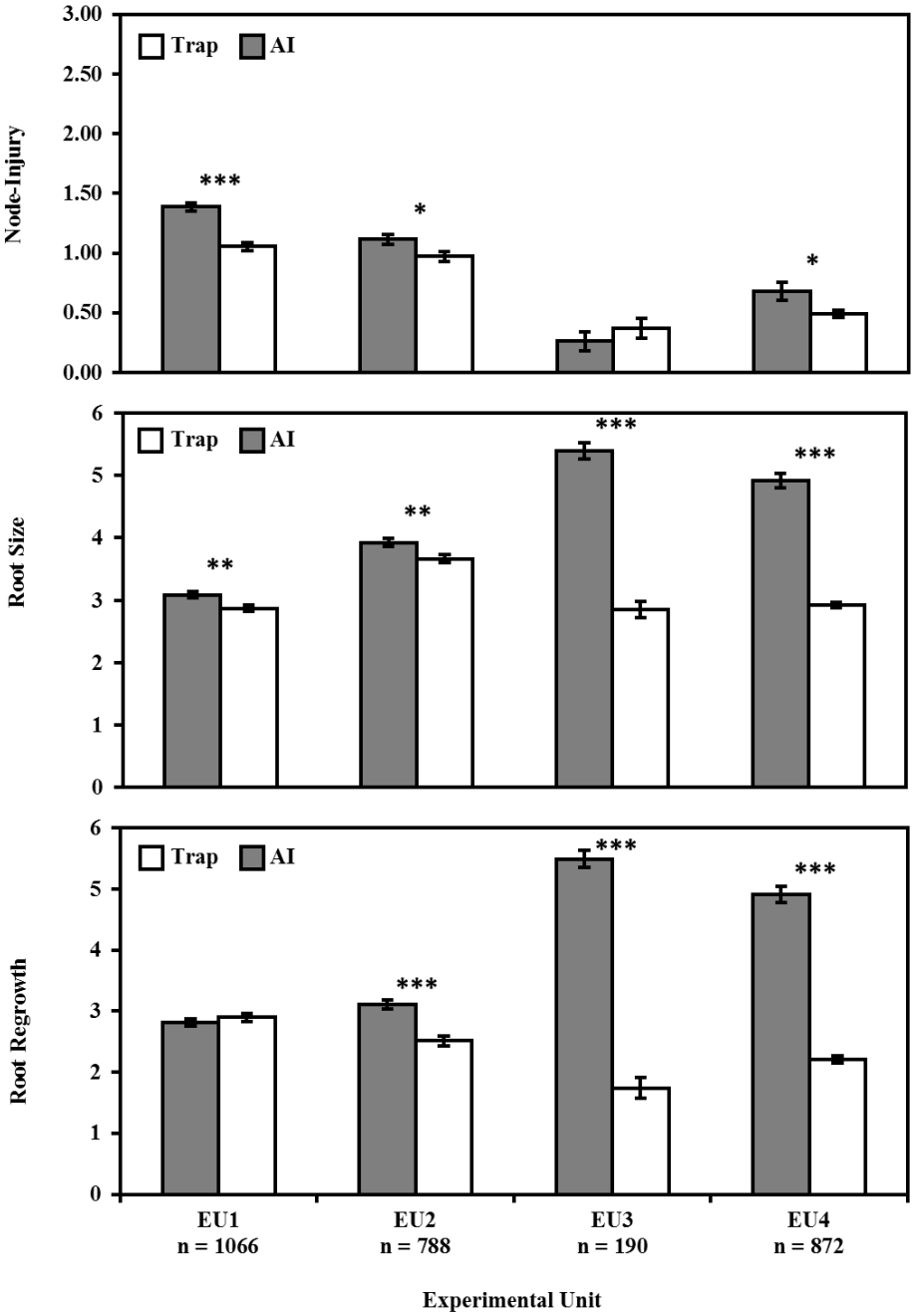

Figure 11. Average node-injury (top), root size (center), and root regrowth (bottom) for artificial infestation and Trap treatments across 4 EUs. Each average is calculated from the mean across *n* plants in an EU and Student's t-tests were used to identify significant treatment differences. Asterisks denote significant differences between AI and Trap treatments.* $P \leq 0.05$, ** $P \leq 0.005$, ** $P \leq 0.0005$.

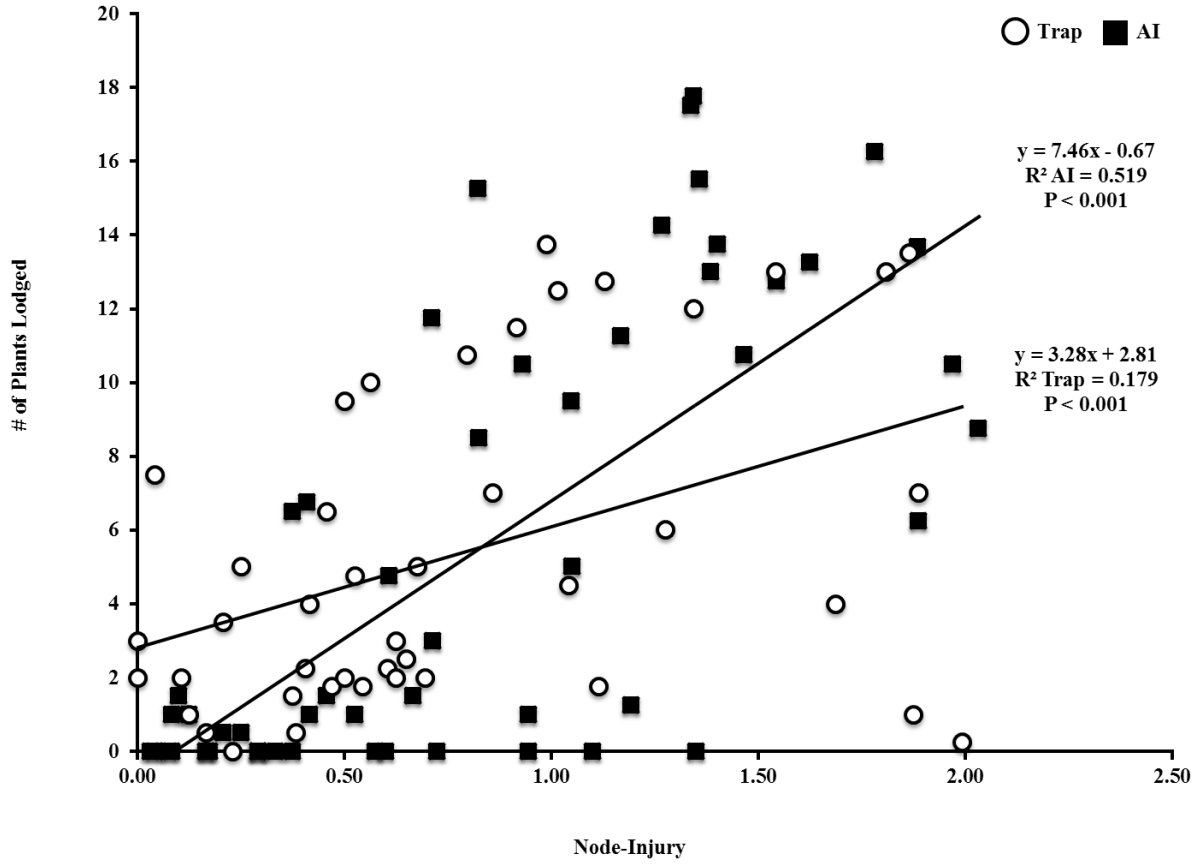

Figure 12. Plot of the relationship between lodging and node-injury under artificial infestation and trap crop treatments across all experimental units. Lodging values are reported as the number of plants per plot displaying either stalk or root lodging and compared to the mean node-injury among the eight plants in the corresponding plot. The accuracy of prediction is reported as the proportion of phenotypic variance explained (R^2).

Table 1. Background information on germplasm sources for experimental units assigned to Trap and Artificial Infestation field treatments during the summer of 2010 and 2011.

Germplasm	Source[†]	Type of Cultivar	Resistance Level[††]	Relevant Publications
EU1				
(AGR_9 x NGSDCRW-1)DH1	AgReliant Genetics	DH	M	none
(AGR_9 x NGSDCRW-1)DH2	AgReliant Genetics	DH	M	none
(AGR_9 x NGSDCRW-1)DH4	AgReliant Genetics	DH	M	none
(AGR_9 x NGSDCRW-1)DH5	AgReliant Genetics	DH	M	none
(AGR_9 x NGSDCRW-1)DH6	AgReliant Genetics	DH	M	none
(AGR_9 x NGSDCRW-1)DH7	AgReliant Genetics	DH	M	none
(AGR_9 x NGSDCRW-1)DH8	AgReliant Genetics	DH	M	none
AGR_9	AgReliant Genetics	SYN	H	none
NGSDCRW1(S2)C4	NCRPI, USDA-ARS, SDSU AES	SYN	M	Kahler et al., 1985; Hibbard et al., 1999; Prischmann et al., 2007; Prischmann, Dashiell, & Hibbard, 2009
CRW8-1	USDA-ARS, Columbia, MO	SYN	H	Russell et al., 1976; Hibbard et al., 1999; Hibbard et al., 2007; Prischmann et al., 2007; Preschmann, Dashiell, & Hibbard, 2009
LH51 (PVPA 8200062)	Holden's Foundation [a]	ExPVP	L	Mikel, 2006
PHZ51 (PVPA 8600132)	Pioneer Hi-Bred International	ExPVP	L	Mikel, 2006
PHG84 (PVPA 8600130)	Pioneer Hi-Bred International	ExPVP	L	Smith et al., 1997; Mikel & Dudley, 2006; Mikel, 2006; Kumar et al., 2012
EU2				
PHZ51 x AGR10	AgReliant Genetics	HYB	H	none
PHZ51 x LH51	AgReliant Genetics	HYB	L	none
PHZ51 x AR17056-16	AgReliant Genetics	HYB	H	none
PHZ51 x NGSDCRW1	AgReliant Genetics	HYB	H	none
PHG84 x PHZ51	Lauter/Hessel	HYB	NC	none
PHG84 x NGSDCRW-1	Lauter/Hessel	HYB	NC	none
PHG84 x LH51	Lauter/Hessel	HYB	NC	none
PHG84 x CRW8-1	Lauter/Hessel	HYB	NC	none
PHG84 x AGR_9	Lauter/Hessel	HYB	NC	none
PHG84 x (AGR_9 x NGSDCRW-1)DH-6	Lauter/Hessel	HYB	NC	none
PHG84 x (AGR_9 x NGSDCRW-1)DH-5	Lauter/Hessel	HYB	NC	none
PHG84 x (AGR_9 x NGSDCRW-1)DH-1	Lauter/Hessel	HYB	NC	none

(**Table 1.** Continued)

Germplasm	Source[†]	Type of Cultivar	Resistance Level[††]	Relevant Publications
EU3				
IBMRI MO010 x B101	Lauter/Scott	HYB	NC	Hallauer & Wright, 1995; Lee et al., 2002
IBMRI MO018 x B101	Lauter/Scott	HYB	NC	Hallauer & Wright, 1995; Lee et al., 2002
IBMRI MO038 x B101	Lauter/Scott	HYB	NC	Hallauer & Wright, 1995; Lee et al., 2002
IBMRI MO055 x B101	Lauter/Scott	HYB	NC	Hallauer & Wright, 1995; Lee et al., 2002
IBMRI MO121 x B101	Lauter/Scott	HYB	NC	Hallauer & Wright, 1995; Lee et al., 2002
IBMRI MO145 x B101	Lauter/Scott	HYB	NC	Hallauer & Wright, 1995; Lee et al., 2002
IBMRI MO186 x B101	Lauter/Scott	HYB	NC	Hallauer & Wright, 1995; Lee et al., 2002
IBMRI MO209 x B101	Lauter/Scott	HYB	NC	Hallauer & Wright, 1995; Lee et al., 2002
IBMRI MO237 x B101	Lauter/Scott	HYB	NC	Hallauer & Wright, 1995; Lee et al., 2002
IBMRI MO263 x B101	Lauter/Scott	HYB	NC	Hallauer & Wright, 1995; Lee et al., 2002
IBMRI MO276 x B101	Lauter/Scott	HYB	NC	Hallauer & Wright, 1995; Lee et al., 2002
IBMRI MO279 x B101	Lauter/Scott	HYB	NC	Hallauer & Wright, 1995; Lee et al., 2002
IBMRI MO282 x B101	Lauter/Scott	HYB	NC	Hallauer & Wright, 1995; Lee et al., 2002
IBMRI MO296 x B101	Lauter/Scott	HYB	NC	Hallauer & Wright, 1995; Lee et al., 2002
IBMRI MO297 x B101	Lauter/Scott	HYB	NC	Hallauer & Wright, 1995; Lee et al., 2002
IBMRI MO357 x B101	Lauter/Scott	HYB	NC	Hallauer & Wright, 1995; Lee et al., 2002
EU4				
[B86 x (FS8-053 x B86)]S1B1 (45413)	Lauter/Hessel	BC1	SEG	Russell et al., 1974; Horner, 1990; Pollak, 2003
[B86 x (FS8-053 x B86)]S1B2 (45414)	Lauter/Hessel	BC1	SEG	Russell et al., 1974; Horner, 1990; Pollak, 2003
[B86 x (FS8-053 x B86)]S1B3 (45415)	Lauter/Hessel	BC1	SEG	Russell et al., 1974; Horner, 1990; Pollak, 2003
[B86 x (FS8-053 x B86)]S1B4 (45416)	Lauter/Hessel	BC1	SEG	Russell et al., 1974; Horner, 1990; Pollak, 2003
[B86 x (FS8-053 x B86)]S1B5 (45417)	Lauter/Hessel	BC1	SEG	Russell et al., 1974; Horner, 1990; Pollak, 2003
[B86 x (FS8-053 x B86)]S2B1 (45452)	Lauter/Hessel	BC1	SEG	Russell et al., 1974; Horner, 1990; Pollak, 2003
[B86 x (FS8-053 x B86)]S2B2 (45453)	Lauter/Hessel	BC1	SEG	Russell et al., 1974; Horner, 1990; Pollak, 2003
[B86 x (FS8-053 x B86)]S2B3 (45454)	Lauter/Hessel	BC1	SEG	Russell et al., 1974; Horner, 1990; Pollak, 2003

[†]Source that developed the germplasm.
[††]Acquired by Monsanto Corporation.
EU, experimental units; NCRPI, North Central Regional Plant Introduction Station ; USDA-ARS, United States Department of Agriculture-Agricultural Research Service; SDSU AES, South Dakota State University, Agricultural Experiment Station.
DH, doubled haploid line; SYN, synthetic variety; ExPVP, expired Plant Variety Protection; HYB, maize hybrid; BC, backcross; NC, not yet characterized; SEG, segregating.

Table 2. Analysis of variance table for a model with *D. virgifera* adult beetle emergence as the response to three main effect terms and three interaction terms.

Model Effect	DF	SS	MS	F-value	Pr > F
Year	1	0.00	0.01	0.00	0.978
Treatment	1	0.50	0.50	0.04	0.834
Genotype	26	656.20	25.24	2.21	0.001
Treatment x Year	1	45.20	45.19	3.96	0.047
Genotype x Year	6	114.90	19.16	1.68	0.124
Genotype x Treatment x Year	24	403.00	16.79	1.47	0.069
Residuals	717	8192.10	11.43		

Pr > F: probability of obtaining an F-value as or more extreme than the calculated value purely by chance.

Table 3. Total larval abundance and adult emergence, and survivorship ratios for different groups of treatment and EU pairings. Total abundances per genotype were averaged within each group and stand errors of the mean are provided.

EU-TRT	μ_L	μ_A	S_{L-A}	S_{E-A}
EU1 AI	46.66 ± 8.54	9.64 ± 1.62	0.207	0.013
EU1 Trap	18.22 ± 3.93	4.44 ± 1.40	0.244	-
EU2 AI	51.25 ± 13.40	8.30 ± 1.50	0.162	0.011
EU2 Trap	20.38 ± 5.67	4.31 ± 1.12	0.211	-
μ_{AI}	48.82 ± 7.52	9.01 ± 1.09	0.185	0.012
μ_{Trap}	19.24 ± 3.28	3.70 ± 0.88	0.192	-
μ_{Tot}	34.03 ± 4.79	6.70 ± 0.79	0.197	-

S_{L-A}: Survivorship ratio from larva to adulthood.
S_{E-A}: Survivorship ratio from egg to adulthood. Note: can only be assessed with accuracy in artificial infestation.
μ_L: Mean of total larval abundance across genotypes.
μ_A: Mean of total adult emergence across genotypes.

Table 4. Proportion of variance in Root Size (RS) and Regrowth (RR) explained by Node-Injury.

EU	**Treatment**	**RS**	**RR**
EU1	AI	0.30***	0.36***
	Trap	0.19***	0.21***
EU2	AI	0.27***	0.41***
	Trap	0.20***	0.29***
EU3	AI	0.17***	0.19***
	Trap	0.10**	0.03
EU4	AI	0.18***	0.19***
	Trap	0.20***	0.09***

** $P \leq 0.005$, *** $P \leq 0.0005$
EU = experimental unit.

Table 5. Restricted maximum likelihood variance component estimates from a standard least squares model containing treatment and year fixed effects and the following random effects for three root traits evaluated for 4 experimental units (EU).

Trait: Root Size						
EU	**Model Parameter**	**Variance Component**	**Lower 95% CI**	**Upper 95% CI**	**% of Total Variation**	**†Model R^2**
1						0.397
	Genotype	0.330	0.025	0.634	21.81	
	Genotype x Treatment	-0.007	-0.221	0.208	0.00	
	Genotype x Year	-0.049	-0.219	0.121	0.00	
	Treatment x Year	-0.017	-0.018	-0.015	0.00	
	Genotype x Treatment x Year	0.215	-0.095	0.512	13.81	
	Residual	0.973	0.894	1.064	64.00	
2						0.29
	Genotype	-58.427	93.200	-23.600	0.00	
	Genotype x Treatment	30.992	27.896	34.088	33.90	
	Genotype x Year	58.535	23.700	93.300	64.03	
	Treatment x Year	0.720	-1.473	2.913	0.79	
	Genotype x Treatment x Year	-30.726	-33.818	-27.634	0.00	
	Residual	1.174	1.064	1.302	1.28	
3						0.676
	Genotype	0.061	-0.080	0.201	5.89	
	Genotype x Treatment	0.054	-0.098	0.206	5.23	
	Residual	0.916	0.743	1.156	88.89	
4						0.347
	Genotype	-0.238	-0.486	0.009	0.00	
	Genotype x Treatment	0.481	0.039	0.924	24.94	
	Residual	1.448	1.320	1.595	75.06	
Trait: Root Regrowth						
EU	**Model Parameter**	**Variance Component**	**Lower 95% CI**	**Upper 95% CI**	**% of Total Variation**	**†Model R^2**
1						0.376
	Genotype	0.435	-0.089	0.958	18.28	
	Genotype x Treatment	-0.001	-0.201	0.199	0.00	
	Genotype x Year	0.257	-0.144	0.657	10.79	
	Treatment x Year	0.030	-0.137	0.196	1.25	
	Genotype x Treatment x Year	0.240	-0.042	0.521	10.07	
	Residual	1.418	1.302	1.550	59.62	
2						0.348
	Genotype	-1.330	-8.250	5.590	0.00	
	Genotype x Treatment	-0.370	-2.220	1.480	0.00	
	Genotype x Year	1.730	-5.190	8.650	42.09	
	Treatment x Year	0.040	-0.370	0.460	1.06	
	Genotype x Treatment x Year	0.790	-1.060	2.640	19.32	
	Residual	1.540	1.400	1.710	37.53	
3						0.770
	Genotype	0.087	-0.159	0.333	5.67	
	Genotype x Treatment	0.171	-0.111	0.453	11.13	
	Residual	1.278	1.037	1.614	83.20	
4						0.416
	Genotype	-0.317	-0.973	0.339	0.00	
	Genotype x Treatment	0.961	-0.045	1.967	28.67	
	Residual	2.392	2.180	2.636	71.33	

(**Table 5.** Continued)

Trait: Node-Injury						
EU	**Model Parameter**	**Variance Component**	**Lower 95% CI**	**Upper 95% CI**	**% of Total Variation**	**†Model R^2**
1						0.417
	Genotype	0.112	-0.012	0.235	12.79	
	Genotype x Treatment	-0.039	-0.174	0.096	0.00	
	Genotype x Year	-0.064	-0.190	0.061	0.00	
	Treatment x Year	0.029	-0.111	0.169	3.32	
	Genotype x Treatment x Year	0.223	0.003	0.443	25.47	
	Residual	0.511	0.469	0.558	58.43	
2						0.435
	Genotype	1.065	0.861	1.268	22.32	
	Genotype x Treatment	-3.071	-4.537	-1.605	0.00	
	Genotype x Year	-1.009	-1.111	-0.907	0.00	
	Treatment x Year	-0.013	-0.116	0.091	0.00	
	Genotype x Treatment x Year	3.305	1.839	4.771	69.29	
	Residual	0.400	0.363	0.444	8.40	
3						0.190
	Genotype	0.003	-0.024	0.031	1.66	
	Genotype x Treatment	0.021	-0.016	0.058	10.44	
	Residual	0.179	0.145	0.226	87.90	
4						0.264
	Genotype	-0.112	-0.241	0.017	0.00	
	Genotype x Treatment	0.249	0.014	0.485	40.92	
	Residual	0.360	0.328	0.397	59.08	

†Proportion of phenotypic variation explained by the full model containing both year and treatment fixed effects and the associated random effects.

CHAPTER 3: GENETIC DISSECTION OF NATIVE RESISTANCE TO WESTERN CORN ROOTWORM LARVAL FEEDING REVEALED IN POPULATIONS OF STIFF-STALK MAIZE: FROM SOURCE SCREENING TO CHARACTERIZATION OF DURABLE QTL ALLELES

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Abstract

Background

The western corn rootworm (*Diabrotica virgifera virgifera* LeConte, WCR) is one of the most damaging pest of maize, resulting in over $1 billion in annual losses in the U.S. due to yield reductions and management costs. WCR has been reasonably well controlled using a combination of insecticides, integrated pest management strategies, and transgenic insecticidal proteins. However, all three of these strategies have shown critical vulnerabilities in recent years. Screening for native resistance has identified some potential sources of resistance, and established that natural variation could be used for crop protection, although dissection of the underlying genetic variation associated with native resistance has been recalcitrant.

Objectives

Our goals were to identify new sources of resistance from exotic germplasm and to characterize the genetic architecture of resistance. Specifically, we report on the screening of diverse germplasm to identify resistance sources, breeding efforts to generate segregating F_2, BC_1, and doubled haploid analysis populations, and the use of these populations for discovering alleles that confer resistance and tolerance to WCR.

Results and Conclusions

From the initial screen, we identified an exotic germplasm source with levels of root injury similar to that observed for the transgenic check, MON863. Mapping populations revealed

extensive variation in both larval feeding and root architecture traits that were traced to discrete chromosomal regions. Several QTL were identified in both populations with strong support for native resistance factors residing on chromosomes 2, 3, 5, and 7. Evidence for both independent and pleiotropic gene action among three native resistance traits was detected. QTL results were validated and recombination events immortalized in a set of doubled haploids. Collectively, we detected 7 QTL that explained 51% of the variance in node-injury, 6 QTL that explained 46% of the variance in root size, and 8 QTL that explained 56% of the variation in root regrowth. In most cases, QTL were found to act additively, suggesting that they could be deployed in hybrids for crop protection. Moreover, we demonstrated that phenotypic performance in the isoline state was correlated with performance in a hybrid state when crossed to a common tester, PHG84. This study represents the first report of genetic characterization of native resistance to rootworm larval feeding in corn. The associated findings and resources provide a new foundation for deploying native resistance alleles in crop protection. In the near term, they facilitate allele mining at the detected QTL and marker assisted selection of the favorable alleles identified. In the longer term, the genetic and germplasm resources developed for this work will serve as a starting point for further mechanistic investigations.

Keywords. western corn rootworm (WCR), quantitative trait loci (QTL), stiff-stalk, nonstiff-stalk, node-injury, recombinant inbred line, single-nucleotide polymorphism, doubled haploid line

Introduction

The western corn rootworm (WCR, *Diabrotica virgifera virgifera* LeConte) is one of the most damaging pests of maize (*Zea mays* ssp. *mays* L.) particularly in the U.S. Corn Belt. Estimates of WCR economic impact approach $1 billion annually at a minimum (Metcalf, 1986), and possibly as much as $2 billion (Frank, Zukoff, et al., 2013) due to costs associated with management and yield reductions. Since the first reports of WCR in the U.S. in the 1860's, its native range has expanded rapidly to include all major corn-producing areas in the U.S. (Chiang, 1973, Gray, Sappington, et al., 2009, LeConte, 1868). An even more dramatic and invasive expansion of Eastern Europe has occurred more recently, and the WCR is projected to become a pest problem in nearly all major areas where corn is produced world-wide (Aragon, Baselga, et al., 2010, Ciosi, Miller, et al., 2008, Gray, Sappington, et al., 2009, Kiss, Edwards, et al., 2005).

Like all coleopteran insects, the WCR possesses several distinct life stages. The larval form of the insect causes the most severe damage by feeding on crown roots, leading to increased root lodging, stunted growth, and reduced yield (Meinke, Sappington, et al., 2009, Spike and Tollefson, 1991). This damage is inflicted between the V4 and V11 stages of plant development (Hibbard, Schweikert, et al., 2008). After larvae pupate, they emerge from the soil as adult beetles and begin feeding on aboveground plant organs including both foliar and reproductive tissues (Branson and Krysan, 1981, Chiang, 1973). The WCR has been difficult to control due to its invasiveness and ability to overcome management strategies, including rotations with soybean, which it overcame by changing egg-laying behaviors (Gray, Sappington, et al., 2009, Levine, Spencer, et al., 2002). However, the WCR typically completes its entire lifecycle on maize, hence its canonical name (Clark and Hibbard, 2004, Meinke, Sappington, et al., 2009).

Several variants of the WCR have been identified including those resistant to crop rotation (Levine, Spencer, et al., 2002) and insecticides (Ball and Weekman, 1962, Meinke, Siegfried, et al., 1998, Wright, Scharf, et al., 2000), demonstrating a need for improved management strategies. More recently, field-evolved resistance to an insecticidal transgene has been reported (Gassmann, Petzold-Maxwell, et al., 2011). Thus, an intensifying need has emerged to develop additional tools and resources that can be used to target the pest, or minimize damage incurred from it. Host-plant resistance remains a viable target in this pursuit. The mechanisms of resistance are likely to take on one of three forms: tolerance, antibiosis, or non-preference (antixenosis), which were first described by (Painter, 1951). Several breeding programs have been conducted to develop native-resistant varieties of maize, but to date, no varieties claim complete resistance (Ivezić, Raspudić, et al., 2009). Nevertheless, attempts to identify native resistance in maize has identified germplasm possessing each of the three resistance mechanisms (Bernklau, Hibbard, et al., 2010, El Khishen, Bohn, et al., 2009, Hibbard, Darrah, et al., 1999, Hibbard, Willmot, et al., 2007, Owens, Peters, et al., 1974). However, Riedell and Evenson (1993) made the observation that most of the characterized resistant maize in the U.S. expresses a tolerance mechanism as opposed to antibiosis. Regardless, the underlying genetic mechanisms of resistance remain largely undescribed.

The extensive spread and adaptability of the WCR necessitates continued research and development in the area of host-plant resistance, and the identification of genetic mechanisms that confer antibiosis or tolerance. Elucidating the genetic architecture of resistance to the WCR

can lead to more directed management options such as marker-assisted selection at resistance loci to improve new and existing varieties. This achievement has been a long sought after goal in the WCR research community (Gray, Sappington, et al., 2009, Ivezić, Raspudić, et al., 2009). Additionally, because of the cost and environmental impact of insecticides, native resistance varieties can provide an effective alternative. Certain markets could also benefit greatly from the use of non-GMO products with elevated resistance, such as use in mandatory refuge areas, or in European countries (Ivezić, Raspudić, et al., 2009, Tollefson, 2007).

One of the most promising places to mine for beneficial alleles involved in insect resistance is in exotic sources that have remained underutilized in breeding programs. The germplasm enhancement of maize project (GEM) is a large-scale collaborative project undertaken by the USDA-ARS, land grant universities, private industry, and non-governmental organizations with the goal of broadening the germplasm base of maize (Pollak and Salhuana, 2001). It was recognized that the large majority of commercially grown and consumed corn in the U.S. encompasses only a small fraction of the potential genetic variation available in maize (Tallury and Goodman, 2001). Much of this variation is retained in exotic germplasm scattered throughout the world. The narrow genetic base associated with modern elite germplasm creates genetic vulnerability, and has resulted in lower responses to selection. Utilization of these exotic lines is beneficial for the direct improvement of commercial maize production, but also for characterizing genetic mechanisms, and the inheritance of quantitative traits in maize.

With the goal of introducing exotic alleles for agronomically important traits into elite germplasm, GEM has established a protocol for maize researchers to develop genetically enriched populations (Pollak and Salhuana, 2001). Several have already been developed for traits associated with leaf blight resistance, oil and starch biosynthesis, and yield components (Pollak, 2003). As part of the project, a set of GEM lines derived from tropical and subtropical sources were repeatedly screened for node-injury and standability from 2002 to 2005. The goal was to identify potential germplasm with natural resistance to important agricultural pests including the WCR, and to provide an initial measure of this resistance so that further investigations could be conducted. Lines included in this screen originated from Chile, Uruguay, Cuba, Argentina, Brazil, and the USA. The screen determined that extensive variation in lodging and root herbivory exists across diverse accessions of maize, and furthermore, that some germplasm sources consistently perform better than others across repeated measurements.

Here, we report on the best varieties that have emerged from the initial GEM screen, and the subsequent efforts to characterize the genetic underpinnings of WCR resistance. Both stiff-stalk (SS) and nonstiff-stalk (NSS) F_2 and BC_1 populations were developed so that alleles could be contributed from more than one heterotic group in future breeding efforts. The use of heterotic groups in modern maize breeding has been instrumentally used to take advantage of the observation that genetically diverse individuals tend to have better combining ability and F_1 performance than crosses from closely related individuals, and this has served as the basis in hybrid breeding programs (Anderson, 1944, Hallauer and Miranda, 1981, Hallauer, Russell, et al., 1988). The traditional Reid Yellow Dent x Lancaster Surecrop correspondence to modern SS and NSS heterotic groups has become blurred over the decades of maize breeding, in part due to the intermixing of ancestral alleles in the development of inbred lines (Gerdes and Tracy, 1993, Smith, Goodman, et al., 1985a, Smith, Goodman, et al., 1985b). Genetic and genomic evidence can still resolve the main heterotic pools of maize, albeit the extent of genetic diversification within each group has extensively increased (Hansey, Vaillancourt, et al., 2012, Lai, Li, et al., 2010, Livini, Ajmone-Marsan, et al., 1992, Lübberstedt, Melchinger, et al., 2000, van Heerwaarden, Hufford, et al., 2012, Wu, Wang, et al., 2000). Here, we take advantage of the widely used SS and NSS heterotic groups combined with the introduction of exotic alleles from GEM germplasm to genetically characterize resistance to the WCR.

Utilizing a quantitative genetics approach to the dissection of root architecture traits and larval feeding damage by the WCR, we developed a series of populations to both positionally map genetic factors and estimate their effects. Quantitative trait locus (QTL) mapping studies with biparental mapping populations require contrasting alleles at loci contributing to the trait being investigated (Mackay, 2001). These alleles segregate in the mapping population, and provide a reference to track co-segregation between genotype and phenotype across a large number of individuals. Within F_2 and BC_1 populations, there should be considerable variation for the trait of interest if subsequent inbreeding or doubled haploidization is to be considered (Bernardo, 2009). The use of isogenic populations for QTL mapping provides a number of benefits which includes providing an immortalized germplasm source that captures recombination events. This allows the particular genetic effects to be interrogated in different environments, years, or by different investigators; which is an important feature that is required for WCR native resistance breeding. With this in mind, the main objectives of this study were to

generate populations of SS and NSS maize segregating for resistance to the WCR, quantify the heritability for native resistance to WCR larval feeding, and genetically characterize the resistance architecture. We also sought to validate QTL results through multiple rounds of analysis in different generations, including doubled haploids, and across different locations to estimate both genetic and environmental variance components.

Materials and Methods

Initial Plant Materials

Founder germplasm chosen for the initial screen included a diverse set of accessions from the GEM Project (Pollak and Salhuana, 2001) that had been repeatedly evaluated as populations in rootworm-infested fields in 2002-2004 and selected as potential native resistance candidates (based on nursery observations from Ed Berry, personal communication, Mike Blanco, 2005). These entries were all derived from six principal founder crosses between a GEM accession and an elite proprietary line, and subsequently selfed for two generations, and balanced-bulked for at least two generations (Pollak, 2003) (**Table 1**). The resulting S_2-balanced bulk populations subsequently underwent 3 years of phenotypic selection under high corn rootworm pressure to create a list of 50 accessions with the most potential for harboring native resistance alleles (shown in **Fig. 1**).

Trap Crop Establishment

Four replicates of 52 entries were planted in 100-plant strip plots in a randomized complete block design on 17 May 2006 at the Iowa State University Bruner Research Farm (42° 3′ 40″ N, 93° 53′ 10″ W). These entries consisted of the 50 accessions from the GEM project, a Mycogen-susceptible control (2Y764), and a *Bt*-resistant check (DKC60-12 YGRW, MON863) that expresses the insecticidal protein Cry3Bb1 (Vaughn, Cavato, et al., 2005). Seven-hundred *Diabrotica virgifera virgifera* LeConte eggs from a diapausing strain were deposited at 7.5 cm below the soil surface for seven plants per entry/replicate on 6 June 2006. Injections were performed by puncturing holes in the soil with a one cm diameter dowel approximately seven cm away from the central stalk. Prior to performing infestations, eggs were suspended in 0.15% agar solution according to (Palmer, Windels, et al., 1977) and calibrated to be ca. 300 eggs/mL.

Females that emerged from these eggs during the summer of 2006 mated with males in the same or nearby fields, and deposited eggs in a neighboring field that was planted late in the

summer to provide a so-called “trap” crop. The eggs overwintered in the soil and hatched the following spring to provide rootworm feeding pressure on developing seedlings. Trapping was maintained in subsequent years in a similar fashion by planting conventional corn-on-corn hybrids late in the season to encourage WCR recruitment and oviposition. All subsequent evaluations of segregating native resistance populations were conducted in this trap crop at the Bruner Research Farm. Standard field management practices for central Iowa were applied to the field including annual application of herbicide and fertilizer (150lbs/acre).

Subsequent Breeding

The most resistant SS and NSS entries from the screen of the 50 top performing GEM S_2-balanced bulks were selected to represent heterotic donor parents of BC_1 and F_2 populations. The SS representative selected was FS8B(S):S0316-053-1-B-B-B (04GEM00161, Set E) and the NSS entry selected was UR13085:N0215-19-2-B (04GEM00452, Set A). The FS8B(S) germplasm was developed originally by E. S. Horner (Horner) using full-sib selection among a wide range of accessions for resistance to southern corn leaf blight (race O of *Bipolaris maydis* (Nisikado) Shoemaker). With the goal of obtaining durable resistance and maximizing discovery of insect resistance alleles, each of these exotic resistance sources was crossed to a variety within its own heterotic group with known resistance to the Lepidopteran, *Ostrinia nubilalis* (European corn borer, ECB). This was done in an effort to combine different resistance mechanisms in one variety, as ECB and WCR target separate maize organs and developmental stages.

FS8B(S):S0316-053-1 (herein FS8) was crossed to a stiff-stalk inbred, B86, with reported resistance to ECB first generation larval feeding (ECB1). B86 (Reg. No.GP-77) arose as the best line from among 200 F_3-lines from the cross of B52 x Oh43 after selection and selfing ear-to-row to the F_5 generation (Russell, Guthrie, et al., 1974). UR13085:N0215-19-2 (herein UR2) was crossed to the nonstiff-stalk inbred Mo47 (Reg. No. GP-300, PI 583352), a line developed for resistance to both leaf feeding and stalk tunneling generations of ECB (Barry, Antonio, et al., 1995, Jampatong, McMullen, et al., 2002). Both recurrent parent lines, B86 and Mo47, have low levels of resistance to WCR larval feeding and thus provided a good parental source for observing segregation of native resistance alleles from our SS and NSS donors.

F_1 plants were planted in 2007 at the Bruner Research Farm trap crop, thinned to optimal density, and ten of the best hybrids based on lodging, goosenecking, and stalk strength were selected for generating F_2 and BC_1 populations within each heterotic group. Because our donor

parents were derived from S_2-balanced bulk populations, the level of heterozygosity achieved should on average be 25%. Each S generation was planted ear-to-row followed by balance bulking within an S_2-derived ear. Therefore, each balanced bulk line had the potential of harboring a unique set of resistance alleles. The subsequent development of F_2 and BC_1 families within each heterotic group thus follows, so that each family manifests a unique set of alleles derived from the F_1 progenitor. This was achieved by tracking single F_1 plants in the generation of F_2's, and the pollination of multiple ears of the recurrent inbred in the case of our BC_1 families. Thus, plants within a BC_1 family sharing the same F_1 plant, but a physiologically different recipient plant, can be called genetic full-sibs but only physiological half-sibs.

Phenotypic Evaluation for Native Resistance in Segregating Populations

F_2 and BC_1 plants were evaluated over the course of two years in 2009 and 2010 at the Iowa State Bruner Research Farm (41° 60′ 35″ N, 93° 44′ 11″ W). In 2009, 2,100 BC_1 and 300 F_2 plants from both SS and NSS germplasm pools were planted using an Almaco 4-row cone planter (0.762 m row spacing) on May 12 at a density of 25 plants per 4.572 m row and 0.914 m alleys. In 2010, 1,000 additional B86 x (FS8 x B86) plants were machine planted on May 25 at the same density as the previous year. In both years, a randomized block design was used with each block corresponding to a different genetic half-sib group. For each heterotic group, two half-sib groups comprising 8 physiological full-sib groups were included. In 2009, 264 BC_1 plants and 120 F_2 plants were randomly selected for root extraction and tagged with a plastic plant tag, avoiding end plants in the selection. These sample sizes were chosen so as to keep the number of discernible recombination events equal between F_2 and BC_1 within a year. The number of BC_1 plants selected in 2010 was 768. Digging of roots and intact plants was done to coincide with an interval of time just after peak larval feeding damage, using 600 degree day units as a starting point (achieved 7 July 2009 and 12 July 2010) (Fisher, Sutter, et al., 1990, Prischmann, Dashiell, et al., 2007). Briefly, roots were excavated using shovels, loaded in batches in the back of a pickup truck, and transported to a centralized station at the farm where 68.137 L totes were set up and filled with water. Roots were soaked overnight so that soil could be washed off the following day using a pressurized hose. After cleaning, the roots were removed from direct sunlight and evaluated on the same day as the plants were washed. Leaf tissue was collected for DNA extraction via standard methods from all 1,152 plants and processed in 96-well plates.

Root damage was assessed by collecting lodging scores (# of plants leaning > 30º from vertical) and evaluating node-injury independently in triplicate on the 0-3 Node-Injury Scale (Oleson, Park, et al., 2005). In 2010, two additional root traits were collected on each of the BC_1 plants on the basis of their power to discriminate different resistance mechanisms and their use in rootworm resistance screening: Root System Size and Secondary Root Development (Rogers, Owens, et al., 1975). Both traits are a measure of the plants ability to tolerate insect feeding, whereas node-injury captures antibiosis or nonpreference, and lodging can be a measure of all three mechanisms (Branson and Sutter, 1989, El Khishen, Bohn, et al., 2009). Secondary root development, now usually called compensatory growth or root regrowth, allows plants to tolerate herbivory, and has been reported to prevent yield loss following larval injury (Ortman, Branson, et al., 1974, Owens, Peters, et al., 1974, Spike and Tollefson, 1989). Both root size and root regrowth were rated on a 0 to 6 scale with 1 being the smallest root system and the lowest level of regrowth and 6 being the largest and most densely branched secondary root systems. A zero corresponds to root systems that have no significant root tissue, either because of heavy feeding damage and no compensatory growth, or because their root systems are so stunted that they fail to be classified as a "1" on the root size scale. In subsequent analyses, these scores were sometimes classified as missing data.

To compare the relative extent of phenotypic variability among and between the FS8 and UR2 populations for lodging, members from two recombinant inbred line (RIL) populations and their inbred parents were included: the intermated B73 x Mo17 RILs (IBMRILs) and NC89 x K55 RILs (INKRILs) (Lauter and Moose, 2008, Lee, Sharopova, et al., 2002). Fifty-two lines each from the IBMRIL and INKRIL populations were randomly selected for inclusion in the study, and planted with the FS8 and UR2 BC_1 and F_2 populations at the Bruner Research Farm trap crop in the summer of 2009.

Genotype Data Collection and Map Construction

Because the results from the initial GEM screen and subsequent evaluation of segregating NSS populations showed no evidence for WCR resistance that reduced node injury, genotyping was performed only for the FS8 x B86 populations. A core set of 1,016 single-nucleotide polymorphisms (SNPs) polymorphic for B73 versus Mo17 (Liu, Chen, et al., 2010), were tested on our SS parents, revealing 379 suitable SNPs. Genotyping was performed using the Sequenom's MassARRAY® iPLEX® platform. One-hundred seventy-five SNPs in the FS8 x

B86 contrast were chosen for F_2 and BC_1 genotyping, and new assays were designed using the MassARRAY® software. These SNPs covered all 10 chromosomes, and were chosen to maximize genomic coverage and inter-marker spacing. A complete list of the SNPs developed and included in a set of 5 multiplex assays, along with their primer sequences, is shown in **Appendix Table 3**. SNP genotypes were converted into a genotypic-class designation of either A, B, or H according to whether the alleles came from the B86 parent, the FS8 parent, or one allele from both parents, respectively. To keep allele designations consistent between F_2 and BC_1 populations, FS8 homozygotes were always assigned a BB genotype, whereas B86 homozygotes were assigned AA genotypes.

Separate genetic maps were constructed for the SS F_2 and BC_1 populations using JoinMap ® 4.0 (Stam, 1993). Initial processing of marker data was performed to identify markers with missing data, and to identify segregation distortion patterns. Markers with more than 75% missing data or X^2 ratio of greater than 20 were removed. This resulted in a trimmed dataset of 136 markers for the BC_1 map, and 141 markers for the F_2 map. The physical map of the B73 genome and the ISU SNP genetic map were used to scaffold the markers to linkage groups and define chromosome starting orders (http://www.maizegdb.org/cgi-bin/displaycompletemaprecord.cgi?id=1234896) (Liu, Chen, et al., 2010, Wei, Zhang, et al., 2009). Grouping of markers into linkage groups was done using an independence LOD score test starting at threshold of 2.0 and ending at 10.0 with a step size of 1.0. The pairwise recombination fraction was tested using the G^2 for independence: $G^2 = 2\sum O \log(O/E)$, where O is the observed number of recombinants and non-recombinants, and E is the expected given that the loci are not linked. The statistic is more robust to segregation distortion than is the normal linkage LOD (van Ooijen, 2006). Any ungrouped loci present after grouping were assigned to one of the existing groups based on their known chromosomal assignments. This validation step was done to cross-reference the IBM SNP map with the observed recombination fractions in the BC_1 and F_2 populations. All markers assigned to a particular linkage group also clustered on a single chromosome in the ISU IBM SNP map. After groups were constructed, pairwise recombination frequencies between markers were calculated using a maximum likelihood algorithm that uses a variety of methods to order loci and compute distances (Jansen, de Jong, et al., 2001). The methods used include: Gibbs sampling to estimate multipoint recombination frequencies; simulated annealing to search for the order that maximizes likelihood; and spatial sampling to

maximize convergence on global optimums rather than local ones. In some cases, the order of markers had changed slightly relative to those assigned in the IBM SNP map. After map construction, the individual linkage groups were integrated into a single map for QTL mapping.

QTL Detection and Mapping

The RStudio (v. 0.97.551) statistical platform was used for mapping of native resistance QTL via the Rqtl package (Broman, Wu, et al., 2003, RStudio, 2012). Marker positions were jittered for any markers residing at the same centiMorgan (cM) position. Genotype probabilities were calculated using a hidden Markov modeling technique in 1 cM steps with an error.prob = 0.01. The Haldane mapping function was used to convert between recombination fractions and genetic distances. Genome scanning was performed first with a single-QTL model (scanone function) using an expectation maximization (EM) algorithm. Composite interval mapping was done in 20 cM windows using the EM method with 3 marker covariates, filling in missing genotypes via imputation. For establishing empirical thresholds for QTL significance, 1,000 permutations were performed in a batch analysis mode. Thresholds were established for 1%, 5%, 10%, and 15% genome-wide significance.

Statistical Methods

Levine's homogeneity of variance test was implemented to test whether variance within a population was different from the variance across all other populations (Levene, 1960). Levine's test shows the F-statistic from an ANOVA in which the response is the absolute value of the difference of each observation and the group mean, as opposed to the squared deviation of each observation and the group mean (Levene, 1960). The test is robust to departures from normality and unequal population sizes. The test statistic has an F-distribution with one numerator degree of freedom and n-k-1 denominator degrees of freedom with an alpha=0.05. The null hypothesis states the populations have equal variance. The alternative hypothesis is that a given population has more or less variance than the combined variance of the other populations.

For developing models to explain variation seen in native resistance traits, a mixed model analysis was run in JMP 10.0 (SAS Institute Inc., 2012). Depending on the particular analysis and comparison sought, the model terms were fit into a standard least squares model as either fixed or random. Year, rep, and location were always treated as fixed effects. Genotype and interaction terms were fit as random effects. For determining main effect significance and making comparisons between levels of a model term, genotype was fit as a fixed effect. Single

marker regression analysis was performed using either R (RStudio, 2012) or JMP 10.0 (SAS Institute Inc., 2012) software under the following model: $y_i = \beta_0 + \beta_1 x_i + \varepsilon_i$, i = 1,…,n; where β_0 is the y-intercept, β_1 is the slope for the i^{th} marker, x_i is the marker effect of the i^{th} marker, and ε_i is the residual effect of the i^{th} marker. Linear regression of marker genotype on phenotype was performed for each marker in the genetic map using R. P-values from linear regression were converted to q-values using Storey and Tibshirani's method (Storey and Tibshirani, 2003) and setting FDR = 0.05. Markers identified as significantly linked to a QTL based on the likelihood ratio test statistic were included in the regression model and used to estimate broad-sense heritabilities.

