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ORIGINAL PAPER

# Mapping QTLs contributing to *Ustilago maydis* resistance in specific plant tissues of maize

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**Abstract** Quantitative trait loci (QTL) contributing to the frequency and severity of *Ustilago maydis* infection in the leaf, ear, stalk, and tassel of maize plants were mapped using an A188 × CMV3 and W23 × CMV3 recombinant inbred (RI) populations. QTLs mapped to genetic bins 2.04 and 9.04–9.05 of the maize genome contributed strongly ($R^2$ = 18–28%) to variation in the frequency and severity of *U. maydis* infection over the entire plant in both populations and within the majority of environments. QTLs mapped to bins 3.05, 3.08, and 8.00 in the A188 × CMV3 population and bin 4.05 in both populations significantly contributed to the frequency or severity of infection in only the tassel tissue. QTLs mapped to bin 1.07 in the A188 × CMV3 population and bin 7.00 in the W23 × CMV3 population contributed to *U. maydis* resistance in only the ear tissue. Interestingly, the CMV3 allele of the QTL mapped to bin 1.10 in the A188 × CMV3 population significantly contributed to *U. maydis* susceptibility in the ear and stalk but significantly increased resistance in the tassel tissue. Digenic epistatic interactions between the QTL mapped to bin 5.08 and four distinct QTLs significantly contributed to the frequency and severity of infection over the entire plant and within the tassel tissue of the A188 × CMV3 population. Several QTLs detected in this study mapped to regions of the maize genome containing previously mapped *U. maydis* resistance QTLs and genes involved in plant disease resistance.

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## Introduction

*Ustilago maydis* (common smut) is a fungus that infects the reproductive and vegetative tissues of maize. Unlike common head smut (*Sporisorium reilianum*) that can infect up to 80% of the plants within a field (White 1999), *U. maydis* normally infects 1–5% of the plants within commercial maize fields (Christensen 1963; Shurtleff 1980), lowering yield by increasing lodging, destroying ear tissue, and subverting plant resources into fungal growth (Immer and Christensen 1928; Billet and Burnett 1978a, b). Sweet corn varieties are often more susceptible to *U. maydis* infection than field corn (Agrios 1988; White 1999). In contrast, *U. maydis* sequesters sugars and other flavors from the plant, creating a culinary delicacy called *huitlacoche* that is

prized for its taste in several regions of the world (Villanueva 1997; Pataky and Chandler 2003). Between 400 and 500 tons of *huitlacoche* is sold in Mexico City annually (Villanueva 1997) and infected ears often cost up to ten times the price of an uninfected ear (A. Munkasci, personal communication). Understanding the genetic architecture of *U. maydis* resistance in maize can assist efforts to produce *U. maydis* resistant populations as well as develop susceptible maize varieties for efficient *huitlacoche* production.

Crosses between *U. maydis* resistant and susceptible lines indicate that *U. maydis* resistance is a polygenic trait and several studies suggest that specific loci may preferentially contribute to *U. maydis* resistance in either the ear or tassel (Hayes et al. 1924; Immer and Christensen 1925; Immer 1927; Hoover 1932; Burnham and Cartledge 1939; Saboe and Hayes 1941; Stringfield and Bowman 1941; Lübberstedt et al. 1998; Kerns et al. 1999). Modern studies mapping *U. maydis* resistance quantitative trait loci (QTL) in maize indicate that several loci weakly contribute to resistance (Lübberstedt et al. 1998; Kerns et al. 1999), while earlier studies suggested the existence of loci strongly contributing to resistance (Immer 1927; Hoover 1932; Saboe and Hayes 1941). Regardless of the genetic effect, both environmental factors and genetic by environmental interactions have been shown to affect the frequency of *U. maydis* infection in maize fields (Christensen and Stakman 1925; Immer and Christensen 1927; Christensen 1963; Lübberstedt et al. 1998; Pataky and Chandler 2003).

In this study, QTLs contributing to variation in the frequency and severity of *U. maydis* infection over the entire plant and within specific plant tissues were mapped using two recombinant inbred (RI) populations. Three QTLs mapped to similar regions in both RI populations and strongly contributed to the frequency and severity of *U. maydis* infection over the entire plant. Depending on the population, several QTLs preferentially contributed to *U. maydis* resistance in the ear, stalk, or tassel tissues. Furthermore, QTL by QTL and QTL by environment interactions significantly contributed to variation in the frequency and severity of infection over the entire plant and within specific plant tissues.

## Materials and methods

### Plant materials and field trials

Two recombinant inbred (RI) populations were developed by crossing the *U. maydis* susceptible inbred, CMV3, to the A188 or W23 inbred lines. Resulting lines were advanced by single seed descent for four generations. Only F5 RI lines with a frequency of *U. maydis* infection greater or less than one standard deviation from the average infection frequency of the F5 RI population were advanced to the F9 generation through single seed descent. This selection process resulted in 126 A188 × CMV3 and 157 W23 × CMV3 F9 RI lines.