Development of Doubled Haploids and QTL Assessment across Locations and Years

To validate QTL detected in the F_2 and BC_1 populations, and to create a set of isolines for further multi-location interrogation of WCR native resistance, we initiated the induction of doubled haploids from F_2, BC_1, and BC_1-intercross segregants. Based on preliminary QTL analysis, the choice to induce only 1882-7 F_1- derived segregants was made. Intercrosses between 1882-7 BC_1 full-sib groups were performed in the summer of 2010 to capture additional recombination events while maintaining a two-allele system. Doubled haploids were developed through the services of the ISU Doubled Haploid Facility (http://www.biotech.iastate.edu/service_facilities/haploid.html). The inducer line RWS was used for haploid induction, with a reported average induction rate of 8.1% (Rober, Gordillo, et al., 2005). The line possesses the dominant anthocyanin marker gene *R1-nj*, allowing for visual screening of haploid kernels which express pigmented endosperm but non-pigmented embryos. For chromosome doubling, a colchicine technique was used as described by (Eder and Chalyk, 2002), which results in a typical doubling rate of ca. 8.4%. In 2010, 225 induction crosses of FS8 BC_1 and F_2 plants were performed, which yielded 67 DH lines with viable seed. Among the intercrosses, an additional 72 DHLs were created. For these populations, an average induction rate of 9.68% and doubling rate of 14.68% was achieved. For each DH line, crosses were made onto the xPVP line PHG84 to test performance in both the isoline *per se* and hybrid state.

Evaluation of DHLs was done over the course of two years in S2012 and S2013. In 2012, seed from FS8 F_2 and BC_1-derived DHLs along with their PHG84 hybrids were grown under high WCR larval feeding pressure in 5 locations in the Midwest. Based on seed availability, 41 DHLs were included at each location, so as to maintain a balanced design, and planted in a

randomized complete block design, with 2 or 4 reps (blocks) per location. The five locations included: 1) ISU Ag Engineering and Agronomy Farm, Boone IA; 2) ISU Bruner Research Farm, Ames IA; 3) ISU Southwest Research Farm, Crawfordsville IA; and 4&5) University of Missouri Bradford Research and Extension Center, Columbia MO. For the three Iowa locations, 4 reps were planted and evaluated, and for the two Missouri locations, two reps were included. Locations 1 and 4 were treated with artificial infestation of WCR eggs (~750 eggs/plant), whereas larval feeding pressure at the other locations was supplied via well-established trap-cropping. At each location, entries were planted in row plots, and 4 plants per plot were tagged and excised for root evaluation at the time of peak larval herbivory as described previously. The three root traits assessed were node-injury (0-3), root size (0-6), and root regrowth (0-6).

Evaluations were repeated in S2013 with the inclusion of 28 BC_1 intercross DHLs that had been bulked in the previous year. Two reps were planted in a randomized incomplete block design with 4 blocks of 18 entries. DHLs and their testcross hybrids were grown in side-by-side plots to improve accuracy of correlations between isoline *per se* and hybrid. The same five locations utilized in 2012 were repeated in 2013, and the same three native resistance traits were collected as described previously for 4 plants per row. In both years, the transgenic check Mir604 (Syngenta, expressing the mCry3A protein) was grown along with four lines with potential native resistance (AGR9 x NGSDCRW-1)DH-6 (AgReliant), CRW8-1 (Prischmann, Dashiell, et al., 2007), and NGSDCRW1(S2)C4 (Kahler, Telkamp, et al., 1985). The inbred B86, and the two expired-PVP lines PHG84 and LH51, were also included in the study as susceptible checks. Each DHL that was developed was cultivated in the USDA-ARS Greenhouse (Ames, IA), and DNA was collected from leaf tissue after 14 days. Genotyping was performed using the same set of markers developed for the F_2 and BC_1 populations. QTL mapping was performed using the Rqtl package as described previously and including nested analyses by year and location.

Results

Germplasm Screen Identified Native Resistance Sources from SS and NSS Heterotic Groups

From the original screen of GEM entries for native resistance to WCR larval feeding we identified 50 of the most resistant, and selected them for further field evaluation in 2006 under artificial infestation. The results of this screen led to the identification of two varieties with

minimal larval feeding damage relative to controls (**Fig. 1**). These lines identified as FS8B(S):S0316-053-1-B-B-B (FS8), and UR13085:N0215-19-2-B (UR2) encompass both SS and NSS heterotic groups of maize, respectively, and are designated with asterisks in **Fig. 1**. The SS variety FS8 had far less root damage than any of the other varieties tested, including UR2. FS8 had an average of only 0.62 ± 0.12 root nodes injured (ni) out of 3.00 and was almost as resistant as the *Bt* check, MON863, (0.41 ± 0.12 ni). UR2 had an average of 2.10 ± 0.21 ni, but was the best among NSS lines tested. Ratings across reps but within an entry were highly consistent. The variance among reps within an entry accounted for only 0.06 ni and had an insignificant effect on node injury ($F_{153,150} = 0.13$, $P \approx 1.0$). The variance between entries on the other hand, was highly significant and accounted for 77% of the total variance ($F_{51,252} = 15.82$, $P < 0.001$). The average among all pairwise correlations between reps was 0.76 and pairwise t-tests determined that none of the reps were significantly different. This provided strong evidence that natural variation between entries accounts for a majority of the variance in node injury.

Root lodging was also quantified among these 52 entries, and again, the only significant main effect observed was for entry, which explained 46% of the variance in lodging ($F_{51, 156} = 1.59$, $P = 0.016$). The top 5 entries with the lowest node injury all had 1 or fewer plants lodged across the four replicates and were indistinguishable from the *Bt* check ($F_{1, 150} = 0.03$, $P = 0.866$). They were, however, different from rest of the entries ($F_{1, 150} = 37.34$, $P < 0.001$). The 5 entries with the most severe node-injury (≥ 2.95 ni) had an average of 11 plants lodged, compared to 8 for all entries. The difference between lodging scores across reps for a given entry was found to be insignificant ($F_{153, 207} = 0.60$, $P > 0.993$). In pairwise t-test comparisons between reps, only 3 entries had significant differences between any two reps. Lodging and node-injury means were 55% correlated, indicating that only part of the ability of plants to stand upright is reflected by rootworm damage. The FS8 source with the lowest node injury also had one of the lowest lodging scores. Only 1 plant out of four 25-plant plots was lodged, whereas for UR2, this number was 7. Based on this evidence of standability and node-injury, FS8 emerged as the strongest candidate for harboring native resistance alleles.

Variability in Genetically Heterogeneous Populations Substantiates Rootworm Resistance Tractability

To evaluate levels of native resistance to larval feeding pressure, variation in the ability of plants to remain upright was assessed among the FS8 and UR2 F_2 and BC_1 populations, and

among two unique RIL populations for comparison (**Table 2**). The NC89 x K55 RILs possessed the largest ranges in lodging, encompassing the entire spectrum of the proportion of plants in the row lodged. They also most closely approached normality relative to the other populations (**Table 3**). The NC89 x K55 RILs had the largest phenotypic variance in the percentage of plants lodged at 1200.56 (**Table 2**). Seventy-eight percent of this variance was due to environmental effects, namely the differences between reps for a given genotype (62% of σ_P^2), and the variance across reps for all genotypes (19% of σ_P^2). For example, in block one, only 38% of plants were lodged, but in block three, 75% were lodged. The only significant source of variation was due to differences between reps ($F_{3,3} = 15.31$, $P < 0.001$). In addition to the NC89 x K55 RILs, the FS8 segregating populations also were highly variable in terms of standability. A significantly larger proportion of variance was explained by genetic effects in the FS8 populations relative to the other populations evaluated (F_2: $H^2 = 95\%$, BC_1: $H^2 = 23\%$). Only the FS8 F_2 population had a greater proportion of variance explained by genetic effects than by environmental effects. The FS8 BC_1 and F_2 populations were the only populations that had detectable differences between genotypes at alpha = 0.05 (**Table 2**), providing evidence that these may be the best source for capturing natural variation in larval feeding resistance.

The (UR2 x Mo47)F_2 population, in contrast to the FS8 populations, had the lowest proportion of lodged plants, and was the least variable with a range of 15%, a mean of 1.40%, and a total phenotypic variance of only 19.56 (**Table 2**). The UR2 BC_1 and the IBMRIL populations also had the low levels of variation as measured by their standard deviation relative to the other groups tested, and in none of these groups was a significant genotypic effect detected. In the case of the IBMRILs, this suggests that the parents of the population likely carry alleles that either 1) do not substantially influence lodging, or 2) are close enough in their contribution to lodging that differences between the alleles cannot be detected. This is further evidenced by the fact that both B73 and Mo17 plots had very little lodging, whereas NC89 was moderately lodged (64%), but K55 was heavily lodged (79%) (**Table 3**). B86 and Mo47 were indistinguishable from one another ($t_{22} = 0.632$, $P = 0.534$). B86 had an average lodging score of 8.91% and standard deviation of 17%, and Mo47 had an average of 5.07% lodged and a standard deviation of 13%, providing validation for their use in a parallel breeding design for SS and NSS groups.

Measures of within and between population variance indicate SS populations are more suitable for capturing variation in lodging than NSS populations. This was evident in the means, phenotypic variances, and ranges, as well as the greater proportion of genetic to environmental variance explaining lodging (**Table 2**). Within each heterotic group, there was more variability in the BC_1 population than in the F_2 population, and this was observed for both SS and NSS groups. The confidence interval for the means was nearly twice as large in the BC_1 groups. In both BC_1 groups, there was significantly more within population variance than variance across all of the populations surveyed (**Table 3**).

Variability in SS and NSS Node-Injury as a Measure of Resistance Mining Potential

When FS8 and UR2 plants were excavated and evaluated for root damage, much of what was observed in lodging was restated in node-injury ratings. The SS population showed the greatest variability in root node-injury in both the F_2 and BC_1 populations (**Table 2, Table 3, Fig. 2**). For both of these populations, the percentage of the nodal roots damaged ranged from 0 to 100%, and the spread about the mean was larger relative to the NSS populations, which had a maximum node-injury percentage of only 67%, and both a median and mode of zero. The FS8 BC_1 population had the largest phenotypic variance, 76% of which was accounted for by genotypic effects. Node-injury was slightly less heritable in the FS8 F_2 population at 67%. This is in stark contrast to the UR2 BC_1 and F_2 populations which had broad-sense heritabilities of 34% and 21%, respectively. Node-injury was different from the lodging data in that all four segregating populations had significant genotypic effects when included in a LSM model, albeit the effect was stronger in the FS8 populations.

The SS populations also more closely approach normality, and had node-injury values at all levels of the rating scale (**Fig. 2**). Homogeneity of variance tests revealed the SS populations had greater levels of variance (smaller F-values), so there was less difference between within-group variance and variance across all groups (**Table 3**). In addition to showing the greatest variability in root node-injury, the SS heterotic group also displayed more severe node damage. Pairwise mean comparisons revealed that the FS8 BC_1 population had the largest mean node-injury at 0.94 ni, followed by the FS8 F_2 at 0.63 ni. The UR2 F_2 and BC_1 group means could not be separated ($t_{570} = 0.1624$, $P = 0.8711$), and both means were less than 0.16 ni. Eighty-seven percent of the (UR2 x Mo47)F_2 population was rated ≤ 1.00. Within the SS heterotic group, the most frequent injury rating was 0.25 ni and 1.00 ni for the F_2 and BC_1, respectively. The most

severe root rating assigned to the UR2 BC_1 population was a 1.75 ni, and the max for the F_2 group was only 1.25. In comparison, 44% of the FS8 BC_1 plants, and 33% of FS8 F_2 plants, had 1.25 or more nodes injured. The R for technical replication between the two reps for a given plant was quite high ($R^2_{rep} = 0.91$), indicating that repeated measurements are very precise. Given that the variation between biological replications is inherently included in a plot main effect, a more accurate estimate of the total genetic variance is 559.35 x 0.91 = 509.01, or 69% of σ_P^2 in the case of FS8 BC_1 population, and 464.54 x 0.91 = 422.73, or 61% of σ_P^2 for the FS8 F_2 population. The UR2 populations both had a technical error rate of 14%. This indicates that the node-injury scale is fairly robust across different germplasm. Given the strong level of WCR larval feeding resistance detected in the FS8 parent, and the greater variability in resistance among the FS8 segregating populations, they represent the best substrates for native resistance mapping. Therefore, the choice was made to prioritize allele discovery and experimental resources on the FS8-derived F_2 and BC_1 populations.

Replicated Evaluation of FS8 BC_1 Plants Reveals Precision in Root Architecture Traits and Variation in Node-Injury across Years

In the summer of 2010, an additional 768 FS8 BC_1 plants were grown and evaluated for node-injury, standability, and two other traits - root size (RS) and root regrowth (RR), to assess other potential modes of resistance and evaluate BC_1 performance across years. These plants were reared in the same high-pressure trap nursery as the previous year and subsequently selected for digging and root evaluation. The R^2 among repeated measurements was high for all three traits, 0.93 for NI, 0.71 for RS, and 0.82 for RR, indicating a high level of precision in technical replication. The level of precision achieved for node-injury was consistent with 2009 levels, indicating again that the 0-3 node-injury scale is both precise and robust.

Fig. 3A and **3B** shows the frequency distributions for these three traits and a collage of some photographic evidence of extreme root characteristics, respectively. For each trait, phenotypes were observed at all levels of the rating scale. RS and RR both showed nearly-normal distributions while NI fit a left-skewed distribution with the majority of the population exhibiting little or no injury. The average node injury across all BC_1 plants was only 0.496 ± 0.03 ni, with a median of 0.25 ni. The most frequent root size rating was 3.0, which was the second most frequent regrowth rating after zero. The mean root size among the BC_1 plants was 2.95 ± 0.05 and mean regrowth was 2.23 ± 0.07. The two root architecture traits, RS and RR, were strongly

correlated with each other (r = 0.768), and they had a negative relationship with NI (**Fig. 3C**). The relationship was stronger for NI and RR than for NI and RS, which would be expected given regrowth is a measure of the response to rootworm larval feeding. Moderate correlations were also detected between lodging and the three root traits with evidence for more damage and lower growth resulting in more severe lodging.

Interestingly, when trait distributions were observed across the two F_1 parental groups and across the 8 full-sib groups some major differences emerged (**Fig. 4**). For example, those progeny groups derived from the F_1 parent 1882-1 all had very little feeding damage and showed a highly left-skewed distribution. Three of the four 1882-1 full-sib groups had significantly lower node-injury at alpha = 0.05 than the mean across all groups of 0.488 ± 0.028 ni (**Fig. 4**). On the other hand, two 1882-7 full-sib groups had group means significantly higher than the overall mean. The full-sib group 45453 had the largest node-injury at 1.159 ± 0.055 (t_{115} = 12.55, P < 0.001) followed by 45455 (t_{94} = 5.11, P < 0.001). In pairwise t-test comparisons, 45453 had significantly higher NI than any other full-sib group and it also fell into the lowest RS and RR mean separation group along with 45454 and 45414 (Tukey HSD, α= 0.05). Analysis of means for variance tests revealed that 45453 and 45455 both had significantly more variance than the rest of the full-sib groups for NI [total MSE = 0.348 , var(45453) = 0.826, UDL(45453) = 0.472; var(45455) = 0.661, UDL(45455) = 0.489]. For both RS and RG, no full-sib group variance exceeded the upper decision limit for variance significance providing evidence that variance was more similar across full-sib groups for these traits (RS: MSE = 1.50, RG: MSE = 2.54). Based on the quantitative segregation patterns among the two BC_1 families, it appears 1882-7 has the greatest amount of variance for all three NR traits. In the case of both RS and NI, phenotypic variance was twice as high in the 1882-7 BC_1family, whereas both variance and distribution were more consistent for RR. Given that quantitative variance is a necessary requisite for mapping of alleles involved in NR, the difference in variance between BC_1 families will likely obscure our ability to detect phenotype-genotype associations unless accounted for as an experimental covariate. Furthermore, the segregation at the phenotypic level across full-sib groups suggests that the different combinations of alleles that were passed to F_1 plants continued to segregate at the BC_1 level.

A model that included year, full-sib group, and year x full-sib interactions explained 64% of the phenotypic variance in node-injury (**Table 4**). Significant effects were found for all three

model terms ($P < 0.001$). Variance among the full-sibs and among full-sib x year interactions accounted for the majority of variance in node-injury. The regression coefficient for year was -0.674 ($t_{1016} = -9.387$, $P < 0.001$), and for year x ear it was 0.445 ($t_{1016} = 4.398$, $P < 0.001$). Node-injury was significantly lower in 2010 than in 2009, and this was true for both BC_1 families ($t_1 = 9.59$, $P < 0.001$). One possible explanation is that the natural abundance of WCRs in trap crops tends to slowly diminish over subsequent years due to competition and migration. Environmental differences between the two years could also have contributed to the disparities in node-injury ratings. The 2010 nursery was planted slightly later in the spring than the 2009 nursery, but node-injury evaluations began on nearly the same calendar date for both years, which is established by degree-day units. Among the 8 full-sib groups, 5 were found to have differences in node-injuries in the two years (**Fig. 5**). The direction of the year effect was not consistent, however, with two full-sib groups receiving more damage in 2009, and three in 2010. Even with the variation across years, the full-sib effect still fit to a regression line and explained 47% of the phenotypic variance for node-injury, which was more predictive than both RS and RR (**Fig. 6**). Significant full-sib group effects were also observed for root size ($t_{754} = -5.634$, $P < 0.001$) and root regrowth ($t_{754} = -4.422$, $P < 0.001$), although these traits were only measured in 2010 so no year effects could be estimated. The full-sib effect explained 17% and 26% of the phenotypic variance for RS and RR, respectively (**Fig. 6**).

Taken together these data provide strong evidence for heritable variation in native resistance traits captured within the FS8 populations. We have revealed differences in phenotypic variation between BC_1 and F_2 families and identified that the 1882-7 F_1-derived populations represent the most likely target for directed allele mining. The technical precision achieved in root phenotyping indicates these traits can be accurately deployed to measure native resistance, with particular value for node-injury. Variation across years, however, justifies the use of both pedigree and year components in subsequent models to explain node-injury. The discovery of natural variation in both larval feeding damage and root architecture has revealed that different resistance mechanisms may be responsible for the phenotypic response to WCR herbivory in the FS8 populations. Genetic dissection of these traits should reveal whether these mechanisms share a similar underlying genetic architecture or if unique regulation exists.

Genetic Dissection of Native Resistance in FS8 Segregating Populations

Genotyping of 170 SNPs in the FS8B(S):S0316 BC_1 and F_2 populations was performed to map genetic associations with native resistance traits. Within the backcross population the marker list was reduced to a set of 136 markers with > 75% non-missing data and for which high-confidence genotype calls were established. Using their IBM2 linkage group assignments and map positions as a scaffold, pairwise recombination fractions were calculated between markers and used to establish a new genetic map (**Fig. 7**). The total map length was 1582.4 cM with an average spacing of 12.6 cM. Markers were relatively evenly spaced across the 10 maize linkage groups and strong linkage disequilibrium between markers close to one another was achieved (**Fig. 8**). More importantly, there were no major marker associations between markers from different linkage groups. The genotype dataset contained 51.3% B86 homozygotes and 48.7% heterozygotes which was not significantly different than the expected frequencies (χ_1^2 = 3.74, P = 0.053) (**Fig. 8**). A similar genetic map was constructed for the F_2 population which resulted in a map length of 1569.0 cM and an average spacing of 13.3 cM. In this case, the dataset consisted of 120 individuals and 141 markers. The genotype frequencies were 0.28, 0.43, 0.29 for AA, AB, and BB genotypic classes, respectively. This deviation from expected was large enough to suggest segregation distortion or genotyping bias may be present in this dataset (χ_1^2 = 300.85, P < 0.001). However, in both BC_1 and F_2 maps the relative marker positions were consistent with those established for the IBM2 map.

Empirical thresholds for QTL significance were established independently for BC_1 and F_2 analysis populations and for the two families comprising each population (**Table 5**). These thresholds converged around a LOD score of 2.56 ± 0.54 and 2.99 ± 0.44 for 10% and 5% genome-wide significance, respectively. Mapping of the three NR traits revealed several QTL above these empirical thresholds (**Table 6**). QTL were detected in each of the analysis populations and for all three NR traits. A total of 31 QTL were independently mapped across all analysis groups, but among these several regions were repeated detected in overlapping genomic regions. A region on chromosome 3 at around 140 cM was among the most frequently detected QTL regions and was identified for NI, RS, and RR. For each case in which it was independently identified, the additive effect direction and magnitude was consistent. The QTL was associated with reduced node-injury, increased root size, and more extensive regrowth with the addition of

an FS8 allele. This confirmed that the FS8 allele, rather than the B86 allele, was contributing to the observed resistance with respect to this QTL. The estimate of additive genetic variance for this QTL was -0.206 for NI, which represents 6.8% of the node-injury scale. Additive genetic variance for RS and RR was 0.346 and 0.394, respectively, and accounted for approximately 3.0% of the variance observed in these traits.

Interestingly, this QTL was only detected in one of the BC_1 families, 1882-7 (**Fig. 9, Table 6**). The QTL likelihood for a locus controlling size, regrowth, and node-injury peaked between markers MAGI_14202 and MAGI_72398 on c3. We see that the likelihood, particularly in the case of RR and NI, increased when independently mapped in the 1882-7 analysis population relative to the joint analysis even though the population size was reduced by nearly half. For both RS and RR, an additional QTL was spotted just upstream of this QTL on c3 that was directly abutting the region. Given the marker densities and level of recombination in our analysis populations it remains uncertain if these two QTL represent separate gene targets or if a true pleiotropic locus has been detected. The strongest marker association for NI was detected for MAGI_14202 (LOD = 2.52), but the peak incidence on c3 for the root architecture traits occurred slightly right-shifted suggesting more evidence for the latter explanation. In addition to being detected in the BC_1 population, a positionally coincident region was detected in the F_2 population as well. In this case, the QTL was localized closest to MAGI_57412 and was positioned in the large gap on the short arm of c3 (**Table 6, Fig. 9**). The QTL support interval did not precisely overlap between BC_1 and F_2 analysis populations, but the additive genetic variance and effect direction of the QTL was similar and in both analysis populations only the 1882-7 family harbored a significant genotype-phenotype association. The QTL most likely acts additively as evidenced by the effects observed in the F_2 1882-7 population.

In addition to the c3 QTL, several other genetic regions also had significant associations with node-injury, although they were only detected in the F_2 population (**Table 6**). These QTL were localized to c2, c5, c6, c7, c8, and c10 and explained between 12% and 26% of σ_p^2. Two of these QTL, q05.024 and q08.125, appeared to act over-dominantly whereby the heterozygote had the lowest level of injury relative to both homozygote classes. When markers included in the 90% support interval for each of the F_2 NI QTL were fit to a model, the total phenotypic variation explained reached 81% ($F_{36,55} = 2.19$, $P = 0.0359$) (**Fig. 10**). The strongest marker association was detected for MAGI_26731 located on c8 at 113.5 cM in bin 8.06 ($P = 0.0023$). The least

squares mean for node-injury among BB and AA homozygotes was 1.185 ± 0.37 and 1.980 ± 0.48, respectively, but significantly lower for the heterozygous class (0.52 ± 0.24). This was among one of the QTL detected that had a fairly strong dominance effect, and in this case over-dominance. This would explain why it was only detected in the F_2 population. The q08.125 locus explained nearly 15% of the total variation for NI.

QTL were also detected exclusive to the two root architecture traits. These included regions on c1 (106-157 cM), c2 (99-147 cM), and c7 (44-111 cM) that were detected for both RS and RR (**Table 6**). An additional QTL (q01.191) was identified for RS only, and was the only QTL that was not shared between the two traits. It was detected in both BC_1 families although the strength of the association was strongest in the 1882-1 family, exceeding the 1% GWT (**Fig. 9**). The largest additive effect detected for RS was observed at this QTL and resulted in an increase of over 1.5 on the 1-6 root size scale. Although the support intervals for the q01.100 and q01.189 QTL were closely adjacent, genotype evidence at multiple markers suggests an association with two separate genes rather than a single-gene explanation. A moderate association was detected for RR in this family, albeit this was the only evidence provided for a RR QTL in this region and the LOD score was considerably lower than for RS. The co-localization of RS and RR QTL confirms that strong genetic correlations exist between these traits, which would be expected given the phenotypic correlations observed. In general, the thresholds for significance were lower for the two root architecture traits relative to node-injury. A likely explanation lays in the phenotypic distributions for these traits whereby the left-skewedness of NI tended to reduce the association of genetic variance with significant phenotypic effects. Collectively, we have demonstrated that all three NR traits are experimentally tractable, and genetically controlled. Phenotypic variance observed in the F_2 and BC_1 populations were mapped to discrete chromosomal regions, and validated with multiple lines of evidence from separate analysis populations.

Phenotypic Evaluation of Doubled Haploid Lines Developed from the 1882-7 F_1 Source

Doubled haploids developed from FS8B(S):S0316 F_2 and BC_1 full-sib groups derived from the 1882-7 F_1 plant were evaluated in the summer of 2012 at three locations in Iowa and two locations in central Missouri. Because of the phenotypic and genetic variance detected in the 1882-7 families, the desire to maximize statistical power, and to validate QTL identified in the segregating populations, we chose to focus haploid induction and DHL development on

members from this F_1 source specifically. Considerable variation in each of the three root traits was captured in the set of 41 DHLs evaluated (**Fig. 11**). DHLs with both less node-injury than the transgenic check, Mir604, and more injury than the susceptible parent B86 were identified. Specifically, DHL026, DHL014, DHL033, DHL009, and DHL053 all had lower levels of injury than the 1.02 ni observed for Mir604. The three partially native-resistant checks included in the study were assigned to intermediate levels of injury, with only (AGR9 x NGSDCRW1)DH6 having less damage than the average among DHLs (1.25 ni). On the reverse side of the spectrum, the xPVP line PHG84 had the highest level of larval feeding (2.29 ni) along with LH51 (2.01). Among the doubled haploids, DHL058, DHL005, and DHL008 had the highest levels of mean injury across the five locations with 1.51 ± 0.04 ni, compared with an average of 0.88 ± 0.03 ni for the top three lines. Thus, the germplasm reported herein captures novel sources of genetic variation on both sides of the root-injury spectrum, and with respect to the lower end, appears more resistant to larval feeding than previously developed native-resistant varieties. Several DHLs had comparable levels of root protection to that conferred by the mCry3A protein present in Mir604.

Although significant differences were detected between individual DHLs, the line effect alone did not explain a significant portion of the total variation due in large part to the variation that existed across locations ($F_{52,518} = 1.23$, $P = 0.144$). The location effect was quite significant ($F_{4,249} = 86.32$, $P < 0.001$). The two Missouri sites and the Crawfordsville site had considerably more feeding damage than the two central Iowa locations (**Fig. 12**). Mean injuries at the Ames and Boone sites were 1.18 ± 0.09 and 0.54 ± 0.09, respectively, versus an average of 2.04 ± 0.09 among the other sites. However, when location was accounted for, the proportion of variance explained by line effects went up considerably (**Fig. 12**). Estimates of heritability for node-injury resistance ranged from 33% in site D to 70% in site A. Not surprisingly, the two artificially-infested sites yielded the highest estimates of heritability (sites A and E), gaining an additional 20% over the average achieved in the Trap crop sites. The relative line effect was fairly consistent between the locations with DHLs performing well in one location also having lower levels of injury in the other locations, and similarly for more susceptible DHLs. In particular, DHL023, DHL034, and DHL026 were among the most resistant in each location, whereas DHL022 was most damaged. The average pairwise correlation between locations was 85%. This

provides more evidence that heritable genetic variation exists for WCR larval feeding in the set of derived DHLs for which is experimentally tractable and potentially agronomically useful.

In addition to larval feeding, variation in root size and regrowth were detected among the set of DHLs (**Fig. 11**). Mir604 had the largest roots with an average size of 4.93 ± 0.09, not surprising given that it is a commercial hybrid line. Among the DHLs and isoline checks, DHL052 had the largest roots across locations with a size of 3.89 ± 0.09. For regrowth, 6 DHLs had regrowth equal to or greater than Mir604 with the highest level achieved for DHL003 and DHL002 (4.23 ± 0.09 and 4.01 ± 0.09). There was a positive correlation between the two root architecture traits ($r = 0.70$, $P < 0.001$), and they both had a negative relationship with NI ($r_{RS:NI} = -0.53$, $r_{RR:NI} = -0.33$, $P < 0.021$). The relatively low correlation between RR and NI suggests these are, at least in part, independent mechanisms. For both RS and RR, the location effect was very significant, as was seen for NI. The locations with the highest level of node-injury had smaller roots and less regrowth. Heritability was highest for RS with an average estimate across locations of 66%. The estimate for regrowth was 55%. The high heritabilities observed nested within location is a result of low levels of rep variation, which for all three traits was not significant ($\alpha = 0.05$). The four reps of a given genotype were highly consistent with each other, allowing for genetic differences between DHLs to be more easily resolved.

This data also provides a good comparison of relative strength of resistance among the experimental checks included in our study. The transgenic check, Mir604, had a clear advantage over the native resistant checks for all three traits (**Fig. 11**). (AGR9 x NGSDCRW-1)DH6 had the lowest level of node-injury and the largest size among the non-transgenic checks, while NGSDCRW-1 had the most secondary root growth. CRW8-1 appears to be the least resistant based on the traits evaluated herein, falling into the bottom 20% for all three traits. We also see that the inbred parent B86 had particularly low levels of regrowth relative to the set of derived DHLs, indicating the acquired variation comes from the FS8 parent.

QTL Mapping in (B86 x FS8) Doubled Haploids across Years and Locations

In order to confirm genetic variance for native resistance at regions detected in F_2 and BC_1 populations, it is important to reproduce results over years and locations. This has been one of the chief challenges in QTL studies, particularly when characterizing resistance to the WCR, but is a necessary step if resistance alleles are to be agronomically useful and mechanistically interrogated. The use of DHLs greatly facilitates this pursuit because it fixes genetic effects and

allows for their assessment in multi-location and multi-year field replications. Using the approach to investigate variation in native resistance among 69 DHLs, we performed QTL mapping in independent and joint analyses by location and for two years of phenotypic evaluation. Among the 175 markers genotyped on the set of DHLs, 164 remained after processing for genotyping errors and missing data. A map length of 1,582.4 cM was achieved with an average marker spacing of 10.3 cM. Linkage group marker densities coincided with the physical length of the 10 maize chromosomes with 27 markers on c1, and 9 markers on c10. Map length and recombination fractions were very similar between the DHLs and the BC_1 map among markers that were retained in both populations. The genotype dataset contained 56.3% B86 homozygotes and 43.7% FS8 homozygotes after imputing missing genotype data. Because of the variation that existed across years and not all DHLs were included in both years of evaluation, QTL mapping was performed independently on the 2012 and 2013 datasets. This also provided a test of the relative penetrance of identified QTL within the set of doubled haploids.

Mapping of native resistance from the DHLs evaluated in 2012 revealed several regions positionally coincident with QTL detected in the backcross and F_2 populations. One of these QTL was a node-injury association on c3 at around 72 cM (**Table 7, Fig. 13**). This QTL was identified independently in Missouri locations as well as jointly, IA1, and when averages across all locations were analyzed. In addition to being broadly detected, it was also one of the most significant QTL, with a LOD that exceeded the 1% GW threshold. The confidence interval placed this QTL on the short arm of c3 between 9.5 cM and 85 cM with the strongest evidence placing it closest to MAGI_99488 (72 cM). This precisely encompasses the NI QTL identified in the F_2 population which had established almost the identical support interval (**Table 6**). The marker MAGI_57412 fell within this support interval in all cases in which the QTL was identified in both F_2 and DHL populations, making it an ideal target for marker-assisted selection. Providing even more compelling evidence for the presence of a QTL is the nearly identical additive effects identified across the different years and analysis populations, all resulting in reduced node-injury upon inheritance of the FS8 allele. The average additive effect resulted in the protection of 0.200 ± 0.026 root nodes over the alternative allele. From the scan of effects across the genome, a large reduction on the short arm of c3 is observed that narrowly overlaps with the peak in LOD incidence and includes support from several markers clustered in the region (**Fig. 13**). Even though this QTL were not detected independently in all 5 locations

using interval mapping, the linear regression of genotype on phenotype still detected a clustering of markers on c3 that all exceeded a P = 0.05 (**Fig. 14**). The markers between 44 cM and 93 cM were consistently associated with changes in node-injury. Although not precisely overlapping, this same q03.072 QTL was also positioned closely to the QTL identified in the BC_1 1882-7 family, and this is likely to represent the same underlying node-injury gene.

Node-injury QTL were also identified on c1 (q01.224), c4 (q04.096), c5 (q05.067), c7 (q07.039), and c9 (q09.107). Among these, only the q07.039 QTL overlapped with the same region in the F_2 analysis. The remaining QTL were only detected within the DHLs, however, q05.067 was positioned close to the c5 QTL detected in the F_2 analysis and may represent the same genetic association. The QTL acted over-dominantly in the F_2 population but had a moderate additive effect in the DHLs and there was a greater difference between homozygote classes in the F_2 population (**Table 6**). Interestingly, this QTL overlapped with the RS QTL detected on the same chromosome which resulted in reduced RS. The q04.096 and q09.107 were the only NI QTL that resulted in a positive additive effect, whereby the FS8 allele increased node-injury relative to the B86 allele, i.e. B86 carried the resistance allele. In the latter case, the QTL was found to act pleiotropically on root regrowth via the opposite resistance response, albeit the effect was quite small. This appears to represent another case in which a negative pleiotropic effect was observed.

For the root architecture traits, QTL were detected on all chromosomes except c4 and c6, with multiple levels of support for many of the QTL detected (**Table 7**). The q02.120 RS QTL that was detected in the BC_1 analysis was resolved to two separate regions on c2, one at around 90 cM and one at 163 cM (**Table 6**). In each case, the QTL was detected in at least two separate analyses and, in the case of the q02.163 region it was detected in 5 including both MO and IA locations. The latter QTL was also unique in that it was a RS-exclusive QTL whereas q02.090 was associated with both RS and RR. The RR QTL was narrowly delineated on the short arm of c2 with support from several markers and a LOD peak that closely mirrored the additive effects across the support interval (**Fig. 13**). Interestingly, more often than not RS and RR QTL did not co-localize, with several regions being detected for only one of the two traits. This suggests separate mechanisms for controlling normal root growth and that specific to regeneration following herbivory, albeit evidence for overlap exists as well.

The q03.091 RS QTL co-localized with the q03.072 NI QTL as was observed in the BC_1 analysis and in both cases a fairly large additive effect was detected that increased root size by nearly 0.5 on the 0-6 scale. In both BC_1 and DHL analyses, the region was also associated with a RR QTL. The RR additive effect was nearly identical between the two analysis populations even though they were evaluated in different years, indicating a lack of dominance and high degree of penetrance. Interestingly, two QTL were identified in the DHLs that co-localized with QTL identified only in the F_2 population (RS: q10.084 and RR: q08.026). For q10.084, the exact same peak in LOD incidence occurred in both the F_2 and DHL analysis populations. This may indicate that these two QTL act recessively as they were not detected in the BC_1 population, however, we failed to detect a NI association in the DHLs, so they likely do not have a direct effect on larval feeding. Among all of the RS and RR QTL detected, only two QTL (q01.063 and q09.107) were lacking evidence from the BC_1 analysis. With respect to the q01.063 QTL, the FS8 allele resulted in a reduction in root size and was only detected in the IA3 location. The validation of QTL results from the earlier analysis provides even more support for native resistance mechanisms against rootworm larval feeding and has confirmed the association of discrete genetic factors associated with reduced node-injury, and increased root size and regrowth.

Repeated Detection of Native Resistance QTL in an Expanded Set of Doubled Haploids

The capturing of genetic variance for native resistance traits in the set of DHLs serves as a validation for both gene presence and relative QTL effect. To take the validation one step further, we repeated the evaluation of DHLs by including the set of intercross-derived lines at each of the locations evaluated in S2012. The analysis has identified 34 independently-mapped QTL exceeding at least a 15% genome-wide significance level, many of which overlapped with the S2012 mapping and the F_2 and BC_1 populations (**Table 8**). Fifteen of the 34 QTL had surpassed the highest level of significance. Of particular interest was the c3 NI QTL that was detected in both of the earlier analyses. The most significant c3 QTL was positioned at 165 cM (q03.165) and was detected in a joint analysis across the 3 Iowa locations. The same QTL was detected in the full analysis across all locations and in the IA3 location. This overlapped with the q03.135 QTL found in the BC_1 mapping population which explained almost 4% of the variation in node-injury. We also identified a NI QTL at ~ 90 cM on c3 that was localized in the Missouri locations and maps closely with the NI QTL detected in both the S2012 DHLs and the F_2 population (**Tables 6 and 7**). The difference in genomic positioning between the two c3 QTL

can be explained by the pedigree of the DHLs analyzed over the two years. In S2012, DHLs derived from F_2 plants comprised 71% of the population while 19% were BC_1-derived. Because the intercross-derived DHLs were made with BC_1 plants, in S2013 the percentage of F_2 and BC_1 plants shifted to become 52% and 48%, respectively. So this can explain why both the q03.086 and q03.165 regions were mapped in S2013. QTL for root size and regrowth were also localized to the same region on c3 between 100 and 150 cM, genomic architecture that recapitulates findings in the previous analysis populations (**Tables 6, 7, and 8**). In all cases, a reduction in node-injury and an increase in root size and regrowth were associated with the QTL.

In addition to confirming the presence of the c3 QTL, genomic co-incidence with other NI QTL was revealed. The q05.065 QTL mapped to almost the exact same position as in the previous year and was close to the c5 NI QTL identified in the F_2 population (**Tables 6, 7, and 8**). A QTL on c2 (q02.108) and c6 (q06.114) mapped to the same locus detected in the F_2 population that explained 12% and 21% of node-injury variation, respectively. Another QTL on c4 was also detected in the S2013 analysis but it had a positive effect on node-injury and was not detected in either the S2012 or F_2/BC_1 analyses. A QTL associated with MAGI_10589 on c7 was detected in both Missouri locations and across all locations. In each case, regressing the single marker on node-injury explained between 10% and 14% of the variance ($F_{2,55} = 4.18$, $P = 0.021$). The FS8 allele was associated with a 0.129 ± 0.04 reduction in root damage. The QTL did overlap precisely with a RS QTL detected in both of the previous analysis populations and was linked to the NI QTL placed near 35 cM (**Tables 6 and 7**). Among all of loci associated with NI, q03.165 and q07.077 had the strongest evidence for harboring resistance alleles. These two QTL were not only detected in multiple analyses in S2013 but they were associated with resistance in previous years of evaluation. Fitting a model that includes only those QTL identified in S2013 that reduce NI upon inheritance of the FS8 allele and using only the closest markers explained 51% of the phenotypic variance ($F_{13,49} = 2.73$, $P = 0.007$) and was associated with a reduction in node-injury of 0.951 ± 0.05. This is very close to the estimate of heritability obtained in both BC_1 analyses and among the DHLs (**Figs. 6 and 12**), and indicates most of the genetic variance has been accounted for in the model.

In conjunction with validation of variance for resistance to larval feeding damage, QTL for root size and compensatory growth were also identified. Hotspots for QTL localization occurred on c2 (q02.027), c3 (q03.137), and c5 (q05.052 and q05.082) (**Table 8**). In each case, QTL were

individually localized in more than one analysis group. The most significant QTL detected in S2013 occurred for RS and was placed between 21 cM and 31 cM on c2 and contributed to a 0.216 ± 0.054 increase in root size with the FS8 allele. The FS8 allele pleiotropically increased regrowth at the same locus. The QTL was also linked to the same region identified as a hotspot for root architecture in the S2012 dataset, although the position of peak incidence was shifted upstream. Validation of the c3 RS QTL was also accomplished. In all analysis populations (BC_1, S2012 DHLs, and S2013 DHLs) a QTL controlling both root size and regrowth was localized to between 100 cM and 120 cM on c3 with a 90% certainty, and in each case, the FS8 allele acted as the high allele. This provides strong evidence for the presence of root architecture gene(s) in this region, especially considering these populations were evaluated in different years between 2009 and 2013.

Another finding that was elucidated was a tradeoff in resistance phenology that occurred at the interface of chromosome 5 QTL at around 70 cM. For instance, evidence was found for reduced node-injury associated with the FS8 allele and increased regrowth (**Tables 7 and 8**), however, a linked region was associated with reduced root size in both S2012 and S2013. One possibility is that two separate genes reside in this genomic region between 14 cM and 85 cM. The FS8 allele at the upstream gene increases injury and decreases size, whereas downstream the FS8 allele reduces injury and increases regrowth. This hypothesis is supported by the q05.024 F_2 QTL that decreased node-injury via additive genetic variance. There is also evidence for a reduction in size upstream as evidenced by the mapped QTL in S2012. The genetic mechanisms operating in this genomic region highlight the importance of evaluating all 3 traits for native resistance and indicate that, at least in some cases, dissociations can occur between the traits, possibly as a result of tradeoffs between obligate growth and defense.

A significant portion of the genetic variance was also captured when a model was fit to RS and RR. Including the markers associated with the 6 QTL identified for RS explained 46% of the phenotypic variation ($F_{12,49} = 2.64$, $P = 0.012$). The largest effect was attributed to the q07.024 QTL, which when accounted for by MAGI_51781 resulted in a genotypic difference of 0.276 ± 0.092. The following model was constructed to explain root size: $3.356 + 0.137x_i + e_i$. For RR, fitting the markers in closest linkage with the 8 QTL identified explained 56% of the phenotypic variance, accounting for slightly more variation than was observed under the models for NI and RS. In the case of regrowth, the most significant locus was MAGI_17375 (q02.027) and fit to the

model $y = 2.082 + 0.627x_i + e_i$. The difference between genotypes was 1.25 ± 0.44 ($t_{15} = 2.87$, $P_{H0: BB>AA} = 0.012$). This difference represents 21% of the root size scale and could potentially translate into meaningful crop improvements. Collectively these results demonstrate that genetic variation exists for native resistance to the western corn rootworm and have revealed key genomic regions responsible for the resistance in FS8B(S):S0316 populations. Loci on c2 c3, c5 and c7 were repeatedly detected as being associated with changes in root architecture or reduced larval feeding damage. Not only were they detected in different years, but after subsequent generations of breeding and in different locations. The evidence suggests that these regions could be used for breeding and improving maize against the WCR and included in models to predict resistance potential.

Correlation Structure Detected Between DHLs and their PHG84 Hybrids

In addition to evaluating isoline performance for native resistance, hybrids generated using PHG84 as the maternal parent were also screened. We were interested in testing to see if resistance potential was maintained in the hybrid and if isoline performance could be used as a predictor for hybrid performance. Given that the majority of genetic variance detected came in the form of additive genetic effects, we would expect a correlation to exist. In fact, this is what was observed between the two states of zygosity, a significant correlation structure was identified (**Fig. 15**). We looked at the mean across the 4 plants per plot as well as max and minimum plot values to get a better idea of which parameter was the better predictor. Not surprisingly, the 3 parameters per trait clustered closely together and mean plot values were the strongest predictor of the other two traits. For instance, mean hybrid NI had an r = -0.526 with mean hybrid size whereas minimum hybrid NI and minimum size had an r = -0.388. One exception to this was with hybrid regrowth in which the highest correlation was achieved between max regrowth and mean NI (r = -0.497). Interestingly, for the DHLs there was considerably lower correlations detected between NI and RS (r = -0.187, P = 0.004) and NI and RR (r = 0.175, P = 0.008), although the correlation between size and regrowth was very high (r = 0.838, P < 0.001).

In the comparison between DHLs and hybrids significant positive correlations existed for each of the traits. A correlation of r = 0.464 existed for NI, r = 0.440 for RS, and r = 0.639 for RR. Max NI in the DHL *per se* actually had a stronger relationship with size in the hybrid than with NI (r = -0.575). Mean size in the DHL *per se* was also predictive of mean regrowth in the

hybrid (r = 0.656). The relationships detected between isolines and their hybrids with PHG84 among the native resistance traits provides an additional level of confirmation that additive genetic variance contributes to the phenotypic variability seen in our doubled haploids. It also indicates that the QTL identified herein can be effectively used for improving maize against the WCR and estimating performance of commercial hybrids based on genotypes of the lines used to derive them.

Discussion

Elucidating genetic factors conferring native resistance to WCR is a difficult and multistep challenge; the traits are difficult to evaluate, adequacy and uniformity of insect pressures are difficult to implement for experiments, and interactions between maize plants and WCRs are dynamic within fields, between fields and across time. Given that billions of dollars have been infused into controlling and managing the pest, the need for elucidating additional resources to add to the management toolkit is a top priority. The ability of the pest to overcome many of the strategies already deployed has been one of its defining characteristics, and further reiterates the urgency for improvement (El Khishen, Bohn, et al., 2009, Gray, Sappington, et al., 2009). Host-plant resistance remains a viable option in this pursuit. Although lines have been developed with partial resistance to larval feeding by the WCR (El Khishen, Bohn, et al., 2009, Gill, Sandoya, et al., 2011, Hibbard, Darrah, et al., 1999, Hibbard, Willmot, et al., 2007), the genetic mechanisms underlying the resistance have not been reported.

Initial Screening for Lodging and Node-Injury Identified Populations Possessing Heritable Variation for Larval Feeding Resistance

This study has identified two novel sources of native resistance to the WCR: FS8B(S):S0316-053-1 and UR13085:N0215-19-2, which represent stiff-stalk and nonstiff-stalk heterotic groups of maize, respectively. From the initial screen of resistance, we revealed that the stiff-stalk source had the greatest resistance potential, exhibiting a level of root protection nearly as strong as the MON863 transgenic check (**Fig. 1**). Subsequently generated F_2 and BC_1 populations confirmed both greater phenotypic variability, and more genetic variance in root node-injury and lodging among the FS8 segregants (**Tables 2 and 3**). This provided good justification for further characterization and allele mining within the FS8 populations. Although, we chose to focus our genetic dissection of native resistance in the FS8 material, evidence for native resistance in the UR2 material was also observed. UR13085:N0215-19-2 had the lowest

node-injury among NSS germplasm, and generally had low levels of larval injury and lodging in segregating populations (**Table 3, Fig. 2**). Even with the lower phenotypic variance, genetic variance was still found to have a significant contribution, albeit much less than was observed for FS8 populations. In contrast to the FS8 populations, the UR2 populations were segregating for silk and kernel feeding by adult WCR beetles and the resistance was attributed to UR13085, rather than Mo47, which is highly attractive to adult beetles (data not shown). The GEM source has been associated with resistance to aflatoxin (Henry, 2013, Henry, Windham, et al., 2012) and ear rot (Hung and Holland, 2012), and has also resulted in high yields in multi-location trials (Pollak and Salhuana, 2001). So it has several beneficial traits that could be exploited for maize improvement and would be a good target for further allele mining.

The variation observed in lodging within the NC89 x K55 RIL population presents an additional source of potential resistance. This variation has been observed in previous years as well (Lauter and Hessel, unpublished results). The underlying resistance could be attributed to an antibiosis, tolerance, or preference mechanism, since using lodging as a measure of resistance cannot resolve these different modes of action (Painter, 1951). Different types of root systems exist between the two parents of the population, hence segregation of tolerance-related genes is most likely (Lauter and Moose, 2008). The population has been successfully utilized to map genes involved in leaf macrohair initiation (Moose, Lauter, et al., 2004). It has levels of per line recombination equivalent to that of the IBMRILs, indicating that fine-mapping is achievable (Lauter and Moose, 2008). Given the widespread variation in this population for lodging, it likely harbors beneficial alleles that could be investigated, and potentially used for mapping WCR resistance.

Variance for larval feeding resistance from the FS8B(S):S0316-053-1 source was detected at multiple levels over the span of 5 years. Among BC_1 and F_2 families, between 47% and 76% of the variance was accounted for by full-sib effects within a given year, and 25% across years. For root size and compensatory growth, 17% and 26% of the variance was explained by full-sib effects, respectively. The lower levels of explained variance for root size and regrowth were partially attributed to evaluation error, with node-injury having the highest level of technical precision. The higher heritabilities may also be due to the more quantitative nature of the node-injury scale versus the categorical values on the size and regrowth scales. Node-injury also has the advantage of capturing antibiosis and/or non-preference, whereas RS and RR are more

reflective of a tolerance response (Branson and Sutter, 1989, Owens, Peters, et al., 1974, Tollefson, 2007). We traced the variance in these traits to particular members derived from the 1882-7 F_1 plant, first at the phenotypic level, and then confirmed with co-segregation at the genotypic level.

Multiple Levels of Evidence for a Chromosome 3 Node-Injury QTL Derived from the1882-7 F_1 Source

Genetic mapping of the causative variation underpinning the phenotypic diversity in native resistance identified discrete chromosomal regions significantly associated with each of the three native resistance traits. In the case of node-injury, differences between the two F_1-derived groups were revealed with members of the 1882-7 group segregating for larval feeding resistance. QTL on chromosome 3 were localized independently in both F_2 and BC_1 families derived from the 1882-7 F_1 parent which were not detected in the 1882-1 families (**Table 6**). In the F_2 population, a QTL was localized to the short arm of chromosome 3, and explained approximately 8% of the variance in node-injury. The QTL in the backcross population was positioned further upstream on the chromosome, but the support intervals were nearly overlapping in the two populations. In both cases, the relevant QTL localized to precisely the same region in the set of doubled haploids, with BC_1-derived DHLs positioning the c3 QTL at around 165 cM, and F_2-derived DHLs placing the QTL around 70 cM. Mapping in the DHLs suggests that two separate node-injury QTL likely reside in this region of chromosome 3, although fine-mapping would have to confirm this. In any case, the resistance allele was confirmed to be FS8-derived and found to act additively. The collective evidence for a gene(s) controlling node-injury on chromosome 3 is very strong. QTL were identified in different analysis populations (BC_1, F_2, and DHL) that were evaluated over the course of several years; as well as in DHLs that were evaluated in geographically separated locations with different levels of larval feeding pressure. The high level of penetrance and mostly additive gene action suggests that this region would be an ideal target for improving resistance of maize against WCR larval feeding. There are several markers reported here that could be used in MAS programs to select for larval feeding resistance.

Possible Candidate Genes Underlying the Chromosome 3 Node-Injury QTL

The QTL on chromosome 3 that affects node-injury by WCR is located in the same genomic region as previously reported insect resistance QTL. Cardinal, Lee, et al. (2001) found several QTL for stalk tunneling by the European corn borer (ECB) on the short arm of c3 that explained

between 5% and 10% of the phenotypic variance. Others have also localized QTL for stalk tunneling to this region (Bohn, Schulz, et al., 2000, Bohn, Khairallah, et al., 1997, Ordas, Malvar, et al., 2010, Papst, Bohn, et al., 2004). Interestingly, the QTL was not found to be associated with leaf feeding by the ECB (Cardinal, Lee, et al., 2001). The coincident location of these QTL leaves open the possibility that a particular genetic factor may confer multiple resistance properties. In this regard, we discuss genes located here which may play a role in multiple modes of herbivory defense. There are known genes in the region between MAGI_51472 and MAGI_72398 on c3 that are thought to be involved in plant defense and regulating sink to source relationships. *Rp*3 is a gene involved in conferring resistance to *Puccinia sorghi* and has been associated with sugarcane mosaic virus resistance (McMullen and Simcox, 1995, Sanz-Alferez, Richter, et al., 1995, Xia, Melchinger, et al., 1999), while *sps2* is involved in sucrose biosynthesis and regulating maize growth (Causse, Rocher, et al., 1995, Cheng, Im, et al., 1996). Both of these genes are interesting in this context because they exert effects that could confer resistance to a broad range of pests.

Another gene of interest is *MEK homolog1* (*mek1*), closely linked to MAGI_72398 (175 cM), which was repeatedly localized with node-injury QTL in the DHLs. This gene is a stress-activated protein kinase (SAPK) important in the wounding response and signal transduction cascades, and is known to be expressed in seedling roots and actively dividing tissue (Hardin and Wolniak, 1998). This candidate offers another potential link between growth and defense. SAPKs can act to transduce auxin signals and respond to osmotic stress in addition to activating downstream defense pathways, hence co-expression of growth and defense can be achieved through their activity (Kovtun, Chiu, et al., 2000, Tena, Asai, et al., 2001). Furthermore, auxins are known to be important for inducing both pre-existing roots, as well as root branching (Overvoorde, Fukaki, et al., 2010). Although we cannot definitively rule out the possibility that the root architecture and node-injury QTL localized to c3 represent different underlying genes, the single-gene hypothesis can be justified from a biochemical perspective.

Other QTL Identified and the Genetic Relationship between Tolerance and Resistance to WCR Larval Feeding

Moderate to low correlations were detected between node-injury and the two root architecture traits. However, there were several cases in each of the years of evaluation where overlap at the genetic level did not exist. DHLs with the largest root sizes did not necessarily

have the lowest levels of node-injury. Even for root size and regrowth, there was not precise overlap between the different genetic loci identified. Thus, from a mechanistic perspective, even if some of the underlying genes are the same between the observed tolerance and resistance to larval feeding by the WCR, there still appears to be two separate physiological mechanisms involved, rather than the correlated product of one mechanism. This provides genetic evidence that confirms the prevailing assumption that different mechanisms are involved in tolerance and resistance to the WCR; and that these mechanisms are reflected in the node-injury and root size/regrowth traits (Branson and Sutter, 1989, Gray, Sappington, et al., 2009, Painter, 1951, Prischmann, Dashiell, et al., 2007).

In addition to the chromosome 3 association with native resistance, several other QTL were identified with repeated evidence for QTL localization on c2, c5, c6, c7, and c8. Among the QTL detected, almost all were found to act additively, whereby the addition of the FS8 allele resulted in reduced node-injury, increased root size, and more extensive regrowth. Two QTL detected in the F_2 population were found to act over-dominantly and located on c5 (q05.024) and c8 (q08.125), and there were a few QTL detected with the resistance allele traced to B86. The 7 unique QTL identified for node-injury in the set of DHLs explained 51% of variance in node-injury. Six QTL were identified that explained 46% of the variance in RS, and 8 QTL explained 56% of the variance in root regrowth. Several marker associations were identified as statistically significant and could be used in MAS breeding programs. The utility of MAS appears to be achievable: the QTL appear to be highly penetrant, largely additive in nature, and expressed in the DHL as well as the hybrid. This correlation detected between isoline and hybrid is very important if the resistance is to have commercial applicability, and it is a necessary requisite that has not always been achieved in WCR native resistance screens (Flint-Garcia, Dashiell, et al., 2009).

Conclusions

We have introduced new germplasm sources which carry alleles that confer resistance to larval feeding by the WCR, and identified regions associated with this resistance in populations of stiff-stalk maize. Doubled haploids developed from segregating populations capture extensive variation at the phenotypic level for the three native resistance traits assessed here. Genetic analysis has confirmed the association of discrete chromosomal regions with each of the traits from earlier analyses. This study serves the WCR research community in three principal ways: 1)

by providing an immortalized source of seed that encompasses extensive variation in node-injury resistance and root architecture, and 2) by demonstrating with empirical evidence that native resistance to the western corn rootworm exists in maize, and 3) identifying regions involved in resistance that can be used for improving maize against the WCR. These regions can now be narrowed to identify causative genes, and mining of alleles at these genes. The significant markers associated with QTLs in our study can now be implemented in marker-assisted selection breeding programs to develop WCR resistant cultivars, and coupled with other agronomically important traits.

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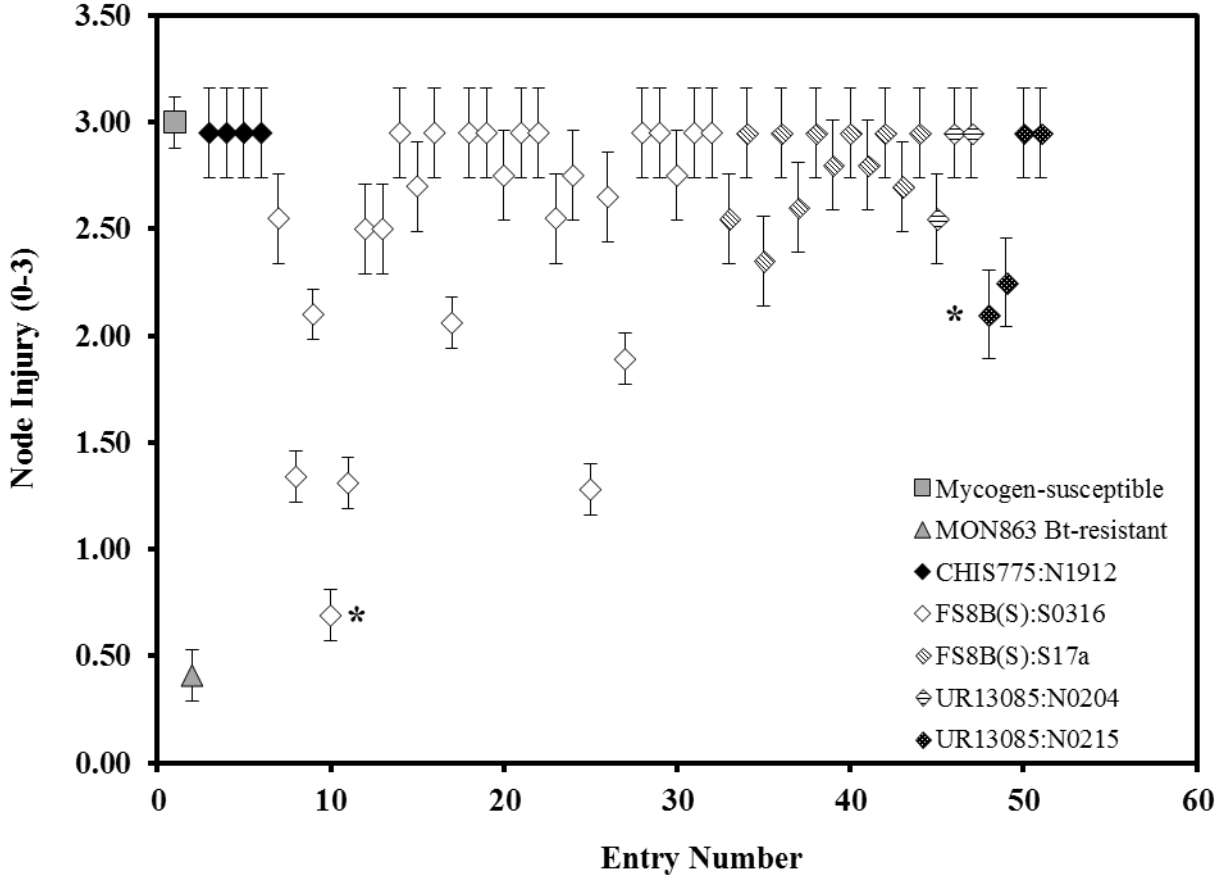


Figure 1. Scaled parameter estimates for a model explaining node-injury that included genotype, rep, and interaction terms. Deviations from the Mycogen-susceptible control are plotted for 52 entries along the x-axis and fill-coded according to entry founder class. Error bars are plus and minus standard errors. Asterisks signify the best stiff-stalk and nonstiff-stalk representatives.

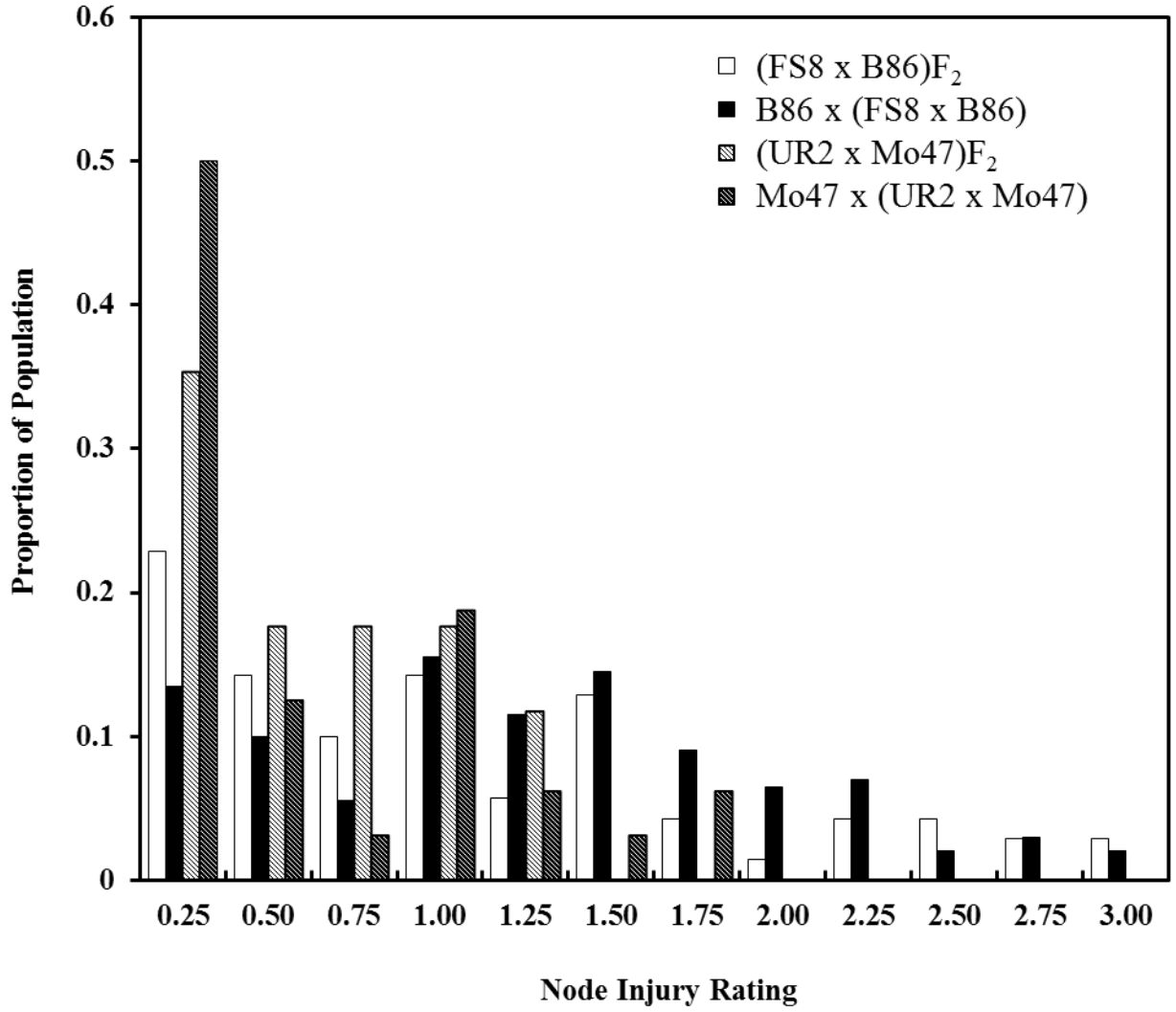
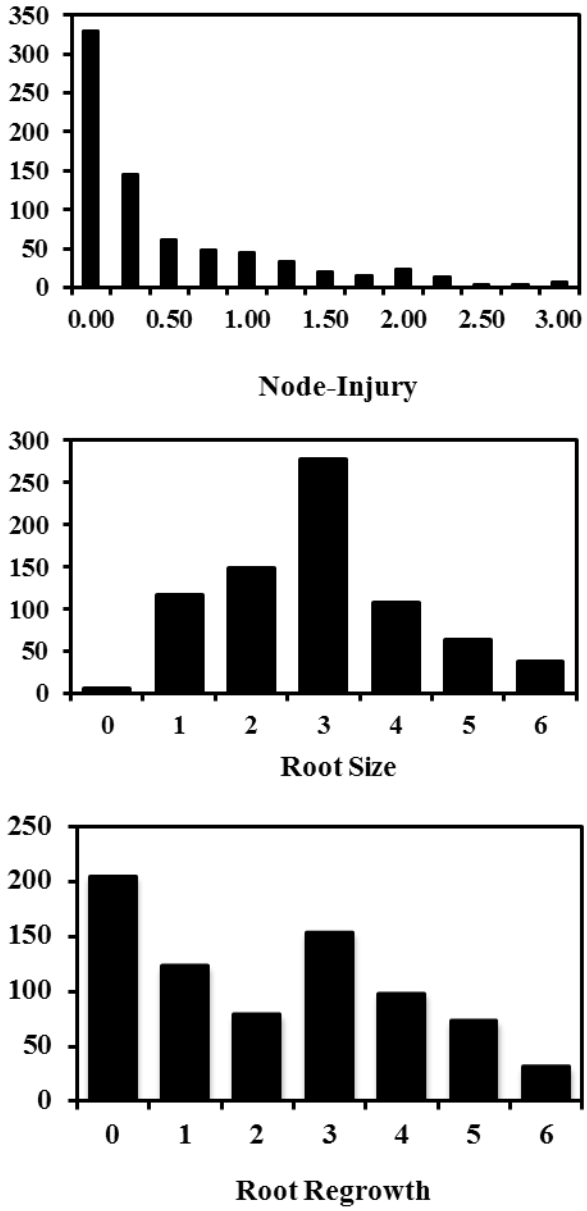


Figure 2. Phenotypic distributions for root node-injury ratings (0-3 scale).

A



B

C

	Stand	LG	NI	RS	RR
Stand					
LG	-0.037				
NI	0.011	0.189			
RS	-0.203	-0.193	-0.294		
RR	-0.118	-0.221	-0.445	0.768	

Figure 3. Phenotypic variability for root characteristics observed among 768 B86 x (FS8 x B86) plants evaluated in 2010. **A)** Frequency distributions for node-injury, root size, and root regrowth (from top to bottom) on their respective scales, **B)** Examples of the range of root phenotypes observed, **C)** Correlation matrix for stand counts, lodging (L), node-injury (NI), root regrowth (RR) and root size (RS). Using a p-value cutoff of 0.01, significance was achieved at |r| = 0.10 for the three root traits and |r| = 0.34 for the two plot-based traits.

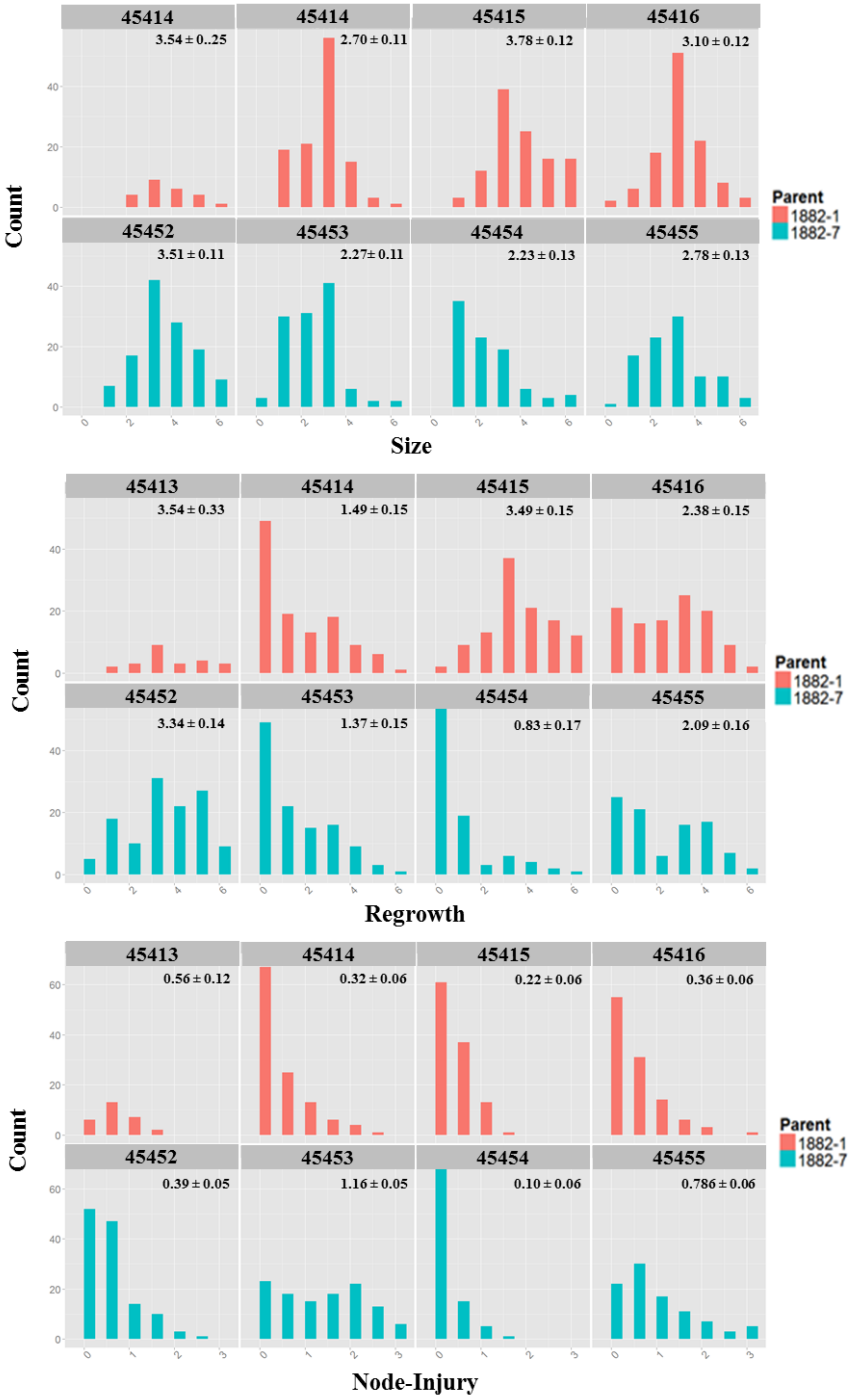


Figure 4. Faceted bar charts showing frequency distributions for node-injury, root size, and regrowth collected among 768 BC_1 plants reared in a high-insect-pressure trap nursery in 2010. Faceting is done by BC_1 family with the upper 4 charts for each trait (colored red) derived from the F_1 parent 1882-1 and the lower 4 full-sib groups (colored green) derived from the F_1 parent 1882-7. Least square means plus and minus standard errors are included for each full-sib group.

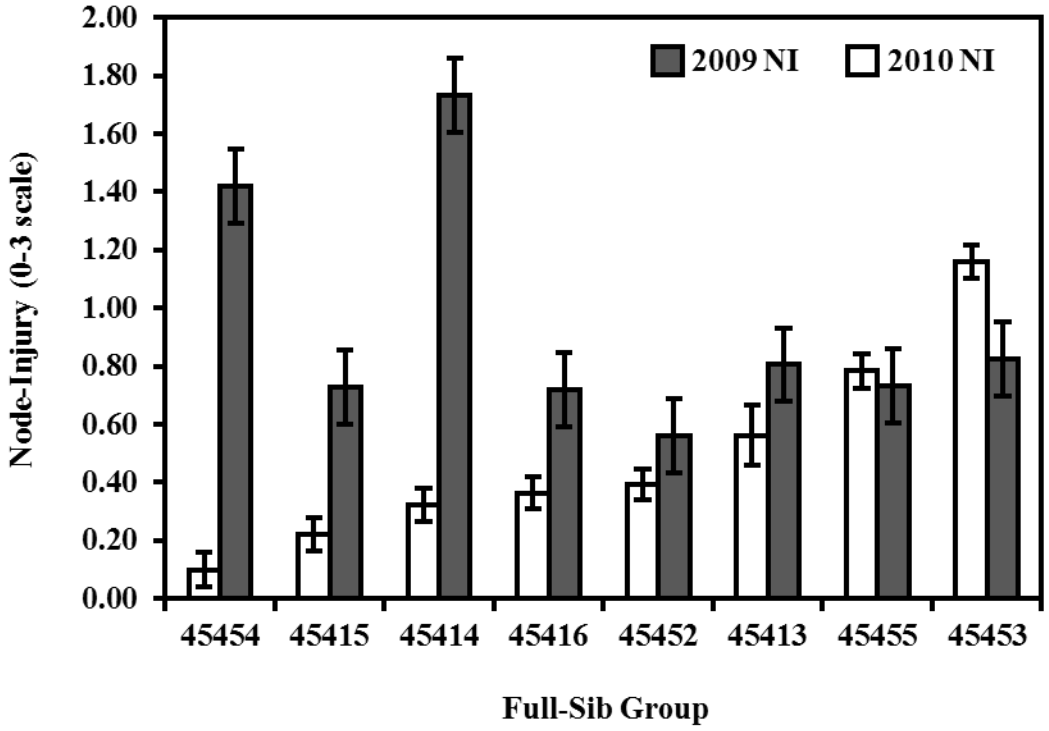


Figure 5. Differences in mean node-injury by year for 8 full-sib BC_1 groups. Error bars show the standard error of the mean.

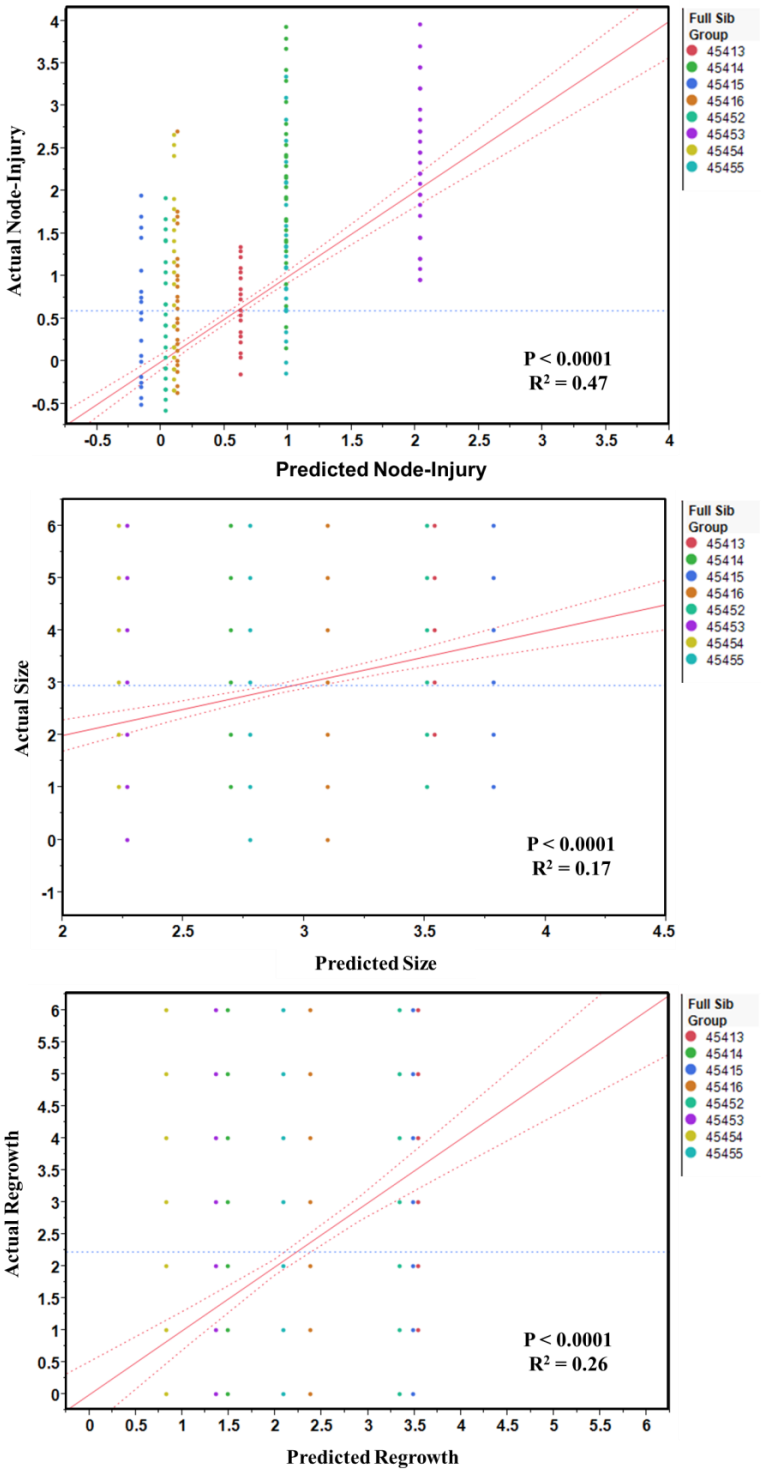


Figure 6. Plots of actual by predicted node-injury, root size, and regrowth for a model including full-sib group as the only main effect and the corresponding p-values and R^2 values for the full model.

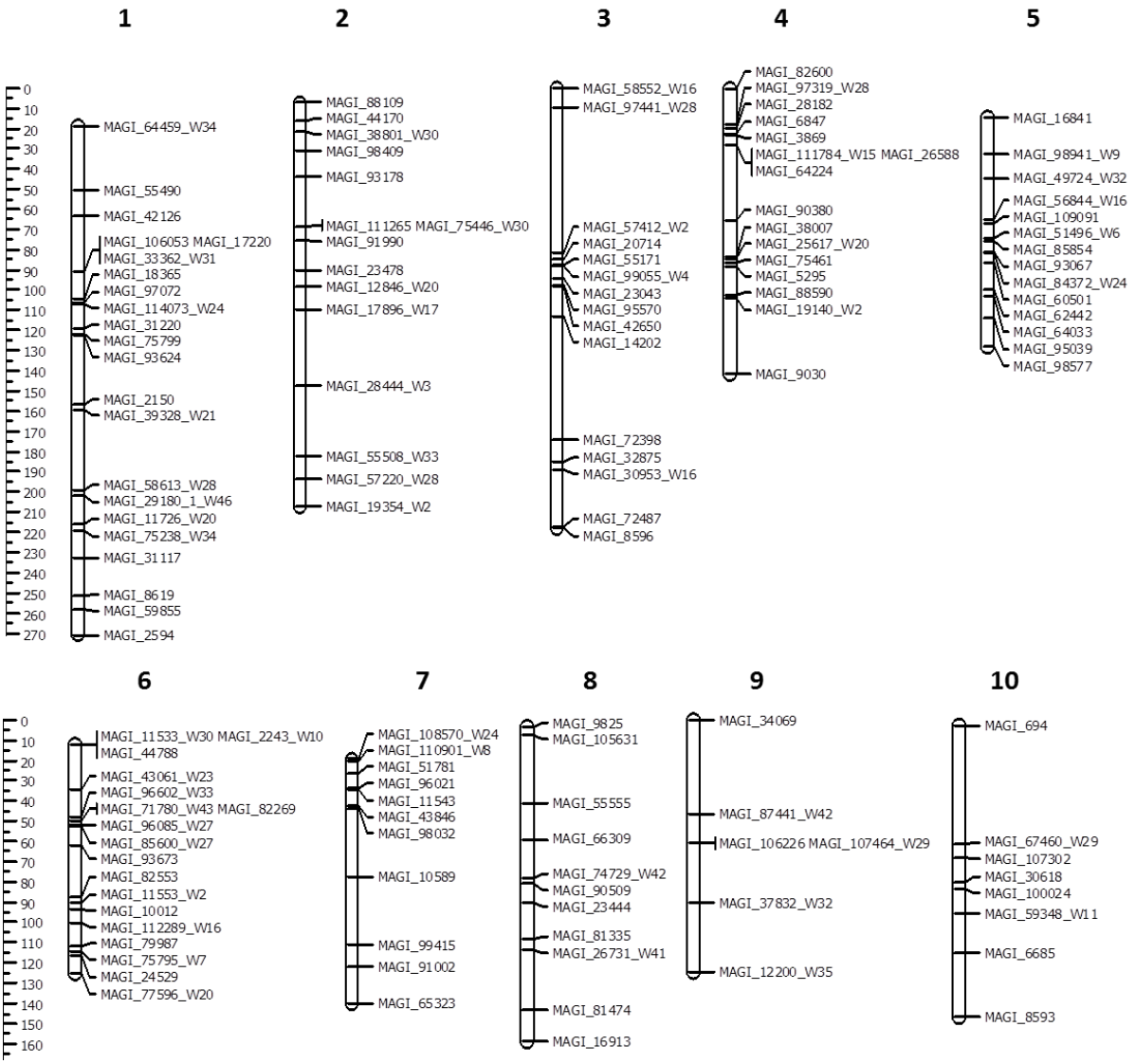


Figure 7. FS8B(S):S0316 x B86 BC_1 map developed using JoinMap software from 1,030 backcross individuals and 136 SNP markers using IBM2 map positions as a scaffold for linkage group assignments.

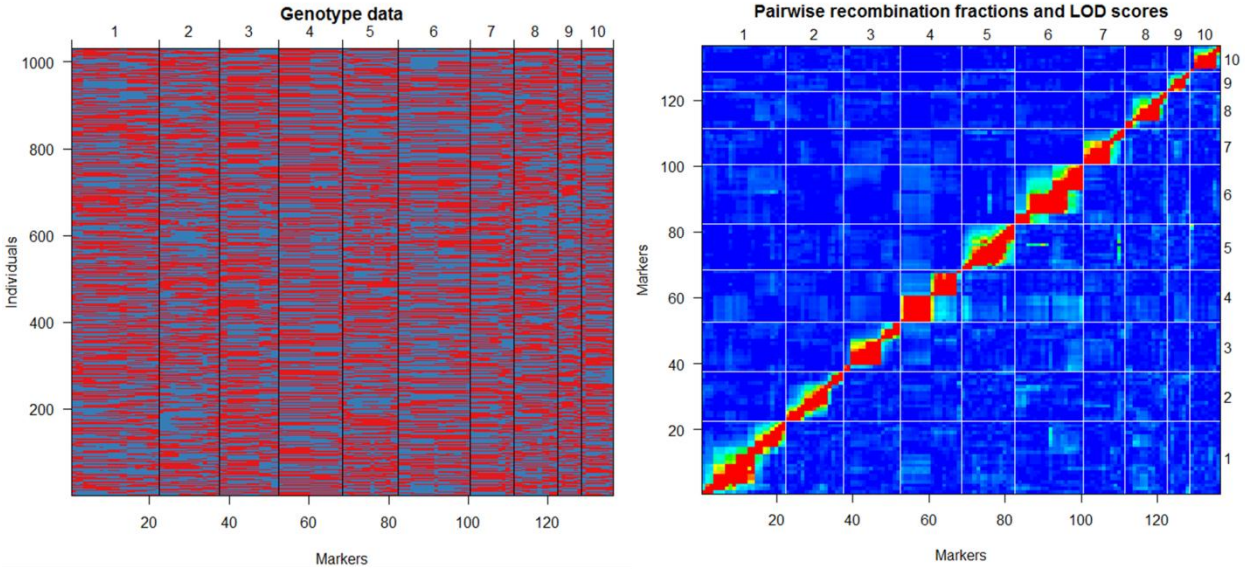


Figure 8. Genotypes for 1,030 B86 x (FS8B(S):S0316 x B86) individuals at 136 SNPs distributed across the maize genome after imputation (left), and linkage disequilibrium observed between markers (right).

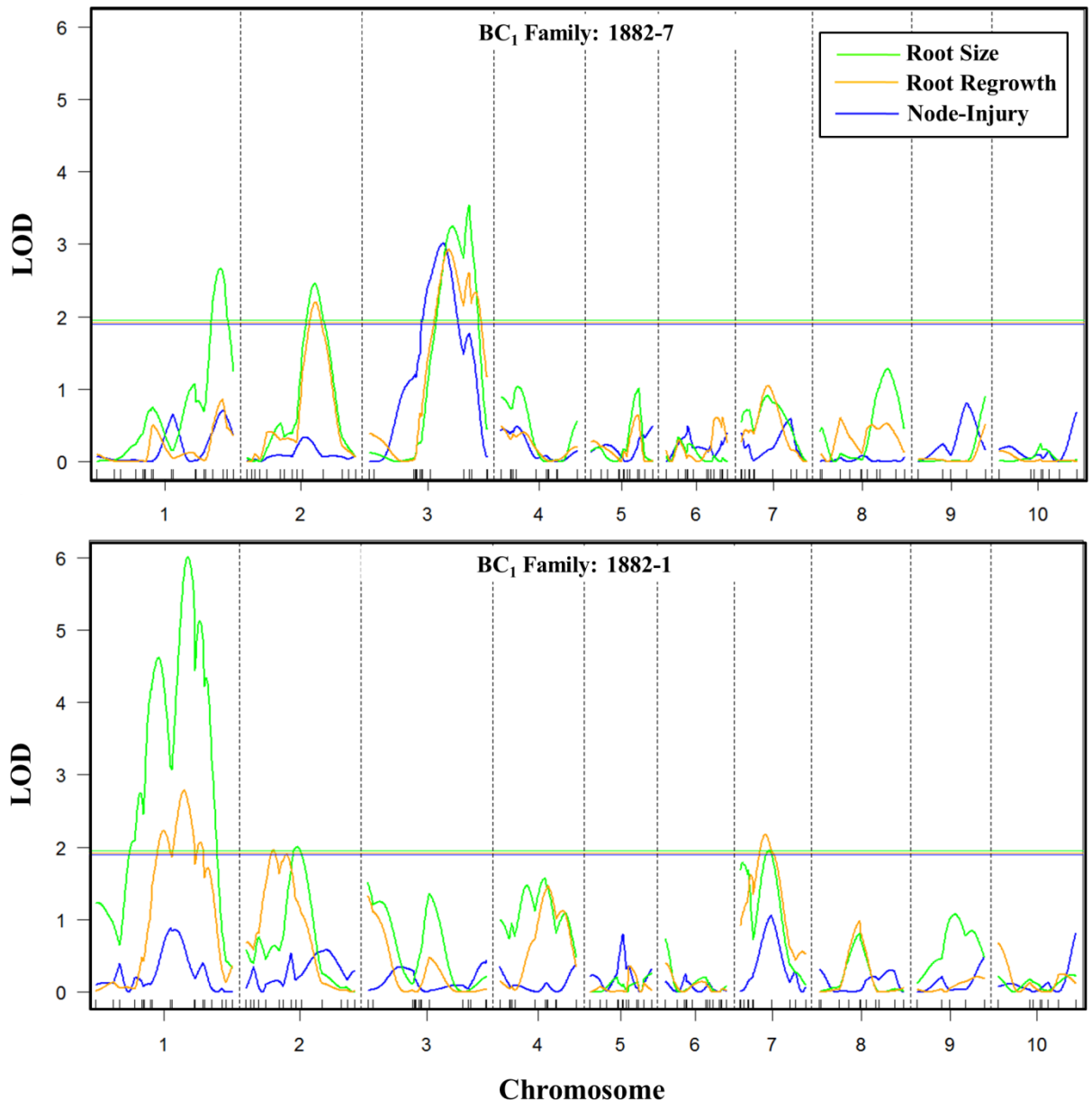


Figure 9. Node-injury, root size, and root regrowth QTL likelihood curves for two independently analyzed backcross subpopulations. Sample sizes for the 1882-7 and 1882-1 families were 534 and 496 individuals, respectively, which included trait data from both years of evaluation. Horizontals represent 10% genome-wide significance thresholds for QTL detection.

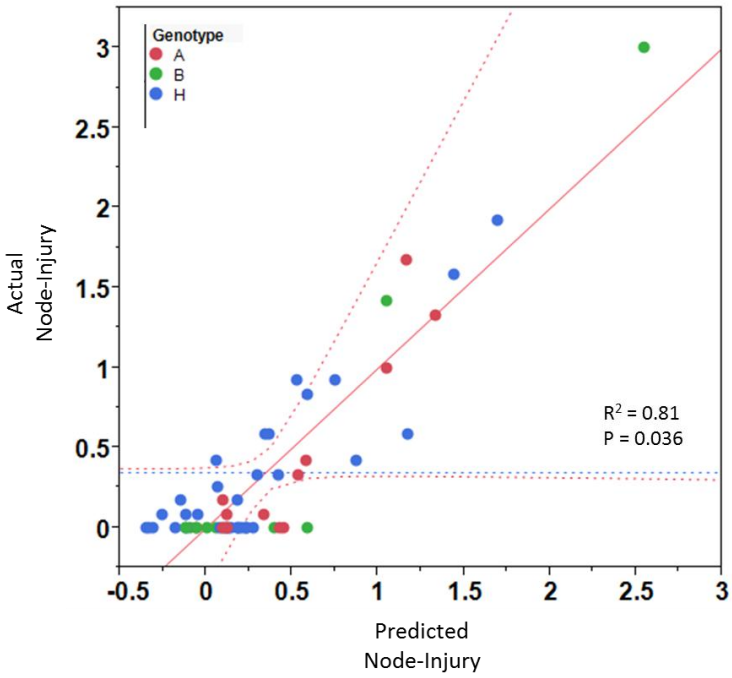


Figure 10. Plot of actual by predicted node-injury in the FS8B(S):S0316-053-1 x B86 F_2 population from a model including 18 markers that lay within QTL support intervals.

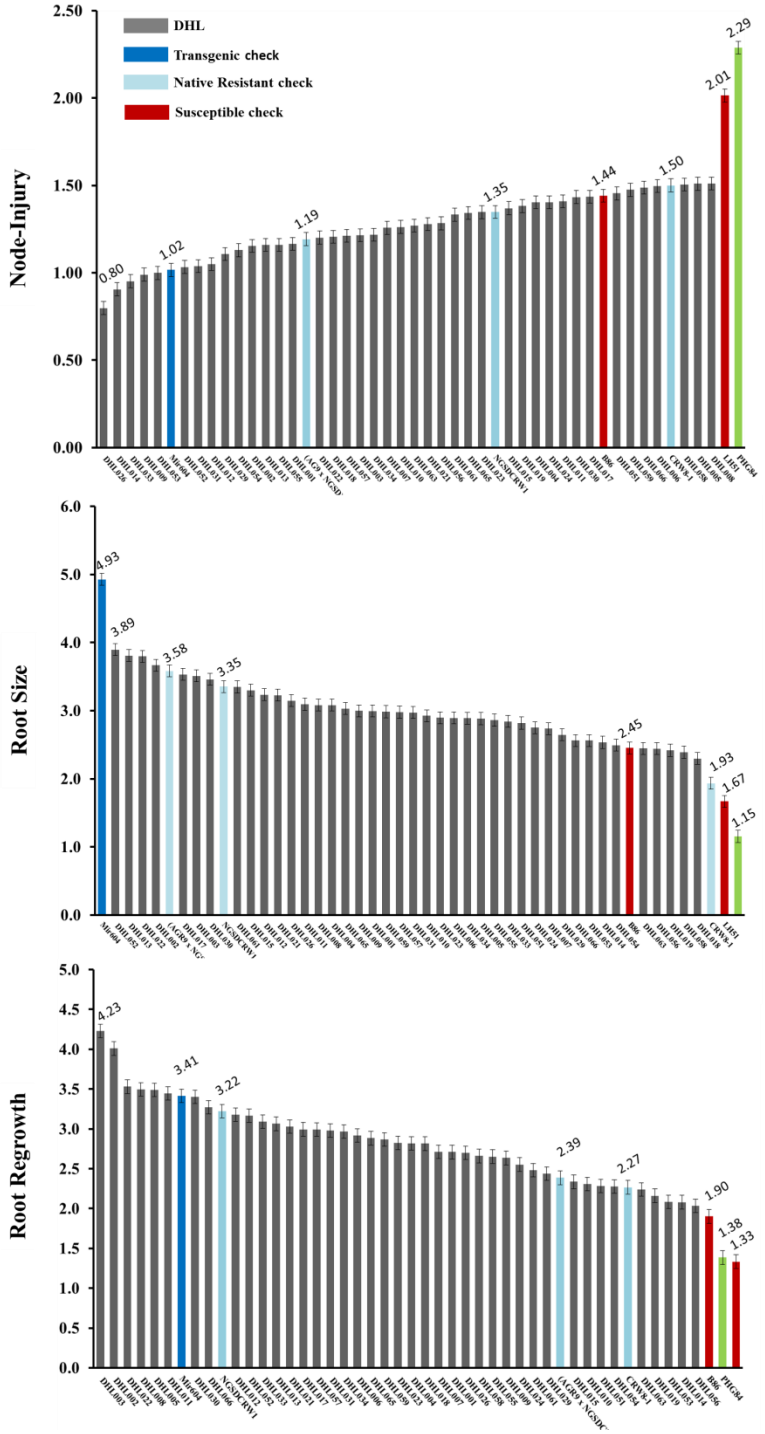


Figure 11. Means for native resistance traits among a set of 41 doubled haploid lines developed from 1882-7 FS8 BC_1 and F_2 plants evaluated in the summer of 2012. Dark blue is Mir604 transgenic check, light blue are other native resistant checks, red are the susceptible isoline checks, and green is PHG84 used as a tester in hybrid crosses.