During the summers of 2000, 2001 and 2002, 20-plant single-row plots of each RI line and the three inbred parents were grown in two randomized, replicate blocks in St Paul, MN and Waseca, MN. Limited seed supply caused by high *U. maydis* infection made it impossible to plant all RI lines from each population in every replicate block. For the purposes of this study, the Waseca and St Paul locations planted each year were considered different environments, creating six separate environments over the study period.

### Inoculation protocol

Half the plants from each RI line were inoculated with equal amounts of sporidia from eight haploid *U. maydis* strains. Diploid teliospores previously collected from several locations were germinated to produce single haploid sporidial cultures. Strains were chosen to be polymorphic at both the *a* and *b* mating loci, allowing each single strain to be sexually compatible with four other strains in the inoculum. Haploid strains were grown individually for 3 days at room temperature in 100 ml potato dextrose broth [6 g potato dextrose medium (Difco Laboratories), 120 µl streptomycin at 10 mg streptomycin/ml]. Growth medium was removed by centrifugation and the remaining sporidia were resuspended in sterile distilled water ($dH_2O$) and brought to a concentration of $10^8$ cells/ml. The eight-sporidial suspensions were combined in equal parts to create the final inoculum. Plants were inoculated by pipetting 1 ml of inoculum into the plant whorl 6 weeks after planting. Noninoculated plants were inoculated with 1 ml sterile $dH_2O$, to provide an experimental control.

### Scoring *U. maydis* Infection

Individual plants were scored for the presence of *U. maydis* infection in the leaf, ear, stalk, and tassel tissue approximately 8 weeks following inoculation. The relative frequency (ratio of infected plants to total number of plants) of *U. maydis* infection in these four tissues was calculated within and across replicate blocks for each RI line. The relative frequency of

infection over the entire plant, regardless of the tissue infected, was also determined for each RI line over the entire study and within each replicate block. For the purposes of calculating infection frequencies, only the presence of *U. maydis* infection was noted and not the number of individual *U. maydis* galls found on an individual plant.

Individual plants were also scored for the severity of *U. maydis* infection within specific plant tissues. The severity of tassel infection was defined as the percentage of each plant's racines (tassel branches) infected with *U. maydis*. The severity of leaf, ear, and stalk infection was defined as the area infected by *U. maydis* and was estimated by the product of the length and width of *U. maydis* infection. Length and width measurements for leaf, ear, and stalk infection were categorized into 5 cm. intervals ranging from 1 (galls measuring between 1 and 5 cm) to 7 (galls measuring between 30 and 35 cm) to facilitate severity scoring. These categorical length and width measurements were multiplied together to estimate the area infected by *U. maydis* and the areas summed together across multiple infection sites within the same tissue. Severity estimates within each plant tissue were then transformed to a standard normal distribution. The standardized severity estimates were summed across tissues to develop an estimate of the total severity of *U. maydis* infection over the entire plant. The average severity of *U. maydis* infection over the entire plant and within the leaf, ear, stalk, and tassel was determined for each RI line by averaging severity estimates across the plants of an RI line within and across replicate blocks.

This division of the phenotypic data created ten estimates of *U. maydis* infection. Each RI line was described by the frequency and the severity of *U. maydis* infection over the entire plant (referred to here as total infection) and specifically within the leaf, ear, stalk, and tassel tissues.

## Data analysis and BLUP estimation

The effect of variation among RI lines on each of the ten infection estimates was assessed using the PROC MIXED procedure implemented in the SAS statistical package (SAS Institute 1997) using restricted maximum likelihood (REML). Each of the ten infection estimates was considered in a univariate analysis using a full factorial model using RI line, year, and location as factors in the analysis. RI line and all interactions involving RI line were treated as random effects and the year and location factors were considered fixed effects. The variance component estimates resulting from these analyses were used to estimate the heritability of each of the ten infection estimates using the equation presented by Hallauer and Miranda (1981):

$$\frac{\sigma^2_{\text{Line}}}{\sigma^2_{\text{Line}} + \sigma^2_{(\text{Line}\times\text{Location}\times\text{Year})}/yl + \sigma^2_{(\text{Line}\times\text{Location})}/y + \sigma^2_{(\text{Line}\times\text{Year})}/l + \sigma^2_{\text{Residual}/ryl}},$$

where $y$ = the number of years, $r$ = number replicates per combination of years and locations, $l$ = number of locations, and $\sigma^2_{\text{Line}}$, $\sigma^2_{(\text{line}\times\text{location}\times\text{year})}$, $\sigma^2_{(\text{line}\times\text{location})}$, $\sigma^2_{(\text{line}\times\text{location}\times\text{year})}$ = line, line by location, line by year, line by year by location variance components, respectively.

Results of these analyses were used to estimate best linear unbiased predictors (BLUPs) of the effect of each RI line on the ten infection estimates over all environments (Searle et al. 1992; Lynch and Walsh 1998). RI line BLUP values were determined separately for each infection estimate and were only calculated for infection estimates significantly influenced by variation among RI lines.