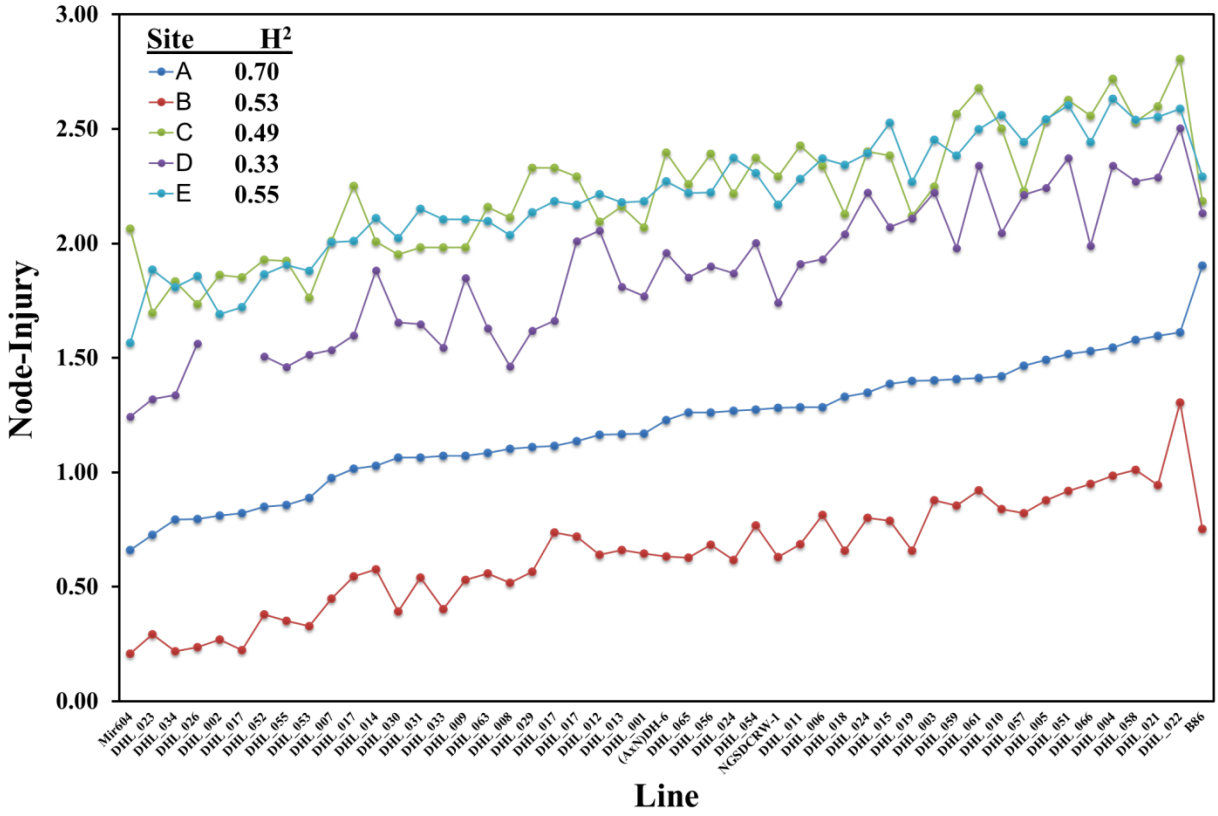


Figure 12. Least squares means for the effect of genotype x location on node-injury. A) Boone, IA; B) Ames, IA; C) Crawfordsville, IA; D) Columbia, MO, Loc1; E) Columbia, MO, Loc2.

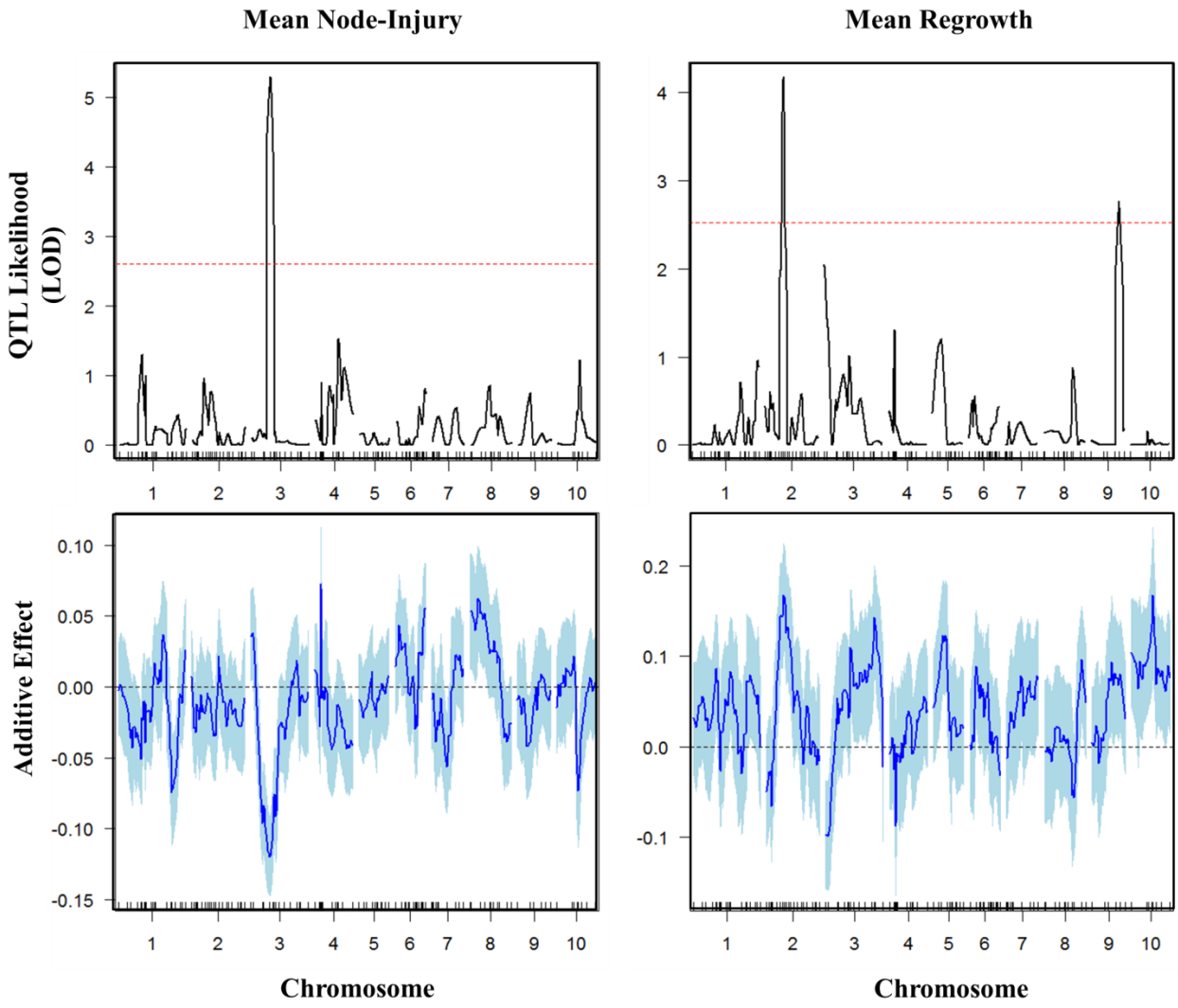


Figure 13. QTL liklihood curves (top) and additive effects (bottom) for mean node-injury and regrowth across all 5 locations evaluated in 2012. Additive effects estimated as ½ (BB – AA). Red horizontals represent 10% genome-wide thresholds based on 1000 permutations.

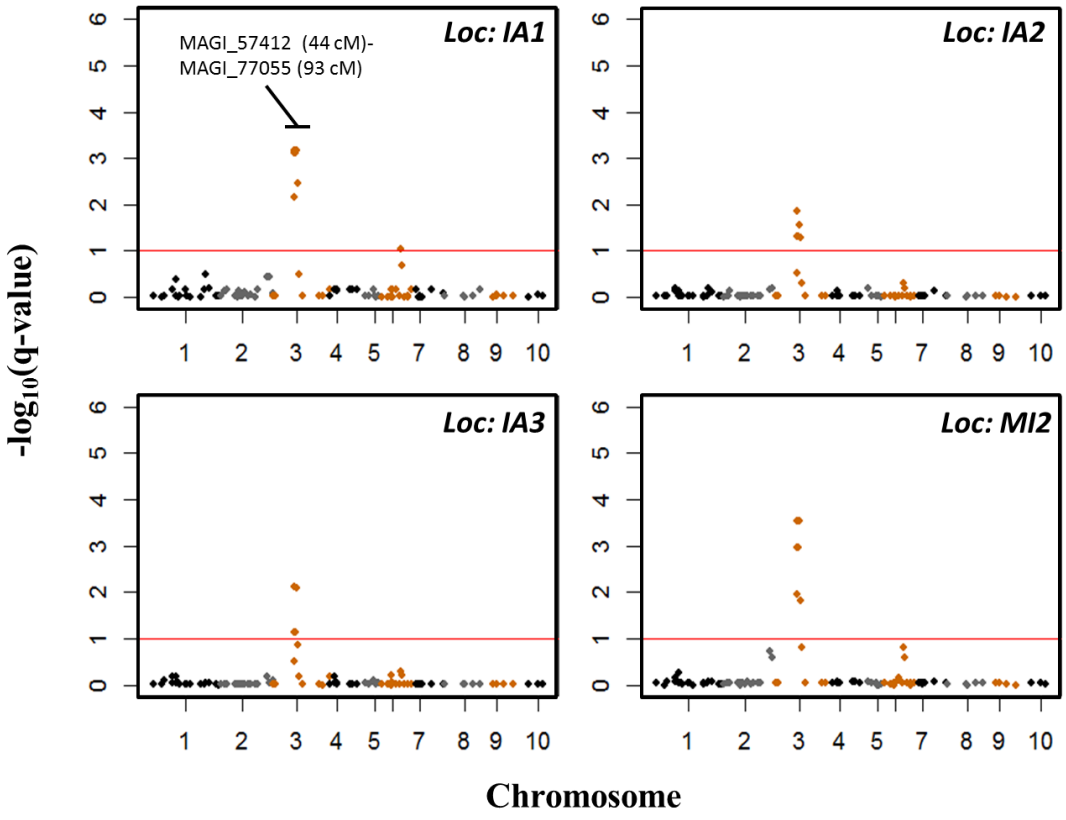


Figure 14. Manhattan plots of the adjusted p-values from linear regression of marker genotype on phenotype across 4 of the 5 locations used to evaluate DHLs in 2012.

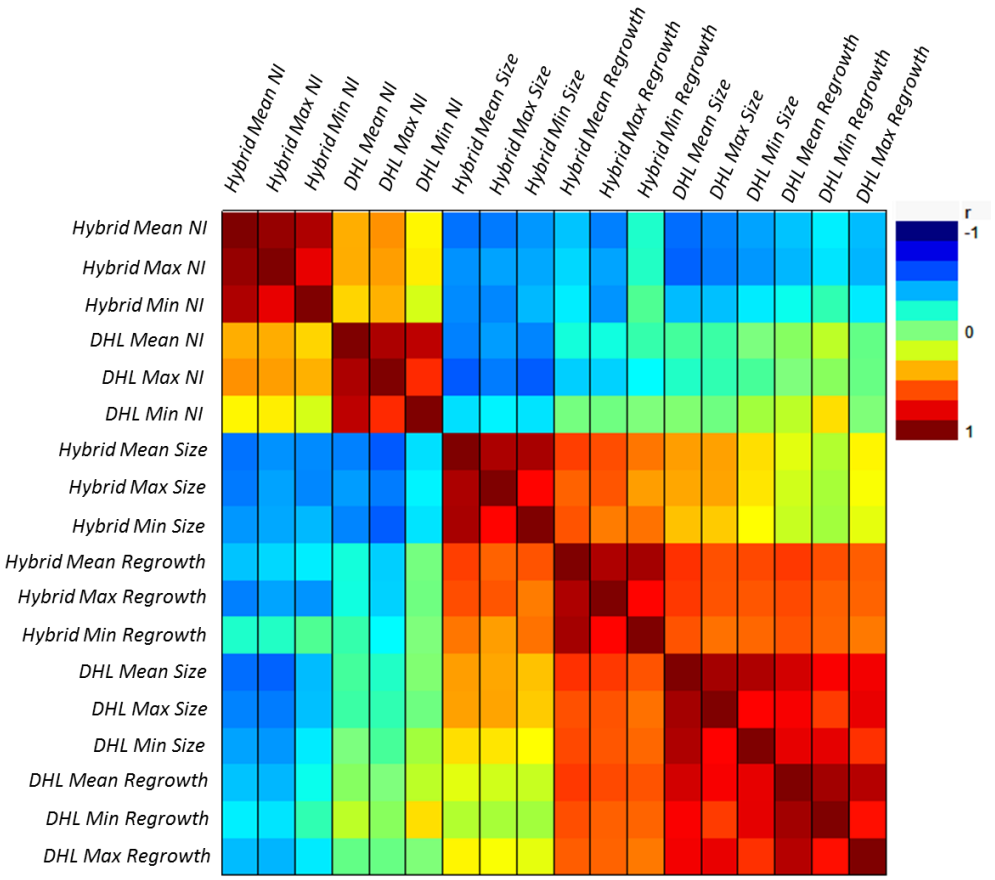


Figure 15. Clustered correlation matrix between plot mean, max, and min native resistance trait values in the set of DHLs and their testcross hybrids with PHG84. The sample size used for the analysis was 230 individuals so using $\alpha = 0.05$ as a threshold of significance, the corresponding correlation cutoff is $|r| = 0.13$.

Table 1. Pedigree information on populations developed from an exotic GEM source and an elite proprietary line and screened for native resistance to western corn rootworm larval feeding.

Founder Cross	Release Year	GEM Accession	Race	Origin
UR13085:N0204	1995	GEM80103	Cateto Sulino	Uruguay
UR13085:N0215	1995	GEM80107	Cateto Sulino	Uruguay
UR13088:S0617	1995	GEM80110	Cateto Sulino	Uruguay
CHIS775:N1912	1998	GEM80097	Tuxpeño	Mexico
FS8B(S):S17a	2001	GEM80004	Mixed	USA
FS8B(S):S0316	2003	GEM80046	Mixed	USA

Table 2. Lodging and root node-injury mean and variance components for two populations of RILs and UR2 and FS8 F_2 and BC_1 populations.

Trait	Population	Range	$\bar{X}$	σ_P^2	σ_G^2	σ_E^2	F_{Model}
Lodging †	NC89 x K55 RILs	0-100	62.70	1200.56	265.44	935.12	0.84
	B73 x Mo17 IRILs	0-60	3.91	165.23	50.20	115.03	1.32
	(FS8 x B86)F_2	0-33	8.10	166.89	159.23	7.66	62.42**
	B86 x (FS8 x B86)	0-91	24.19	704.70	162.64	542.06	1.96*
	(UR2 x Mo47)F_2	0-15	1.40	19.56	3.42	16.14	0.85
	Mo47 x (UR2 x Mo47)	0-47	5.68	113.79	9.40	104.39	0.57
Node-Injury ††	(FS8 x B86)F_2	0-100	21.04	690.16	464.54	225.62	32.94***
	B86 x (FS8 x B86)	0-100	31.25	740.27	559.35	180.92	11.58***
	(UR2 x Mo47)F_2	0-38	5.21	105.11	22.22	82.89	5.01**
	Mo47 x (UR2 x Mo47)	0-67	4.64	148.00	50.10	97.90	1.97*

†Expressed as the percentage of plants in a row lodged, ††Expressed as the percentage of nodal roots damaged
*P ≤ 0.05, **P ≤ 0.005, ***P ≤ 0.0005
σ_P^2 = total phenotypic variance
σ_G^2 = phenotypic variance attributable to genetic variance
σ_E^2 = phenotypic variance attributable to environmental variance
F_{Model} = F-statistic for the model including genotype as the only main effect; Genotype for RILs is simply the line effect, for F_2 and BC_1 populations this is the full-sib group effect (in the case of Lodging) and plot effect (in the case of Node-Injury)
Note: The estimates of genetic and environmental variance for Root Node Injury are slightly over and underestimated respectively by including plot as the main effect because both genetic and environmental effects common to a plot are included in the Genetic estimate of variance and only environmental effects across plots are represented in the environmental variance estimate.

Table 3. Lodging and root node-injury summary statistics for recombinant inbred line, FS8 and UR2 F_2 and BC_1 populations, as well as their inbred progenitors.

Trait	**Population/Inbred**	**Range**	$\bar{x}$	***Mo***	***Md***	***S***	$F_{(0.05,\ k-1,\ n-k)}$
Lodging	NC89 x K55 RILs	0-100%	62.70%	100%	71.10%	34.74%	201.01***
	B73 x Mo17 IRILs	0-60%	2.93%	0%	0.00%	8.52%	536.84***
	(FS8 x B86)F_2	0-33%	8.10%	0%	0.00%	14.44%	5.55
	B86 x (FS8 x B86)	0-91%	24.19%	0%	17.16%	26.55%	17.36***
	(UR2 x Mo47)F_2	0-15%	1.40%	0%	0.00%	4.64%	24.21***
	Mo47 x (UR2 x Mo47)	0-47%	5.68%	0%	0.00%	10.73%	142.73***
	NC89	0-100%	64.50%	N/A	61.10%	29.82%	0.34
	K55	46-100%	78.90%	N/A	85.00%	23.72%	1.92
	B73	0-50%	13.26%	0%	0.00%	19.54%	4.23
	Mo17	0-33%	8.64%	0%	0.00%	12.89%	8.63*
	B86	0-56%	8.91%	0%	0.00%	16.76%	11.54**
	Mo47	0-43%	5.07%	0%	0.00%	12.69%	17.35***
Node-Injury	(FS8 x B86)F_2	0-100%	21.04%	0%	8.33%	26.38%	0.06
	B86 x (FS8 x B86)	0-100%	31.25%	0%	33.33%	27.26%	52.62***
	(UR2 x Mo47)F_2	0-38%	5.21%	0%	0.00%	10.34%	69.93***
	Mo47 x (UR2 x Mo47)	0-67%	4.64%	0%	0.00%	12.21%	148.53***

* $P \leq 0.01$, ** $P \leq 0.001$, *** $P \leq 0.0001$

$\bar{x}$ = mean, Mo = Mode, Md = median, S = standard deviation

$F_{(0.05,\ k-1,\ n-k)}$ = F-statistic based on Levene's Homogeneity of Variance Test

Table 4. Variance in node-injury and percent of total phenotypic variance accounted for by year, full-sib, and year x full-sib interactions

Effect	**σ^2**	**% Total**	**Model R^2**
Year	0.044 ± 0.14	3.71	0.644
FSgrp	0.306 ± 0.30	25.84	
FSgrp x Year	0.439 ± 0.24	37.11	
Residual	0.395 ± 0.02	33.35	
Total	1.184 ± 0.33	100.00	

Table 5. Genome-wide permutation thresholds (GWT) for node-injury (NI), root size (RS), and root regrowth (RR) for six independent analysis populations derived from FS8B(S):S0316-053-1 and B86 parents.

Population[†]	Trait	# of Individuals	# of Markers	1% GWT	5% GWT	10% GWT	15% GWT
BC1ALL	NI	1030	136	3.69	2.64	2.27	2.10
BC1ALL	RS	1030	136	3.33	2.62	2.32	2.12
BC1ALL	RR	1030	136	3.41	2.63	2.31	2.13
BC1_1882.1	NI	496	136	3.24	2.57	2.27	2.10
BC1_1882.1	RS	496	136	3.33	2.54	2.22	2.04
BC1_1882.1	RR	496	136	3.41	2.59	2.29	2.12
BC1_1882.7	NI	534	136	3.30	2.65	3.30	2.06
BC1_1882.7	RS	534	136	3.15	2.67	2.35	2.11
BC1_1882.7	RR	534	136	3.19	2.54	2.28	2.10
F2ALL	NI	120	141	4.41	3.63	3.20	2.93
F2_1882.1	NI	60	97	4.37	3.75	3.29	3.02
F2_1882.7	NI	60	124	4.49	3.49	3.20	3.01

[†]Because FS8B(S):S0316-053-1 is not a true-breeding line, independent analyses were conducted using all B86 x (FS8B(S):S0316-053-1 x B86) individuals [BC1ALL], all (FS8B(S):S0316-053-1 x B86) F_2 individuals [F2ALL], as well as with BC_1 and F_2 subpopulations derived from each of the two F_1 plants [1882.1 and 1882.7].

Table 6. Significant QTL for node-injury, root size, and root regrowth.

Pop.	Trait	Locus (Chr.Pos)	LOD[†]	a ± SE[††]	d ± SE[††]	$R_{Q'IL}^2$	Flanking Markers[†††]		90% Support Interval
							Left	Right	
BC1all	NI	q03.139	2.40[c]	-0.100 ± 0.051	-	1.90%	MAGI_14202	MAGI_72398	113 - 175
BC1all	RR	q01.116	2.31[c]	0.379 ± 0.169	-	2.59%	MAGI_93624	MAGI_2150	123 - 157
BC1all	RR	q02.122	2.42[c]	0.336 ± 0.149	-	1.38%	MAGI_17896	MAGI_28444	110 - 147
BC1all	RR	q03.134	2.28[d]	0.351 ± 0.145	-	1.31%	MAGI_14202	MAGI_72398	113 - 175
BC1all	RR	q07.066	2.98[a]	0.426 ± 0.181	-	1.71%	MAGI_98032	MAGI_99415	44 - 111
BC1all	RS	q01.130	4.27[a]	0.356 ± 0.180	-	6.61%	MAGI_97072	MAGI_2150	106 - 157
BC1all	RS	q01.191	5.94[a]	0.622 ± 0.517	-	6.92%	MAGI_39328	MAGI_59855	159 - 258
BC1all	RS	q02.120	3.26[b]	0.233 ± 0.310	-	2.80%	MAGI_12846	MAGI_28444	99 - 147
BC1all	RS	q03.135	3.20[b]	0.338 ± 0.212	-	1.49%	MAGI_42650	MAGI_72398	102 - 175
BC1all	RS	q07.068	2.52[c]	0.286 ± 0.109	-	1.60%	MAGI_98032	MAGI_10589	44 - 77
BC1_1882.1	RR	q01.142	2.32[c]	0.451 ± 0.225	-	3.45%	MAGI_93624	MAGI_2150	123 - 157
BC1_1882.1	RR	q01.182	2.87[b]	0.929 ± 0.451	-	3.68%	MAGI_39328	MAGI_29180	159 - 202
BC1_1882.1	RS	q01.189	6.23[a]	1.569 ± 0.649	-	14.13%	MAGI_114073	MAGI_8619	107 - 251
BC1_1882.1	RS	q01.100	2.68[b]	0.872 ± 0.502	-	2.82%	MAGI_33362	MAGI_97072	91 - 106
BC1_1882.7	NI	q03.135	3.00[b]	-0.206 ± 0.135	-	3.75%	MAGI_42650	MAGI_72398	99 - 175
BC1_1882.7	RR	q03.146	2.62[b]	0.394 ± 0.194	-	2.87%	MAGI_14202	MAGI_72398	113 - 175
BC1_1882.7	RR	q03.185	2.63[b]	1.020 ± 0.654	-	4.02%	MAGI_72398	MAGI_30953	175 - 189
BC1_1882.7	RS	q01.245	2.77[b]	0.161 ± 0.302	-	0.50%	MAGI_31117	MAGI_59855	233 - 258
BC1_1882.7	RS	q02.129	2.59[c]	0.282 ± 0.149	-	2.80%	MAGI_17896	MAGI_28444	110 - 147
BC1_1882.7	RS	q03.150	2.98[b]	0.346 ± 0.145	-	3.24%	MAGI_14202	MAGI_72398	113 - 175
BC1_1882.7	RS	q03.185	3.58[a]	0.790 ± 0.476	-	6.43%	MAGI_72398	MAGI_72487	175 - 217
F2_all	NI	q02.121	6.62[a]	-0.309 ± 0.105	-0.017 ± 0.189	12.32%	MAGI_12846	MAGI_28444	99 - 147
F2_all	NI	q03.050	6.58[a]	-0.176 ± 0.096	0.293 ± 0.150	8.03%	MAGI_97441	MAGI_57412	10 - 82
F2_all	NI	q05.024	5.27[a]	0.263 ± 0.094	-0.055 ± 0.152	14.23%	MAGI_16841	MAGI_98941	14 - 32
F2_all	NI	q06.098	6.65[a]	-0.220 ± 0.158	0.204 ± 0.199	21.05%	MAGI_82553	MAGI_75795	88 - 114
F2_all	NI	q07.024	8.07[a]	-0.260 ± 0.102	-0.101 ± 0.153	10.04%	MAGI_110901	MAGI_51781	20 - 26
F2_all	NI	q08.125	6.63[a]	0.250 ± 0.105	-0.129 ± 0.195	14.51%	MAGI_26731	MAGI_81474	114 - 143
F2_all	NI	q10.084	3.69[a]	-0.178 ± 0.106	0.420 ± 0.149	25.93%	MAGI_30618	MAGI_59348	80 - 95
F2_1882.1	NI	q02.097	8.98[a]	-0.154 ± 0.195	0.903 ± 0.282	8.89%	MAGI_105144	MAGI_111265	89 - 103
F2_1882.7	NI	q03.050	3.50[b]	-0.173 ± 0.143	-0.081 ± 0.241	3.34%	MAGI_97441	MAGI_57412	10 - 82
F2_1882.7	NI	q08.026	4.14[b]	-0.248 ± 0.180	-0.061 ± 0.271	10.80%	MAGI_105631	MAGI_55555	8 - 41

[†]QTL significant at [a]1% GWT, [b]5% GWT, [c]10% GWT, [d]15% GWT
[††]Additive (a) and dominance (d) genetic variance plus/minus standard error
[†††]Markers flanking the 90% Bayesian support interval

Table 7. Joint and location-specific QTL for native resistance traits identified using doubled haploids evaluated in 5 locations in 2012.

Trait	Location	Locus (Chr.Pos)	LOD†	Lower 90% CI	Uppper 90% CI	Nearest Marker		
						Name	Pos. (cM)	a ± SE
RS	IA3	q01.063	8.25[a]	52.60	71.10	MAGI_42126	63.10	-0.232 ± 0.045
NI	All IA	q01.224	2.71[c]	199.10	234.10	MAGI_87281	221.00	-0.080 ± 0.030
RR	All	q02.077	4.18[a]	72.10	80.10	MAGI_91990	75.70	0.164 ± 0.056
RS	IA3	q02.087	3.97[a]	69.10	90.60	MAGI_23478	90.20	0.085 ± 0.045
RS	MO2	q02.090	3.09[b]	75.70	207.00	MAGI_11864	90.20	0.204 ± 0.066
RR	IA3	q02.092	4.86[a]	85.60	95.10	MAGI_11864	90.20	0.235 ± 0.063
RS	IA1	q02.156	3.76[b]	149.60	162.10	MAGI_21401	159.80	0.246 ± 0.076
RS	All MO	q02.161	3.73[b]	151.60	171.10	MAGI_21401	159.80	0.279 ± 0.061
RS	MO1	q02.161	3.77[a]	152.60	172.10	MAGI_21401	159.80	0.383 ± 0.086
RS	All	q02.163	3.95[b]	12.10	207.00	MAGI_21401	159.80	0.195 ± 0.049
RS	All IA	q02.192	4.93[a]	178.60	191.60	MAGI_57369	194.00	0.195 ± 0.056
NI	IA1	q03.037	6.53[a]	29.50	45.00	MAGI_57412	44.00	-0.170 ± 0.042
NI	MO2	q03.044	3.18[b]	9.50	72.00	MAGI_57412	44.00	-0.165 ± 0.061
NI	All	q03.072	5.85[a]	47.00	85.00	MAGI_99488	72.00	-0.120 ± 0.027
NI	All MO	q03.072	4.66[a]	62.00	79.50	MAGI_99488	72.00	-0.246 ± 0.068
NI	MO1	q03.072	4.34[a]	61.00	80.00	MAGI_99488	72.00	-0.331 ± 0.116
RS	IA2	q03.091	3.03[b]	83.00	102.00	MAGI_77055	92.90	0.403 ± 0.132
RR	IA2	q03.119	2.58[c]	99.50	131.00	MAGI_23043	125.00	0.350 ± 0.137
NI	All MO	q04.096	4.10[a]	93.00	111.50	MAGI_5295	88.20	0.116 ± 0.061
NI	MO1	q04.096	4.89[a]	93.00	117.00	MAGI_88590	102.40	0.151 ± 0.121
NI	IA2	q05.067	2.73[c]	53.70	70.20	MAGI_109091	66.80	-0.080 ± 0.038
RS	MO1	q05.074	3.44[b]	71.20	88.70	MAGI_51496	73.80	0.134 ± 0.125
RS	All MO	q05.076	3.77[b]	70.20	88.70	MAGI_85854	75.50	-0.247 ± 0.066
RS	IA3	q05.076	2.56[d]	69.70	87.70	MAGI_85854	75.50	-0.077 ± 0.046
NI	IA1	q07.039	2.66[c]	23.70	41.20	MAGI_11543	34.50	-0.105 ± 0.052
RS	IA3	q07.087	2.73[c]	68.70	100.20	MAGI_10589	77.40	0.104 ± 0.047
RR	IA1	q08.156	3.02[b]	7.50	158.70	MAGI_16913	158.70	0.114 ± 0.086
RR	All	q09.107	2.77[c]	95.00	117.00	MAGI_105195	106.50	0.081 ± 0.059
NI	IA2	q09.107	4.13[a]	98.50	118.50	MAGI_105195	106.50	0.102 ± 0.036
RS	MO1	q10.084	3.15[b]	72.70	92.00	MAGI_100024	83.60	0.451 ± 0.113

†QTL significant at [a]1% GWT, [b]5% GWT, [c]10% GWT, [d]15% GWT

CI = Bayesian support interval; Chr = Chromosome; Pos = position in centiMorgans; a = QTL additive genetic variance; IA1 = ISU Ag Engineering and Agronomy Research Farm (Boone, IA); IA2 = ISU Bruner Research Farm (Ames, IA); IA3 = ISU Southeast Research Farm (Crawfordsville, IA); MO1 = MU Bradford Research and Extension Center Location 1 (Columbia, MO), MO2 = MU Bradford Research and Extension Center Location 2 (Columbia, MO).

Table 8. Joint and location-specific QTL for native resistance traits identified using doubled haploids evaluated in 5 locations in 2013.

Trait	Location	Locus (Chr.Pos)	LOD†	Lower 90% CI	Upper 90% CI	Nearest Marker		
						Name	Pos. (cM)	a ± SE
NI	All MO	q01.116	3.31[b]	111.10	120.10	MAGI_107844	115.20	0.067 ± 0.030
RS	MO2	q01.219	3.38[b]	213.10	225.10	MAGI_75238	219.20	0.155 ± 0.044
RS	All	q02.026	6.16[a]	21.40	30.80	MAGI_17275	27.00	0.216 ± 0.054
RR	All	q02.027	3.21[b]	22.60	30.80	MAGI_17275	27.00	0.129 ± 0.045
RS	All IA	q02.027	4.26[a]	25.10	29.10	MAGI_17275	27.00	0.206 ± 0.059
RS	IA1	q02.027	4.33[a]	24.60	28.60	MAGI_17275	27.00	0.269 ± 0.073
NI	IA2	q02.108	5.24[a]	104.10	115.10	MAGI_17896	109.50	-0.164 ± 0.050
NI	MO2	q03.086	3.58[b]	77.00	91.00	MAGI_77055	81.00	-0.146 ± 0.056
NI	All MO	q03.093	3.37[b]	87.00	101.00	MAGI_77055	92.90	-0.105 ± 0.035
RS	IA1	q03.108	3.12[b]	103.50	119.50	MAGI_14202	113.20	0.151 ± 0.090
RR	IA3	q03.137	3.45[b]	127.50	149.00	MAGI_14202	113.20	0.224 ± 0.102
RS	All	q03.153	3.07[b]	132.00	156.00	MAGI_14202	141.00	0.101 ± 0.068
NI	All IA	q03.165	4.86[a]	165.00	169.50	MAGI_72398	174.50	-0.073 ± 0.029
NI	All	q03.168	3.20[b]	165.00	179.00	MAGI_72398	174.50	-0.065 ± 0.025
NI	IA3	q03.171	3.24[b]	161.00	180.00	MAGI_72398	174.50	-0.157 ± 0.055
NI	IA1	q04.010	3.60[a]	3.50	15.50	MAGI_82600	0.00	0.086 ± 0.039
RS	All	q05.044	2.89[b]	39.50	54.50	MAGI_49724	44.20	-0.188 ± 0.057
RS	All MO	q05.047	3.47[b]	41.50	54.50	MAGI_49724	44.20	-0.231 ± 0.069
RS	MO1	q05.052	4.79[a]	47.50	54.00	MAGI_49724	44.20	-0.171 ± 0.052
NI	IA1	q05.065	4.66[a]	60.00	66.50	MAGI_56844	64.50	-0.136 ± 0.041
RR	IA1	q05.067	2.94[b]	56.50	73.50	MAGI_109091	66.80	0.363 ± 0.145
RR	All	q05.072	5.70[a]	66.80	78.50	MAGI_51496	73.80	0.184 ± 0.058
RR	All MO	q05.082	5.32[a]	73.80	81.60	MAGI_84372	81.60	0.094 ± 0.024
RR	MO2	q05.082	4.02[b]	76.00	81.60	MAGI_84372	81.60	0.182 ± 0.040
NI	IA1	q06.052	3.90[a]	50.60	57.10	MAGI_85600	52.40	-0.105 ± 0.038
NI	IA2	q06.114	3.16[b]	101.10	118.10	MAGI_75795	114.20	-0.110 ± 0.049
RS	All IA	q07.024	3.96[a]	19.20	25.70	MAGI_51781	26.30	0.124 ± 0.068
RR	IA1	q07.052	4.83[a]	47.20	52.20	MAGI_43846	42.20	0.324 ± 0.146
NI	All	q07.077	3.44[b]	70.20	86.70	MAGI_10589	77.40	-0.067 ± 0.024
NI	MO1	q07.077	3.50[b]	69.20	86.20	MAGI_10589	77.40	-0.069 ± 0.024
NI	MO2	q07.077	3.44[b]	68.20	91.20	MAGI_10589	77.40	-0.133 ± 0.047
RS	All	q08.114	3.70[a]	112.50	122.00	MAGI_26731	113.50	-0.096 ± 0.029
RR	MO1	q08.124	4.38[a]	124.00	128.50	MAGI_26731	113.50	0.046 ± 0.018
RR	IA2	q09.047	2.91[c]	33.00	53.00	MAGI_87441	46.70	0.257 ± 0.079

†QTL significant at [a]1% GWT, [b]5% GWT, [c]10% GWT, [d]15% GWT

CI = Bayesian support interval; Chr = Chromosome; Pos = position in centiMorgans; a = QTL additive genetic variance; IA1 = ISU Ag Engineering and Agronomy Research Farm (Boone, IA); IA2 = ISU Bruner Research Farm (Ames, IA); IA3 = ISU Southeast Research Farm (Crawfordsville, IA); MO1 = MU Bradford Research and Extension Center Location 1 (Columbia, MO); MO2 = MU Bradford Research and Extension Center Location 2 (Columbia, MO).

CHAPTER 4: GENETIC ANALYSIS OF COMPENSATORY RESPONSES TO WESTERN CORN ROOTWORM HERBIVORY IN HYBRID MAIZE POPULATIONS

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Abstract

Plants respond to herbivory through suites of biochemical and physiological changes that often extend well beyond the point of infliction. These changes have been observed in the context of both aboveground and belowground herbivore pressure, although the mechanisms by which these changes occur are not always the same. Research targeted at aboveground herbivory has yielded some important results, but responses from belowground herbivory have been harder to resolve. However, the crosstalk that occurs between root and canopy physiology is increasingly identified as an important feature of the defense response. Here, we describe the physiological response to root herbivory in maize using a pest system of major agricultural importance, the western corn rootworm (WCR). Using a high resolution hybrid population derived from crossing a recombinant inbred line population with a tester, we evaluated 18 traits spanning different physiological classes, under both high and low WCR rootworm larval feeding pressure over the course of two years. For each trait and for each line, tests were performed to identify differences in phenotypic performance under high and low WCR treatments. QTL mapping was conducted in both treatments, independently and jointly, to detect genotype x treatment associations. The findings have identified specific physiological mechanisms involved in under-, over-, and neutral compensation to WCR herbivory, and have revealed the genetic architecture being selected upon. The overcompensation response was found to be targeted at changes in plant growth and ear architecture. These changes were genetically tractable, and in several cases, penetrant across replications and years within the study. Substantial genotype by treatment effects were detected for 20 and 53 QTL that were associated with overcompensation and undercompensation responses, respectively. For suites of related traits, we examined the co-localization (or lack thereof) for QTL that may act pleiotropically. Of particular interest was a

QTL on chromosome 3 (361 cM) that accounted for over 10% of the phenotypic variation in plant height, ear height, and the ratio of ear height to plant height. Genetic variation at this QTL had a significantly stronger association with plant architecture traits in the high WCR treatment than in the low treatment, and resulted in increased growth under high WCR pressure. A stringent positional confidence interval for this QTL contains 39 predicted maize genes, at least one of which is an interesting candidate for the observed response; the *sucrose phosphate synthase2* (sps2) gene has been shown to play an important role in allocating energy to preserve yield under stress in maize, and is thus implicated as a candidate gene for harboring a polymorphism that accounts for the response to high WCR pressure. In this vein, we place our QTL results in the context of plant defense theory and propose a model for specifically describing how plants may respond to root herbivory.

Keywords: western corn rootworm, herbivory, tolerance, overcompensation, undercompensation, recombinant inbred line, quantitative trait locus, resource availability

Introduction

Plant defense has long been thought of as an extremely complex process that involves numerous genetic and environmental factors, many of which interact with each other. Different plant organs and tissues often experience a different set of stressors and have different metabolic resources available to them. However, a growing body of evidence suggests that extensive cross-talk occurs between aboveground and belowground defense mechanisms (Gassmann 2004; Gill et al. 2011; Tiffin 2000; van Dam 2009; Watts et al. 2011). Collectively, these biotic and abiotic factors interact to manifest in the overall health of the plant.

Several models have been brought forth to explain the defense response to herbivory. These models seem to converge around increasing the photosynthetic and/or growth rate, and balancing resource allocations for defense, reproduction, and growth (Strauss and Agrawal 1999). The carbon/nutrient balance model suggest that plants can cater their defense strategies based on nutrients available to them, and that this balance is dependent on carbon and nitrogen availability (Bryant et al. 1983). Thus, plants possess the capacity to allocate nutrients to different physiological processes based on the C/N balance. An alternative model, the resource availability model, states that plant defense is ultimately dependent on the availability of resources and the

balance between growth and defense (Coley et al. 1985). This model suggests that defense and growth processes are in a perpetual battle for the available resources; plants that have slower growth rates may be able to better shunt resources to herbivory defense. A third model that has been proposed is the Growth/Differentiation model (Herms and Mattson 1992; Loomis 1953). This proposes that resources are balanced between adding additional tissue (growth) and specializing existing tissue (differentiation), and both processes compete for the same pool of carbohydrates. The assumption is that plants that invest their energy into specialized defense processes will consequently need to sacrifice growth as a result. However, if plants can invest their energy into physiological changes that both reduce the negative effects of herbivory and also aid in their growth, reproduction, or resource acquisition, then the return on investment can be multifaceted and perhaps synergistic by comparison to the alternative.

Tolerance, or the ability of plants to compensate for herbivore damage, most likely serves as an important mechanism for mitigating losses under herbivore pressures. Many definitions for tolerance exist and some regard it as a form of resistance, while others suggest it is a separate phenomenon (Núñez-Farfán et al. 2007; Strauss and Agrawal 1999; van Dam 2009). Tiffin (2000) defines tolerance as changes in plant traits that minimize or reduce the damaging effects of herbivory on plant fitness. Strauss and Agrawal (1999) refer to tolerance in terms of plant fitness in a damaged state relative to an undamaged state. Under this definition, tolerance is best assessed using individuals that are genetically similar, and preferably isogenic, so that the same genetic variabilities are tested under both conditions. However, even if herbivory damage is completely compensated for, genotypes with lower tolerance to herbivory may still have higher fitness, so there is not always a direct relationship. In fact, research suggests there can be fitness costs to tolerance (Agrawal et al. 1999; Simms and Triplett 1994; Strauss and Agrawal 1999).

From an evolutionary perspective, plants that have evolved a particular resistance should not undergo selection for tolerance because they receive minimal damage and would not be selected to compensate for it, an idea originally proposed by van der Meijden et al. (1988). Several experimental cases have been reported that support the idea of negative trade-offs between tolerance and resistance (Agrawal et al. 1999; Fineblum and Rausher 1995; Stowe 1998). For example, if defensive compounds are relatively costly to produce, as is often the case, then more resistant plants may have reduced fitness even though they accrue minimal damage. Alternatively, preferential selection of host-plants by the insect can also contribute to an

observed negative relationship between tolerance and resistance. If herbivory is specifically targeted at plants with greater vigor or resource efficiency, and for which are more tolerant, than it may appear less resistant than nutritionally inferior plants (Price 1991). Positive relationships between resistance and tolerance are also known to exist (Mauricio et al. 1997). Depending on the type and severity of herbivory, a host-plant may be both tolerant and resistant. For example, if a plant is genetically adapted for high nitrogen-use efficiency, then allocation of nitrogen to both defense and growth processes may be possible. Another possible explanation is that defense chemicals may act pleiotropically on development and/or regeneration. One example of this is proteinase inhibitors, which have both antibiosis and protein storage functions (Strauss and Agrawal 1999).

The degree of tolerance exhibited by host plants can depend on the type of herbivore, the frequency and severity of damage, and the site of infliction. The extent to which tolerance is achieved is related to which of the three main categories of fitness responses are adopted (Strauss and Agrawal 1999); 1) If fitness is reduced in the presence of herbivory relative to its absence, then the plant is said to undercompensate for the herbivory; 2) When fitness in the damaged state is equal to fitness in the undamaged state, the plant is said to fully compensate for herbivory; and 3) If plant fitness is increased in the presence of herbivory, then the plant is said to have overcompensated. All three compensatory mechanisms have been observed, but perhaps the most intriguing is the overcompensation response.

The idea that herbivory might actually result in fitness benefits for the host plant is counterintuitive, but nevertheless, increasingly found to be an important response strategy. For instance, studies in scarlet gilia, *Ipomopsis aggregata*, found that 95% removal of aboveground tissue by mammalian herbivory resulted in 2.4 times more seed production and seedling survival relative to uneaten controls (Paige and Whitham 1987). A separate study using *I. aggregata*, found both more flowers and more fruits on plants exposed to herbivory pressure than on undamaged plants (Paige 1999). Interestingly, this effect was observed for both naturally-grazed and experimentally-clipped herbivory. For both of these studies, the herbivory occurred relatively early in the vegetative growth phase, so timing of herbivory relative to reproductive development likely plays an important role. Studies in other species have also identified overcompensation as a functional response to herbivory including *Gentianella campestris* (Lennartsson et al. 1997), *I. arizonica* (Maschinski and Whitham 1989), *Erysimum strictum*

(Rautio et al. 2005), and *Arabidopsis thaliana* (Mauricio et al. 1997; Siddappaji et al. 2013). Furthermore, evidence suggests that genetic variation, in addition to resource availability, plays a role in overcompensation (Agrawal 2000). One gene involved in plant metabolism, G6PDH1, has even been elucidated as being specifically involved in overcompensation in Arabidopsis (Siddappaji et al. 2013). Increases in transcriptional activity of defense and metabolically-related genes has been proposed as a genetic mechanism for overcompensation, possibly resulting from endoreduplication in cells that have terminal fates (Scholes et al. 2013; Siddappaji et al. 2013). Although recent progress has been made in explaining the genetic regulation of overcompensation, no studies to our knowledge have addressed this issue in maize, which affords an opportunity to examine the expression of these genetic mechanisms in an agriculturally important host-herbivore interaction.

Here, we address the issue of tolerance in maize (*Zea mays* L.) to an extremely important agricultural pest, the western corn rootworm (*Diabrotica virgifera virgifera* LeConte, WCR). To do so, we employed genetic variation present within a set of highly recombinant inbred lines to genetically characterize the response to herbivory. Reciprocal hybrids were generated between 250 members of the intermated B73 x Mo17 Recombinant Inbred Line population (IBMRIL) and the inbred B101, and were evaluated under both high and low WCR larval feeding pressure. Eighteen different agronomically important traits were phenotyped over the span of two summer seasons in each treatment, representing multiple developmental and physiological processes including germination and establishment, root growth and development, plant architecture, ear architecture, and grain-filling. Tests were performed to reveal the genetically-conditioned physiological responses to WCR root herbivory by comparing performance in low and high WCR treatments, first by replicate and then by line. These analyses identified the traits most responsive to WCR herbivory, and also revealed cases of each of the three tolerance outcomes i.e. overcompensation, undercompensation, and neutral compensation.

Genetic analyses were performed to identify genomic regions underlying the response to herbivory using a multiple QTL mapping approach, and comparing QTL likelihood and QTL effects between high and low WCR treatments. QTL mapping of the phenotypic response to root herbivory using the IBMRILs is a high-resolution approach to identifying candidate regions involved in insect stress response. Because of the additional recombination events that are captured during intermating, the IBMRILs improve resolution by up to 50-fold compared to

conventional RIL populations, and has been effectively deployed to identify QTL for other agronomically important traits (Balint-Kurti et al. 2007; Lauter et al. 2008; Lee et al. 2002). The relatively high marker density of the IBMRILs and the whole-genome sequence availability of both B73 and Mo17 provide further justification for its use in mapping stress response loci.

Our results have identified specific genetically-conditioned physiological changes that accompany the response to WCR herbivory and that these changes generally result in reduced agronomic performance. The response extends well beyond the root interface and encompasses both belowground and aboveground plant organs and tissues. We also revealed that both tolerance (successful compensation) and overcompensation to WCR larval feeding exists, and that the mechanisms of response tend to be targeted at changes in plant growth and ear architecture. The phenological changes underlying the response to herbivory were traced to discrete chromosomal regions and their QTL effects were revealed. Large genotype by treatment interactions were identified for the traits that were most commonly overcompensated. Several of these QTL were independently detected through multiple rounds of analysis, in reciprocal populations, and across years of the study. Finally, we place these results in the context of plant defense theory and discuss possible candidate genes and physiological mechanisms underlying overcompensation.

Materials and Methods

Plant Materials

The 250 RILs from the intermated B73 x Mo17 recombinant inbred line population (Lee et al. 2002) used for this experiment were quality controlled with molecular markers as described by Lauter et al. (2008). All hybrids were generated by reciprocal crossing with B101 (Hallauer and Wright 1995) in a single season at one location, thus reducing ear-parent effects. B101 seed for this project was obtained by self-pollinating several hundred plants in an isolation block that was inspected for phenotypic uniformity for a suite of common naked-eye polymorphisms.

Field Design, Treatments, and Phenotypes Collected

In 2010, 220 IBMRIL x B101 hybrids were grown in an alpha lattice design with two replications per treatment (4 reps total) and 14 blocks per rep, each consisting of 16 randomly assigned entries. Four hybrid checks obtained from AgReliant Genetics were also included as entries in each rep for assessing the extent of the rootworm pressure. The low pressure treatment

(LP) consisted of a conventional nursery located at the Iowa State University Agronomy and Ag Engineering Research Farm (42° 0′ 60″ N, 93° 46′ 11″ W) in Boone, Iowa. Traditional field management procedures were applied including the use of annual crop rotation from corn to soybean, herbicide application, and fertilizer applied at a concentration of 100 lbs N/acre.

The high pressure treatment (HP) consisted of a trap crop located at the Iowa State University Bruner Research Farm (41° 60′ 35″ N, 93° 44′ 11″ W) located in Ames, Iowa. The trap crop had been maintained since 2006 using corn-on-corn rotation and was originally established by artificial infestation of diapausing western corn rootworm (WCR, *Diabrotica virgifera virgifera* LeConte) eggs on individual plants according to Palmer et al. (1977) at a concentration of 750 eggs per plant. In subsequent years, the trap crop was planted late in the season adjacent to the previous year's trap, typically around the second week of June, to encourage migration of adult rootworms and subsequent egg-laying. Herbicide was applied as needed to manage weeds and fertilizer was applied at a concentration of 150 lbs N/acre. Plots were planted at a density of 25 seeds per 4.572 m row on 24 May and 25 May for HP and LP, respectively. In 2011 the experiment was expanded by including the evaluation of 238 IBMRIL x B101 and 241 B101 x IBMRIL hybrids planted in the same LP and HP treatments and using the same field management practices. The hybrids included were chosen based on seed availability and planted in a randomized incomplete block design on 18 May and 19 May, 2011 for HP and LP, respectively.

For each treatment, 18 different traits were phenotyped, covering germination, establishment, root development, plant and ear architecture, and grain filling physiological processes. **Table 1** provides a summary of all of these traits and the abbreviations that will be used throughout this paper. GERM, LG, STAND, BS, EW, GW, and CW were all collected on a row basis so only one trait value is available per plot whereas the remaining traits were assessed with 5 plants per plot. STAND, AEPH, EH+PH, and EH/PH are derivative traits so they were not directly assessed but rather calculated from direct traits as described in **Table 1**. With the exception of EH, the plant architecture traits were only collected in 2010 so only data on the IBMRIL x B101 hybrids is available. EL was added in 2011 because of the potential it provided in resolving the ear architecture response, therefore, only one year of data is available. KRF was calculated based on the proportion of kernel rows aborted and not the proportion of missing or insect damaged kernels so it was a measure of the relative number of ovules brought to maturity.

Phenotypic Analysis

For each trait collected, mean values were calculated per genotype per rep in both HP and LP, and used for statistical analysis unless otherwise stated. In all analyses, the same genotypes for a given population were evaluated in both HP and LP to maintain a balanced design. The only exception was for the EH trait in 2011, which was only evaluated in the HP treatment. Differences between reps were calculated using a least squares mean model that included rep as the single model parameter. Rep 1 and rep 2 were assigned to LP, rep 3 and rep 4 were assigned to the HP. Individual pairwise comparisons between reps were computed using Student's t-tests on rep least squares means. Reps were statistically different using $\alpha = 0.05$.

For comparing the differences between HP and LP per genotype we perform independent multiple t-tests to test the null hypothesis that performance under HP is equal to performance under LP (H_0: HP = LP) for a given trait. P-values obtained from t-tests were converted to q-values for multiple test correction using Storey and Tibshirani's method of q-value determination (Storey and Tibshirani 2003). Based on these adjusted q-values, three outcomes were established for each hypothesis test by setting FDR = 0.05. In tests that resulted in a failure to reject the null hypothesis, a tolerant outcome was established. Tests in which a given hybrid performed superior under LP relative to HP were assigned an undercompensation outcome (H_a : LP > HP). Tests in which performance under HP was superior to that under LP were assigned an overcompensation outcome. This process was done for each trait to identify lines falling into each outcome category. For the traits evaluated on a per plot basis, linear regression estimates were performed instead of independent two-sample t-tests because there was not a large enough sample size per treatment to perform the test. In this case, p-values were obtained from the linear regression of genotype x treatment on mean plot values. All statistical tests were performed using either R or JMP® Pro 10.0.0 statistical software (R Development Core Team 2008; SAS Institute Inc. 2012).

Molecular Marker Dataset and QTL Analysis

The mapping population used for this study was the IBMRIL population and corresponding IBM2 genetic map (Lee et al. 2002). The Cogenfito webtool component of MaizeGDB was used to get marker scores for the entire mapping population including 2,023 markers across 302 RILs (Hessel et al. 2010; Lawrence et al. 2008). The dataset was reduced to include only those IBMRILs used as hybrids in our study, and genetic markers identified as problematic by Lauter

et al. (2008) were removed. The total genetic map length was 7,090 cM with an average marker spacing of 3.52 cM. Among the 91.1% non-missing genotypes, 51.4% were B73-derived (AA), and 48.6% were MO17-derived (BB). Initial tests for genotype-phenotype associations were performed using a single-marker linear regression model of marker genotype by phenotype. For each trait, the linear regression was performed on each population (2010 IBMRIL x B101, 2011 B101 x IBMRIL, and 2011 IBMRIL x B101) and for each treatment (HP and LP) as well as for the combined datasets. P-values from linear regression were converted to q-values using Storey and Tibshirani's method of q-value determination (Storey and Tibshirani 2003). Significance was established by controlling FDR = 0.05.

Prior to performing interval mapping, the dataset was adjusted so that markers at the same position were moved to be slightly off from one another and missing genotypes were imputed using a minimum probability of 0.1. QTL mapping was performed using the Rqtl package (Broman et al. 2003). Additive effects of markers and QTL were estimated using the effectscan function of Rqtl, which estimates the additive effect in RIL populations as half the difference between the phenotypic averages for the two genotypic classes. In this case, since each RIL was evaluated in a hybrid state with B101, the difference is represented as $\frac{1}{2}[(p_2p_3) - (p_1p_3)]$, where p_2p_3 represents Mo17/B101 heterozygotes and p_1p_3 represents B73/B101 heterozygotes. In this derivation, the B101 alleles cancel out, leaving just the B73-to-Mo17 allele substitution effect. Therefore, using the RIL model, the additive effects reported here are actually $\frac{1}{2}[a]$, and are a conservative estimate of the true mid-parent average. Multiple QTL mapping was performed as described by Arends et al. (2010). Briefly, for each trait, a single genome scan was first performed to identify potential candidate regions and test the single QTL model using the $\log_{10}$ likelihood ratio test statistic. A multiple QTL model was then tested through the mqmscan function of Rqtl, and is analogous to composite interval mapping done in other mapping software. An automatic cofactor selection process was performed using an initial starting set of 50 markers that takes into account marker density, followed by backward elimination to identify the most probable cofactor list. This list was then used as the set of cofactors for multiple QTL mapping. Bayesian confidence intervals for QTL were derived using the bayesint function of Rqtl and using a probability coverage of 90% (Broman et al. 2003). Significance thresholds were established by using 1,000 permutation datasets and setting 5% and 10% genome-wide thresholds, which were used as the final criteria for classifying significant QTL.

Results and Discussion

Phenotypic Response to Rootworm Pressure

Collecting information on traits that span different developmental periods and different tissue-types allows for a comprehensive understanding of the phenotypic response to root herbivory by the WCR. Among the 17 traits evaluated across 4 reps, all had significant differences between high and low pressure treatments and less variation was observed between the two reps within a treatment than across treatments (**Table 2, Fig. 1**). The difference between the two reps within a treatment, however, was more evident under high WCR pressure. Under high pressure, 8 of the 17 traits were different between reps, whereas only 4 were different between reps under low pressure. The grain-filling traits (EW, GW, and CW) were particularly vulnerable to this rep variation under heavy pressure. The proportion of total height accounted for by ear height (EH/PH) was the only trait that was non-significant between reps across treatments. The four traits that were assessed on a row basis (GERM, LG, STAND, and BS) were not different between reps within either treatment, suggesting these are more robust to intra-field variation. The differences observed between low and high treatments suggest that a physiological response to high WCR pressure exists and manifests in both aboveground and belowground plant characteristics. Furthermore, under high pressure, additional variation is introduced that has measurable effects on grain-filling traits.

When the mean value for each of the traits was compared across all 4 reps, the variation between the HP and LP treatments was easily observed (**Fig. 1**). Plants grown under high pressure had more severe lodging, reduced standability, and more broken stalks than those under low pressure. The plants in high pressure also had statistically lower values for all of the plant architecture and grain-filling traits. STAND, GW, EW, LG, and KPR were the traits most affected by treatment application ($F_{1, 874} > 300$, $P < 1.0e\text{-}20$). Thus, a clear consequence of increased root herbivory pressure is a reduction in viable seed set. In addition to having fewer KPR in the high pressure treatment, plants also had a lower proportion of their kernel rows filled. Plants in LP had an average of 5.60 ± 0.27 more kernels per row and an increase of 4.80% ± 0.34% of the available kernel rows filled relative to plants in HP. This reduction in both KPR and KRF under high pressure is likely responsible for the 45.01 g ± 1.51 g reduction in GW seen in HP relative to LP. EH/PH, EH, and GERM were among the traits with the least significant differences between treatments. The primary mechanism of response appears to be directed at

ovule production, along both poximodistal and radial ear axes, and grain-filling, and thus has direct effects on yield potential.

Another major mechanism of response was a change in plant architecture. Plants under high pressure tended to have reduced plant height, ear height, and fewer NLAE, but they also had smaller spacing between phytomers as measured by EIL (**Table 2, Fig. 1**). However, the proportion of total height that was accounted for by ear height was only slightly different between treatments. Thus, it appears that the plants maintained their relative proportions under stress, but were slightly stunted under WCR herbivore pressure relative to normal conditions. This evidence indicates that the increased larval feeding under high pressure consequentially affects other plant traits, possibly by shunting resource allocations from development and reproductive processes to defense. The two main mechanisms of response that are supported from these data are a reduction in mature kernels and vegetative growth.

A correlation structure was also detected among related traits, particularly those falling within the same physiological class (**Fig. 2**). EW, GW, and CW were strongly correlated with each other ($r \geq 0.67$), as were EH and PH ($r = 0.86$), and KPR and KRF ($r = 0.63$). There were also some correlations detected across physiological classes. For example, KPR and KRF had a direct relationship with EW, CW, GW, and EL (all with $r > 0.7$). For the most part, the correlation structure between traits was maintained in both treatments with the same magnitude and direction, but a few relationships were only detected in one of the treatments. A negative relationship between lodging (positive with standability) and ear height was only detected in HP. Plants exposed to larval feeding are more susceptible to lodging, and if weight is distributed higher up on the plant, the effects are exacerbated. A similar architectural phenomenon can explain the positive correlation between ear length and broken stalks that was only present in HP. As weight extends further from the central stalk, the likelihood of stalk breakage from wind force increases. It remains unclear, however, why this relationship was only detected under heavy WCR pressure. One possibility is that the strength of the stalk is reduced under heavy feeding pressure. Alternatively, there may be more compensatory root growth or root branching following larval feeding, which leads to stronger root-to-shoot ratios. Another interesting feature is the stronger correlations detected between PH, NLAE, and AEPH with EW, GW, and CW under heavy pressure. This suggests that under normal conditions, plant height and the number of

leaves above ears don't relate directly to yield, but under nutrient-limited or biotic stress, these traits become more relevant.

Detection of Genotypes with Under-, Over-, and Neutral-Compensatory Responses

In order to assess the phenotypic response to herbivory and to assign over, under, and neutral tolerance, we compared the performance of a given hybrid under high versus low WCR pressure, and tested the null hypothesis that performance was equal between the two treatments. **Fig. 3** shows the results of the two-sample independent t-tests for each of the traits collected from multiple plants per IBMRIL hybrid genotype. Although 40% of the tests were significant based on the one-tailed probability distributions, only 34% remained significant after q-value adjustment. For each trait, a normal distribution of t-values was observed, with a tendency towards more positive t-values than negative, resulting from a higher trait value under LP than HP. The effect of q-value adjustment was more profound for some traits than others, for instance EH/PH, which resulted in only 5% of the originally significant tests retained after adjustment. On the other hand, AEPH retained nearly 83% of the significant tests. For most traits, the multiple test correction resulted in very low p-values being adjusted upward and higher p-values being adjusted downward. In a few cases the multiple test correction resulted in non-significant tests becoming significant. This was most relevant for PH, in which an additional 36 IBMRIL hybrids (IBMRILH) were identified as significantly different between HP and LP treatments.

Table 3 displays the null and two alternative hypotheses that were tested for each IBMRILH genotype and across all traits. Lines for which the null hypothesis could be rejected were tested against each of the alternative hypotheses, and the number of tests falling into each category is shown in **Table 3,** before and after multiple test correction. All three mechanisms of compensation were detected in the set of IBMRILHs. The test correction was a more stringent criteria for deeming significance in the case of both under and overcompensation and resulted in 171 fewer significant tests. Among the 7,671 tests, 82% failed to detect a significant difference between HP and LP, and thus, were classified as physiologically-tolerant to WCR larval feeding. The remaining tests were classified as either over-compensated (1%) or under-compensated (17%). The over-compensated lines are perhaps the most biologically interesting as they represent lines that had superior performance under insect feeding stress.

For several IBMRILHs, an overcompensation response was detected for more than one trait. Fifteen IBMRILHs had overcompensated for two or more traits, and 3 IBMRILHS (MO014,

MO079, and MO150) had overcompensated for three traits (**Table 4**). Moreover, for most lines, multiple traits within the same physiological class were overcompensated, providing further support for a true physiological response. **Fig. 4** shows the overcompensation effect for two such lines, MO014 and MO205. MO014 had more kernels per row (q = 0.020), but also a greater proportion of the kernel rows filled (q = 0.028) in HP than in LP. MO014 did not however, have a difference in the total number of kernel rows (μ_{HP}: 19.20 ± 0.49, μ_{LP}: 18.40 ± 0.40, q = 0.658). Thus, this line overcompensated by allocating resources to ear growth along the poximodistal axis exclusively. Ear length was also significantly overcompensated (μ_{LP}: 5.55 ± 0.33, μ_{HP}: 8.00 ± 0.24, q = 0.008), which further supports this mechanism.

MO205 responded in a different manner to overcompensate for larval feeding via a change in plant architecture. This line had both a taller ear height and total plant height under heavy pressure (**Fig. 4**), however, the EH/PH proportion was not different between the treatments (μ_{LP}: 0.51 ± 0.01, μ_{HP}: 0.54 ± 0.01, q = 0.178). This maintenance of the EH/PH proportion is consistent with the mean separation tests across reps (**Table 2**), and provides further evidence that lines under or overcompensate for root larval feeding damage via a change in EH, PH, or both, but the relative position of the upper female inflorescence is maintained. Some lines overcompensated across physiological classes, for instance, MO298/MO238 (EIL and KRF), and MO334/MO379 (KRN and PH) (Table 3). For these lines, they may possess a genetic architecture that changes resource availability under stress, and that available resources can be more broadly allocated, versus to only one physiological process. This data provides evidence that tolerance mechanisms exist in the IBMRILH population and that the path of response follows a change in plant architecture and/or yield potential.

Physiological Mechanisms of Response to WCR Herbivory

Among the set of 240 IBMRILHs, 75 displayed an overcompensation response, and at least one line responded by overcompensating in 12 of the 18 traits (**Appendix Table 4**). The most common mechanism of overcompensation was an increase in the proportion of kernel rows filled. KRF accounted for 37% of all significant tests for overcompensation and was independently detected for 36 IBMRILHs (**Table 5**). Interestingly, only two of these IBMRILHs also overcompensated for EL or KPR (MO014 and MO223, **Table 4**). In fact, 29 of the hybrids had actually undercompensated for either EL or KPR, so they produced fewer kernels and had shorter ears under stress, but a greater proportion of the kernels were filled out. So the response

was specifically directed at bringing the available ovules to maturity rather than increasing the total number of ovules or their size.

An increase in the mean kernels per row was also one of the most common overcompensation mechanisms and was detected for 13 separate IBMRILHs (**Appendix. Table 4**). These lines compensated by adding an average of 7.76 ± 0.97 kernels per row under heavy pressure relative to low rootworm pressure. Six of these lines overcompensated for other traits as well (**Table 4**). One of these IBMRILHs, MO014 had the largest mean difference between treatments for KPR (16.40 ± 5.19), and also overcompensated for KRF and EL (**Fig. 4**). Thus, three separate ear traits point to a stress response directed at allocation of resources to ear growth and grain-filling. It is also important to note that KPR was one of the most common traits of response for both under and overcompensation, so it clearly plays a role in the stress response (**Table 5**). It also was the most common response trait detected across different experimental populations (**Table 6**). For six of the IBMRIL genotypes, the same mechanism was detected in 2010 for IBMRIL x B101 hybrids as well as the for the reciprocal hybrids evaluated in 2011. Therefore, KPR is a highly heritable trait for this genetic contrast and shows a very consistent response to the high herbivory pressure treatment.

The third most common overcompensated trait was an increase in plant height (**Appendix Table 4, Table 5).** This is in line with the hypothesis that the normal stress response would target a change in plant architecture and vegetative growth, but interestingly, the response can apparently work in both directions. As with KPR, PH was among the top three mechanisms detected for both over- and undercompensation categories. Over half of the lines that overcompensated for PH also overcompensated for other traits. For instance, MO079 was 2.50 ± 0.79 cm taller, had 10.6 ± 3.35 more kernels per row, and had 1.35 ± 0.43 cm more in ear length under heavy pressure than under low pressure, so the overcompensation response extended beyond physiological class.

In addition to the three most common overcompensated traits, IBMRILHs were also detected that overcompensated for EL (7), EH/PH (7), EH (5), EIL (5), STAND (4), KRN (4), BS (3), CW (2), EW (1), and GW (1). There were only three traits that had no RILs perform better under high pressure (LG, AEPH, and NLAE). The expectation that considerably more root larval feeding by the WCR was accrued in the high-pressure WCR trap crop relative to the conventional nursery is supported by the lack of evidence for overcompensation in lodging,

which is consistent with the mean distributions across reps (**Fig. 1**). We did, however, see 174 cases in which a line had significantly more lodging under heavy pressure, which provides additional confirmation for the increased pressure (**Table 5**). Interestingly, no cases were found of IBMRILHs that had a larger EH to PH ratio under LP, even though seven IBMRILHs had a statistically smaller EH/PH ratio. It was also interesting that undercompensation for EW, GW, or CW was not detected, especially considering that KPR and KRN were largely undercompensated. Collectively, these data provide strong evidence for the existence of distinct mechanisms of response to WCR larval feeding pressure, and that changes in plant and ear architecture are the main physiological classes involved. PH and KPR are among the most responsive traits for both compensation mechanisms, whereas KRF appears more important for overcompensation and KRN for undercompensation. Detecting these phenotypic responses is a major achievement, especially given the economic importance of the pest; but determining their heritable basis and genetic underpinnings is a necessary step in mechanistic understanding which is what is described in the next sections.

Identification of Genetic Loci for Stress Response Traits

To confirm the phenotypic response to biotic rootworm pressure, genetic association tests were first performed to identify significant QTL for each of the traits evaluated. Using three separate analysis pipelines of higher-order accuracy, significant QTL we detected, mapped, and effects determined. The first approach was to perform single-marker linear regression (SMR) of genotype on phenotype using an FDR = 0.05 to identify significant genetic associations. **Fig. 5** shows the transformed adjusted q-values from this analysis for several traits from different physiological classes. Strong genetic associations were detected for each physiological class evaluated. For instance, for BS, two regions had significant marker associations: one on c7 centered at ndk1 (383 cM), and another on c9 at the bronze1 locus (bz1, 90 cM). A large clustering of markers at around 331 cM on the long arm of c9 was associated with germination and had a peak probability at marker ufg63.

Evidence for pleiotropic gene action was also detected among several traits, some of which crossed physiological boundaries. A positionally coincident region on the long arm of c1 was detected for both EH and CW. In both cases, a peak in QTL likelihood occurred at around cM position 756.50, and included a region of significant marker associations between 720 and 785 cM. This same interval was detected in genome scans for PH, EH/PH, GW, and EW, evidence

which suggests the presence of an upstream or more broadly-acting genetic element(s), and possibly a stress responsive gene. Interestingly, an overlapping region was localized for goosenecking and standability in 2011 (q-values < 0.02), providing further support for its comprehensive role.

Among the most penetrant QTL identified, was a strong association on c3 with plant architecture, which was repeatedly detected for all of the plant architecture traits evaluated, including PH, EH, NLAE, and AEPH, EIL, and EH/PH. The two middle plots of **Fig. 5** show where this c3 association was localized for AEPH and EH. Peaks occurred at 361 cM (q-value = 5.95E-17) and 344 cM (q-value = 5.95E-17) for the two traits, respectively. The interval between 303 cM and 384 cM on c3 is where 95% of all significant marker associations for this QTL were detected among the 6 plant architecture traits. This same region was also detected in 2011 for EH, which was the only plant architecture trait evaluated in the second year of the study (**Fig. 6**). It was independently detected for both the IBMRIL x B101 hybrids as well as the B101 x IBMRILs, pointing to the absence of material effects in regulating this locus and the presence of an effect that can be detected across years.

As a confirmatory step, SMR was performed on each rep from the 2010 experiment separately, and QTL likelihood estimates were found to vary significantly by treatment. Genetic variance around the locus csu636 had a much stronger association with the EH/PH ratio under heavy pressure than under low rootworm pressure (**Fig. 7**). This G x T interaction was detected in both reps grown under high pressure, and although there was a significant association at the same locus under low pressure, the strength of the association was much lower. The same genetic effect was also observed for EH (**Fig. 8**). A peak in marker significance occurred at the same locus, csu636 at the 361 cM position. For both EH/PH and EH additional clusters of significant marker associations were seen on c1 at two separate positions, one at locus csu3 (405 cM) and another around locus AY109506 (811 cM). These two regions appear to be more penetrant with EH than with the EH/PH ratio as they were detected for EH in each of the 4 reps. The detection of significant marker associations with several agronomically important traits and the identification of genetic interactions between high and low rootworm pressure treatments provides an informative framework for more intensive confirmational analysis and positional refining of the underlying genetic variation.

Interval Mapping Positionally Confirms QTL Regulating the Phenotypic Response to Larval Herbivory

Standard interval mapping was able to confirm the presence of QTL identified in the SMR analysis and also revealed additional loci associated with trait variation. A total of 91 QTL were detected across 15 of the 18 traits using a 10% genome-wide threshold (**Table 7**). QTL were localized to each of the 10 maize linkage groups with c1 and c3 containing the most QTL at 15 each. The Bayesian confidence intervals surrounding QTL were quite variable and ranged from less than 1 cM (CW: q03.445, CW: q08.315, EH: q02.094, and EH/PH: q10.393) to over 1,000 cM (KRF: q01.103) depending on the trait, with an average support interval of 211.17 ± 25.19 cM. For many of the QTL identified, the nearest marker had been mapped to the physical B73 genome, providing a physical scaffold for mining potential gene candidates in QTL regions.

The majority of QTL were detected for traits falling into the plant architecture physiological class (**Table 7**). EH, EIL, and EH/PH had the greatest number of QTL detected at 11, 9, and 9, respectively. The highest LOD score obtained was for the q03.361 EH/PH QTL (LOD = 20.33) which was localized to only a 3 cM interval. This same QTL, which is centered on csu636 was also detected for EH (LOD = 19.85) and is placed precisely within chromosome bin 3.05. This was the same marker detected in the earlier linear regression analysis of EH and EH/PH (**Fig. 7 and 8**). Just as in the SMR analysis, this QTL was detected using interval mapping in both years for EH, but interval mapping was able to more finely delineate the QTL boundaries. A novel QTL for EH on c9 was also detected across both years of the study and was positioned at the marker umc1258 (q09.196). The same peak position in QTL likelihood and the highly overlapping confidence intervals detected for both the q03.361 and q09.196 QTL provide strong evidence for robust genetic control of EH at these loci, given that they were narrowly delineated independently across different years.

A strong QTL for PH (LOD = 8.42) was also localized to the same c3 position and had the strongest association with csu636. QTL for both EIL and AEPH were also mapped to bin 3.05 and had overlapping confidence intervals. This confirms the pleiotropic effect of this locus on regulating multiple traits associated with plant architecture. Interestingly, a QTL on c3 for CW (q03.037) also had an overlapping confidence interval with the q03.361 QTL, although the CI was very large, and the 2011 CW QTL for c3 fell outside the q03.361 CI (**Table 7**). There may be separate but closely linked underlying genes involved. The BS QTL detected from SMR on c9

was also confirmed through interval mapping and placed with 90% certainty between positions 74.8 cM and 101.00 cM, which is precisely where the bz1 locus is mapped.

Several other genetic regions were found to be positionally coincident across traits, and tended to be hotspots for QTL localization. The region on c1 between 401 cM and 425 cM was significantly associated with QTL for PH, EH, EW, GW, and CW. Given the phenotypic correlation between the traits within these two physiological classes, it is not surprising that QTL were localized to the same region. However, it is more surprising to localize traits across both grain-filling and plant architecture traits to such a narrowly defined interval, and points to the identification of a locus that acts in many biological pathways, and in different tissues and developmental stages. Another QTL hotspot occurred on c9 and spanned the region between 195 cM and 253 cM. This included the q09.196 EH QTL detected in both 2010 and 2011, and also QTL for KRF and STAND that were localized independently in both years of the experiment. The region also housed QTL for EH/PH, KPR, and PH, and appears to be another locus involved in multiple physiological modes of action, possibly as a stress responsive locus. This analysis confirms the earlier locations of QTL identified in SMR, and provides strong support for genetic regulation of the key traits involved the stress response to rootworm herbivory. The underlying genetic architecture points to several loci involved in each trait analyzed, but only a few QTL hotspots that seem to act basally on many different traits that span physiological classes.

Significant Genotype x Treatment Interactions Identify the QTL that Underlie Differential Responses to Rootworm Herbivory

Revealing genetic variation underlying traits that are critically involved in plant health, and responding to larval herbivory by the WCR is both agronomically useful and scientifically revealing. If a QTL is truly involved in responding to larval herbivory, the expectation follows that genetic variation would give rise to a difference in genetic effect in the presence of rootworm pressure (HP treatment) than in its absence (LP treatment). This is precisely what was observed for several of the QTL identified. A significant difference in additive gene effect between the HP and LP treatments was identified for 4 of the 6 plant architecture traits (**Fig. 9**). The QTL hotspot on c3 at around position 361 cM had the greatest difference in additive effect between treatments. Significant differences were observed for EIL, EH, and EH/PH at this QTL. In the case of EIL, the additive effect, or the effect of adding an additional Mo17 allele under heavy rootworm pressure resulted in 0.134 ± 0.04 additional cm of growth per internode than

under low pressure; and this explained 4% of the phenotypic variation in both treatments. The effect was in the opposite direction for EH and EH/PH, and resulted in reduced ear height, and lowered the EH/PH ratio in the HP treatment relative to LP. For EH, 13% of the variation was explained by this single QTL (q03.361) in HP, but only 8% was explained under low pressure. This translated into a mean genotypic difference between AA and BB of 7.00 ± 0.09 cm in LP and 10.01 ± 0.09 cm in HP, with the B73 allele acting as the high allele in both treatments. For the EH/PH ratio in HP, AA individuals had ears on average 2.80% ± 0.24% higher on the plant than BB individuals whereas under low pressure the genotypic difference accounted for only a 1.70% ± 0.24% proportional increase. This treatment difference resulted in the q03.361 QTL accounting for 14% of the total variation in the EH/PH ratio under heavy pressure but only 7% under low rootworm pressure.

Several of the QTL identified for the other physiological classes were also found to have a significantly different effect under the two treatments (**Fig. 10**). The q03.453 and q07.489 QTL for KPR both resulted in an additive effect with significantly more KPR under heavy pressure than low pressure, or rather a less negative allele substitution effect. The genotypic difference at the q04.744 KRF QTL resulted in a 2.0% ± 0.3% difference in ear filling under heavy pressure and only a 0.61% ± 0.3% difference under low pressure. The QTL explained 3% and 1% of the total phenotypic variation in kernel row filling in the HP and LP treatments, respectively. The q07.518 QTL for KRN had a treatment interaction that actually changed the additive effect from negatively regulating KRN under low pressure to positively adding to the row number under heavy pressure (**Fig. 10**). The allele substitution effect under high pressure added an additional 0.15 ± 0.07 kernel rows whereas the effect under low pressure reduced the kernel row number by 0.29 ± 0.08 kernel rows. This was the only QTL identified for which a change in effect-direction was observed.

Two of the QTL for CW and GW were also found to overcompensate for rootworm pressure. The q03.769 QTL for GW overlapped with the q03.361 QTL hotspot that was repeatedly detected for the plant architecture traits and was found to have a significant G x E interaction (**Table 7, Fig. 10**). This effect manifested in the preservation of 4.73 g ± 1.3 g of grain weight under heavy pressure. An overcompensation response was also detected for the q08.315 pleiotropic QTL affecting all three grain-filling traits in the same direction and with a proportional effect. The result was a smaller reduction in grain-filling between the two genotypic

classes in HP versus LP, as if the genotypic effect had been masked in the presence of high rootworm pressure. This would be expected in a mechanism that preserves yield potential and is consistent with the earlier findings from the phenotypic overcompensation of the KRF trait.

This analysis has identified genetic variation associated with traits important for the response to rootworm herbivory. Confirmation of these genetic associations and positional refining has localized QTL to discrete chromosomal regions, many of which are in strong linkage disequilibrium with one or more core bin markers. Furthermore, we have identified the genetic effects administered by these QTL, which has revealed regions with significantly different effects in the presence and absence of WCR pressure. This provides strong support for a novel genetic architecture that orchestrates the response to WCR herbivory, one in which a few QTL pleiotropically affect several response traits.

Changes in QTL Likelihood by Treatment Reveals Sources of Genetic Variation Important in the Response to Rootworm Larval Feeding

The identification and confirmation of QTL involved in traits that orchestrate the phenotypic response to rootworm larval feeding is a major achievement. To further validate the presence of treatment-response QTL and identify additional regions that may have been masked in the genome scan using the single-QTL model requires the use of a model that accounts for multiple segregating QTL. If a given QTL is truly involved in the response itself, the likelihood should be different in the presence and absence of the biotic stress, because it is the stress that drives manifestation of phenotypic variation. To test this hypothesis, multiple QTL mapping was performed on each treatment separately, and the QTL likelihoods compared.

The analysis revealed a total of 20 QTL that had a substantially higher likelihoods under heavy rootworm pressure, and 53 QTL that had a higher likelihood under low pressure (**Tables 8 and 9**). The majority of these QTL were detected in the earlier SMR and single-QTL model analyses. However, not surprisingly, some novel QTL were also identified. The use of cofactors in QTL mapping allows for linked genetic variance to be accounted for when individual intervals are tested, which is why all of the novel QTL identified using the MQM approach resided on linkage groups with associated QTL in the previous analyses. For those QTL having a higher LOD value in HP, 4 additional QTL were identified (**Table 7**). One QTL pleiotropically effecting both PH and EH was localized to c2 at 600 cM. Another QTL was found on c5 at 440 cM that was again detected for both PH and EH, and a third QTL for both traits was localized to

c8 at position 370 cM. For each of these QTL, the LOD was only above 10% genome-wide thresholds for the HP treatment.

One of the most revealing insights from the comparison of treatments was the localization of the q03.361 QTL hotspot, which had a significantly higher QTL likelihood probability in the HP treatment. All of the c3 QTL shown in **Table 8** overlap with this QTL hotspot and the LOD difference between treatments was highest at the QTL localized to 360 cM among all of the treatment comparisons. This treatment difference was detected for EH, PH, and the EH/PH ratio. The largest LOD difference occurred for the EH/PH ratio, where the QTL likelihood in HP was 16.151 versus 9.787 in LP (**Fig. 11**). The nearest marker associated with this QTL was csu636 and is only 1.1 cM away from the peak. This was the same marker association detected in the earlier analyses and serves as an ideal candidate for marker assisted selection, both because it pleiotropically effects many beneficial plant architectural traits, but also because it represents a novel marker strongly associated with the phenotypic response to rootworm herbivory.

In addition to genetic variation specifically associated with the high rootworm pressure treatment, we also observed QTL that were only detected in the conventional low pressure nursery. In fact, there were over twice as many QTL in this category than in the latter (**Table 8**). Almost 80% of these LP-only QTL were previously identified in the single-QTL genome scan, and for the majority of them, multiple traits mapped to the same region. Not surprisingly, the pleiotropic pattern tended to follow the phenotypic correlations between traits. In addition to providing confirmation for earlier detected QTL, twelve new loci were also revealed. Three of these were localized to the short arm of c3, which were likely masked in the single-QTL scan because of the large q03.361 QTL.

A few of the previously identified QTL were also resolved into two separate regions. For instance, the q07.377 EIL QTL identified in the single-QTL scan had large CI, which upon mqm analysis revealed two separate QTL regions (**Table 7, Table 9**). The two largest LOD differences among the LP-only QTL were 7.686 and 6.893, and occurred at q01.880 (STAND) and q08.loc310 (CW). These differences were much smaller than the LOD differences observed for the HP-only QTL. The HP-only QTL were enriched in plant architecture traits and only 1 grain-filling QTL was observed for the single trait, CW (umc1594). For the LP-only QTL, the traits encompassed were more comprehensive and included multiple QTL for every trait except BS. The three grain-filling traits were also proportionally much more abundant. This is likely due

to the reduced seed set in the high pressure treatment that masked some of the genetic variation associated with grain-filling that was present in the LP treatment.

Taken together, these results provide the first reported case of QTL specifically responsive to stress induced by WCR larval feeding. Not only has the identification of overcompensation as a tolerance mechanism been detected, but ascertainment of the underlying genetic architecture has also been elucidated. We have localized genomic regions important for several agronomic traits that were identified to be over, under, or neutrally-compensated. These QTL were confirmed through multiple rounds of phenotypic and genotypic analyses, and their locations resolved to discrete chromosomal regions. The analysis has revealed that both changes in plant architecture and grain-filling are the primary targets of the response to herbivory. Several sources of genetic variation were found to be only penetrant in the high WCR pressure treatment, and this G x T effect was further confirmed through analysis of QTL likelihood. This information provides a solid framework for further scientific inquiry of the mechanisms used by maize to defense against the WCR. It also serves as a starting point for investigating whether or not this response is executed against other types of biotic and abiotic stress.

Characterization of Specific Phenotypic Responses to WCR Herbivory

The results from this study have identified that there are discernible physiological consequences of rootworm herbivory that manifest not only at the root interface, but also in other organs that contribute to the overall health of the plant. The effects of WCR herbivory tend to drive changes that reduce agronomic performance for both belowground and aboveground plant characteristics relative to performance in an undamaged state. The mechanism of response was directed particularly at plant architecture and grain-filling. Plants in the high WCR treatment had extensively more lodging and reduced standability, but they were also reduced in size, had lower grain weight, and fewer kernels.

This is consistent with the idea of plant defense being an energy-constrained process in competition with both reproductive and growth processes, a subject of extensive research (Orians et al. 2011; Strauss and Agrawal 1999; Tiffin 2000). For instance, Carmona et al. (2011) identified from a comprehensive review of the literature that life history traits, including plant growth, are among the most strongly associated with susceptibility to herbivory, and that genetic variation for these traits can serve as a target for herbivore selection. In fact, many studies point

to a change in phenology in addition to allelochemical changes that occur following herbivory (Ohgushi 2005; Tiffin 2000; Wu and Baldwin 2010).

Although much research has focused on phenological changes associated with aboveground herbivory, less has focused on the consequences of belowground herbivory, despite the observation that root herbivory may have a larger impact on fitness than shoot herbivory (Maron 1998; Reichman and Smith 1991). In terms of resource allocations, roots can require a large amount of the available fixed carbon to drive new root turnover and provide defense at the root interface (Farrar and Jones 2003; Lynch 2007). When more resources are being driven to root defense, fewer are available for use in aboveground growth and development. This is even more important for nitrogen, which serves as a major component of many defense compounds, but is also critical for plant growth (Rubio and Lynch 2007; Tiffin 2000). This would explain the observed reduction in plant growth (EH, PH, EIL, AEPH, NLAE), and grain-filling (EW, GW, CW) that occurred under high rootworm pressure. We also observed that the EH/PH ratio was relatively unchanged across treatments, so a general trend towards growth reduction was detected, rather than tissue-specific reduction.

The response to herbivory also had a clear consequence on yield. Plants in the high pressure treatment had fewer ovules produced, and this resulted in reduced grain weight. This is consistent with the work by Spike and Tollefson (1991), which showed reductions in both ear height and yield when infested with WCR eggs. They found a strong negative correlation between lodging and yield, and hypothesized that lodging caused by WCR larval feeding results in reduced photosynthetic efficiency. This is more consistent with the idea of a resource-availability model rather than resource allocation (Bryant et al. 1983; Coley et al. 1985). This might explain why we observed a high abundance of lodged plants that were overcompensated and no cases of undercompensation. Evidence further supported by our identification of genetic variance for lodging only in the low pressure treatment. Lodging was more uniform and persistent under heavy pressure, and less of the variation was attributed to genetic differences between lines. The plant architecture traits, on the other hand, like EH, PH, and EIL had the greatest genetic variation under high pressure. A likely result of increased selection intensity on plants trying to compete for sunlight and other resources.

Overcompensation as a Driver of Resource Availability Constraints

Detection of lines that actually overcompensate for root herbivory is an interesting and potentially broadly impacting phenomenon. In the case of our IBMRILH populations, filling of the available ovules, increasing kernels per row, and increasing plant height were the most commonly overcompensated traits. We further traced genetic variation for plant architecture traits to discrete chromosomal positions and identified that both genetic effects and likelihood of QTL presence was elevated under high herbivore pressure. The idea of plants performing better under herbivory is not a new phenomenon, albeit much less common than the reverse scenario (Paige and Whitham 1987). In this study, we only detected a rate of overcompensation of about 1%, relative to that of undercompensation (17%). Therefore, herbivory generally results in detrimental effects on the plant but in rare cases, can provide a fitness benefit. From an evolutionary perspective, plants that maintain a mutualistic benefit with their herbivore counterparts can persist as long as the response gain outweighs the costs of herbivory (Agrawal 2000). It is also possible for a plant to overcompensate for one beneficial trait but undercompensate for another, so fitness advantage tends to be a relatively concept. Furthermore, there is evidence that overcompensation is genetically controlled and a likely target for natural selection (Agrawal 2000; Scholes et al. 2013; Strauss and Agrawal 1999).

The actual manifestations of overcompensation is likely to come in the same form of response as the negative effects of herbivory. Overcompensation has often been associated with increased photosynthetic rate, compensatory growth, and plant architectural changes (Strauss and Agrawal 1999; Tiffin 2000). The results described here point to a change in plant architecture as a means of increasing photosynthetic rate. Those plants that overcompensated did so in large part by increasing plant height and ear height, and the genetic variance had a larger contribution to the phenotypic variation in the presence of herbivory. This is probably due to the by-products of larval herbivory rather than a direct consequence of it. Lodging, which is the by-product, provides a substrate for selection of genetic variation that increases the ability of plants to obtain necessary photosynthates. Detection of elevated photosynthates in taller plants would have to confirm this, but evidence suggests this is a viable explanation. Additionally, the number of overcompensated lines for kernel row filling would seem to support the idea of a better utilization of resources under pressure. These plants generally did not produce more kernels than their undamaged counterparts but had a greater proportion of their available ovules filled to

maturity. Given this information, we propose the idea of an allocation-selection model for overcompensation to WCR herbivory that is based on resource allocation and subsequent selection on plant architecture traits. Under heavy pressure, the need to allocate resources effectively becomes more important, and as such, available resources are directed towards preserving the yield potential. Because resources are more narrowly allocated, genetic variance for traits that increase resource acquisition, like plant architecture, become more important and are selected upon, an idea similar to the conclusion by Carmona et al. (Carmona et al. 2011). Although a balance must exist between allocation of energy for growth to provide more resources and energy for yield preservation, thus the model includes elements of both the resource allocation and defense-growth models (Bryant et al. 1983; Coley et al. 1985; Herms and Mattson 1992).

Candidate Gene Identification at QTL with Clear Roles in Overcompensation Responses

One of the strongest genetic associations with a response to herbivory was identified on c3 with a peak around 361 cM. This region was found to pleiotropically affect many plant architectural traits and was robustly penetrant across years and experimental replications. We also found that there was not a difference in maternal effect for this QTL. Interestingly, within its narrow confidence interval (between 358 cM and 361 cM) lies the gene sps2, *sucrose phosphate synthase2*, which has been implicated in regulating source-to-sink relationships in maize leaves and the biosynthesis of sucrose (Cheng et al. 1996). More specifically, it has been identified as being important for maize vegetative growth and heterosis (Causse et al. 1995; Rocher et al. 1989). This is a good target for the gene underlying the q03.361 QTL detected in this experiment, and it adds to our current understanding of how energy is allocated and changes with biotic stress. Furthermore, the importance of sps2 in heterosis might shed light on how heterosis is affected by biotic stress, and vice versa, sets up a testing framework for evaluating the relationship between these two phenomena in maize.

Another region that was found to pleiotropically effect both PH and EH only under heavy WCR pressure was the q05.360 QTL mapped to bin 5.04. The marker nearest to the peak for this QTL is myb3 (Mp1), which encodes a WD-repeat protein. This protein is expressed throughout the plant, and is thought to be involved in the signal transduction pathway that regulates the flavonoid pathway (Hernandez et al. 2000). Flavonoids, in addition to being important for auxin transport (Brown et al. 2001), and UV protection (Stapleton and Walbot 1994), are also major

components of the host-defense system (Dooner et al. 1991; Schoonhoven et al. 2005). Thus, one possible biochemical explanation for the greater genetic variation observed under high rootworm pressure may be due to increased gene expression at the Mp1 locus as a means of up-regulating defensive flavonoids. This would go towards explaining the mechanism of increased vegetative growth observed in lines overcompensating for WCR herbivory, as a bi-product of flavonoid production. An idea further supported by the role of PAC1, a close paralog of Mp1, in regulating plant height (Carey et al. 2004).

Another locus that was found to have a G x T interaction between the high and low WCR treatments was the q09.100 QTL for broken stalks (90% CI: 75 cM - 101 cM), which peaked at the bronze1 (bz1) marker. Despite the fact that a significantly higher proportion of plants had broken stalks in the high pressure treatment and there were twice as many cases of undercompensation, this QTL was only detected under low rootworm pressure. Bronze1 has been a widely studied for its importance in the anthocyanin pathway and for its use as a classical gene dating back to the research of Barbara McClintock (McClintock 1946, 1947). It has since been reported to also be important in flavonoid biosynthesis (Larson and Coe 1977), maysin accumulation (Byrne et al. 1996), and endosperm development (Dooner and Nelson 1979). It was found to encode the enzyme UDPG-flavonol 3-0-glucosyl transferase, an important component in flavonoid biosynthesis (Larson and Coe 1977). Additionally, double mutants Bz1/Pl1 have been observed to be shorter and more brittle than their wild-type counterparts (based on comments from domain experts via MaizeGDB Locus Lookup, (Andorf et al. 2010)). The Bz1 protein is a structural enzyme in the anthocyanin pathway, so it would seem to be a good fit for stalk strength since its expression is also known to exist in stalk tissue (Dooner and Nelson 1979). There have also been other stalk strength QTL localized to the short arm of c9 (Flint-Garcia et al. 2003), although it is unclear if they represent the same underlying genes as in the case here. Nevertheless, the bz1 locus is one potential candidate for the gene underlying the LP-only q09.100 QTL for broken stalks.

In conclusion, we have demonstrated that discernible, genetically-conditioned, physiological changes take place in response to WCR herbivory that generally reduces the agronomic performance of the host plant. These changes cross physiological classes, and are likely the result of resource availability constraints. We found strong evidence for the case of overcompensation to WCR herbivory, and identified the phenotypic mechanisms by which this occurs. We also

provide the strongest case to date for genetic regulation of overcompensation to insect herbivory, and have revealed several QTL hotspots that act pleiotropically in the presence of high rootworm larval feeding. Lastly, we introduce the idea of the allocation-selection model for overcompensation, and propose possible genic pathways through which changes in plant architecture are orchestrated in the context of our QTL findings. This study sheds light on the importance of plant-insect interactions and should encourage others to dig deeper into this interesting phenomenon. Collectively, these results provide strong evidence for overcompensation that is physiologically targeted, genetically tractable, and penetrant across years and experimental replications. This knowledge will add to our current understand of tolerance and herbivory, and contribute to the growing body of knowledge providing evidence for overcompensation. An additional benefit is that the mechanisms have been revealed using an economically important pest system, so the potential for applied crop improvements also exists.