## Creating genetic maps of the A188 × CMV3 and W23 × CMV3 RI populations

DNA was extracted from a bulked sample of ten seedlings from 106 F7 A188 × CMV3 RI lines and 126 F7 W23 × CMV3 RI lines using the CTAB protocol described by Sagai-Maroof et al. (1984). Approximately 80–90 SSRs, RFLPs, and gene sequences previously mapped within the maize genome (http://www.maizegdb.org/map.php) were amplified by PCR and surveyed for polymorphisms using 3% agarose gels. A list of markers and map positions used in this study are provided as supplementary material. MapMaker 3.0 (Lincoln et al. 1992) was used to group markers into linkage groups using a LOD threshold of 3.0. Genetic maps of loci mapped to the same linkage group were developed using the COMPARE command in MapMaker 3.0.

## QTL mapping

The position and effect of QTLs contributing to variation among the ten *U. maydis* infection estimates were

first estimated using the ten RI line infection estimates averaged across all replicate blocks, years, and locations of this study. QTLs were then mapped using RI line BLUP values for each *U. maydis* infection estimate. QTLs were mapped using PLABQTL (Utz and Melchinger 1997) analyzing each of the ten-infection estimate separately. RI line estimates with a studentized residual of greater than 3.5 (PLABQTL default parameter) were considered outliers and the analysis of QTL position re-run without these values. QTLs were initially mapped using single interval mapping (SIM) and then mapped using composite interval mapping (CIM). The stepwise regression method found in the PLABQTL software was used to select markers with a partial *F* statistic of 3.5 as marker co-factors for CIM. The significance of each QTL was determined through comparison to 5% LOD thresholds generated from 1,000 permutations of the observed data. The positions of QTLs are reported as the genetic bins (Gardiner et al. 1993) containing the flanking markers of the QTL.

### QTL by environment and QTL by QTL interactions

QTL by environment interactions (Q × E) contributing to the ten infection estimates were first estimated using the standard regression method implemented in PLABQTL. Q × E interactions were then assessed by comparing the position and effect of QTLs significantly contributing to the ten *U. maydis* infection estimates within each environment.

The multiple regression method implemented in PLABQTL was used to determine the significance of additive × additive epistatic interactions between QTLs found to significantly contribute to RI line averages or BLUP values (Utz and Melchinger 1997). Interactions between QTLs with a *P*-value of the less than 0.05 in this multiple regression method were considered significant.

## Results

Approximately, 10,000 *U. maydis* galls were characterized across 30,000 plants from the A188 × CMV3 and W23 × CMV3 RI populations. Variation between RI lines significantly contributed to variation in all ten infection estimates in the W23 × CMV3 RI population and to all infection estimates except the frequency and severity of leaf infection and the severity of stalk infection in the A188 × CMV3 RI population (Table 1). Furthermore, large variation was found in RI line raw averages and BLUP estimates for the majority of the ten infection estimates in both RI populations (Table 1).

### Genetic maps of the two recombinant inbred populations

The final genetic maps of the A188 × CMV3 and W23 × CMV3 were approximately 1471.3 cM and 1598.6 cM long, respectively (Fig. 1). The genetic map

**Table 1** Minimum and maximum values of RI line averages and BLUP estimates for the frequency (*I*) and severity (*S*) of infection over the entire plant (*T*) and in the leaf (*L*), ear (*E*), stalk (*St*), and tassel (*Ts*) of the two RI populations

| | A188 × CMV3 | | | | | | W23 × CMV3 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Min | | Max | | *P*-value | $h^2$ | Min | | Max | | *P*-value | $h^2$ |
| | Avg. | BLUP | Avg. | BLUP | | | Avg. | BLUP | Avg. | BLUP | | |
| TI | 0 | 0.21 | 0.95 | 0.72 | ** | 0.84 | 0 | 0.04 | 0.83 | 0.64 | ** | 0.61 |
| LI | 0 | NS | 0.45 | NS | | NS | 0 | 0 | 0.45 | 0.28 | ** | 0.62 |
| EI | 0 | 0.01 | 0.41 | 0.3 | ** | 0.63 | 0 | 0.02 | 0.33 | 0.29 | * | 0.38 |
| StI | 0 | 0.01 | 0.69 | 0.49 | * | 0.57 | 0 | 0.01 | 0.76 | 0.43 | ** | 0.64 |
| TsI | 0 | 0 | 0.89 | 0.66 | ** | 0.80 | 0 | 0 | 0.69 | 0.4 | ** | 0.59 |
| TS | 0 | 2.5 | 125 | 111 | ** | 0.85 | 0 | 0.9 | 102 | 48.9 | * | 0.85 |
| LS | 0 | NS | 29 | NS | | NS | 0 | 0 | 26.3 | 15.2 | ** | 0.41 |
| ES | 0 | 0 | 32.8 | 27.6 | * | 0.48 | 0 | 0.1 | 30 | 23.9 | * | 0.45 |
| StS | 0 | NS | 90 | NS | | NS | 0 | 1.5 | 142.5 | 44 | ** | 0.64 |
| TsS | 0 | 0.35 | 0.56 | 0.7 | ** | 0.54 | 0 | 0.21 | 0.2 | 0.4 | ** | 0.61 |