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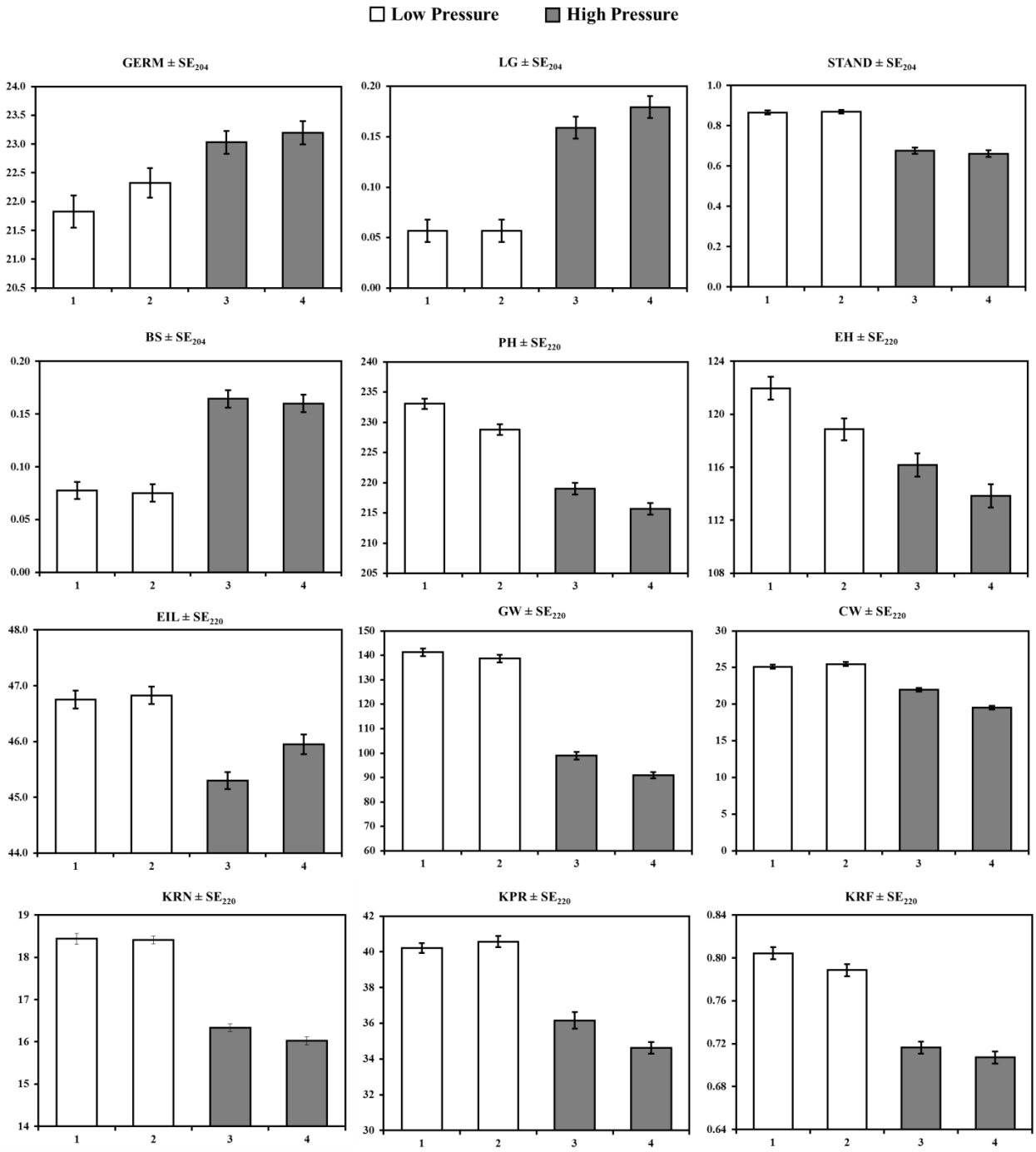

Figure 1. Bargraphs showing mean values ± SE for 12 agronomically important traits evaluated in two reps under low WCR pressure and two reps under high WCR pressure. The number of unique IBMRIL x B101 hybrids assessed for each trait is subscripted on the SE notation. Vertical axes correspond to the units of measure for each trait as described in Table 1.

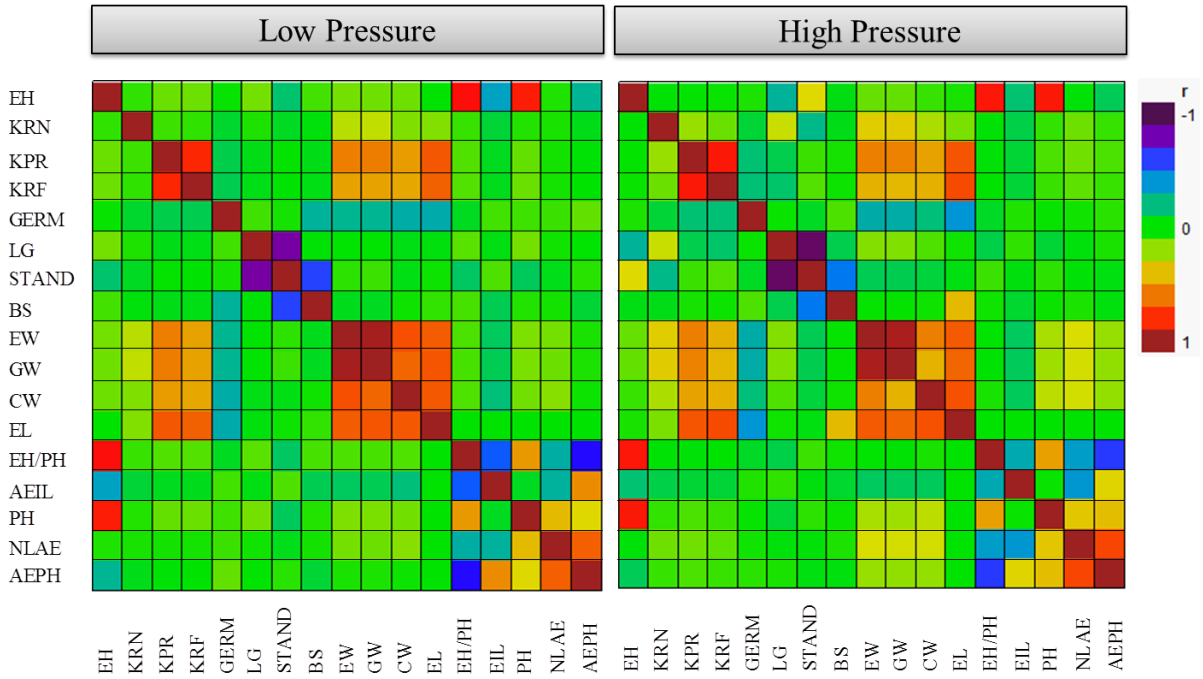

Figure 2. Correlation matrix for 17 different traits assessed under low (left) and high (right) WCR pressure. Directions and magnitudes of the Pearson correlation coefficients are color-coded according to the key at the right. Any color in the matrix deviating from the green zero color-code is significant using $\alpha = 0.001$.

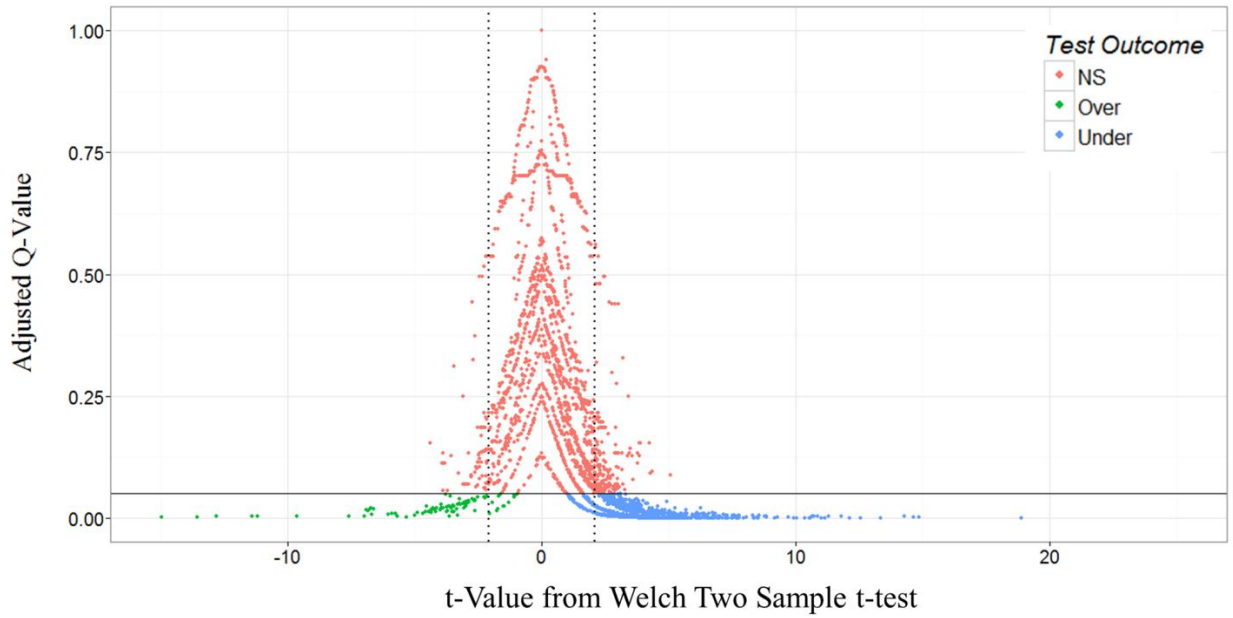

Figure 3. Adjusted q-value plotted against the t-value from independent two-sample t-tests for 10 independently analyzed traits. Tests falling below the horizontal line were significant using an FDR = 0.05. Tests falling above or below the dashed vertical lines were significant tests before multiple test correction using $t_{19} = |2.10|$ (1330 tests). Red indicates tests that were not significantly different (tolerant outcome), blue are tests that had an undercompensation outcome, and green are tests significant for an overcompensation outcome in a comparison between high and low pressure treatments.

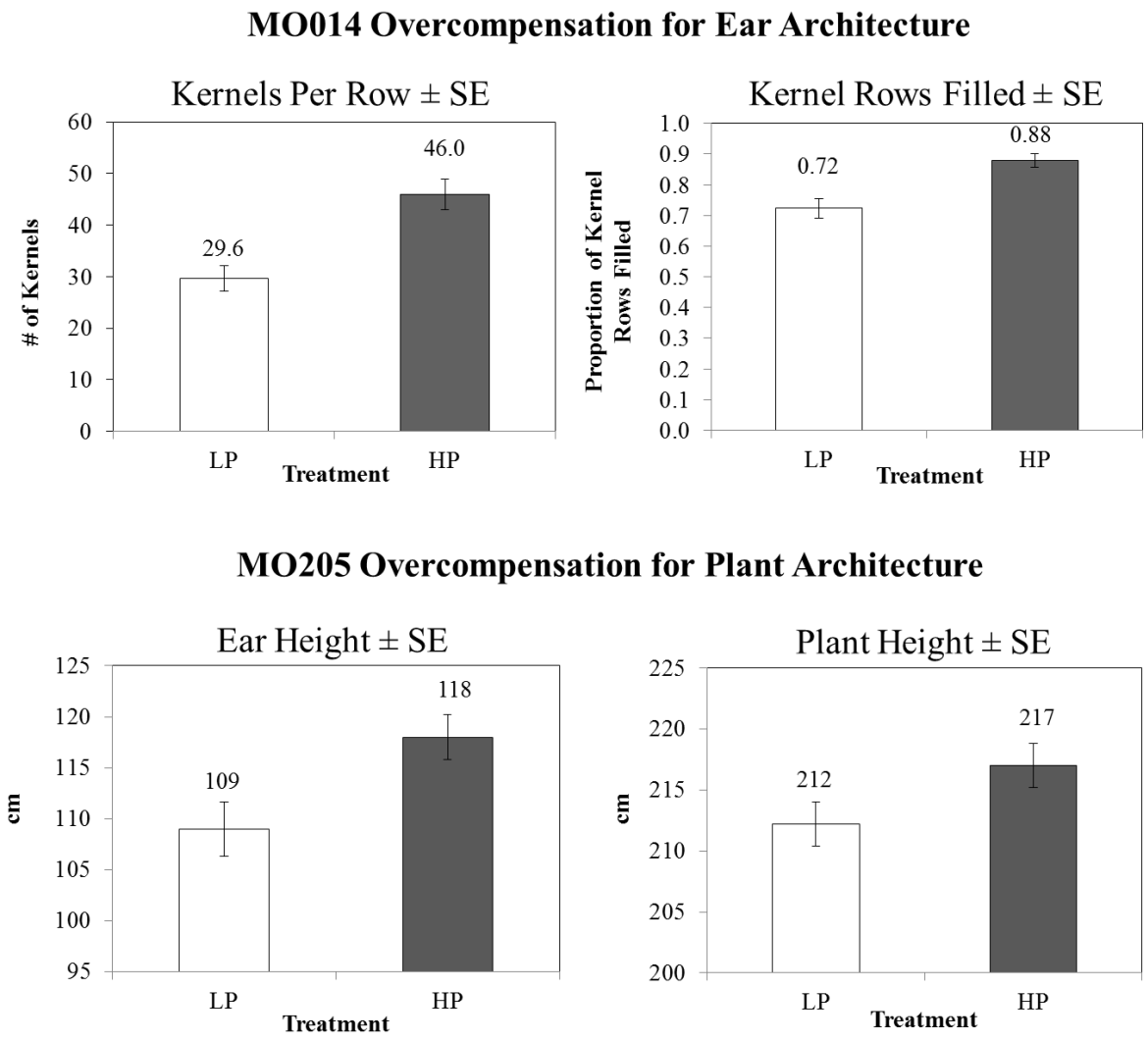

Figure 4. Two examples of IBMRILHs that overcompensated for more than one trait by performing better under high herbivory pressure. The top panel shows the case for two ear architecture traits and the bottom panel is an example for plant architecture.

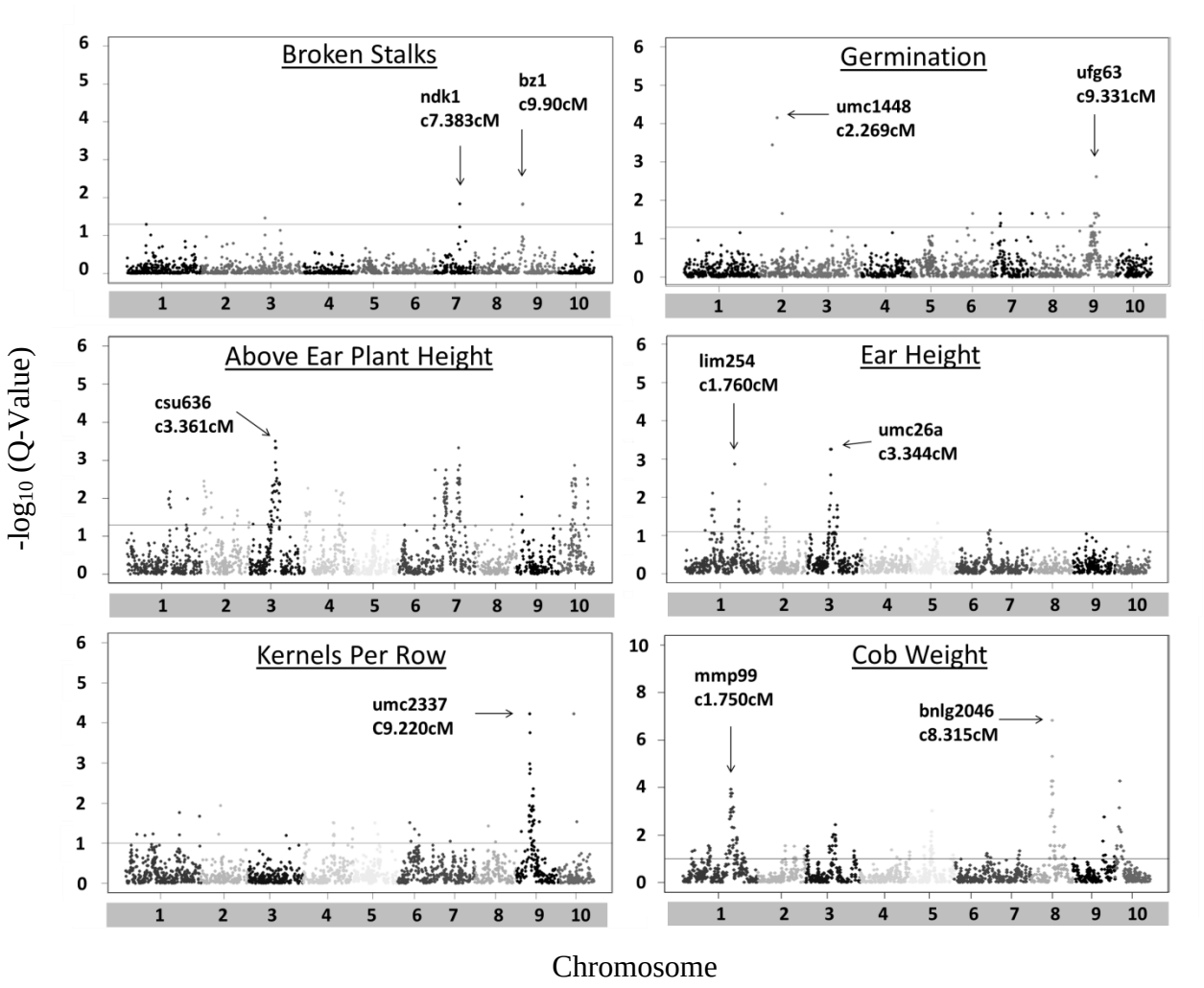

Figure 5. Significant QTL detected by linear regression of marker genotype on phenotype across 6 traits from different physiological classes. Manhattan plots show the $-\log_{10}$ q-values of the corrected p-values from the regression analysis across 2019 IBM2 markers. Chromosome numbers are shown across the x-axis and q-values are shaded to distinguish adjacent chromosomes. The horizontal line depicts the genome-wide threshold of significance after q-value adjustment (FDR = 0.05).

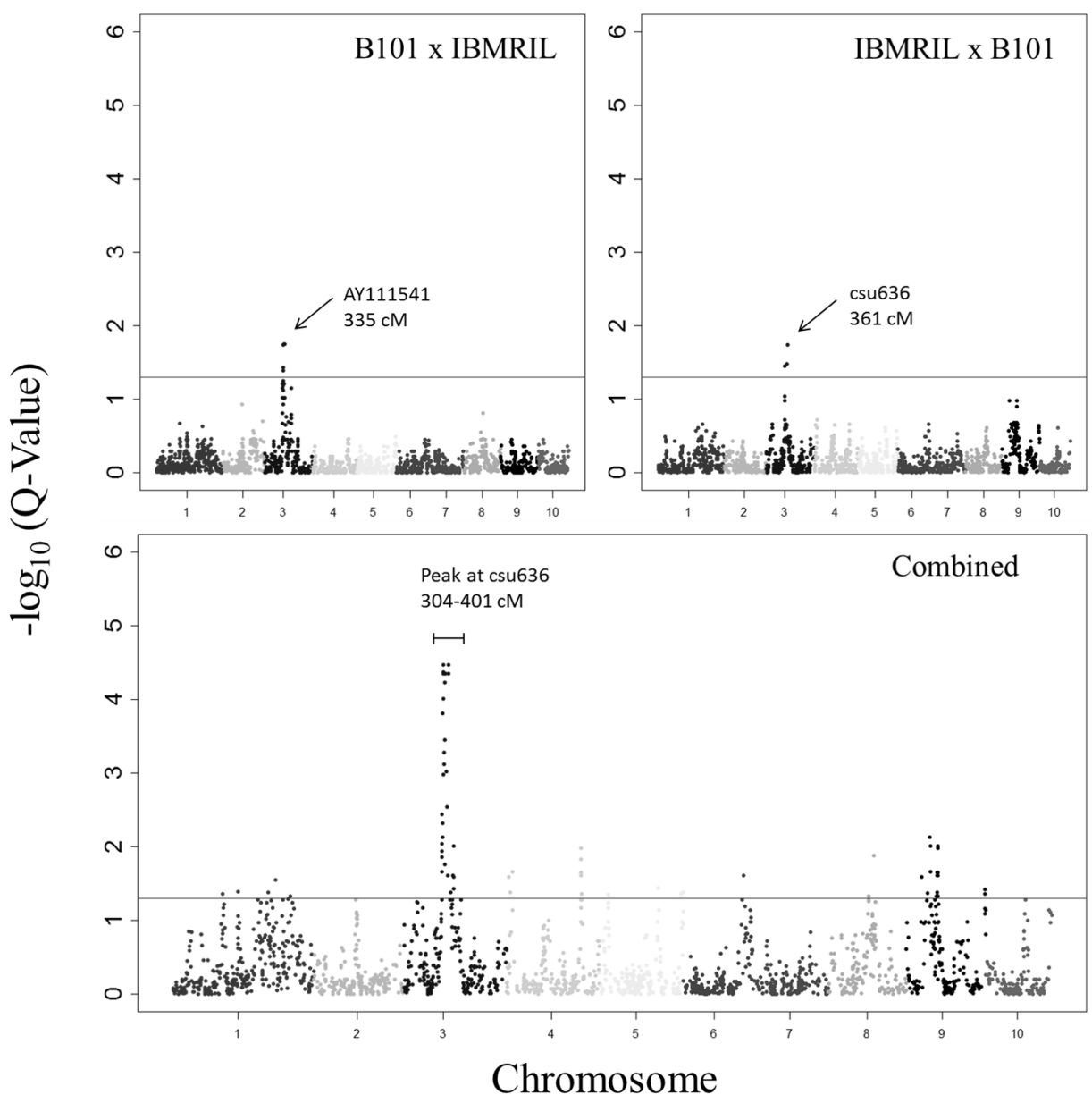

Figure 6. Significant EH QTL detected in the 2011 high pressure treatment by linear regression across two reciprocal hybrid populations between the cross of 240 IBMRILs and B101. The lower panel plot shows the combined dataset including both hybrid populations evaluated in the high pressure treatment. The $-\log_{10}$ Q-Values adjusted from the linear regression of marker genotype on EH are shown on the y-axis. Chromosome numbers are shown across the x-axis and q-values are shaded to distinguish adjacent chromosomes. Horizontals depict the genome-wide thresholds using FDR = 0.05 after q-value adjustment.

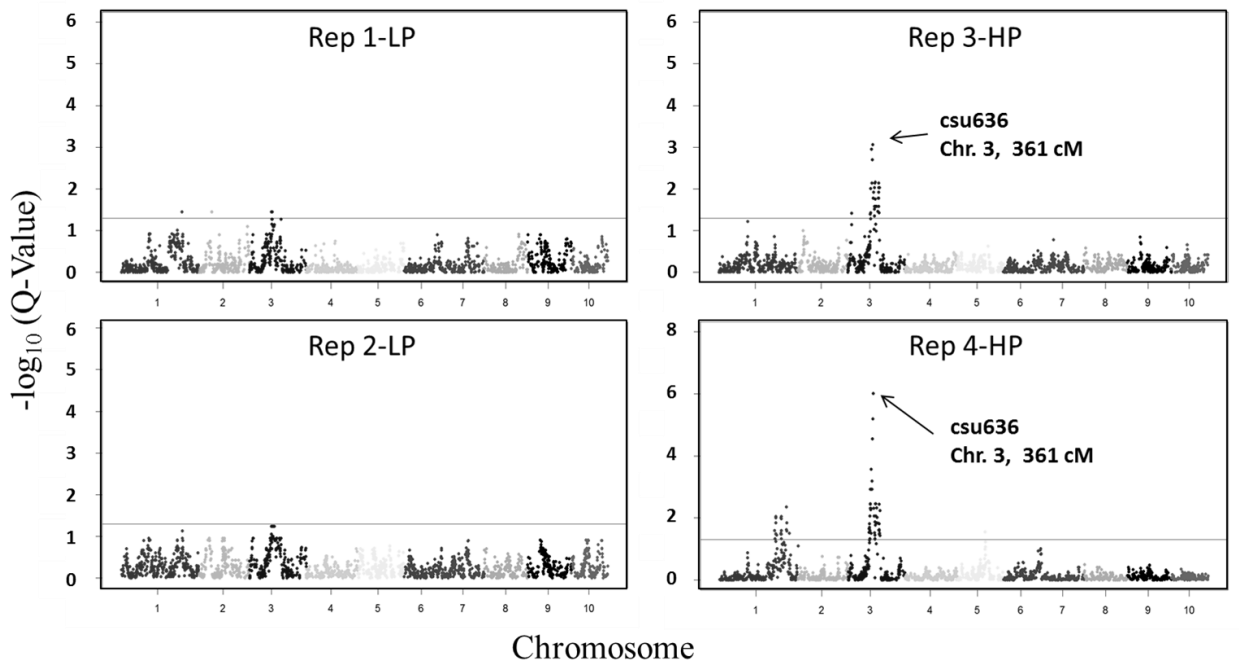

Figure 7. Significant EH/PH QTL detected by linear regression across 4 IBMRIL x B101 reps evaluated in 2010. The $-\log_{10}$ Q-Values adjusted from the linear regression of marker genotype on the EH/PH ratio are shown across the y-axis. Reps 1 and 2 comprised the low-pressure treatment (LP), and reps 3 and 4 the high-pressure treatment (HP). Chromosome numbers are shown across the x-axis and q-values are shaded to distinguish adjacent chromosomes. Horizontals depict the genome-wide thresholds using FDR = 0.05 after q-value adjustment.

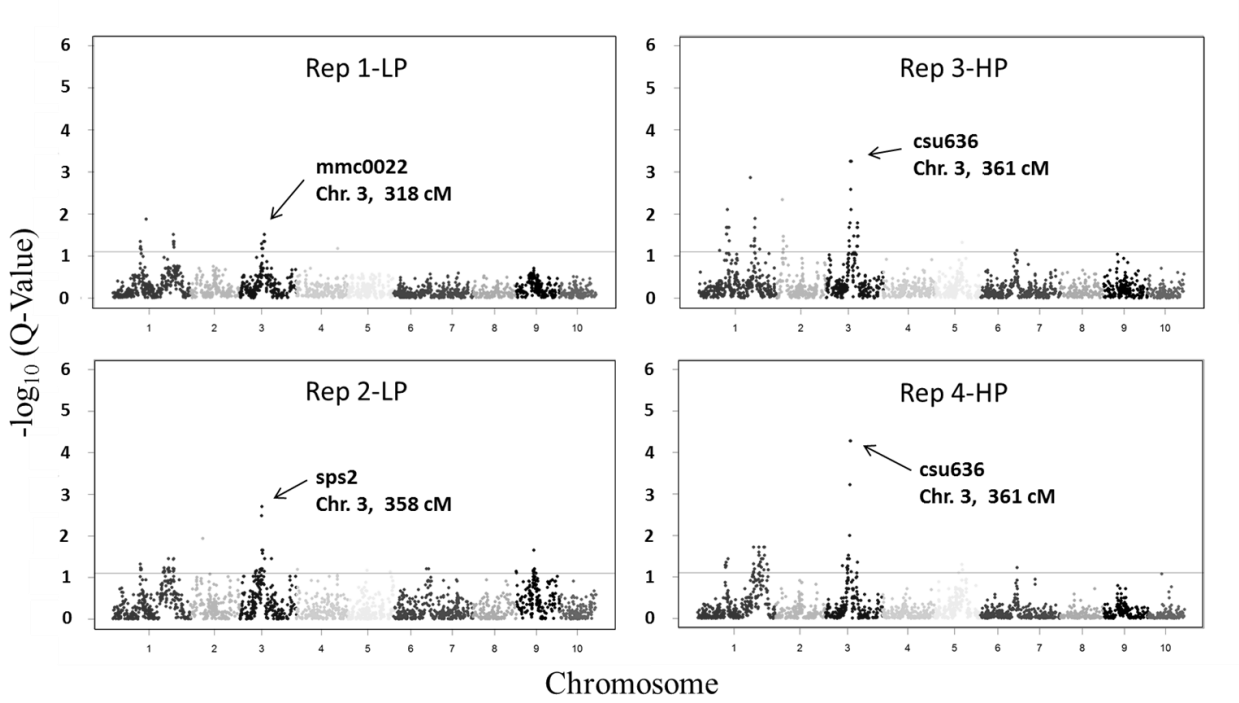

Figure 8. Significant EH QTL detected by linear regression across 4 IBMRIL x B101 reps evaluated in 2010. The $-\log_{10}$ Q-Values adjusted from the linear regression of marker genotype on the EH are shown on the y-axis. Reps 1 and 2 comprised the low-pressure treatment (LP), and reps 3 and 4 the high-pressure treatment (HP). Chromosome numbers are shown across the x-axis and q-values are shaded to distinguish adjacent chromosomes. Horizontals depict the genome-wide thresholds using FDR = 0.05 after q-value adjustment.

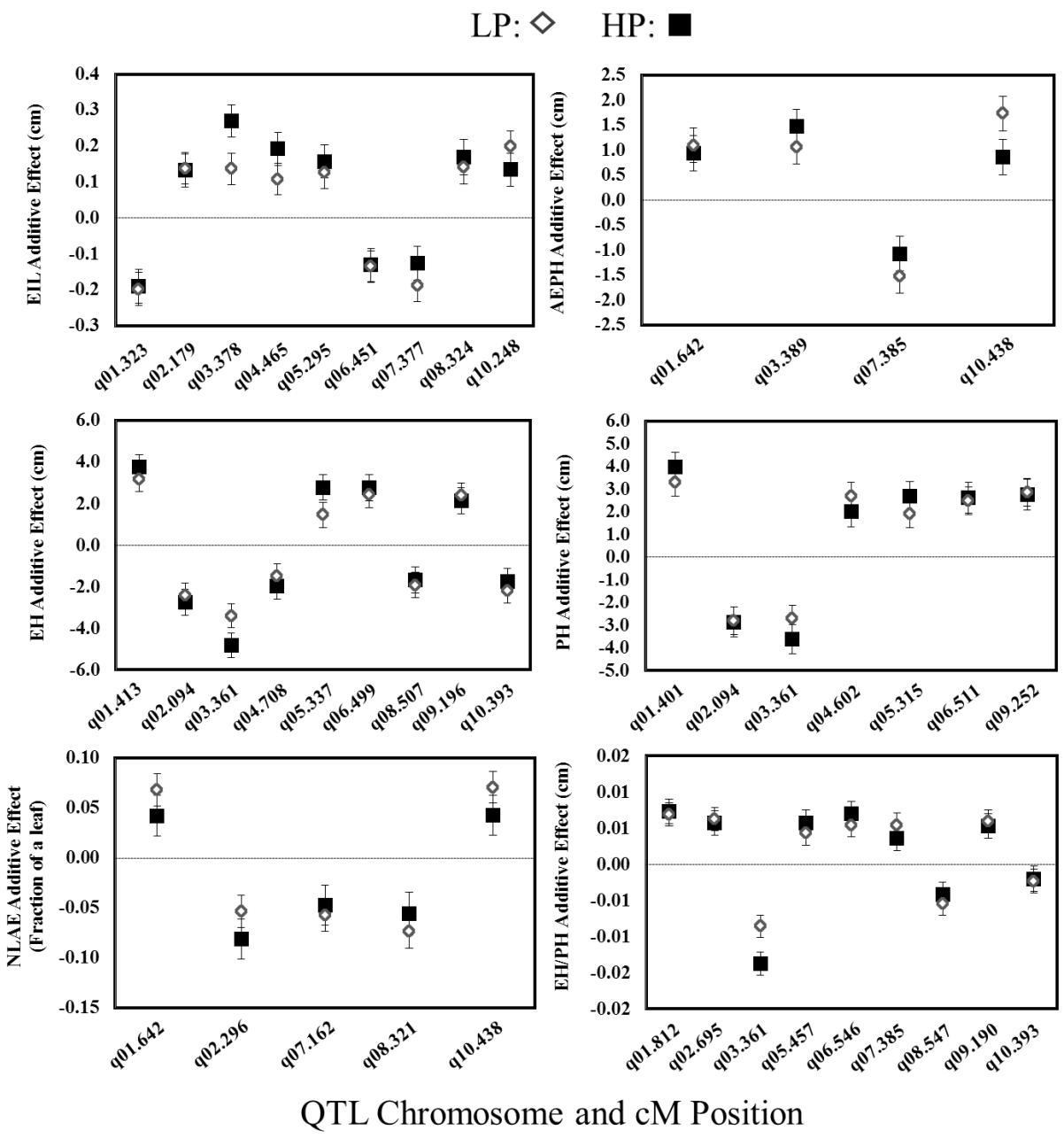

Figure 9. QTL additive effects under high (HP) and low (LP) rootworm pressure for significant plant architecture QTL. The additive effect for each of the six traits is shown on the y-axis in units consistent with their phenotyping scheme. QTL for each trait are labeled on the x-axis using chromosome.cM designations. Error bars are plus and minus the standard error of the additive effect.

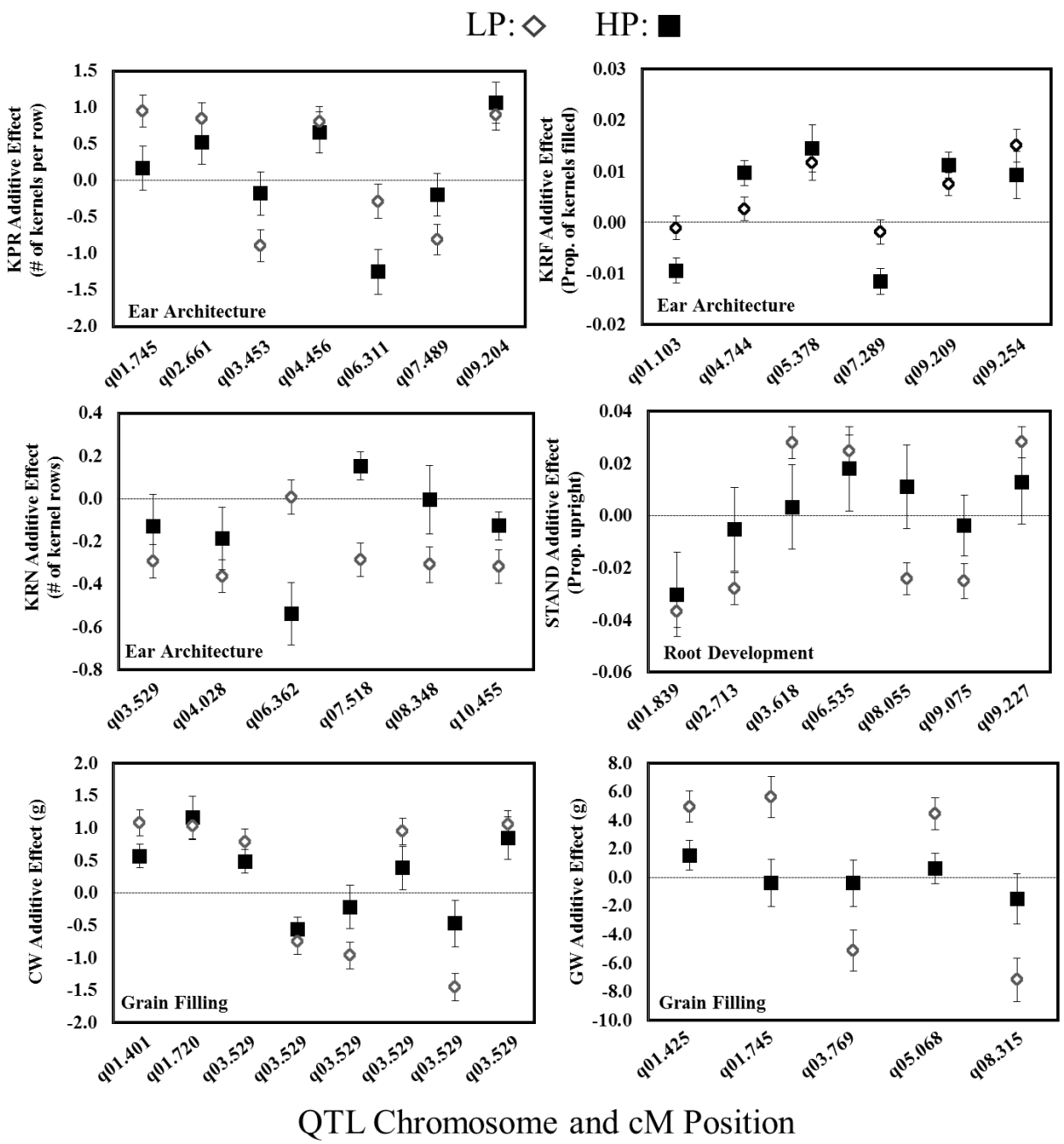

Figure 10. QTL additive effects under high (HP) and low (LP) rootworm pressure for the most significant ear architecture, root development, and grain-filling QTL. The additive effect for each of the six traits is shown on the y-axis in units consistent with their phenotyping scheme. QTL for each trait are labeled on the x-axis using chromosome.cM designations. Error bars are plus and minus the standard error of the additive effect.

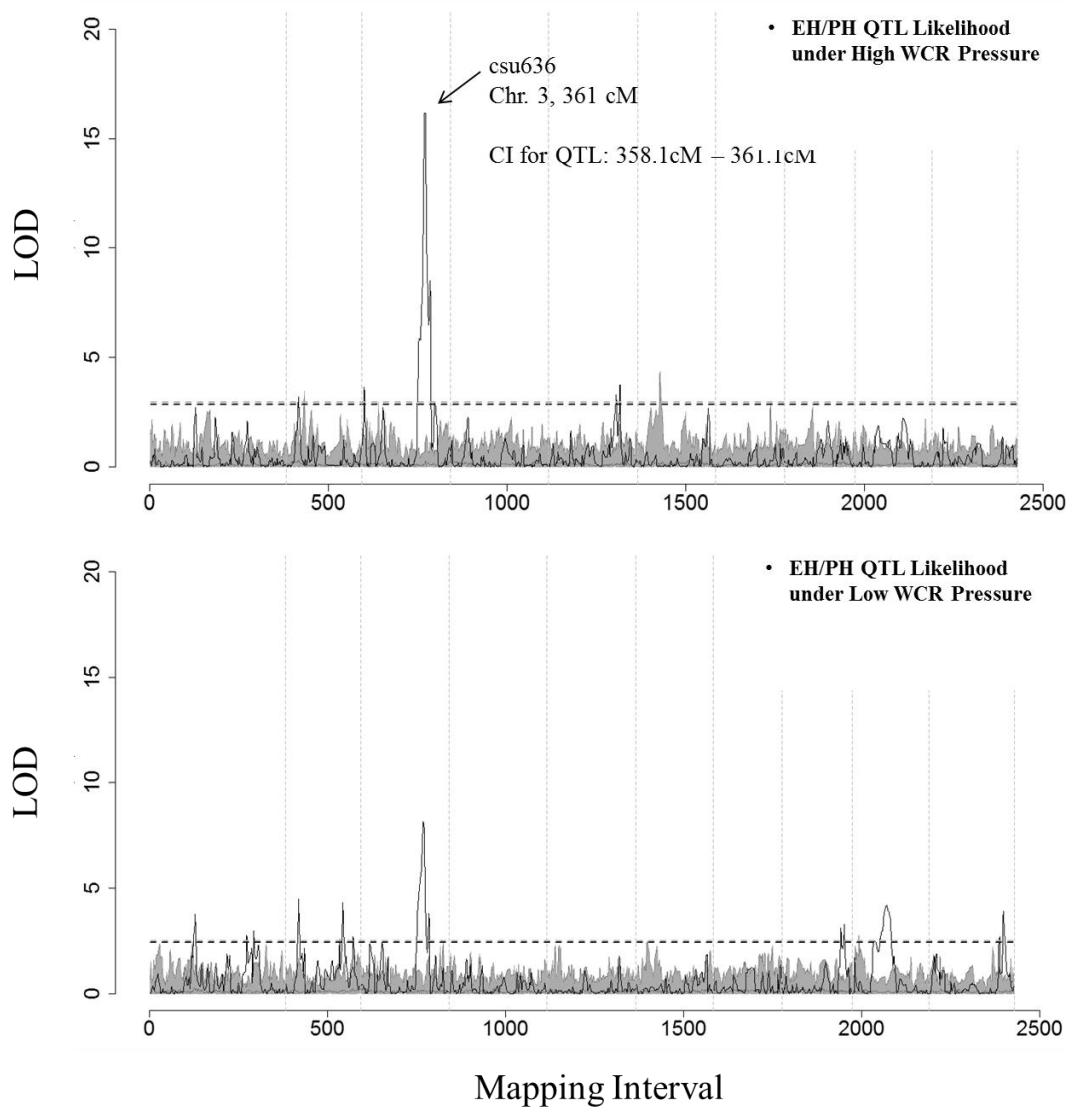

Figure 11. EH/PH QTL likelihood plots from multiple QTL mapping for IBMRILHs evaluated under high rootworm pressure (top) and low rootworm pressure (bottom). Vertical dashed lines separate chromosomes. The horizontal lines designates 10% and 5% genome-wide thresholds. LOD values from 1000 permutation datasets (shaded) are plotted with the actual QTL likelihood curve.

Table 1. List of traits collected in high and low western corn rootworm treatments, description of how each trait was assessed, and the physiological class to which the trait belongs.

Trait Abbreviation	Full Name	Description	Physiological Class
GERM	Germination	# of plants established out of 25	Seed
LG	Lodging	Proportion of row leaning > 30 ° from vertical	Root Development
STAND	Standability	Proportion of row standing straight up	Root Development
BS	Broken Stalks	Proportion of row with snapped stalks	Stalk Strength
PH	Plant Height	Distance from soil surface to base of peduncle	Plant Architecture
EH	Ear Height	Distance from soil surface to the stalk-peduncle attachment of uppermost ear	Plant Architecture
NLAE	# of Leaves Above Upper Ear	# of leaves above uppermost ear including flag leaf	Plant Architecture
AEPH	Above Ear Plant Height	Difference between PH and EH	Plant Architecture
EH + PH	Ear Height + Plant Height	Length of the addition between EH and PH	Plant Architecture
EH/PH	Ear Height/Plant Height	Proportion of plant height represented in ear height	Plant Architecture
EIL	Ear Internode Length	Distance between the first two nodes giving rise to a female inflorescence	Plant Architecture
EW	Ear Weight	Total dry weight of 5 ears per row	Grain Filling
GW	Grain Weight	Dry kernel weight from 5 ears per row	Grain Filling
CW	Cob Weight	Dry weight of 5 cobs per row	Grain Filling
KRN	Kernel Row #	Total # of kernel rows per ear	Ear Architecture
KPR	Kernels per Row	# of kernels per kernel row	Ear Architecture
KRF	Kernel Rows Filled	Proportion of kernel rows filled	Ear Architecture
EL	Ear Length	Length from ear base to ear tip	Ear Architecture

Table 2. Least squares means 4-way significance tests among 4 reps across two treatments for 17 different traits collected from a hybrid population of 220 IBMRILs x B101. Levels not connected by the same letter are significantly different ($\alpha = 0.05$).

	Low-WCR Pressure		High-WCR Pressure	
Trait	**Rep1**	**Rep2**	**Rep3**	**Rep4**
GERM	A	A	B	B
LG	A	A	B	B
STAND	A	A	B	B
BS	A	A	B	B
PH	A	B	C	D
EH	A	B	C	C
NLAE	A	B	C	D
AEPH	A	A	B	B
EH + PH	A	A	B	B
EH/PH	AB	B	C	AC
EIL	A	A	B	C
EW	A	A	B	C
GW	A	A	B	C
CW	A	A	B	C
KRN	A	A	B	C
KPR	A	A	B	C
KRF	A	B	C	C

Table 3. Number of independent tests failing to reject one of three hypotheses before and after multiple test correction and the outcome of the test on the tolerance to herbivory assumption.

Hypothesis	**# of Tests Before MTC**[†]	**# of Tests After MTC**[†]	**Assumption**
H_0: LP = HP	6104 (79.6%)	6295 (82.1%)	Tolerant
H_A: LP > HP	1427 (18.6%)	1277 (16.6%)	Not tolerant (undercompensation)
H_A: LP < HP	140 (1.8%)	99 (1.3%)	Not tolerant (overcompensation)
Total	7671	7671	

[†]Multiple test correction using Storey and Tibshirani's method of q-value determination.

Table 4. List of IBMRILHs with an overcompensation response detected for multiple traits. Means ± SE are reported for both high (HP) and low pressure (LP) along with the t-ratio and 2-sided p-value from independent t-tests and adjusted q-values for multiple test correction.

Trait	IBMRIL	Mean in LP ± SE	Mean in HP ± SE	t Ratio	Prob>|t|	q-value
EL	MO014	5.55 ± 0.77	8.00 ± 0.77	-6.03	4.56E-04	7.57E-03
KPR	MO014	29.60 ± 5.19	46.00 ± 5.19	-4.28	2.90E-03	2.00E-02
KRF	MO014	0.72 ± 0.03	0.88 ± 0.03	-3.98	5.11E-03	2.78E-02
EL	MO079	7.60 ± 0.43	8.95 ± 0.43	-4.57	4.31E-03	1.25E-02
KPR	MO079	37.80 ± 3.35	48.40 ± 3.35	-2.99	1.74E-02	2.35E-02
PH	MO079	244.00 ± 0.79	246.50 ± 0.79	-0.97	3.43E-01	4.83E-02
BS	MO128	0.39 ± 0.25	0.09 ± 0.04	3.53	4.62E-04	2.99E-02
GERM	MO128	10.00 ± 5.06	22.50 ± 5.06	-4.23	2.72E-05	9.51E-03
EH	MO150	104.80 ± 2.88	113.90 ± 2.88	-3.46	2.97E-03	1.24E-02
EH/PH	MO150	0.47 ± 0.02	0.51 ± 0.02	-5.67	3.02E-05	3.46E-03
KRF	MO150	0.85 ± 0.01	0.91 ± 0.01	-4.37	5.73E-03	9.90E-03
EH	MO157	113.50 ± 4.74	128.50 ± 4.74	-5.33	4.84E-05	1.13E-03
EH/PH	MO157	0.53 ± 0.02	0.60 ± 0.02	-4.89	1.78E-04	1.02E-02
KPR	MO172	38.80 ± 2.50	46.70 ± 2.50	-3.63	1.94E-03	3.11E-03
KRN	MO172	16.40 ± 0.32	17.40 ± 0.32	-1.52	1.46E-01	2.43E-02
EH	MO177	114.70 ± 4.52	129.00 ± 4.52	-2.53	2.15E-02	4.33E-02
PH	MO177	225.50 ± 1.68	230.80 ± 1.68	-1.08	2.93E-01	4.23E-02
EH	MO205	109.00 ± 2.85	118.00 ± 2.85	-2.61	1.80E-02	3.78E-02
PH	MO205	212.20 ± 1.52	217.00 ± 1.52	-1.51	1.51E-01	2.35E-02
KPR	MO223	28.20 ± 3.25	36.40 ± 3.29	-3.74	5.79E-03	1.32E-02
KRF	MO223	0.75 ± 0.03	0.85 ± 0.03	-2.15	6.39E-02	4.46E-02
EIL	MO238	17.53 ± 0.54	19.47 ± 0.33	-3.07	7.85E-03	3.21E-02
KRF	MO238	0.82 ± 0.05	0.98 ± 0.02	-3.48	2.54E-02	2.53E-02
EH	MO289	94.10 ± 6.61	115.00 ± 6.61	-3.35	5.82E-03	1.81E-02
EH/PH	MO289	0.41 ± 0.03	0.51 ± 0.03	-4.35	1.06E-03	1.73E-02
EIL	MO298	18.00 ± 0.22	19.58 ± 0.41	-3.44	4.06E-03	2.34E-02
KRF	MO298	0.84 ± 0.05	0.98 ± 0.02	-3.10	3.62E-02	3.01E-02
EL	MO332	6.70 ± 0.40	7.95 ± 0.40	-3.00	1.71E-02	2.86E-02
KPR	MO332	36.60 ± 2.97	46.00 ± 2.97	-3.25	2.64E-02	3.02E-02
KRN	MO334	10.20 ± 1.45	14.80 ± 1.45	-6.64	1.63E-04	1.76E-02
PH	MO334	208.90 ± 1.74	214.40 ± 1.74	-1.78	9.24E-02	1.53E-02
KRN	MO379	10.60 ± 1.20	14.40 ± 1.20	-6.72	1.50E-04	1.95E-02
PH	MO379	213.20 ± 1.90	219.20 ± 1.90	-1.28	2.16E-01	3.23E-02

Table 5. Percentage of the total significant overcompensated and undercompensated tests represented by each trait.

Trait	% of Over (total)†	% of Under (total)†
KRF	**37.37 (37)**	6.42 (82)
KPR	**13.13 (13)**	**19.89 (254)**
PH	**9.09 (9)**	**14.72 (188)**
EL	7.07 (7)	8.14 (104)
EH/PH	7.07 (7)	0.00 (0)
EH	5.05 (5)	3.92 (50)
EIL	5.05 (5)	3.21 (41)
STAND	4.04 (4)	0.31 (4)
KRN	4.04 (4)	**15.27 (195)**
BS	3.03 (3)	0.47 (6)
CW	2.02 (2)	0.00 (0)
EW	1.01 (1)	0.00 (0)
GW	1.01 (1)	0.00 (0)
GERM	1.01 (1)	0.00 (0)
LG	0.00 (0)	13.63 (174)
AEPH	0.00 (0)	11.28 (144)
NLAE	0.00 (0)	2.74 (35)
Total	100.00 (99)	100.00 (1277)

†Numbers in parentheses are the total number of tests for each trait falling into that response category. Bolded numbers signify the top three most frequent traits in each category.

Table 6. List of IBMRILs included in all three experimental populations with the same response mechanism detected in at least two of the three populations.

Line	Trait	2010 IBMRIL x B101	2011 IBMRIL x B101	2011 B101 x IBMRIL	Mechanism
MO093	EL†	-	Yes	Yes	under
MO120	EL†	-	Yes	Yes	under
MO237	EL†	-	Yes	Yes	under
MO288	EL†	-	Yes	Yes	under
MO380	EL†	-	Yes	Yes	under
MO017	KPR	Yes	No	Yes	under
MO021*	KPR	Yes	Yes	Yes	under
MO022	KPR	Yes	No	Yes	under
MO023	KPR	Yes	Yes	No	under
MO026	KPR	Yes	Yes	No	under
MO029	KPR	Yes	Yes	No	under
MO032	KPR	Yes	Yes	No	under
MO046	KPR	Yes	No	Yes	under
MO051	KPR	Yes	Yes	No	under
MO056	KPR	Yes	Yes	No	under
MO066	KPR	Yes	Yes	No	under
MO068	KPR	Yes	Yes	No	under
MO077	KPR	Yes	Yes	No	under
MO080	KPR	Yes	Yes	No	under
MO081	KPR	Yes	Yes	No	under
MO082	KPR	Yes	Yes	No	under
MO088	KPR	Yes	Yes	No	under
MO090	KPR	Yes	Yes	No	under
MO097*	KPR	Yes	Yes	Yes	under
MO101	KPR	No	Yes	Yes	under
MO106	KPR	Yes	No	Yes	under
MO111	KPR	Yes	No	Yes	under
MO130	KPR	Yes	Yes	No	under
MO134	KPR	Yes	Yes	No	under
MO145	KPR	Yes	Yes	No	under
MO150	KPR	Yes	Yes	No	under
MO154	KPR	Yes	Yes	No	under
MO156	KPR	Yes	Yes	No	under
MO160	KPR	Yes	Yes	No	under
MO164	KPR	Yes	Yes	No	under
MO167	KPR	Yes	Yes	No	under
MO176	KPR	No	Yes	Yes	under
MO177	KPR	Yes	Yes	No	under
MO188	KPR	Yes	No	Yes	under
MO200	KPR	No	Yes	Yes	under
MO206	KPR	Yes	Yes	No	under
MO209	KPR	Yes	Yes	No	under
MO222	KPR	Yes	Yes	No	under
MO224	KPR	Yes	Yes	No	under
MO230	KPR	Yes	No	Yes	under
MO232	KPR	Yes	Yes	No	under
MO237	KPR	No	Yes	Yes	under
MO238	KPR	Yes	Yes	No	under
MO250	KPR	Yes	Yes	No	under
MO258	KPR	Yes	Yes	No	under
MO266	KPR	Yes	Yes	No	under
MO267	KPR	Yes	Yes	No	under
MO270	KPR	Yes	Yes	No	under
MO271*	KPR	Yes	Yes	Yes	under
MO274	KPR	Yes	Yes	No	under
MO278	KPR	Yes	Yes	No	under
MO298	KPR	Yes	Yes	No	under
MO304	KPR	Yes	Yes	No	under
MO311	KPR	Yes	Yes	No	under
MO315	KPR	Yes	Yes	No	under
MO326	KPR	Yes	No	Yes	under
MO331	KPR	Yes	Yes	No	under
MO338	KPR	Yes	Yes	No	under
MO340*	KPR	Yes	Yes	Yes	under
MO341	KPR	Yes	Yes	No	under
MO349	KPR	Yes	Yes	No	under
MO369*	KPR	Yes	Yes	Yes	under
MO374	KPR	Yes	Yes	No	under
MO380*	KPR	Yes	Yes	Yes	under
MO096	KRF	No	Yes	Yes	under
MO161	KRF	No	Yes	Yes	over
MO169	KRF	No	Yes	Yes	under
MO266	KRF	No	Yes	Yes	under
MO335	KRF	No	Yes	Yes	under
MO337	KRF	No	Yes	Yes	under
MO101	KRN	Yes	Yes	No	under
MO021*	LG	Yes	Yes	Yes	under
MO086*	LG	Yes	Yes	Yes	under
MO128	STAND	Yes	Yes	No	over

†EL was included only in 2011 evaluations.

* IBMRILs with the same response trait and direction detected in all three populations.

Table 7. Complete list of QTL detected above 10% genome-wide thresholds by standard interval mapping using a single-QTL model across all traits evaluated in both 2010 and 2011.

Trait	QTL (Chr.Pos)[†]	Locus[††]	Bin	Mapped[‡]	LOD	Lower 90% CI	Upper 90% CI	Year
EIL	q01.323	cdo938a	1.02	no	7.39	320.10	324.30	2010
EIL	q02.179	mmc0231	2.03	no	3.72	163.50	269.60	2010
EIL	q03.378	ay106230	3.05	no	8.91	358.30	377.90	2010
EIL	q04.465	bnl7.65	4.08	no	4.41	443.20	539.00	2010
EIL	q05.295	mmp58	5.03	no	4.14	284.30	428.30	2010
EIL	q06.451	ph299852	6.07	no	3.42	420.40	504.80	2010
EIL	q07.377	bnlg155	7.03	no	4.76	347.20	382.60	2010
EIL	q08.324	gta101d	8.04	no	4.32	282.70	464.00	2010
EIL	q10.248	umc1246	10.04	no	5.56	244.60	248.20	2010
AEPH	q01.642	csu374b	1.07	no	3.65	630.60	916.70	2010
AEPH	q03.389	mmp184	3.05	yes	5.25	358.30	434.30	2010
AEPH	q07.385	umc1112	7.03	yes	4.58	13.80	390.50	2010
AEPH	q10.438	bnl7.49a	10.07	no	4.52	244.60	442.20	2010
CW	q01.401	bnlg2086	1.04	yes	6.30	401.20	405.00	2010
CW	q01.720	npi447a	1.07	no	5.94	720.30	755.20	2011
CW	q02.496	bcd808a	2.08	no	3.54	409.30	498.30	2010
CW	**q03.037**	**php20905**	3.01	no	3.87	30.50	828.90	2010
CW	**q03.445**	**umc2267**	3.06	yes	4.84	445.00	445.00	2011
CW	q05.333	mmp19	5.04	yes	4.49	328.50	332.70	2011
CW	q08.315	bnlg2046	8.04	no	9.83	315.20	315.20	2011
CW	q10.081	umc2018	10.01	no	5.75	64.10	81.10	2011
EH	q01.413	mmp61	1.05	yes	12.78	401.20	412.60	2010
EH	q02.094	ay109516	2.02	yes	7.38	94.40	94.40	2010
EH	**q03.358**	**sps2**	3.05	no	6.34	319.20	361.10	2011
EH	**q03.361**	**csu636**	3.05	no	19.85	358.30	361.10	2010
EH	q04.708	umc2289	4.10	yes	3.46	18.70	750.20	2010
EH	q05.337	ay110906	5.04	no	5.00	68.10	404.00	2010
EH	q06.499	ay109797	6.07	yes	6.99	498.70	542.70	2010
EH	q08.507	bnlg1828	8.07	no	3.61	457.20	628.20	2010
EH	**q09.196**	**umc1258**	9.03	yes	5.77	185.20	603.50	2010
EH	**q09.196**	**umc1258**	9.03	yes	3.68	116.60	633.20	2011
EH	q10.393	umc2122	10.06	no	4.46	325.10	533.20	2010
EH/PH	q01.812	cdj2	1.09	yes	8.13	401.20	886.90	2010
EH/PH	q02.695	mmp183	2.09	yes	5.68	122.40	694.60	2010
EH/PH	q03.361	csu636	3.05	no	20.33	358.30	361.10	2010
EH/PH	q05.457	mmp104	5.05	no	3.63	68.10	469.60	2010
EH/PH	q06.546	cdo345c	6.08	no	5.55	498.70	545.80	2010
EH/PH	q07.385	umc1112	7.03	yes	4.33	354.90	393.10	2010
EH/PH	q08.547	umc1673	8.08	no	3.56	457.20	546.90	2010
EH/PH	q09.190	lim286	9.02	no	4.96	185.20	314.30	2010
EH/PH	q10.393	umc2122	10.06	no	5.62	392.50	392.50	2010
EW	q01.425	mmp39	1.05	yes	4.85	390.80	425.20	2010
EW	q01.745	cdo98b	1.08	no	3.77	720.30	760.30	2011
EW	q05.068	umc1260	5.00	yes	3.68	54.00	520.10	2010
EW	q07.489	bnlg2259	7.04	no	3.22	471.40	494.80	2010
EW	q08.315	bnlg2046	8.04	no	5.02	312.40	315.20	2011

[†]QTL chromosome number and centiMorgan position. Bolded QTL highlight overlapping regions independently detected in both years for a given trait.
[††]Nearest marker on the IBM2 genetic map.
[‡]Mapped to the B73 genome physical map.

Table 7. (Continued)

Trait	QTL (Chr.Pos)[†]	Locus[††]	Bin	Mapped[‡]	LOD	Lower 90% CI	Upper 90% CI	Year
GERM	q09.332	c9.loc166	9.05	no	3.64	238.90	364.00	2010
GW	q01.425	mmp39	1.05	yes	4.68	390.80	430.60	2010
GW	q01.745	cdo98b	1.08	no	4.17	720.30	756.50	2011
GW	q03.769	AY110567	3.09	no	3.68	21.80	823.50	2011
GW	q05.068	umc1260	5.00	yes	3.51	54.00	520.10	2010
GW	q08.315	bnlg2046	8.04	no	5.33	295.30	315.20	2011
KPR	q01.745	cdo98b	1.08	no	4.23	401.20	817.30	2011
KPR	q02.661	umc36a	2.09	no	3.57	302.60	708.10	2011
KPR	q03.453	umc60	3.06	yes	3.63	21.80	738.70	2011
KPR	q04.456	mmp3	4.08	no	3.22	46.60	672.40	2010
KPR	q06.311	AY110260	6.05	yes	3.90	277.10	342.70	2011
KPR	q07.489	bnlg2259	7.04	no	3.21	53.30	593.40	2010
KPR	q09.204	ufg71	9.03	no	5.49	199.70	208.50	2010
KRF	q01.103	umc1685	1.01	yes	3.10	22.80	1039.70	2010
KRF	q04.744	umc1197	4.11	no	3.31	408.70	750.20	2010
KRF	q05.378	umc1966	5.04	yes	3.59	323.10	383.80	2011
KRF	q07.289	umc116a	7.03	no	4.33	285.40	300.00	2010
KRF	**q09.209**	**mmp170b**	9.03	yes	4.33	199.70	240.60	2010
KRF	**q09.254**	**AW257883**	9.03	yes	4.64	236.10	266.00	2011
KRN	q03.529	umc3b	3.06	no	2.86	499.00	823.50	2011
KRN	q04.028	csu509	4.05	no	4.95	277.80	310.70	2011
KRN	q06.362	uaz400	6.05	yes	3.21	362.00	378.40	2011
KRN	q07.518	aw267377	7.04	yes	2.92	2.70	518.90	2010
KRN	q08.348	mmp15	8.05	yes	3.05	135.60	415.70	2011
KRN	q10.455	npi208b	10.07	no	3.63	217.80	470.90	2010
NLAE	q01.642	csu374b	1.07	no	4.31	417.00	942.40	2010
NLAE	q02.296	umc2249	2.04	yes	4.94	43.30	306.30	2010
NLAE	q07.162	ay105589	7.02	yes	3.21	13.80	471.40	2010
NLAE	q08.321	ay104017	8.04	yes	4.08	320.60	353.90	2010
NLAE	q10.438	bnl7.49a	10.07	no	3.62	422.70	442.20	2010
PH	q01.401	bnlg2086	1.04	yes	10.65	401.20	405.00	2010
PH	q02.094	ay109516	2.02	yes	6.70	88.02	94.40	2010
PH	q03.361	csu636	3.05	no	8.42	354.00	361.10	2010
PH	q04.602	umc1650	4.09	no	4.24	18.70	605.50	2010
PH	q05.315	umc2300	5.04	no	4.35	68.10	404.00	2010
PH	q06.511	bnlg1740	6.07	no	5.26	483.50	510.60	2010
PH	q09.252	umc2087	9.03	yes	6.52	249.20	587.90	2010
BS	q09.101	umc1170	9.02	yes	4.17	74.80	101.10	2010
STAND	q01.839	phi011	1.09	no	7.71	833.00	879.70	2011
STAND	q02.713	AY109586	2.10	yes	4.50	600.70	713.10	2011
STAND	q03.618	umc1915	3.08	no	4.44	77.00	632.40	2011
STAND	q06.535	mmp105	6.07	yes	3.59	66.40	548.70	2011
STAND	q08.055	umc1327	8.01	no	3.27	44.40	361.20	2011
STAND	**q09.075**	**umc2335**	9.01	yes	3.72	65.20	556.40	2010
STAND	**q09.227**	**bcd1421**	9.03	no	4.57	223.90	235.50	2011

[†]QTL chromosome number and centiMorgan position. Bolded QTL highlight overlapping regions independently detected in both years for a given trait.
[††]Nearest marker on the IBM2 genetic map.
[‡]Mapped to the B73 genome physical map.

Table 8. QTL identified with significantly higher likelihood of odds ratios in the high rootworm treatment (HP) than in the low treatment (LP).

Trait	QTL (Chr.Pos)[†]	Bin	10% GW Threshold[††]	LOD LP	LOD HP	LOD Diff.[‡]	Year
EH/PH	q02.590	2.09	3.28	0.707	3.634	2.927	2010
EH	q02.600*	2.09	3.25	0.625	3.715	3.090	2010
PH	q02.600*	2.09	2.93	0.528	3.715	3.187	2010
EH	q03.360	3.05	3.25	10.329	15.533	5.204	2010
EH/PH	q03.360	3.05	3.28	9.787	16.151	6.364	2010
PH	q03.360	3.05	2.93	10.329	15.533	5.204	2010
AEPH	q03.380	3.05	3.59	3.097	4.085	0.988	2010
CW	q03.829	3.09	3.09	1.836	3.592	1.756	2010
PH	q04.330	4.06	2.93	2.551	2.971	0.420	2010
EH	q05.360	5.04	3.25	1.267	4.176	2.909	2010
PH	q05.360	5.04	2.93	1.267	4.176	2.909	2010
EH/PH	q05.440	5.05	3.28	1.666	3.744	2.078	2010
EH	q05.440*	5.05	3.25	1.947	3.568	1.621	2010
PH	q05.440*	5.05	2.93	0.304	3.568	3.264	2010
EIL	q05.670*	5.09	2.82	0.509	3.195	2.686	2010
KPR	q06.310	6.05	3.40	1.497	3.945	2.448	2011
KRN	q06.360	6.05	2.80	0.256	3.082	2.826	2011
KRF	q07.290	7.03	2.95	0.089	4.264	4.175	2010
EH	q08.370*	8.05	3.25	2.565	3.416	0.851	2010
PH	q08.370*	8.05	2.93	2.565	3.416	0.851	2010

[†]QTL chromosome number and centiMorgan position.
[††]10% Genome-wide permutation threshold based on 1000 permutations.
[‡]Absolute value of the difference in LOD score between high and low treatments. The difference was classified as significant if the LOD exceeded 10% genome-wide thresholds under HP but not LP or if the LOD difference was greater than 3.00.*Indicates novel QTL not identified in the genome scan using the single QTL model.

Table 9. QTL identified with significantly higher likelihood of odds ratios in the low rootworm treatment (LP) than in the high treatment (HP).

Trait	QTL (Chr.Pos)[†]	Bin	10% GW Threshold[††]	LOD LP	LOD HP	LOD Diff.[‡]	Year
STAND	q01.090*	1.01	3.30	4.450	0.395	4.055	2011
NLAE	q01.110*	1.01	3.34	3.859	0.041	3.818	2010
CW	q01.400	1.04	3.09	6.667	1.672	4.995	2010
EH/PH	q01.410	1.05	3.28	4.371	2.710	1.661	2010
EW	q01.410	1.05	3.03	4.609	0.688	3.921	2010
GW	q01.420	1.05	3.11	4.093	0.660	3.433	2010
NLAE	q01.640	1.07	3.34	3.806	1.335	2.470	2010
CW	q01.720	1.07	3.00	6.624	2.959	3.664	2011
KPR	q01.740	1.08	3.40	5.054	1.083	3.971	2011
PH	q01.740	1.08	2.93	3.146	1.144	2.002	2010
GW	q01.740*	1.08	3.30	4.488	0.545	3.943	2011
STAND	q01.880	1.09	3.30	9.071	1.385	7.686	2011
EH	q01.890*	1.10	3.25	4.821	2.575	2.246	2010
PH	q01.890*	1.10	2.93	4.821	2.575	2.246	2010
EH/PH	q02.130	2.02	3.28	4.316	3.169	1.146	2010
EH/PH	q02.430	2.07	3.28	4.425	1.232	3.193	2010
KPR	q02.660*	2.09	3.40	4.374	1.835	2.539	2011
EW	q03.020*	3.01	3.30	3.539	1.180	2.359	2011
PH	q03.050*	3.01	2.93	3.656	2.463	1.193	2010
EIL	q03.060*	3.02	2.82	3.363	0.307	3.055	2010
KRN	q03.530	3.06	2.80	2.952	0.966	1.986	2011
STAND	q03.620	3.08	3.30	3.772	1.468	2.304	2011
GW	q03.770	3.09	3.30	3.940	1.160	2.780	2011
KRN	q04.280	4.05	2.80	5.288	0.785	4.503	2011
EW	q05.060	5.00	3.03	3.333	1.450	1.883	2010
GW	q05.060	5.00	3.11	3.324	1.692	1.632	2010
CW	q05.330	5.04	3.00	4.267	0.215	4.052	2011
STAND	q06.540	6.08	3.30	4.959	1.921	3.038	2011
AEPH	q07.160	7.02	3.59	3.681	2.322	1.359	2010
EIL	q07.280*	7.02	2.82	3.716	1.031	2.684	2010
AEPH	q07.370	7.03	3.59	3.807	2.121	1.687	2010
EW	q07.490	7.04	3.03	3.163	0.502	2.662	2010
KPR	q07.490	7.04	3.11	3.376	1.200	2.176	2010
CW	q08.310	8.03	3.00	7.269	0.376	6.893	2011
EW	q08.320	8.04	3.30	3.940	1.159	2.781	2011
GW	q08.320	8.04	3.30	3.932	2.289	1.642	2011
EIL	q08.330	8.05	2.82	3.654	2.397	1.257	2010
NLAE	q08.350	8.05	3.34	4.451	1.442	3.009	2010
EH/PH	q08.500	8.07	3.28	3.816	1.248	2.568	2010
STAND	q09.070	9.01	2.99	3.400	0.914	2.487	2010
BS	q09.100	9.02	3.13	3.783	1.631	2.152	2010
KPR	q09.210	9.03	3.11	3.546	2.920	0.626	2010
EH	q09.250	9.03	3.25	6.723	3.537	3.186	2010
EH/PH	q09.250	9.03	3.28	4.491	2.234	2.257	2010
PH	q09.250	9.03	2.93	6.723	3.537	3.186	2010
KRF	q09.260	9.04	3.30	4.884	1.223	3.660	2011
PH	q09.600*	9.08	2.93	4.360	2.151	2.209	2010
CW	q10.060	10.01	3.00	5.377	1.155	4.222	2011
EIL	q10.250	10.04	2.82	3.912	0.843	3.069	2010
NLAE	q10.430	10.06	3.34	5.980	1.009	4.971	2010
EH/PH	q10.440	10.07	3.28	3.621	1.348	2.273	2010
AEPH	q10.440*	10.07	3.59	6.412	2.148	4.264	2010
KRN	q10.460	10.07	2.77	3.353	2.330	1.023	2010

[†]QTL chromosome number and centiMorgan position.
[††]10% Genome-wide permutation threshold based on 1000 permutations.
[‡]Absolute value of the difference in LOD score between high and low treatments. The difference was classified as significant if the LOD exceeded 10% genome-wide thresholds under HP but not LP or if the LOD difference was greater than 3.00.*Indicates novel QTL not identified in the genome scan using the single QTL model.

CHAPTER 5. GENERAL CONCLUSIONS

General Discussion

In this dissertation I present evidence that native resistance to western corn rootworm larval herbivory has a genetically tractable basis and that a favorable allele can confer resistance that is stable across years and environments. In Chapter 2, the main sources of variation controlling differences between naturally and artificially infested treatments were identified in the context of both rootworm population dynamics and host-plant phenology. In addition to experientially guiding the framework for the remaining experiments, the results also demonstrate that both treatments can be effective in providing enough larval feeding pressure for genotypic differences to be observed. However, a higher level of larval feeding pressure was achieved under AI and there was greater correspondence between larval densities and root phenotypes. Higher larval densities were associated with more severe node-injury and increased lodging. This improvement in the ability to capture node-injury under AI conditions is due largely to a minimization of environmental variance inherently associated with natural infestations. Because year, environment, and G x E variation have been among the main challenges in native resistance screens (Ivezić, Raspudić, et al., 2009a, Ivezić, Raspudić, et al., 2009b, Simić, Ivezić, et al., 2007), the use of AI can lead to more accurate estimations of genetic variance. We confirmed this in the heritability estimates of node-injury among doubled haploids which were highest at AI locations in the genetic study involving doubled haploid lines phenotyped in 10 different trials.

We also detected evidence for both intra and interspecies competition among rootworm populations. In the latter case, the larger-sized roots of hybrids were able to support greater numbers of larvae, but fewer survived to adulthood and this was observed at concentrations of 750 eggs/plant. This is consistent with levels of density-dependent mortality reported by Hibbard, Meihls, et al. (2010) and Onstad, Hibbard, et al. (2006). Furthermore, we observed that only a small fraction of the eggs survived to adulthood. Although there were almost certainly adults that were not accounted for using our sampling approach, the results are consistent with an estimate of density-independent mortality of between 91% and 97% reported by (Hibbard, Meihls, et al., 2010). There was also a negative relationship between western corn rootworm larval densities and those of northern corn rootworms. This interspecific interaction is not

surprising given their similarity in habitats and feeding behavior (Chiang, 1973, Chiang and Raros, 1968). Clearly, there are natural constraints that restrict rootworm population densities. Therefore, infestation levels may only correspond to node-injury up to a certain point and infestations beyond this point may not be necessary. It is also important to consider levels of host-plant resistance within a particular germplasm pool, as higher larval feeding pressures may be too intense to allow for detection of more moderate effect resistance alleles. Of course, there are always trade-offs that each investigator must evaluate when choosing to perform resistance screens in trap, AI, or both treatments. Natural populations can save time and resources but can have greater levels of environmental variance. One option would be to conduct a preliminary screen in a trap crop using a greater number of lines to identify resistance candidates and then follow-up with AI experiments on a smaller scale among the best and worst candidates.

Understanding how plants defend against and how they respond to root herbivory is an overarching theme of this dissertation. Returning to the three main concepts of defense developed by Painter (1951), the results presented herein provide evidence that both tolerance and resistance mechanisms exist, and furthermore, they have genetic etiologies. We demonstrate with reproducibility that regions on maize chromosomes 2, 3, 5, and 7 contain genes associated with node-injury (NI) resistance, root size (RS) or root regrowth (RR). For several of the QTL detected, co-localization for two or all three native resistance traits was observed. While this is expected for RS and RR because they are regarded as interdependent tolerance mechanisms (Tollefson, 2007), these observations do not rule out overlap among the underlying genetic controls of tolerance and resistance in the forms of antibiosis or antixenosis. However, since the resolution in our mapping populations is moderate at best, we cannot reliably indicate the relative probabilities of multiple-polymorphism versus pleiotropic polymorphism architectures underlying these co-located QTL.

As expected, we detected strong correspondence between RS and RR QTL, and in general, a greater number of QTL were detected for these traits. This is a good genetic explanation for why most of the native resistant varieties developed have been tolerant rather than resistant to larval herbivory (Ivezić, Raspudić, et al., 2009b, Owens, 1974, Riedell and Evenson, 1993). The greater number of genes available for selection increases the probability of capturing genetic

variation in germplasm screens. RS and RR have been reported in some cases to be better predictors of yield than node-injury (Spike and Tollefson, 1989), so this could also explain why tolerance has been predominant.

This work challenges the idea that growth and defense are mutually exclusive or counter-acting processes (Herms and Mattson, 1992, Meijden, Wijn, et al., 1988). In addition to the co-localization of NI and biomass QTL reported in Chapter 3, we also observed changes in growth patterns under heavy WCR pressure in Chapter 4. If growth was unrelated to the defense response, as suggested by the results from Assabgui, Arnason, et al. (1995), then we would have expected equivalent plant architectures (ear height, plant height, internode length) under high and low WCR pressure. Alternatively, if the processes were negatively related, we would observe stunted phenotypes under heavy pressure. This is generally what we observed; plants under heavy herbivory stress tended to be smaller both vegetatively and reproductively. Although cases were also observed in which herbivory stress, which should induce a defense response, resulted in enhanced growth relative to performance under low herbivory stress. Canonically referred to as overcompensation, we traced this phenotype x treatment interaction to discrete genetic regions, one of which localized to a 3 cM locus on chromosome 3. The *sucrose phosphate synthase2* gene was presented as a possible candidate in this response, particularly with respect to plant height and ear height, and has been implicated in regulating source-sink relationships in the plant (Cheng, Im, et al., 1996). For WCR defense, growth at the root interface is an important parameter (Ivezic, Tollefson, et al., 2006) and as such, resource allocation towards growth may directly or indirectly improve the ability to withstand larval feeding damage. Interestingly, although different biparental populations and genetic maps were used between the tolerance study (Chapter 4) and the native resistance study (Chapter 3), the narrowly localized QTL detected in Chapter 4 maps to the peak of the broadly localized QTL from Chapter 3. Together, these results strongly indicate an important role for this region of chromosome 3 in conferring meaningful and robust protection from crop losses due to WCR. Thus, further experiments to better localize and functionally characterize the effects of these QTL are certainly warranted.

Significance

This represents the first study to definitively associate genetic variation at particular loci with native resistance to the WCR larval feeding. The results have several important implications for both basic and applied agricultural science, and broadens our understanding of WCR population dynamics and host-plant defense against herbivory. It also sets the stage for further experiments. One of the main products of this work is a set of more than 70 (B86xFS8) doubled haploid lines that as a population segregate for WCR native resistance traits. Because the lines are genetically immortalized and have been genotyped, they can now be used by others to extend these results and to investigate other traits. Within the set of DHLs, we observed several lines with stronger levels of node-injury resistance and regrowth than three previously reported native-resistant varieties, and at least eight lines that were equal to or more resistant than the transgenic check, Mir604 (Syngenta, expressing mCry3A). In the initial screen of the FS8B(S):S0316-053-1, we also showed that resistance levels were similar to that achieved for MON863, possessing a different transgenic protein, Cry3Bb1 (Vaughn, Cavato, et al., 2005). These top performing lines are suitable starting materials for more detailed mechanistic investigations or for use in commercial breeding programs. Finally, the QTL locations reported herein can be used for marker-assisted selection or for mining additional resistance alleles from diverse maize germplasm.

Recommendations for Future Research

There are several experiments that could be conducted to extend the research of this dissertation. Although, we did assess QTL main effects, we did not report on epistatic interactions between QTL and it would be interesting to conduct searches for QTL x QTL interactions. The QTL that were identified can be targeted more directly to identify specific genes and determine if node-injury and root architecture are separately regulated. It would also be important to test the antibiosis efficacy of the DHLs by measuring larval abundance and head capsule width. These traits in turn could be mapped genetically to reveal additional antibiosis QTL. Lastly, we focused our native resistance genetic mapping on the stiff-stalk side of the maize pedigree, but we also generated nonstiff-stalk F_2 and BC_1 populations derived from the UR13085:N0215-19-2 source. Within these populations we observed segregation of silk feeding

resistance during one summer of observation. Further evaluation of these populations and mapping of silk-feeding QTL could potentially lead to combining adult and larval native resistance across heterotic groups.

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APPENDIX

This Appendix contains data tables with supplemental information for Chapters 2-4.

Appendix Table 1. (Chapter 2) Correlation matrices between three root traits, larval and adult rootworm abundance, and standability for AI and Trap treatments across 4 experimental units (EUs).

EU1

AI†† \ Trap†	RS	RR	NI	Mu A	Mu L	LG
RS		**0.773**	**-0.541**	-0.146	-0.222	-0.301
RR	**0.767**		**-0.508**	-0.221	**-0.538**	-0.355
NI	**-0.726**	**-0.741**		0.013	**0.484**	**0.689**
Mu A	0.179	0.101	0.064		0.249	0.030
Mu L	0.092	0.050	0.110	**0.731**		0.339
LG	**-0.570**	-0.234	0.391	0.064	0.018	

EU2

AI†† \ Trap†	RS	RR	NI	Mu A	Mu L	LG
RS		**0.823**	-0.371	0.232	**-0.431**	**-0.631**
RR	**0.608**		**-0.633**	**0.614**	-0.421	-0.305
NI	**-0.435**	**-0.732**		**-0.503**	0.329	0.308
Mu A	-0.397	-0.081	0.184		-0.360	-0.030
Mu L	**-0.586**	**-0.433**	0.334	**-0.578**		0.335
LG	-0.081	**-0.538**	**0.626**	**0.559**	0.174	

EU3

AI†† \ Trap†	RS	RR	NI	LG	BS
RS		**0.728**	-0.144	**-0.670**	0.126
RR	0.389		-0.016	**-0.537**	**0.446**
NI	-0.209	-0.376		0.114	0.227
LG	0.400	0.046	0.304		-0.102
BS	**-0.734**	-0.144	0.055	-0.180	

EU4

AI†† \ Trap†	RS	RR	NI	LG	BS
RS		**0.977**	-0.318	**0.944**	-0.333
RR	**0.507**		-0.167	**0.960**	-0.283
NI	**-0.737**	**-0.627**		**0.710**	**0.701**
LG	-0.222	-0.456	0.296		**0.677**
BS	-0.366	0.352	0.209	-0.078	

† The upper panel for each EU corresponds to correlations within the Trap treatment, †† The lower panel corresponds to correlations within AI treatment. The mean genotype values per treatment were taken for each trait and the correlation between means was used to generate the matrices below. Bolded values are Pearson correlations with P > 0.05. EU = experimental unit,, Mu A = mean adult emergence, Mu L = mean larval abundance, RS = root size, RR = root regrowth, NI = node-injury, LG = root or stalk lodging, BS = broken stalks.

Appendix Table 2. (Chapter 2) Mean genotype values ± SE for 4 ear morphology traits and three grain-filling traits collected from 5 ears in both AI and Trap treatments.

Genotype	KRN		KPR		KNF		EL		EW		GW		CW	
	AI	Trap	AI	Trap	AI	Trap	AI	Trap	AI	Trap	AI	Trap	AI	Trap
(AGR_9 x NGSDCRW-1)DH-2	13.6 ± 0.6	14.4 ± 0.6	16.4 ± 3.2	19.0 ± 3.2	17.2 ± 6.1	16.4 ± 6.1	16.5 ± 1.5	13.2 ± 1.5	92 ± 16	54 ± 16	70 ± 14	34 ± 14	23 ± 2	20 ± 2
(AGR_9 x NGSDCRW-1)DH-4	15.6 ± 0.6	14.8 ± 0.6	21.2 ± 1.3	19.4 ± 1.3	4.2 ± 0.9	8.2 ± 0.9 *	11.2 ± 0.5	10.4 ± 0.5	73 ± 16	47 ± 16	6 ± 14	39 ± 14	12 ± 2	8 ± 2
(AGR_9 x NGSDCRW-1)DH-5	13.6 ± 1.1	15.2 ± 1.1	14.0 ± 2.3	24.2 ± 2.3 *	8.6 ± 1.5	6.0 ± 1.5	12.2 ± 1.3	14.2 ± 1.3	55 ± 16	77 ± 16	41 ± 14	65 ± 14	13 ± 2	13 ± 2
(AGR_9 x NGSDCRW-1)DH-6	12.8 ± 1.1	8.4 ± 1.1 *	13.0 ± 2.0	7.6 ± 2.0	11.8 ± 2.3	23.8 ± 2.3 *	14.2 ± 0.5	13.0 ± 0.5	62 ± 16	21 ± 16	44 ± 14	7 ± 14	19 ± 2	13 ± 2
(AGR_9 x NGSDCRW-1)DH-8	13.2 ± 1.4	9.0 ± 1.4	12.4 ± 1.9	8.8 ± 1.9	14.2 ± 2.2	20.2 ± 2.2	14.0 ± 0.5	11.7 ± 0.5 *	38 ± 16	12 ± 16	26 ± 14	4 ± 14	13 ± 2	7 ± 2
AGR_9	12.8 ± 1.2	11.2 ± 1.2	17.2 ± 2.1	16.0 ± 2.1	10.0 ± 3.0	16.8 ± 3.3	13.2 ± 0.8	13.3 ± 0.8	60 ± 16	-	43 ± 15	-	17 ± 2	-
NGSDCRW-1	11.2 ± 0.3	14.0 ± 0.3 ***	7.0 ± 1.6	22.8 ± 1.6 ***	14.8 ± 2.1	7.8 ± 2.1 *	15.7 ± 3.8	15.0 ± 3.8	41 ± 16	53 ± 16	28 ± 14	41 ± 14	12 ± 2	13 ± 2
CRW8-1	13.6 ± 1.0	2.0 ± 1.2 *	8.2 ± 3.6	0.0 ± 4.6	32.0 ± 3.7	19.3 ± 4.7 *	17.0 ± 1.3	8.4 ± 1.3 *	18 ± 16	3 ± 16	-	3 ± 15	-	0
LH51	10.0 ± 1.6	8.4 ± 1.6	6.6 ± 2.0	10.4 ± 2.0	34.8 ± 2.6	27.2 ± 2.6	18.3 ± 0.5	17.8 ± 0.5	28 ± 16	13 ± 16	14 ± 14	2 ± 14	14 ± 2	11 ± 2
B86	0.0 ± 0.4	3.0 ± 0.5 *	0.0 ± 0.4	3.0 ± 0.5 *	ALL	32.5 ± 8.5	10.4 ± 1.3	12.2 ± 1.8	13 ± 16	5 ± 16	-	3 ± 15	-	8 ± 2
Mo47	13.6 ± 0.4	13.6 ± 0.4	16.0 ± 1.8	16.6 ± 1.8	21.6 ± 2.7	21.4 ± 2.7	15.0 ± 0.5	15.2 ± 0.5	61 ± 16	46 ± 16	43 ± 14	31 ± 14	18 ± 2	15 ± 2
PHG84	14.0 ± 1.1	15.2 ± 1.1	12.8 ± 5.9	23.0 ± 5.9	23.8 ± 7.2	19.6 ± 7.2	16.5 ± 0.8	18.5 ± 0.8	60 ± 16	71 ± 16	27 ± 14	37 ± 14	32 ± 2	35 ± 2
PHZ51	11.3 ± 0.6	12.8 ± 0.5	16.0 ± 2.3	22.2 ± 1.8	8.3 ± 1.7	5.8 ± 1.3	11.9 ± 0.3	13.2 ± 0.3 *	63 ± 16	57 ± 16	47 ± 14	43 ± 14	17 ± 2	14 ± 2
PHG84 x (AGR_9 x NGSDCRW-1)DH-1	15.6 ± 0.6	15.2 ± 0.8	41.8 ± 2.6	42.8 ± 3.7	3.6 ± 0.6	7.0 ± 0.9 *	19.1 ± 0.8	20.6 ± 1.0	232 ± 16	229 ± 16	198 ± 10	196 ± 14	34 ± 1	34 ± 2
PHG84 x (AGR_9 x NGSDCRW-1)DH-5	16.0 ± 0.6	16.8 ± 0.6	46.2 ± 2.9	39.6 ± 2.9	3.4 ± 2.5	9.0 ± 2.5	23.6 ± 0.5	23.6 ± 0.5	293 ± 12	231 ± 16	248 ± 14	185 ± 14	47 ± 2	45 ± 2
PHG84 x (AGR_9 x NGSDCRW-1)DH-6	16.0 ± 0.5	16.4 ± 0.5	35.8 ± 5.9	33.2 ± 5.9	7.4 ± 1.5	13.8 ± 1.5 *	21.1 ± 0.5	19.1 ± 0.5 *	265 ± 16	197 ± 16	224 ± 14	160 ± 14	43 ± 2	35 ± 2
PHG84 x (AGR_9 x NGSDCRW-1)DH-7	15.2 ± 0.5	14.8 ± 0.5	42.0 ± 1.5	30.0 ± 1.5 **	4.4 ± 1.4	13.2 ± 1.4 **	21.3 ± 0.5	17.5 ± 0.5 **	261 ± 17	-	219 ± 15	-	43 ± 2	-
PHG84 x (AGR_9 x NGSDCRW-1)DH-8	16.8 ± 0.7	17.2 ± 0.7	39.6 ± 0.9	41.0 ± 0.9	7.6 ± 0.7	11.6 ± 0.7 *	20.8 ± 0.8	20.1 ± 0.8	272 ± 16	236 ± 16	228 ± 14	197 ± 14	44 ± 2	39 ± 2
PHG84 x AGR_9	14.8 ± 0.5	14.8 ± 0.5	44.0 ± 1.6	41.2 ± 1.6	4.0 ± 0.8	8.6 ± 0.8 **	21.6 ± 0.8	19.3 ± 0.8	263 ± 16	-	224 ± 15	-	40 ± 2	-
PHG84 x NGSDCRW-1	15.6 ± 0.4	16.4 ± 0.4	47.0 ± 2.4	40.2 ± 2.4	5.2 ± 2.0	9.6 ± 2.0	23.9 ± 1.0	21.1 ± 1.0	299 ± 16	236 ± 16	252 ± 14	196 ± 10	50 ± 2	37 ± 2
PHG84 x CRW8-1	14.8 ± 0.9	16.4 ± 0.9	44.4 ± 2.8	40.8 ± 2.8	4.0 ± 0.7	9.4 ± 0.7 **	23.4 ± 0.8	22.4 ± 0.8	280 ± 16	240 ± 16	223 ± 14	177 ± 10	57 ± 2	63 ± 2
PHG84 x PHZ51	14.4 ± 0.4	14.8 ± 0.4	46.8 ± 1.7	42.0 ± 1.7	4.2 ± 0.8	7.0 ± 0.8 *	23.4 ± 0.5	20.6 ± 0.5 *	307 ± 16	236 ± 16	243 ± 10	186 ± 10	66 ± 2	48 ± 2
Mean	**13.0 ± 0.5**	**12.6 ± 0.4**	**24.4 ± 1.0**	**24.2 ± 1.0**	**13.0 ± 1.0**	**14.7 ± 0.9**	**17.5 ± 0.5**	**16.3 ± 0.3 ***	**142 ± 7**	**116 ± 7 ***	**114 ± 7**	**90 ± 6 ***	**29 ± 1**	**25 ± 2 ***

* $P \leq 0.05$, ** $P \leq 0.005$, *** $P \leq 0.0005$

KRN = Kernel Row Number, KPR = Kernels Per Row, KNF = Kernels Not Filled, EL = Ear Length, EW = Ear Width, GW = Grain Weight, CW = Cob Weight

Appendix Table 3. (Chapter 3) List of SNPs between FS8B(S):S0316-053-1 and B86 and their primer sequences designed in 5 multiplex genotyping assays.