Additive effects for leaf, ear, and stalk severity is given in $cm^2$. Tassel severity is given by the percentage of total racines infected by *U. maydis*. The estimate of the total severity of infection represents the sum of the individual tissues severity estimates after they have been standardized to a standard normal distribution. The frequency of infection in all tissues is given as the percentage of infected plants to the total number of plants within an RI line

*NS* not significant

* < 0.05, ** < 0.01

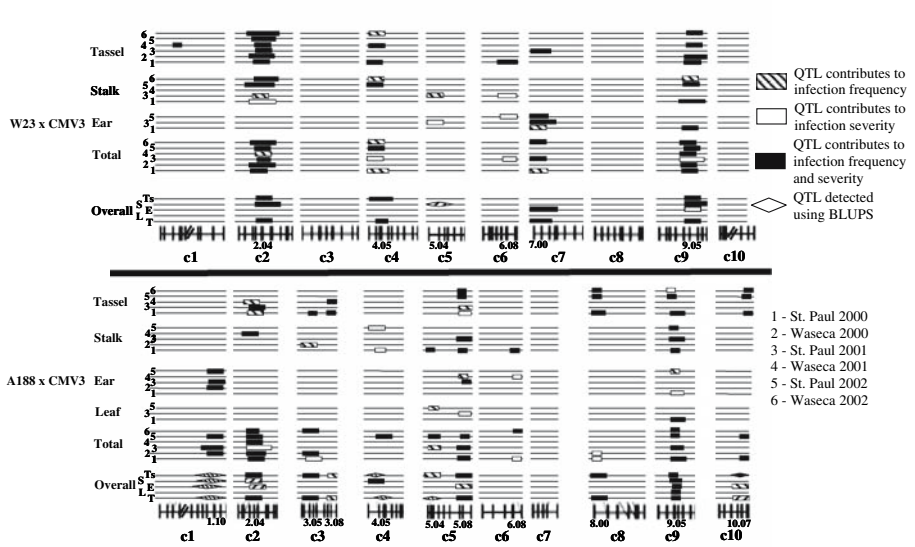


**Fig. 1** Position of QTLs contributing to the frequency and severity of *Ustilago maydis* infection over the entire plant (total; *T*) and in the leaf (*L*), ear (*E*), stalk (*S*), and tassel (*Ts*) tissues over the entire experiment (overall) and within specific environments (listed as numbers 1–6). Genetic maps of each chromosome of the two RI populations are given as hashed lines with each hash representing a genetic marker. Genetic bin location is given for each detected QTL. *Bars* represent QTLs contributing to *U. maydis* resistance in specific plant tissues within a given environment

used for mapping QTL in the A188 × CMV3 RI population contained 82 SSR markers and the map of the W23 × CMV3 RI population contained 90 SSR markers. The two genetic maps had 31 SSR markers in common. Markers previously mapped to chromosome 1 or 10 fell into two linkage groups in the W23 × CMV3 RI population, as did markers from chromosome 4 of the A188 × CMV3 RI population.

## QTLs contributing to total frequency and severity of *U. maydis* infection

QTLs contributing to the total frequency and severity of *U. maydis* resistance were first mapped using the total frequency and severity of *U. maydis* infection over the entire experiment. QTLs significantly contributing to variation in RI line averages and BLUP values for the total frequency and/or severity of infection mapped to genetic bins 2.04, 4.05 9.04–9.05 (Table 2; Fig. 1) in both RI populations. Additional QTLs contributing to the total frequency or severity of infection mapped to bins 1.10, 3.05, 3.08, 5.04, 5.08, 8.00, and 10.07 in the A188 × CMV3 population and bin 7.00 within the W23 × CMV3 RI population. (Table 2; Fig. 1). Allelic variation at several of these QTLs strongly contributed to variation in the total frequency or severity of infection in both populations. For example, the resistant allele of the QTLs mapped to bins 2.04 and 9.05 in both populations decreased the total frequency of infection by up to 13% from the population mean and the total severity of infection by approximately 9 $cm^2$ (Table 2). Surprisingly, while A188 was considered a resistant parent in this study, A188 contributed alleles increasing the frequency of *U. maydis* infection at the 1.10, 3.08, 4.05, and 10.07 QTLs. In contrast, W23 contributed only alleles at QTLs significantly influencing *U. maydis* infection that decreased the frequency and severity of *U. maydis* infection.

## QTLs contributing to *U. maydis* resistance in specific plant tissues

### *W23 × CMV3 RI population*

QTLs mapped to bin 2.04 significantly contributed to variation in RI line averages and BLUP values for the frequency and severity of infection of stalk and tassel tissue infection in the W23 × CMV3 RI population. Similarly, QTLs mapped to bins 9.05 contributed to the frequency and severity of ear, stalk, and tassel infection (Table 2; Fig. 1). QTLs mapped to genetic bin 7.00 contributed to the frequency and severity of infection in only the ear tissue of W23 × CMV3 RI lines. A QTL mapped to bin 4.05 contributed to variation in RI line averages and BLUP values only for the frequency and severity of tassel infection in the W23 × CMV3 RI population. Finally, a QTL mapping to bin 5.04 significantly contributed to variation in BLUP values for the frequency of stalk infection (Table 2; Fig. 1).

**Table 2** Position and effect of QTLs significantly contributing to the frequency (*I*) and severity (*S*) of *U. maydis* infection over the entire plant (*T*) and in the leaf (*L*), ear (*E*), stalk (*St*), and tassel (*Ts*) of the A188 × CMV3 (*A*) and the W23 × CMV3 (*W*) populations

| Genetic bin | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Trait | Pop. | | 1.07 | 1.10 | 2.04 | 3.05 | 3.08 | 4.05 | 5.04 | 5.08 | 7.00 | 8.00 | 9.05 | 10.07 |
| TS | W | $R^2$ | | | 18.1 | | | 10.4[b] | | | 14.6[b] | | *9.0* | |
| | | Add. | | | 7.3 | | | 4.5 | | | 4.3 | | *4.0* | |
| | A | $R^2$ | | | 13.9[b] | 11.5[b] | **38.3**[a] | | | 14.7[b] | | 13.0[b] | 11.2[b] | |
| | | Add. | | | 8.8 | 9.5 | **-5.0** | | | 8.3 | | 10.5 | 6.8 | |
| TI | W | $R^2$ | | | 27.1 | | | 9.5[b] | | | 10.2 | | 18.5[b] | |
| | | Add. | | | 0.13 | | | 0.08 | | | 0.08 | | 0.09 | |
| | A | $R^2$ | | **30.0** [a] | 25.4[b] | 10.7[b] | **33.2**[a] | **11.7** | **33.5**[a] | 22.1[b] | | **24.3**[a] | 13.8[b] | **14.1** |
| | | Add. | | **–0.05** | 0.10 | 0.10 | **-0.05** | **–0.10** | **0.08** | 0.12 | | **0.05** | 0.10 | **-0.06** |
| LS | A | $R^2$ | | | | | | | | | | | *10.9* | |
| | | Add. | | | | | | | | | | | *2.0* | |
| LI | A | $R^2$ | | | | | | | | | | | *10.8* | |
| | | Add. | | | | | | | | | | | *0.03* | |
| ES | W | $R^2$ | | | | | | | | | 14.7 | | *9.9* | |
| | | Add. | | | | | | | | | 2.0 | | *2.0* | |
| | A | $R^2$ | | **18.1** | **11.9** | | | | | | | | **12.8** | |
| | | Add. | | **2.0** | **1.5** | | | | | | | | **1.5** | |
| EI | W | $R^2$ | | | | | | | | | 12.9 | | *10.9* | |
| | | Add. | | | | | | | | | 0.09 | | *0.08* | |
| | A | $R^2$ | **26.7**[a] | **23.9** | | | | | | **19.9** | | | | **39.1**[a] |
| | | Add. | **0.02** | **0.03** | | | | | | **0.03** | | | | **–0.02** |
| StS | W | $R^2$ | | | *9.1* | | | | | | | | 11.8 | |
| | | Add. | | | *4.3* | | | | | | | | 4.8 | |
| | A | $R^2$ | | | 19.5[b] | | | *10.1* | | | | | *19.5*[b] | |
| | | Add. | | | 7.50 | | | *3.25* | | | | | *7.50* | |
| StI | W | $R^2$ | | | *13.4* | | | | **10.8**[a] | | | | 9.5 | |
| | | Add. | | | *0.06* | | | | **–0.06** | | | | 0.06 | |
| | A | $R^2$ | | **13.8** | | | | **17.9**[a] | | | | | **17.5**[a] | |
| | | Add. | | **0.05** | | | | **–0** | | | | | **0.04** | |
| TsS | W | $R^2$ | | | 13.5[b] | | | *10.8* [b] | | | | | 17.2 | |
| | | Add. | | | 0.07 | | | *0.04* | | | | | 0.05 | |
| | A | $R^2$ | | | *11.6* [b] | 14.9[a,b] | **25.7**[a] | | **16.8** | 12.6[b] | | 12.3[b] | 10.9[b] | |
| | | Add. | | | *0.07* | 0.03 | **-0.10** | | 0.03 | 0.03 | | 0.03 | 0.03 | |
| TsI | W | $R^2$ | | | 17.6 | | | **9.3** | | | | | 11.3 | |
| | | Add. | | | 0.08 | | | **0.04** | | | | | 0.03 | |
| | A | $R^2$ | | **24.1**[a] | 13.5[b] | *11.3*[a,b] | **34.5**[a] | *17.6*[b] | **30.2**[a] | 16.1[b] | | 13.4[b] | 12.0[b] | 18.9[a,b] |
| | | Add. | | **–0.10** | 0.06 | *0.05* | **-0.10** | *–0.10* | **0.07** | 0.09 | | 0.08 | 0.08 | -0.08 |