SNP Name[†]	Mutliplex Group	Reverse Primer Sequence	Forward Primer Sequence	Extension Primer Sequence	SNP Call (B86/FS8)
LLMAGI_90380	1	ACGTTGGATGCACAAACTTTTAGCCGCGTG	ACGTTGGATGTCGCGGATCGTAAATTAGTG	CACTCTCCACAGCAC	C/T
LLMAGI_44788	1	ACGTTGGATGTTATACCGTGACCGTAGACC	ACGTTGGATGCCAGCTACAAAATCCGAAAC	TCGCAGCGGAAAGAT	A/T
LLMAGI_3869	1	ACGTTGGATGTTATCACAGGCCTAGTGTCC	ACGTTGGATGCCCGGATAGCCATTTACTAC	TCGCCCAAAGAAATCG	T/C
LLMAGI_2594	1	ACGTTGGATGGCCTTTAGCTTATGGTGCTC	ACGTTGGATGTGGACAACACAGGAAACCTC	TCTGAACATCCTCTCAC	A/T
LLMAGI_694	1	ACGTTGGATGTCCCAGTTTTATCGCCTGTG	ACGTTGGATGAGTGCATGTTCAACACGACC	CGCCTGTGAAATGAAAG	C/T
LLMAGI_79987	1	ACGTTGGATGGTCGAGTTGAGTTCAGTTCC	ACGTTGGATGTGCAATGAATGTGTTGGCTG	aaAATGCGTAGGCAAGC	T/C
LLMAGI_17220	1	ACGTTGGATGTCAATCCTTACATTGCACGG	ACGTTGGATGTTAACAAGATGTAGCATCG	ccCATTGCACGGCAAATA	T/C
LLMAGI_23043	1	ACGTTGGATGAAGTGGAAGTGTCTCCACTG	ACGTTGGATGGGGTTCTATTCTGTTGTCGG	cGTCTCCACTGGTGAACA	G/A
LLMAGI_44170	1	ACGTTGGATGGTTGAACTGGGAAATGCGTG	ACGTTGGATGCACTATCTTCAGACCCAAAC	gggCTGTAGTGTGGTCGC	T/C
LLMAGI_55171	1	ACGTTGGATGAAGAAATCAAAGCTACCGCC	ACGTTGGATGGCAATTCAGTTGCCCATGTG	ccccGCTACCGCCGTCTCT	A/C
LLMAGI_95356	1	ACGTTGGATGACTGTGTAGAGATCGGAAGG	ACGTTGGATGCTGCATTCTAAGCTGCCTAC	cAGGGCATTGCATTTCTGG	A/T
LLMAGI_16913	1	ACGTTGGATGAAAACAAAGCAGCAACCGCC	ACGTTGGATGAGTATAGCGCTTGCTGTTTG	CAACCGCCACATTAATACTT	C/T
LLMAGI_93178	1	ACGTTGGATGGTAGTTGCATGCTTACCCAC	ACGTTGGATGCATGGTGTCGACCAATCTTG	CCCGACAAATAACATACACG	C/T
LLMAGI_5295	1	ACGTTGGATGCAAGAATAAACTAGACAAGG	ACGTTGGATGCTACCATTCTGCGATTACCG	gAAACTAGACAAGGGCATAG	C/T
LLMAGI_25617	1	ACGTTGGATGGAAGAAAACTACCAGCAGCC	ACGTTGGATGAAACCAATCAGCCTCAACGG	GCCGTTTAGCCTGAATAATTG	T/C
LLMAGI_82600	1	ACGTTGGATGGTACCATTTTTCATGCTAGGG	ACGTTGGATGGCACACAGTTCAGGAAAACG	cgGCTAGGGTTCTAATCTGTT	A/G
LLMAGI_95039	1	ACGTTGGATGGCCGGGATAAAAATTCTCCA	ACGTTGGATGTGGCATAGGTCTAATCCCTC	cTCTCCATATGAATCTCCTCCC	T/G
LLMAGI_29672	1	ACGTTGGATGCTGATTGTTGATGTCCCTCC	ACGTTGGATGACAATCCCCAAGGCATCATC	cttgGATGTCCCTCCTATTCTG	T/G
LLMAGI_98409	1	ACGTTGGATGTGCGCTTGTTATGGATCTCG	ACGTTGGATGTCAACGAGACGTTTCCTTTG	AACATGTCGATGATGTGTTAAA	G/A
LLMAGI_99415	1	ACGTTGGATGTATGGCTCGCTTACGTTCAC	ACGTTGGATGTGTATGAACATGGTAATGGC	aCAACATAGTAAACCTCTGGTAC	A/G
LLMAGI_98032	1	ACGTTGGATGTGCTATATGGTGTAGCGTCC	ACGTTGGATGGTACTTGATGACTGTTGACG	tctgGCCTGTAAAGAAATCCCAT	G/A
LLMAGI_42126	1	ACGTTGGATGCAGACCTATGGTCATGGAAG	ACGTTGGATGAAAGGCCGTCATGTGATCAG	tgtgtAAGCCAAGGATGAATTCC	C/T
LLMAGI_24529	1	ACGTTGGATGTACTTGTGCCTGTCTTGTA	ACGTTGGATGATGCGTACATACCGTTCCG	gaggTGATTGCTGAACTGATATG	A/C
LLMAGI_105631	1	ACGTTGGATGGGCAACAAGTTCATGGGTTC	ACGTTGGATGCTCCATGTTCTTCTGCTGAG	gcaaCCCCGACCATACACCCAAAA	C/A
LLMAGI_6847	1	ACGTTGGATGGAATTCAATCTGGCCTCCTC	ACGTTGGATGCACACAGCAACCATTATCCG	cctaCTCCTCTTCGTTCCGAGACTG	G/A
LLMAGI_64033	1	ACGTTGGATGCATTCGCTGCTGGACTATTG	ACGTTGGATGGCTGAGGTTGTGTTAAGGTG	ccCTTTAGAAATTCACCCTTAGATA	T/C
LLMAGI_93624	1	ACGTTGGATGGATCAGGCAATAAGAAACA	ACGTTGGATGGCTGCTAGATTGTACCGAAA	GATCAAATATTCAAATGAGATCCTC	C/A
LLMAGI_20181	1	ACGTTGGATGGGCTAGTACGGTGAATGATG	ACGTTGGATGACCGCACAGCTAACACATTG	gggtTCCATCTGTACAAGTGTGTAAC	G/T
LLMAGI_10012	1	ACGTTGGATGGAGGATGACTGCTGCTTTTG	ACGTTGGATGGTCTGGTAAAAGTCAGCCC	cccccTCCTTGGCCCATCTGCTGTAGT	C/A
LLMAGI_107302	1	ACGTTGGATGTGTAGTGAAAAAAGTCTGGG	ACGTTGGATGGTGTCCATCAACTCAAAGCC	ccggtATTTCAATTCTCAAATGTTAGC	T/C
LLMAGI_8619	1	ACGTTGGATGAAGCTACTCATGGCATGCTC	ACGTTGGATGTGTAGCTCACTACAGCAACG	cattCTCTTTAAAGTTTAAATCGAGCA	A/G
LLMAGI_16841	1	ACGTTGGATGCTGCCAAAAACAGAAGCACG	ACGTTGGATGCTTCCAGACACCTATTACAC	aggtAGTGTTGAGAATCGATATACTT	T/C
LLMAGI_60501	1	ACGTTGGATGCTGACACTGTCCATGACAAC	ACGTTGGATGGTACATTGTGTCTGGCGTTC	aaaAACGAAGAAACACAATATACATACT	A/G
LLMAGI_79083	1	ACGTTGGATGTCAGGTCCGAACCTGTACAC	ACGTTGGATGATGCCGCCTGATACCTTTTG	ggtaaGAACCTGTACACTGGTATATATG	T/C
LLMAGI_98577	2	ACGTTGGATGGCTGATGTTTTAGATCTCGG	ACGTTGGATGTTTATCTGAAGACGGCAAGG	ACCTGCTGGTAGCAT	A/G
LLMAGI_8593	2	ACGTTGGATGGATGTTCAGCACATCTGACC	ACGTTGGATGATATCCTGCTGTTAGTACGG	ACATCTGACCTCTCCT	C/T
LLMAGI_66309	2	ACGTTGGATGGAACAGAACACCTGTATCTC	ACGTTGGATGTGCTTGACAGGACCCATAAC	aCTCTTTTGGCGACGC	T/G
LLMAGI_55555	2	ACGTTGGATGCTGTATTGAGACAGGACGAC	ACGTTGGATGGTTTCTAATCCGAAAGATGG	ACAGGACGACGTGAGA	T/C
LLMAGI_85854	2	ACGTTGGATGCTACTTCCTTGGTTGTTCAG	ACGTTGGATGGCGCGCCTTCTTTTTTTCAC	TGGTTGTTCAGGATTCG	G/C
LLMAGI_93868	2	ACGTTGGATGTGTGTGCCATTCTGAACCTG	ACGTTGGATGACTTGTGCATTGCAGCTTAG	cccGCTAGTTCATCGCCC	C/T
LLMAGI_65323	2	ACGTTGGATGCCCCTATTTTCAAACTTCTC	ACGTTGGATGAGGCTCAATCAGATTCAGAC	CTTCTCTCTGCAAAGAGT	T/C
LLMAGI_9825	2	ACGTTGGATGGATCACCTTACTCGTCATGG	ACGTTGGATGTGAACGATCTGGTGTCTGAG	CCTCGTACTCCTCCTCATC	C/T
LLMAGI_23444	2	ACGTTGGATGAGAAAGCCAGAAAACCACGC	ACGTTGGATGTGTAGGTGCGATGCTTGTTG	tAAAACCACGCATTATTCT	G/C
LLMAGI_72487	2	ACGTTGGATGTACTAACGGTGTACCTTCGG	ACGTTGGATGTTGGAGATTAGCATCCAGTC	AAGAGTAAAAATACACCGC	A/G
LLMAGI_88590	2	ACGTTGGATGTGCAACAACACACTACAGCC	ACGTTGGATGTGTTATCTCAAGTTGCGCCG	ACAGCCGTTATAAGGAAAA	G/A
LLMAGI_75461	2	ACGTTGGATGCACCTCCATGGTTTGAACTC	ACGTTGGATGGCAACATTAGCGGAAGGATG	aggggACTCGTCGGGGACTT	T/C
LLMAGI_9084	2	ACGTTGGATGACTCGACACATGTGTGCAAC	ACGTTGGATGTGTAAGCATCCCTCTATCGG	acGCAACTCATTTAACACACC	A/G
LLMAGI_91002	2	ACGTTGGATGATCCAGTCTGCGTAAACCAC	ACGTTGGATGTGACCCATGTCATGTGAAAC	cTCTAGATACCAGACTTCAAC	T/G
LLMAGI_30618	2	ACGTTGGATGACACGTACTCCACCTTAACC	ACGTTGGATGATCCTTCTCCCGGTTCCTTC	GTTCTGAGGGTATCACAAGAA	A/G
LLMAGI_100024	2	ACGTTGGATGTGTTGTCGTCGTGGCAAATG	ACGTTGGATGCAACTGGCCTTTTGCCTTAG	gggcATATGAGGTCGTACCGT	T/C
LLMAGI_2150	2	ACGTTGGATGCGGAGTACCAAACGAAAATG	ACGTTGGATGTTGGGCAGCACTGTATTGAG	caccTGTGTAAATGACACCAGT	C/A
LLMAGI_95181	2	ACGTTGGATGCGGCATAGGAGTATAGACTG	ACGTTGGATGGAGTTGAGTTGCAGGCATGT	ccgcTCTTGAAATGTTCCGTTCT	T/G
LLMAGI_31220	2	ACGTTGGATGTGGAACGTGTACTAACAGAG	ACGTTGGATGGGGCAAAAACCAACACTTTC	GAACTTGGTTTAAATGTTAGCCT	A/C
LLMAGI_8596	2	ACGTTGGATGCTAGTACATTTTGTGTTGTC	ACGTTGGATGCCACTTGGCGCAAATCAATC	AGAACAAAGAAATTCAGAGGAAA	G/T
LLMAGI_62442	2	ACGTTGGATGTAGCATCCACAATGTCAATG	ACGTTGGATGAAACTACGTTTCAGTCTCCC	cataCACAATGTCAATGTCAAACA	C/A
LLMAGI_38007	2	ACGTTGGATGACCGTGCACATGTCAAGTTC	ACGTTGGATGATTTGGATCGTCCTGAGTTC	ccctcAAACCAATATTGATCCCCAG	C/T
LLMAGI_42650	2	ACGTTGGATGCCCCCGGATGCTATAATTTG	ACGTTGGATGACTGCTTGCCAATGAGAGAC	cccccCAATAAGGTAACGCCAAATC	G/T
LLMAGI_21401	2	ACGTTGGATGCACAGGGCAGCAAACAATTC	ACGTTGGATGGGAATTGCCGTTTCGTGTTG	catACAGTTAAGAACAAAGCATGAC	T/C
LLMAGI_10969	2	ACGTTGGATGGTTAGCATTGCTATGATGAG	ACGTTGGATGCAAAACATACAGCTCCAAGG	agaATTGCTATGATGAGAAACAATA	C/T
LLMAGI_18365	2	ACGTTGGATGCGTCCTGTCCTATGTTAACC	ACGTTGGATGCAGGTTTTAACAGCAGACAG	ccCCTATGTTAACCTACATAAGAAAG	G/A
LLMAGI_34069	2	ACGTTGGATGCATGAGAAACGAACATTGAGC	ACGTTGGATGCATGAAAAGCACGTTCTCGG	acttcAGTGGAAGTTCAACCATATCC	C/T
LLMAGI_9030	2	ACGTTGGATGACAAACAATGTCCACCTCGG	ACGTTGGATGCATCTTTGCAAGTTGGTTGG	gggaGGGTTACATAATAACATCCTAA	A/G
LLMAGI_93067	2	ACGTTGGATGACGGCGTACAGAATTCAGAG	ACGTTGGATGGCGAAGGCAACTACTGAATC	cgacCCTGTGCACCACGTTTACTTCTC	T/C
LLMAGI_32875	2	ACGTTGGATGTGCAGCTCGATCAACTCATC	ACGTTGGATGCCGTTGTGTCACTGACACTG	tttagCTGTGGTCACCAACAACATAAG	G/T
LLMAGI_93673	2	ACGTTGGATGACCAAAGAAAGCTAAAGCCG	ACGTTGGATGTCTGTTAGCCTAGACATCTG	gggatTCATCATTATTTGGGACAAGAT	C/T
LLMAGI_23478	2	ACGTTGGATGCGCCAACACGGTCTAGTTTT	ACGTTGGATGCTGTAAATAGCTTCTCCGTC	cccccGTTTTATCATACAGGTTCGGTTC	C/T
LLMAGI_81335	2	ACGTTGGATGGTACATGTGTTTACATGACG	ACGTTGGATGCAGCTGAACAAAAATGCGCC	accTGACGTGTAATCTGTACATGAGACC	T/C
LLMAGI_95570	2	ACGTTGGATGGGTTCTCACTCTCACAACTG	ACGTTGGATGGGTTCTTGTTGCTAAAAGGG	ggtggCAGCAACAAAAGACTAGATCAAA	C/T

[†]Given an "LL" designator with the associated GSS contig

Appendix Table 3. (Continued)

SNP Name†	Mutliplex Group	Reverse Primer Sequence	Forward Primer Sequence	Extension Primer Sequence	SNP Call (B86/FS8)
LLMAGI_6685	3	ACGTTGGATGCTATACATCCAAAGGCCAGC	ACGTTGGATGATGTGCAAATGTTGGGTGGG	AACCAACCACCCTCA	A/G
LLMAGI_91990	3	ACGTTGGATGGACAAGGTGCAAAAGACAGG	ACGTTGGATGCTGCCGTTAAGGCAAATCAG	CAACGGCCCCCAATA	G/A
LLMAGI_88109	3	ACGTTGGATGGATGTCGGCACCTCAATTTG	ACGTTGGATGCACGAGCATACAACAAATGAC	GACAGAGGCGCCATA	A/G
LLMAGI_90509	3	ACGTTGGATGTGAATGGCAACGATGGTAGG	ACGTTGGATGGTGTGTATCCCTGATCCATC	ATGGTAGGCCGACGA	G/A
LLMAGI_88913	3	ACGTTGGATGGCAAGAACATGGTTCCATCG	ACGTTGGATGTCCTGCTTGTGTACAAAAAC	CATCGGTCCATCGAAC	T/G
LLMAGI_96021	3	ACGTTGGATGCCGGTTCATTGTTTCTGTCG	ACGTTGGATGCAGTCTGGATCAGAATGTGG	gGGTCTCAAGGGAGGT	A/C
LLMAGI_14202	3	ACGTTGGATGTGTCTCGATCTGCCCAGTAG	ACGTTGGATGGACGGAAGCAGACAATTCTC	TGTGGTACCACCAATTT	T/C
LLMAGI_97072	3	ACGTTGGATGCAGTACGTAGCCTCTATTCG	ACGTTGGATGAGACCGTGTGTTTCGCTATC	TCGGCTCATGTTCATTG	G/A
LLMAGI_77055	3	ACGTTGGATGTTGTGAAATGCGATCCAGCG	ACGTTGGATGTCAGAGTTTTACGGTGTGGG	aAGCGGGGAAAAGAAGG	T/G
LLMAGI_82269	3	ACGTTGGATGCCAGTATCCTTTCTTGCACG	ACGTTGGATGTGCTTCAGTTGACATGTGGG	CACGCTGGGTGTCGAATA	G/A
LLMAGI_72398	3	ACGTTGGATGTAACCGACATATCACACGAC	ACGTTGGATGTTCTGCTGCATGTGGTTTTG	cAGGGGAAACTGTACATT	T/C
LLMAGI_106226	3	ACGTTGGATGTATCAGCTCAGACACTTCCC	ACGTTGGATGTCTAGCTATGGTTCACACTG	CAGACACTTCCCAAACATA	G/T
LLMAGI_26588	3	ACGTTGGATGCTATCACGATCAAGCTCCAG	ACGTTGGATGTGCAGGGTGTGATTATTGGG	caGACCGACCACTCATATT	T/C
LLMAGI_43846	3	ACGTTGGATGAGTATATATAGGAGGAGTGC	ACGTTGGATGGGACTGTAGTGTGCGTACTG	AGGAGGAGTGCATACATAA	T/C
LLMAGI_55490	3	ACGTTGGATGAGTCATCGACGATACATCGC	ACGTTGGATGTGATGTGAAGTGATCCAAGG	caACACGTTTATTGCCATAG	T/C
LLMAGI_17275	3	ACGTTGGATGCACCACACACAACACAAGAC	ACGTTGGATGCTCTGCCACATCTGAATCTG	atACGTACGGGAGAGACATT	G/C
LLMAGI_51781	3	ACGTTGGATGATCCAAGAGTTGCCAGCAAG	ACGTTGGATGACCCTGTTCCATGCTGTTTG	tcACGTAACATCTACTGGCAT	C/A
LLMAGI_109091	3	ACGTTGGATGTTGTTATTTCGAGGCTTGAG	ACGTTGGATGAGCATAGAAATCAGCTCCCG	ATTTCGAGGCTTGAGACGGAG	C/A
LLMAGI_81474	3	ACGTTGGATGAGCACCACGAAATCTGACTG	ACGTTGGATGGACATAATCTTTGCAGCGTG	gGGCAGAAACAGAAGGCAAAT	T/G
LLMAGI_105195	3	ACGTTGGATGCTTGCAGCAGGGATAAAACG	ACGTTGGATGTGTCACAATTGTGACATGAG	AGAGAACTAATACATCAGCACA	T/C
LLMAGI_28182	3	ACGTTGGATGGGAATGTGGGTCTCTATGTG	ACGTTGGATGCTTGGAGTGTGCCTTTTGAC	tcTGCATTCTCTAGCTGCTGTCC	T/C
LLMAGI_10589	3	ACGTTGGATGTCATGGTTTCTGAGCAACCG	ACGTTGGATGGATATGCTCCACCTCAGATA	TCTGAGCAACCGCCGGGTCCATA	T/G
LLMAGI_64224	3	ACGTTGGATGCAGCGTGGTTGTTTTTGCTC	ACGTTGGATGTATTAGAGCCATGGAACACC	CTAGTAAGTTGACGGATAATGAT	G/C
LLMAGI_18573	3	ACGTTGGATGCTTAGCCGTATTCACATCTG	ACGTTGGATGGAGAGTTCGATGTGTTTGGG	ccttcTTCACATCTGATTCAACGA	T/A
LLMAGI_20714	3	ACGTTGGATGTGCCCCTAGCTAAATGGATG	ACGTTGGATGACTGCTTTTTGACGGTACTG	ggAATGGATGAATTGAACTGCCAA	C/A
LLMAGI_111265	3	ACGTTGGATGTTCTAATACGCGCTCGACTG	ACGTTGGATGACGTTGCCTTACTGGCATAG	GCTCGACTGAACATATTTCTTTTTG	G/C
LLMAGI_111335	3	ACGTTGGATGCCCAATCCATTTTAATACAC	ACGTTGGATGGGCCTTTGATTACACTACAC	CCCAATCCATTTTAATACACTTCAAT	G/A
LLMAGI_82553	3	ACGTTGGATGTACACTCCTGTCTCCAGTTG	ACGTTGGATGCCAGCAAGATCTTCCTCAAC	cCCTGTCTCCAGTTGCTCTCCAGTTT	G/A
LLMAGI_59855	3	ACGTTGGATGGACGATAGCATTGGATCCTC	ACGTTGGATGCAATGCAAGTCGCACCATAC	AAGATCTGACAACCGATATGTCTCTA	A/G
LLMAGI_107844	3	ACGTTGGATGCTGTTTTGTTGTGCGCAAGG	ACGTTGGATGCTCCCCTAATTCCATTCCAC	AGTGCATTGCGTTGTAATACACTAAT	C/T
LLMAGI_11543	3	ACGTTGGATGTGTGGCAAGGCTAACAATGG	ACGTTGGATGTGAACAACAAATTGACACCC	cTGGAAGACATATAGTCTTTTGATTG	C/A
LLMAGI_81738	3	ACGTTGGATGGTTACAACATGCCATCAAGC	ACGTTGGATGTTGACTGGTTTCGTTATCGG	ctCATCAAGCATGCTATTTTACCGTTT	A/C
LLMAGI_31117	3	ACGTTGGATGGCAGGCTTGGTTGTAACTTG	ACGTTGGATGATCTTGAACCTTCCAGGCTC	gcATGTTTGTTGCTGGGCGGATTTTGT	A/C
LLMAGI_54824	3	ACGTTGGATGTTGGAAAAGCTTCAGGCCAG	ACGTTGGATGTGGTTGTATCGGGTACTGAC	ccCCATACTTCTTTTTGACGAACACAGG	C/T
LLMAGI_106053	3	ACGTTGGATGATCTGATATCTAACCCGGAG	ACGTTGGATGTTCCCATCACTAATGTCTTG	CCGGAGTTCAAATAACTATCTTTGAGTC	C/T
LLMAGI_75799	3	ACGTTGGATGCCAGAGCTCTAATAAGCAAG	ACGTTGGATGCTGCAGGATATTTATGGTGG	AGCAAGAGTAAAAATAACCAACGAACCA	C/T
LLMAGI_77596	4	ACGTTGGATGCGAGCGCTTCTCTTTGATTG	ACGTTGGATGGTGATTAGATTCCTGGGTGC	AGCCAACTGCCAAGA	A/C
LLMAGI_92611	4	ACGTTGGATGCCTGGAATAATTGGAGCTTG	ACGTTGGATGACGGTTCAAAGCAGCATGTG	AAACCGTGCGGATAA	T/C
LLMAGI_2243	4	ACGTTGGATGCATGTTCAGACTGTTTGTGG	ACGTTGGATGTCCAGTTAACTTCCCACCAC	TCCCACCACTTCTACA	C/T
LLMAGI_26731	4	ACGTTGGATGCGAGCAGAAAGATGAGAAAC	ACGTTGGATGTGATGACCCTACACGCAATC	TCGTCTGATCCTCCAC	A/G
LLMAGI_49724	4	ACGTTGGATGTGTGGGTGCTTCGTTTTGAG	ACGTTGGATGTGAGTTATTCTCAGGCTGCG	TGGAAGTACCCAGCAT	C/T
LLMAGI_28444	4	ACGTTGGATGAGCGTGTACGAAATGACAGC	ACGTTGGATGATCGGATCGAGACCAGATAC	ATACACAGCACACTCCA	C/G
LLMAGI_38962	4	ACGTTGGATGCATTAGCTGGGTGTACCTTC	ACGTTGGATGAGCGCCTGCTATTTCATGAC	TGACCTGATCGCACAAG	G/A
LLMAGI_84372	4	ACGTTGGATGTAACGAAGCTTTGCCCACTG	ACGTTGGATGGATTAAGAGAAGTGTTTGGGC	gGTGTTTGGGCTGCCGA	A/G
LLMAGI_11864	4	ACGTTGGATGAGAATTGTGTCCTAGCCAGC	ACGTTGGATGAGGGTAGTAGTAGGACTAGG	ATCATCATCAGCAGGTCA	A/G
LLMAGI_98941	4	ACGTTGGATGGCTTCAAGAGCTGCAGAATC	ACGTTGGATGACAATGATCTGGGTCGAGTG	ttGCATGTGTACCCACAG	T/C
LLMAGI_58334	4	ACGTTGGATGACCCATACGTAACGGCTTAG	ACGTTGGATGTATGGTGGAACCGTGGAATC	GATGACTAGAAGCAGGTA	A/G
LLMAGI_77815	4	ACGTTGGATGCCCCACAAAAGCATTTTCAG	ACGTTGGATGACCGTTTCATTTTGGCACAG	ccAAACTTACGCTGCTCCT	G/A
LLMAGI_19354	4	ACGTTGGATGGCGGCGGATGTTTGTAATTC	ACGTTGGATGGCCAAATTAGAATGATGCAG	gggACCGGAAACCAGCAAG	G/A
LLMAGI_96085	4	ACGTTGGATGTTGTTCATTGGGTTGTGGTG	ACGTTGGATGGAAAACTTAACCCACTCTCC	TCCAATTCCATTGGCCACTA	G/A
LLMAGI_38801	4	ACGTTGGATGGGTGCTGATTGGTTGCTTTC	ACGTTGGATGATTCATTGTGCCAGGTTTAC	aGTTTACAAACATGTCTCGT	C/A
LLMAGI_97441	4	ACGTTGGATGGGTTCGCATATAGGTCCTTG	ACGTTGGATGCGGAGACACAACATGTTAGC	tccTGTTAGCTACAACCTCCT	G/A
LLMAGI_56844	4	ACGTTGGATGCGTATGAGGTTGAAAAAGGC	ACGTTGGATGTTTCCCGTTCTGTGCAAACC	GCAAACCTGAGTTTAGAAGAA	G/A
LLMAGI_85600	4	ACGTTGGATGTGTCAAGTGTGTGCTCTAAG	ACGTTGGATGCCAGGTATCAAACAGTTGGG	cccACTCTGGTCTCTATTTTCA	A/G
LLMAGI_30953	4	ACGTTGGATGGATATCTAGATCGAAGCGGG	ACGTTGGATGCATGGCTGATGGATCAAAAG	tTTTGTACTAGCTTCCTATCGT	G/A
LLMAGI_58552	4	ACGTTGGATGGTTGATGAGCAAGTCGTCAC	ACGTTGGATGACTCATGAACCCAGCAGACG	cccAATTGCAAAGCAAACTACA	G/A
LLMAGI_99488	4	ACGTTGGATGATGCAGACTCTCCAGTTGAC	ACGTTGGATGAAGAGGGCAAAGCTACAAGG	ggAATCAAACAGAAAAGCTTCA	C/A
LLMAGI_74729	4	ACGTTGGATGGGTGACGACGGAGTATCTAT	ACGTTGGATGCAGACATTTTTCTTTCTCCG	TCTCCGTTGCTAACAAAAAGAAT	T/A
LLMAGI_75795	4	ACGTTGGATGACGATAAGAATGGTGCCGTG	ACGTTGGATGCTTGTTGAAGAGAACGCTGG	gcTCTGGGGAGTCGATTTCGTAG	T/C
LLMAGI_71780	4	ACGTTGGATGTCCCGAAGACAGTAGAAATC	ACGTTGGATGAGCACGTCGAGTTTTACAGC	ccTATTCCCAATCATCTGAATGTA	A/G
LLMAGI_25617_2	4	ACGTTGGATGGAAGAAAACTACCAGCAGCC	ACGTTGGATGAAACCAATCAGCCTCAACGG	caCTCAACGGTAACCTATCATTTA	T/C
LLMAGI_111784	4	ACGTTGGATGTTCTGGAGGACATGTCTACG	ACGTTGGATGCTTTCGGATCGAAATGTCGG	cGATGATGATGGTTGTTATTGTTC	G/A
LLMAGI_39328	4	ACGTTGGATGCCTTCCCCCAGTCAATCATC	ACGTTGGATGACTCCAGTGCAATTCTTGGG	aTCTACAAAAATCAGTGTCGCACAA	G/C
LLMAGI_114073	4	ACGTTGGATGTGGCTAGAGACTATGTGGTG	ACGTTGGATGAGCCTTTCTGCTTCAATGCC	aAGACATACATACCAAAGCAAGAGG	C/T
LLMAGI_99055	4	ACGTTGGATGTATCGACCCTATTACGTGCC	ACGTTGGATGGTGCGTCCTTGTTTCTTTTC	cGAGAGAGAGAGAGCTGGCTAAAGA	C/T
LLMAGI_12846	4	ACGTTGGATGTAATCGGCTGCAAATGCCAC	ACGTTGGATGTGTCCGCGAAGATTTATTGG	TTGTGTACTAGTAGTCAATTGTTATA	A/T
LLMAGI_105144	4	ACGTTGGATGGACTCGACTAGTCGAGTTTC	ACGTTGGATGAATGAGTGAAGCACGTTCGG	cACCAGCACGTGCCCAACCATCATACT	A/T
LLMAGI_59348	4	ACGTTGGATGAGCAGAATGGGTTACGTGAG	ACGTTGGATGACATTTCCTCTGTAATTGGC	cATTAAGCTTACAACTTATATTAGTCG	A/G
LLMAGI_64459	4	ACGTTGGATGGACCTGGAAGTTGCAATGAC	ACGTTGGATGTGACGGTTCCTGTGATATTG	caaacATTTAGTCAGCTCCAGAATTACA	C/G
LLMAGI_75446	4	ACGTTGGATGCCACTCGGAGAAAGGAAGAC	ACGTTGGATGTAGGTTTCTACTGCGACAGC	gGACAGCTGCGGCGCAGAACTTCAATTC	C/G
LLMAGI_29945	4	ACGTTGGATGCTTACTATTACATGTGGGAGC	ACGTTGGATGGTCGAAAAAATAGGTACCATC	gcAAAAATAGGTACCATCGAAATAGAAT	G/A
LLMAGI_11726	4	ACGTTGGATGGACTCCAGATGTAGATTATG	ACGTTGGATGGCTAGGTCTTTGTGGCATAC	gggGAATCAAGGGTTCTAAGTACAAAAC	C/T

†Given an "LL" designator with the associated GSS contig

Appendix Table 3. (Continued)

SNP Name[†]	Mutliplex Group	Reverse Primer Sequence	Forward Primer Sequence	Extension Primer Sequence	SNP Call (B86/FS8)
LLMAGI_97319	5	ACGTTGGATGGGATGATGACCTGAATCACC	ACGTTGGATGGGTAATAATATAGCAACCCC	CAACCCCATGCAAGA	C/A
LLMAGI_17896	5	ACGTTGGATGATGGATTGCCAAACACCCAG	ACGTTGGATGAAATTCACGGCGCAGATGTC	GGCGGGCAAGGAAAT	T/C
LLMAGI_14134	5	ACGTTGGATGAACAATACGCAGCAGTAGGG	ACGTTGGATGTGTAGCTACACTCTGGCTTC	CTGTGATCTCCAGAGC	C/T
LLMAGI_57369	5	ACGTTGGATGCATCATAAGGGTCTCTTGGG	ACGTTGGATGGTTTCCCTCTACTCATCTTG	TCTACGCTACAGGGAA	A/G
LLMAGI_29180	5	ACGTTGGATGTGCTAGGGTTTGCTCTATTG	ACGTTGGATGAGGGTTTCAAAGCGTTGGAC	AGACTGGTGGAGTACA	C/T
LLMAGI_57412	5	ACGTTGGATGGGTTGTTGCTGTCTAGCAAA	ACGTTGGATGATCATCGGCTACGGTGTAAC	AACCTATCCATGTCCCA	T/A
LLMAGI_75238	5	ACGTTGGATGGGGTTGTGAATTGTGAAATG	ACGTTGGATGCGCAGAGTGTCCTGATCATT	tcCCCATCGGATCGAATT	G/C
LLMAGI_87281	5	ACGTTGGATGTTCTTGCCATCCATTGTCCC	ACGTTGGATGAAGCGTGCAAGAAACGTAGC	GTAGCAGAAATCGTCTCA	C/T
LLMAGI_37832	5	ACGTTGGATGGGTTAGAAGAGGATTGAGGG	ACGTTGGATGTTGCTGCCCTTTCAGTAGAG	gAGTAGAGCGCAAAGTAT	A/T
LLMAGI_67460	5	ACGTTGGATGTTGTGCGTCACACTTTGTGG	ACGTTGGATGCCGACCAAATTGCCAGATAC	acACACGATTACAAAGCGA	T/A
LLMAGI_55508	5	ACGTTGGATGAAACGCTTTGACAGTCAATC	ACGTTGGATGGGTTATGTAGTTGCCCTGTG	ccatTTGCCCTGTGTAACTT	T/G
LLMAGI_81231	5	ACGTTGGATGGAATGGCAGTGCAGGTTTAC	ACGTTGGATGCCCCCAATTTTTTGTTGCCC	ggAGGACATACATACGGTCA	G/C
LLMAGI_96602	5	ACGTTGGATGGCTCGTGCTATTTGTTTTGC	ACGTTGGATGAGTAGGACCAATCCTCGTTC	ggatGAATGGATCGGCACAA	T/G
LLMAGI_17170	5	ACGTTGGATGGTTGTAACAGGTTACAGGGC	ACGTTGGATGCTAGTGCTATCCATCTCTGC	ctATCTCTGCTCAGAATATCA	G/A
LLMAGI_11152	5	ACGTTGGATGAGTGGTTTCGTCGGTATCGG	ACGTTGGATGCCATTCTCCTGCTAAACTCC	CTAAACTCCACAAACAGATAG	C/G
LLMAGI_51496	5	ACGTTGGATGCAAGAACATCTACATCTGTG	ACGTTGGATGAACAAATGGTACACCGGGAC	ggCACCGGGACACATTTATTAA	G/A
LLMAGI_93873	5	ACGTTGGATGGTACCTCTCAACTGACCATC	ACGTTGGATGGCTAGGAACTGGGAACAAAG	ttagACAAAGAGTACGGCCACT	C/T
LLMAGI_11533	5	ACGTTGGATGTGGATCTTGTAGCCAGTTTC	ACGTTGGATGACACTTGGTGATGGAGCAAC	acggGTCCACAGGAAGAGGAAG	T/C
LLMAGI_43061	5	ACGTTGGATGCTTTCATGCATGGCTACGAC	ACGTTGGATGTCTTCACCGATGAAACACTC	ccCACAAACGCACACAACCAAGG	C/T
LLMAGI_33362	5	ACGTTGGATGGTCAAACACATCAACACCCC	ACGTTGGATGGTCTTCTGATGGTTAGGGAG	gtagGGAACGCCTACAGTAGTTT	C/G
LLMAGI_73225	5	ACGTTGGATGAATGGCCCTGTTTTGTAGCG	ACGTTGGATGTAGTAGTAGCGGTGGTAAGG	tGGTGGTAAGGACATGGAAGATG	T/C
LLMAGI_58613	5	ACGTTGGATGGTTGCCTGTGTAGCTGTTAG	ACGTTGGATGTCTTCTTGGTACCATCCACG	ttgtTACCATCCACGGTTCAAGAA	C/T
LLMAGI_107464	5	ACGTTGGATGCAAGCGGTATAATACAAGCC	ACGTTGGATGTATGGCTTGTGAGAGCAAAC	gaacACGTCTGTATTCATTTTCAG	T/C
LLMAGI_84629	5	ACGTTGGATGTTGGAGGATGATACCTCAGC	ACGTTGGATGTACTGTGGAGTGGATGGAAC	ggagCACAAGGAAATAGCTTTCAG	G/A
LLMAGI_71931	5	ACGTTGGATGTCTGGCTACTGTATAGCCTG	ACGTTGGATGCCAAACCACTCAGGAATGTC	ggaaCACTCAGGAATGTCCTACGGC	C/A
LLMAGI_112289	5	ACGTTGGATGCAAGACATCGATTGAGAGAC	ACGTTGGATGCTGCTGTTTGTCAAGCATGG	gaTGTTTGTCAAGCATGGACGAATG	C/G
LLMAGI_7690	5	ACGTTGGATGCAAAAAGAAGAGCGTGTGAG	ACGTTGGATGGGTCTTATTGTCTCGACTAC	tcCTAATTCTGTAAATATCCAAATCA	T/C
LLMAGI_3838	5	ACGTTGGATGTTCATTTGTTGGCCCTGCTG	ACGTTGGATGGCGCCAAATGTTCCAGTATC	ggTCCAGTATCATATATGCATAGACA	A/G
LLMAGI_110901	5	ACGTTGGATGTCAAACCAACTACTTGCACG	ACGTTGGATGTCAGGCTGGTGTTAGAACTC	gggGTACTACTTGTAGGCTTAGGTGA	C/A
LLMAGI_57220	5	ACGTTGGATGAGTTGTGGCACACAATGTGG	ACGTTGGATGGAGCACGAAACTTATATCAC	gaacCTTATATCACTTATCAGCGGATC	C/T
LLMAGI_11553	5	ACGTTGGATGGGTTGATAGGGAAAACGGTG	ACGTTGGATGCACTCACATGTAAAAGTACG	AAAAGTACGATGAAGATACATTATATT	A/C
LLMAGI_87441	5	ACGTTGGATGTAGAAATATGTTTATTAGCC	ACGTTGGATGAAGCTGCATACCAGCCTAAC	catcCCAGCCTAACTGCTTATGTATCAT	A/G
LLMAGI_19140	5	ACGTTGGATGCTACATGTTCAGCATGAGCG	ACGTTGGATGTTGGGATGATCGTCGTTGTC	cGTCCTTAGATTATCTTAAGACCTAGTA	T/G
LLMAGI_108570	5	ACGTTGGATGTGTTTGTGCATTTCCGCGAG	ACGTTGGATGGTGCACAAATCCAAGCACAG	gcaaAAATCCAAGCACAGTTCAGACAAC	C/A
LLMAGI_12200	5	ACGTTGGATGTCAGAGTGAGCTGTCTGAAC	ACGTTGGATGCAGATATTGCAGGAGTTAACA	gagcTATTGCAGGAGTTAACATATTATT	G/A

[†]Given an "LL" designator with the associated GSS contig

Appendix Table 4. (Chapter 4) Complete list of IBMRILs with an overcompensation response mechanism detected.

Trait	IBMRIL	Mean in LP ± SE	Mean in HP ± SE	t Ratio	Prob>|t|	q-value
EIL	MO125	18.36 ± 0.46	19.82 ± 0.46	-3.06	7.11E-03	3.18E-02
EIL	MO145	17.19 ± 0.52	18.83 ± 0.52	-4.99	2.42E-04	5.80E-03
EIL	MO238*	17.53 ± 0.61	19.47 ± 0.61	-3.07	7.85E-03	3.21E-02
EIL	MO298*	17.99 ± 0.50	19.58 ± 0.50	-3.44	4.06E-03	2.34E-02
EIL	MO378	17.27 ± 0.88	20.06 ± 0.88	-3.71	1.93E-03	1.47E-02
BS	MO025	0.45 ± 0.06	0.07 ± 0.06	4.12	4.65E-05	5.43E-03
BS	MO128*	0.39 ± 0.06	0.09 ± 0.06	3.53	4.62E-04	2.99E-02
BS	MO300	0.42 ± 0.06	0.07 ± 0.06	7.45	8.40E-08	1.08E-05
CW	MO304	28.00 ± 5.09	101.00 ± 5.09	-7.19	3.08E-11	2.85E-08
EH	MO150*	104.80 ± 2.88	113.90 ± 2.88	-3.46	2.97E-03	1.24E-02
EH	MO157*	113.50 ± 4.74	128.50 ± 4.74	-5.33	4.84E-05	1.13E-03
EH	MO177*	114.70 ± 4.52	129.00 ± 4.52	-2.53	2.15E-02	4.33E-02
EH	MO205*	109.00 ± 2.85	118.00 ± 2.85	-2.61	1.80E-02	3.78E-02
EH	MO289*	94.10 ± 6.61	115.00 ± 6.61	-3.35	5.82E-03	1.81E-02
EH/PH	MO001	0.49 ± 0.01	0.54 ± 0.01	-3.98	9.39E-04	1.73E-02
EH/PH	MO113	0.50 ± 0.02	0.58 ± 0.02	-4.37	4.69E-04	1.25E-02
EH/PH	MO150*	0.47 ± 0.02	0.51 ± 0.02	-5.67	3.02E-05	3.46E-03
EH/PH	MO157*	0.53 ± 0.02	0.60 ± 0.02	-4.89	1.78E-04	1.02E-02
EH/PH	MO289*	0.41 ± 0.03	0.51 ± 0.03	-4.35	1.06E-03	1.73E-02
EH/PH	MO305	0.50 ± 0.02	0.56 ± 0.02	-4.19	5.47E-04	1.25E-02
EH/PH	MO327	0.53 ± 0.02	0.60 ± 0.02	-4.46	3.48E-04	1.25E-02
EL	MO005	6.45 ± 0.11	6.80 ± 0.11	-3.30	1.60E-02	2.76E-02
EL	MO014*	5.55 ± 0.77	8.00 ± 0.77	-6.03	4.56E-04	7.57E-03
EL	MO056*	6.55 ± 0.32	7.55 ± 0.32	-4.26	4.22E-03	1.75E-02
EL	MO079*	7.60 ± 0.43	8.95 ± 0.43	-4.57	4.31E-03	1.25E-02
EL	MO197	7.15 ± 0.21	7.80 ± 0.21	-2.74	2.98E-02	3.88E-02
EL	MO332*	6.70 ± 0.40	7.95 ± 0.40	-3.00	1.71E-02	2.86E-02
EL	MO347*	6.55 ± 0.88	9.33 ± 0.88	-6.84	6.04E-03	1.50E-02
GERM	MO128*	10.00 ± 5.06	22.50 ± 5.06	-4.23	2.72E-05	9.51E-03
KPR	MO014*	29.60 ± 5.19	46.00 ± 5.19	-4.28	2.90E-03	2.00E-02
KPR	MO016	30.90 ± 3.07	40.60 ± 3.07	-3.30	4.86E-03	5.51E-03
KPR	MO079*	37.80 ± 3.35	48.40 ± 3.35	-2.99	1.74E-02	2.35E-02
KPR	MO092	30.60 ± 2.91	39.80 ± 2.91	-2.65	2.18E-02	1.44E-02
KPR	MO165	37.40 ± 1.11	40.90 ± 1.11	-1.96	6.65E-02	3.13E-02
KPR	MO171	33.60 ± 1.83	39.40 ± 1.83	-2.89	2.75E-02	3.07E-02
KPR	MO172*	38.80 ± 2.50	46.70 ± 2.50	-3.63	1.94E-03	3.11E-03
KPR	MO178	39.80 ± 1.58	44.80 ± 1.58	-3.24	1.21E-02	3.87E-02
KPR	MO199	34.10 ± 1.68	39.40 ± 1.68	-1.71	1.08E-01	4.41E-02
KPR	MO223*	28.20 ± 2.59	36.40 ± 2.59	-3.74	5.79E-03	1.32E-02
KPR	MO332*	36.60 ± 2.97	46.00 ± 2.97	-3.25	2.64E-02	3.02E-02
KPR	MO347*	44.00 ± 2.00	50.33 ± 2.00	-6.76	1.12E-02	1.91E-02
KPR	MO351	39.90 ± 1.11	43.40 ± 1.11	-1.63	1.20E-01	4.84E-02
KRF	MO009	0.93 ± 0.02	0.98 ± 0.02	-2.89	4.47E-02	3.46E-02
KRF	MO011	0.90 ± 0.02	0.98 ± 0.02	-3.74	2.02E-02	2.21E-02
KRF	MO013	0.87 ± 0.02	0.98 ± 0.02	-3.90	1.75E-02	2.09E-02
KRF	MO014*	0.72 ± 0.03	0.88 ± 0.03	-3.98	5.11E-03	2.78E-02
KRF	MO024	0.90 ± 0.01	0.95 ± 0.01	-2.63	4.73E-02	3.53E-02

* IBMRILs for which multiple traits were overcompensated.

(**Appendix Table 4.** Continued)

Trait	IBMRIL	Mean in LP ± SE	Mean in HP ± SE	t Ratio	Prob>|t|	q-value
KRF	MO028	0.90 ± 0.02	0.98 ± 0.02	-3.96	1.66E-02	2.02E-02
KRF	MO045	0.86 ± 0.01	0.90 ± 0.01	-2.27	5.95E-02	4.20E-02
KRF	MO071	0.85 ± 0.03	0.97 ± 0.03	-3.21	3.27E-02	2.81E-02
KRF	MO106	0.84 ± 0.02	0.93 ± 0.02	-3.56	1.64E-02	2.02E-02
KRF	MO115	0.81 ± 0.02	0.89 ± 0.02	-2.65	4.47E-02	3.46E-02
KRF	MO121	0.78 ± 0.02	0.98 ± 0.02	-9.64	6.48E-04	3.27E-03
KRF	MO150*	0.85 ± 0.01	0.91 ± 0.01	-4.37	5.73E-03	9.90E-03
KRF	MO156	0.73 ± 0.03	0.91 ± 0.03	-4.54	3.96E-03	2.31E-02
KRF	MO161*	0.79 ± 0.01	0.99 ± 0.01	-14.97	1.16E-04	1.67E-03
KRF	MO188	0.71 ± 0.03	0.81 ± 0.03	-2.21	5.80E-02	4.14E-02
KRF	MO201	0.78 ± 0.02	0.91 ± 0.02	-3.52	8.15E-03	1.19E-02
KRF	MO206	0.82 ± 0.02	0.98 ± 0.02	-5.75	4.54E-03	9.18E-03
KRF	MO213	0.77 ± 0.01	0.88 ± 0.01	-7.59	9.64E-05	3.72E-03
KRF	MO216	0.88 ± 0.01	0.99 ± 0.01	-6.69	2.59E-03	7.40E-03
KRF	MO223*	0.75 ± 0.03	0.85 ± 0.03	-2.15	6.39E-02	4.46E-02
KRF	MO224	0.84 ± 0.01	0.93 ± 0.01	-4.70	5.20E-03	9.64E-03
KRF	MO229	0.70 ± 0.02	0.92 ± 0.02	-3.79	1.27E-02	4.92E-02
KRF	MO238*	0.82 ± 0.04	0.96 ± 0.04	-3.48	2.54E-02	2.53E-02
KRF	MO248	0.89 ± 0.03	0.97 ± 0.01	-2.98	4.07E-02	3.34E-02
KRF	MO278	0.86 ± 0.01	0.99 ± 0.01	-6.90	2.31E-03	1.72E-02
KRF	MO286	0.78 ± 0.01	0.99 ± 0.01	-11.40	3.38E-04	2.40E-03
KRF	MO288	0.75 ± 0.04	0.91 ± 0.04	-3.05	1.62E-02	2.02E-02
KRF	MO297	0.81 ± 0.01	0.99 ± 0.01	-13.58	1.70E-04	1.86E-03
KRF	MO298*	0.84 ± 0.04	0.96 ± 0.04	-3.10	3.62E-02	3.01E-02
KRF	MO308	0.90 ± 0.02	0.98 ± 0.02	-3.66	2.15E-02	2.30E-02
KRF	MO326	0.88 ± 0.02	0.98 ± 0.02	-3.65	2.17E-02	2.30E-02
KRF	MO342	0.83 ± 0.02	0.98 ± 0.02	-11.18	3.65E-04	2.40E-03
KRF	MO358	0.92 ± 0.01	0.99 ± 0.01	-5.92	4.08E-03	9.18E-03
KRF	MO360	0.70 ± 0.02	0.89 ± 0.02	6.62	1.73E-04	8.91E-03
KRF	MO368	0.76 ± 0.02	0.98 ± 0.02	-12.80	2.15E-04	3.72E-03
KRF	MO374	0.80 ± 0.03	0.95 ± 0.03	-3.62	1.08E-02	4.40E-02
KRN	MO172*	16.40 ± 0.32	17.40 ± 0.32	-1.52	1.46E-01	2.43E-02
KRN	MO187	16.10 ± 0.22	16.80 ± 0.22	-1.07	2.99E-01	4.58E-02
KRN	MO334*	10.20 ± 1.45	14.80 ± 1.45	-6.64	1.63E-04	1.76E-02
KRN	MO379*	10.60 ± 1.20	14.40 ± 1.20	-6.72	1.50E-04	1.95E-02
PH	MO079*	244.00 ± 0.79	246.50 ± 0.79	-0.97	3.43E-01	4.83E-02
PH	MO111	234.20 ± 1.33	238.40 ± 1.33	-1.29	2.15E-01	3.23E-02
PH	MO177*	225.50 ± 1.68	230.80 ± 1.68	-1.08	2.93E-01	4.23E-02
PH	MO205*	212.20 ± 1.52	217.00 ± 1.52	-1.51	1.51E-01	2.35E-02
PH	MO258	228.60 ± 2.94	237.90 ± 2.94	-1.73	1.10E-01	1.79E-02
PH	MO301	233.50 ± 1.58	238.50 ± 1.58	-1.48	1.56E-01	2.41E-02
PH	MO334*	208.90 ± 1.74	214.40 ± 1.74	-1.78	9.24E-02	1.53E-02
PH	MO349	232.00 ± 2.53	240.00 ± 2.53	-2.07	5.44E-02	9.31E-03
PH	MO379*	213.20 ± 1.90	219.20 ± 1.90	-1.28	2.16E-01	3.23E-02
STAND	MO060	0.55 ± 0.11	0.79 ± 0.11	-7.45	1.67E-12	2.11E-09
STAND	MO061	0.44 ± 0.11	0.91 ± 0.11	-6.15	3.30E-09	2.39E-06
STAND	MO195	0.55 ± 0.11	0.79 ± 0.11	-7.02	2.32E-11	2.35E-08

* IBMRILs for which multiple traits were overcompensated.