All QTLs displayed were significant using RI line BLUPs or infection estimates averaged over the entire experiment. QTL effects in bold indicate QTLs only detected using RI line BLUP estimates and effects in italics indicate QTLs found only using raw data averages. All other QTLs were found significant using both data averages and BLUPs with only the results of RI line averages represented here. Significant QTL by environment interactions at the listed QTLs indicate that while the QTL significantly contributed to aspects of *U. maydis* infection over entire experiment, QTL effect significantly varied between the seven environments in the study. Additive effects for leaf, ear, and stalk severity is given in $cm^2$. Tassel severity is given by the percentage of total racines infected by *U. maydis*. The estimate of the total severity of infection represents the sum of the individual tissues severity estimates after they have been standardized to a standard normal distribution. The frequency of infection in all tissues is given as the percentage of infected plants to the total number of plants within an RI line

[a] QTL only detected using composite interval mapping (CIM)

[b] QTL by environment interactions detected at $P < 0.05$ significance

### *A188 × CMV3 RI population*

In the A188 × CMV3 population, QTLs mapped to bin 9.05 significantly contributed to variation in RI line averages or BLUP values for all infection classes except for the frequency of ear infection (Table 2; Fig. 1). Similarly, QTLs mapped to genetic bin 2.04 contributed to variation in the frequency and severity of ear, stalk, and tassel infection in this population. A QTL mapped to bin 1.07 significantly contributed to the frequency of ear infection among A188 × CMV3 RI lines. QTLs mapped to bins 4.05, 5.08, and 10.07 contributed to the frequency and severity of infection in either the stalk, ear, or tassel. QTLs mapped to bins 3.05, 3.08, 5.04, and 8.00 contributed to variation in the frequency or severity of infection in only the tassel

tissue of the A188 × CMV3 populations (Table 2; Fig. 1). Surprisingly, the CMV3 allele of the QTL mapped to bin 1.10 significantly increased the frequency and severity of *U. maydis* infection in the ear and the frequency of infection in the stalk, but actually decreased BLUP estimates concerning the frequency of tassel infection.

### *QTL × environment interactions*

The significance of Q × E interactions were first examined by using the multiple regression method in PLABQTL and using data averaged over both replicate blocks within individual location–year combinations. In the A188 × CMV3 population, Q × E interactions contributed to variation in all infection classes except the frequency and severity of leaf infection (Table 2). In the W23 × CMV3 population, significant Q × E interactions were found for the QTLs mapped to bins 2.04, 4.05, and 9.05, but these interactions only contributed to the frequency or severity of total and tassel infection. No significant Q × E interaction was detected for the QTL mapped to bin 7.00 contributing to only the frequency and severity of ear infection (Table 2) among W23 × CMV3 RI lines.

QTLs contributing to variation in the ten *U. maydis* infection estimates were mapped using data from each environment and compared between environments to further examine the stability of QTL significance and effect across environments. QTLs mapped to bins 2.04 and 9.05 significantly contributed to the total frequency and severity of infection in all environments of the W23 × CMV3 RI population. Similarly, the QTL mapped to bin 2.04 contributed to the total frequency and/or severity of infection in all environments of the A188 × CMV3 RI population. QTLs mapped to bins 5.08 and 9.04–9.05 in the A188 × CMV3 population significantly contributed to the total frequency of infection in four or more environments (Fig. 1). While the QTLs mapped to bins 2.04, 5.08, and 9.04–9.05 significantly contributed to *U. maydis* resistance in several environments in both RI populations, the effect of these QTLs did vary between environments in both RI populations. However, the effects of these QTLs were strongest in environments where the frequency of *U. maydis* infection was the highest (data not shown).

Within each RI population, several QTLs were found to preferentially confer resistance in specific plant tissues across several environments. For example, the QTL mapped to bin 7.00 in the W23 × CMV3 RI population strongly contributed to ear resistance in four environments (Fig. 1) while the QTLs mapped to bins 3.05, 3.08, and 8.00 only significantly contributed to variation in tassel infection in the A188 × CMV3 RI population. However, the QTL mapped to bin 7.00 in the W23 × CMV3 RI population that contributed exclusively to ear infection was found to weakly contribute to the frequency and severity of *U. maydis* infection in other tissues in some environments, suggesting that the resistance conferred by these loci is not entirely exclusive to one plant tissue.

### Epistasis between QTLs contributing to *U. maydis* resistance

Several QTL by QTL epistatic interactions significantly contributed to the frequency or severity of infection over the entire plant and the tassel tissue of both RI populations (Table 3). Epistatic interaction between the QTLs mapped to bin 2.04 and 9.05 contributed weakly to infection estimates concerning the frequency of infection over the entire plant and in the tassel tissue (Table 3) in the W23 × CMV3 RI population. In contrast, several interactions between QTLs were found to contribute to variation in RI line averages and BLUP values concerning the frequency and severity of infection over the entire plant and in the tassel tissue in the A188 × CMV3 RI population. Interestingly, nine of the ten significant epistatic interactions found in the A188 × CMV3 RI population involved a QTL mapped to bin 5.08 (Table 3).

## Discussion

Two RI populations segregating for *U. maydis* resistance were developed to map QTLs contributing to variation in the frequency and severity of *U. maydis* infection over the entire plant and within specific plant tissues. We observed 12 QTLs with significant effects on at least one of the ten infection estimates considered in this study with QTLs contributing to *U. maydis* resistance over the entire plant mapping to genetic bins 2.04, 4.05, and 9.04–9.05 in both RI populations. More importantly, the QTLs mapped to bins 2.04 and 9.04–9.05 in both populations as well as the QTL mapped to bin 5.08 in the A188 × CMV3 RI population strongly and consistently contributed to variation in *U. maydis* resistance over the entire plant and within specific plant tissues in all or nearly all environments. Several additional QTLs contributed to resistance over the entire plant and specifically within the ear and tassel tissues in both RI populations.

Six previous studies, spanning nearly a century, have mapped *U. maydis* resistance QTLs in several populations, using morphological traits, translocation

**Table 3** Additive by additive epistatic interactions between QTLs significantly contributing to the frequency and average severity of *Ustilago maydis* infection over the entire plant (total infection) and in the tassel of the A188 × CMV3 and W23 × CMV3 populations

| Population | Trait | QTL #1 | QTL #2 | Add.[a] | $R^2$ | Method[b] |
|---|---|---|---|---|---|---|
| A188 × CMV3 | Tassel frequency | 8.00 | 5.08 | 0.03 | 6.4 | R |
| A188 × CMV3 | Tassel frequency | 9.04 | 5.08 | 0.05 | 4.7 | R |
| A188 × CMV3 | Tassel severity | 3.05 | 5.08 | 0.03 | 12.9 | B |
| A188 × CMV3 | Tassel severity | 9.04 | 5.08 | 0.06 | 7.3 | R, B |
| A188 × CMV3 | Tassel severity | 8.00 | 5.08 | 0.02 | 13.7 | B |
| A188 × CMV3 | Total frequency | 2.04 | 5.08 | 0.06 | 5.8 | R |
| A188 × CMV3 | Total severity | 3.05 | 5.08 | 5.60 | 6.1 | B |
| A188 × CMV3 | Total severity | 3.05 | 9.05 | 7.13 | 17.6 | B |
| A188 × CMV3 | Total severity | 9.04 | 5.08 | 6.25 | 11.3 | B |
| W23 × CMV3 | Tassel severity | 9.05 | 2.04 | 0.04 | 4.2 | R |
| W23 × CMV3 | Tassel frequency | 9.05 | 2.04 | -0.06 | 3.9 | R |

QTL × QTL interaction detected using RI line averages (R) or RI line breeding values (B)

testers, and molecular markers (Immer 1927; Hoover 1932; Burham and Cartledge 1939; Saboe and Hayes 1941; Lübberstedt et al. 1998; Kerns et al. 1999). While several of these studies mapped *U. maydis* resistance QTLs before dense genetic maps of the maize genome were created, the position of the translocations and morphological markers used in these classic studies have been incorporated in genetic maps (http://www.maizegdb.org), allowing comparison between *U. maydis* resistance loci mapped in early and more modern studies. Comparison of these studies show that while no region of the maize genome contains *U. maydis* resistance QTLs mapped in all seven studies, *U. maydis* QTLs were mapped to genetic bins 1.06, 2.03–2.04, 3.05, 7.00–7.02, and 9.05 in five or six studies. Furthermore, QTLs for *U. maydis* resistance mapped to bins 2.04 and 9.05 in nine of the ten populations used to map QTLs in seven different studies.

Few previous studies have mapped QTLs for *U. maydis* resistance in specific plant tissues. Hoover (1932) found linkage between the *ramosa1* gene (bins 7.00–7.01) and *U. maydis* infection in the ear of plants, similar to the position of the QTL contributing to ear resistance in the W23 × CMV3 RI population. Both Hoover (1932) and Immer (1927) found linkage between the *su1* (genetic bin 4.05) locus and increased resistance in the tassel, similar to the QTL contributing to tassel resistance in both the A188 × CMV3 and W23 × CMV3 RI populations. Comparison of these studies demonstrates that loci contributing to *U. maydis* resistance over the entire plant and in specific plant tissues map to similar regions of the maize genome, strengthening the significance of results reported in this study.

Several genes shown to confer pathogen resistance in maize map to the same regions of the maize genome as the *U. maydis* resistance QTLs mapped in the W23 × CMV3 and A188 × CMV3 populations. For example, the NBS-LRR resistance gene analog (RGA) *pic17* (Collins et al. 1998), the pathogenesis-related protein, *prp2* (Cordero et al. 1994a), a chitinase, *cta1* (Cordero et al. 1994b), and QTLs conferring *U. maydis* resistance in both RI populations all map to genetic bin 2.04. Other NBS-LRR RGAs, such as the *rp3* resistance gene complex (Webb et al. 2002), and *U. maydis* resistance QTLs map to bins 3.05 and 7.00–7.01 (Collins et al. 1998). Additionally, *U. maydis* resistance QTLs in both RI populations mapped to genetic bin 4.05 map near the wound-inducible protein, *wip1* (bin 4.03) (Rohmeir and Lehle 1993), and the basal antifungal protein, *bap2* (bin 4.05) (Hueros et al. 1995). These loci could be considered candidate genes in future studies examining the loci conferring *U. maydis* resistance within these two RI populations.

Several of the QTLs found to weakly contribute to variation in the frequency and severity of *U. maydis* infection over the entire plant were found to strongly contribute to resistance in specific tissues of the plant. This result suggests that the QTLs overall contribution to overall plant resistance comes from providing resistance within a specific plant tissue. The biological cause of this tissue-specificity is not readily apparent in this study. Several of genes conferring resistance such as the *bap2* (Hueros et al. 1995) gene have been shown to be preferentially expressed within specific plant tissues. However, the tissue-specific resistance provided by these QTLs could also result from control of morphological or maturity traits, such as husk cover or flowering, limiting access of the fungus to specific plant tissues.

Surprisingly, the CMV3 allele of the QTL mapped to bin 1.10 of the A188 × CMV3 RI population significantly increased RI line BLUP estimates concerning the frequency of *U. maydis* infection in the ear and

stalk tissue, but decreased RI line BLUP estimates for the frequency of tassel infection. It should be noted that the QTL contributing to stalk and ear infection was mapped using SIM while the QTL contributing to the frequency of tassel infection was detected using CIM, possibly suggesting that differences between analytical approaches may contribute to the observed allelic associations. However, this shift in allele effect could also be caused by linkage between genes conferring tissue-specific *U. maydis* resistance or different alleles of the same gene conferring resistance in separate tissues. Further recombination within this genomic region of the A188 × CMV3 RI population is needed to distinguish whether this variable allelic effect is caused by a single gene or linkage between several different genes.

QTL by environment interactions were found to contribute to the frequency and severity of *U. maydis* infection over the entire plant and within specific plant tissues in both RI populations. However, the QTLs mapped to bins 2.04 in both populations and the QTL mapped to bin 9.05 in the W23 × CMV3 RI populations significantly contributed to total *U. maydis* resistance in all environments. Furthermore, QTLs mapped to bins 5.08 and 9.04–9.05 strongly contributed to *U. maydis* resistance in the A188 × CMV3 population in the majority of environments. These results indicate that these QTLs are relatively stable across environmental conditions and could be useful in studies trying to modify *U. maydis* resistance within these RI and related populations. However, significant Q × E interactions were found to contribute to several infection estimates in both RI populations. The significance of these Q × E interactions seems to be driven by QTLs only significantly contributing to variation in infection estimates in specific environments and not a reversal in the effect of a QTL's allele.

The biological cause of this variable response of QTLs to environments is not immediately apparent from this study, but variation in precipitation, temperature, and genetic structure of *U. maydis* populations can affect *U. maydis* infection (Immer and Christensen 1928; Kostandi and Geisler 1989; Stakman and Christensen 1926; Sandoval and Corcuera 1998). Furthermore, limited infection in a specific plant tissue would limit the detection of QTLs contributing to resistance in that specific tissue.

Additive × additive epistatic interactions were found to significantly contribute to *U. maydis* resistance in both RI populations. An interaction between alleles at the 2.04 and 9.05 QTL significantly contributed to the frequency of infection over the entire plant in the W23 × CMV3 RI population. A similar epistatic interaction involving a QTL mapped to chromosome bin 2.04 contributed to *U. maydis* resistance in two European flint populations of maize (Lübberstedt et al. 1998). In contrast to the W23 × CMV3 RI population, epistatic interactions between the QTL mapped to bin 5.08 and several other QTLs significantly contributed to the frequency and severity of *U. maydis* infection over the entire plant and within the tassel tissue

In this study, QTLs for *U. maydis* resistance were mapped using two populations consisting of RI lines selected for *U. maydis* resistance or susceptibility over the entire F5 generation. This selective genotyping process was an attempt to limit the number of lines needed for phenotyping and genotyping. While this method of selective genotyping increases the power of QTL detection, it must be noted that QTL effect estimates can often be biased by this form of sampling (Darvasi and Soller 1992). Due to this bias, estimates of the additive effect of the QTLs presented in this study need to be carefully evaluated. However, the distribution of the frequency of *U. maydis* infection in both populations over the entire experiment did not show a bimodal distribution, indicating that RI line selection had not created two different phenotypic classes in the two RI populations (data not shown).

*Ustilago maydis* infection can be viewed in a positive or negative light, depending on the intended use of the plant and fungus. *U. maydis* infection lowers grain yield by destroying ear tissue, increasing stalk lodging, and sterilizing plants through ear and tassel infection, especially in sweet corn populations. In contrast, *U. maydis* infection is sold as a delicacy called *huitlacoche* in several regions of the world. In this study, QTLs strongly contributing to the frequency and severity of *U. maydis* infection in several different tissues mapped to similar genomic regions in two RI populations. Interestingly, many QTLs only significantly contributed to resistance in discrete tissues of the plant, suggesting that maize populations could be created with *U. maydis* resistance within only specific plant tissues. Furthermore, regions of the genome containing QTLs strongly contributing to *U. maydis* resistance, such as genetic bins 2.04 and 9.05, can be used in future mapping and marker assisted selection studies manipulating *U. maydis* resistance in specific maize populations.

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