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Precise Mapping of Quantitative Trait Loci for Resistance to Southern Leaf Blight, Caused by *Cochliobolus heterostrophus* Race O, and Flowering Time Using Advanced Intercross Maize Lines

P. J. Balint-Kurti,*,†,[1] J. C. Zwonitzer,† R. J. Wisser,† M. L. Carson,‡ M. A. Oropeza-Rosas,§ J. B. Holland,§ and S. J. Szalma§**

**U.S. Department of Agriculture–Agricultural Research Service (USDA–ARS) Plant Science Research Unit and* †*Department of Plant Pathology, North Carolina State University, Raleigh, North Carolina 27695-7616,* ‡*USDA–ARS, Cereal Disease Laboratory, St. Paul, Minnesota 55108 and* ***USDA–ARS Plant Science Research Unit and* §*Department of Crop Science, North Carolina State University, Raleigh, North Carolina 27695-7620*

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ABSTRACT

The *i*ntermated *B*73 × *M*o17 (IBM) population, an advanced intercross recombinant inbred line population derived from a cross between the maize lines B73 (susceptible) and Mo17 (resistant), was evaluated in four environments for resistance to southern leaf blight (SLB) disease caused by *Cochliobolus heterostrophus* race O. Two environments were artificially inoculated, while two were not inoculated and consequently had substantially lower disease pressure. Four common SLB resistance quantitative trait loci (QTL) were identified in all environments, two in bin 3.04 and one each in bins 1.10 and 8.02/3. There was no significant correlation between disease resistance and days to anthesis. A direct comparison was made between SLB QTL detected in two populations, independently derived from the same parental cross: the IBM advanced intercross population and a conventional recombinant inbred line population. Several QTL for SLB resistance were detected in both populations, with the IBM providing between 5 and, in one case, 50 times greater mapping resolution.

QUANTITATIVE trait locus (QTL) mapping using biparentally derived populations has historically been imprecise, with the support or confidence interval for a QTL position spanning 10–30 cM or comprising 1–3% of a genome (Kearsey and Farquhar 1998; Dekkers and Hospital 2002; Salvi and Tuberosa 2005). Reasons for this level of imprecision include insufficient marker density and limited opportunities for recombination between closely linked loci due to the relatively small size of many mapping populations (often 100–200 individuals). Increasing QTL resolution while maintaining a manageable population size can be achieved through the development of advanced intercross lines (AILs), as proposed by Darvasi and Soller (1995). The *i*ntermated *B*73 × *M*o17 (IBM) population is an AIL maize population developed by including four generations of random mating following the formation of the F_2 generation and prior to the development of inbred lines (Lee *et al.* 2002). The increased opportunity for recombination has had the effect of expanding the genetic map approximately fourfold compared to nonintermated, conventional, recombinant inbred line (RIL) populations (Lee *et al.* 2002). The IBM population consists of a relatively large number of lines (302) that have been densely genotyped with >2000 molecular markers (Coe *et al.* 2002).

Cochliobolus heterostrophus (Drechs.) Drechs. (anamorph, *Bipolaris maydis* (Nisikado) Shoemaker; synonym, *Helminthosporium maydis* Nisikado) is a necrotrophic plant pathogen and the causal agent of southern leaf blight (SLB). This disease is frequently found in hot, humid maize-growing areas and was not considered an important pathogen until 1970 when *C. heterostrophus* race T became prevalent in the U.S. Corn Belt. Race T was highly pathogenic on Texas male-sterile cytoplasm (cms-T), causing a major disease epidemic in 1970 and 1971 (Ullstrup 1972). Since that time, cms-T has been eliminated from elite germplasm and effective polygenic resistance has been introduced. Most of this resistance is quantitative and can be additive or recessive in effect (Scott and Futrell 1975; Lim and Hooker 1976; Burnette and White 1985; Holley and Goodman 1989; Balint-Kurti *et al.* 2006) although one qualitative recessive gene, *rhm*, which primarily conditions resistance in preanthesis growth stages, has been mapped to the distal end of the short arm of chromosome 6 (bin 6.00) (Thompson and Bergquist 1984; Zaitlin *et al.* 1993). The disease, predominantly caused by race O, is still a

[1]*Corresponding author:* Department of Plant Pathology, 3418 Gardner Hall, Box 7616, North Carolina State University, Raleigh, NC 27695-7616. E-mail: peter_balintkurti@ncsu.edu

significant problem in the southern Atlantic coast area of the United States and parts of India, Africa, and Western Europe. It has the potential to cause grain yield losses of ≥40% (Fisher *et al.* 1976; Gregory *et al.* 1979; Byrnes *et al.* 1989). However, use of more resistant germplasm, especially in the United States and western Europe, has largely controlled yield losses due to SLB.

To date, three studies have been published on mapping quantitative trait loci for field resistance to SLB in maize (Jiang *et al.* 1999; Carson *et al.* 2004; Balint-Kurti *et al.* 2006). In one of these (Jiang *et al.* 1999) only one SLB QTL was detected and this was observed in only one environment. Another study identified SLB resistance QTL in juvenile maize plants (Balint-Kurti and Carson 2006). All of these studies reported QTL spanning large genomic regions. This study reports SLB resistance QTL identified in the IBM population and compares the results to SLB resistance QTL identified in a conventional RIL mapping population derived from the same parents as the IBM population. QTL identified for time to anthesis are also reported.

MATERIALS AND METHODS

Plant materials: Phenotypic data were collected from the IBM mapping population composed of 298 $F_{7:8}$ RILs derived from the cross of maize inbred lines B73 (relatively susceptible parent) and Mo17 (relatively resistant parent). This population had been intermated four times at the F_2 stage before inbred lines were derived (Lee *et al.* 2002). Seed of IBM lines was received from the Maize Genetic Stock Center and also as gifts from A. Stapleton and O. Hoekenga.

The other population referred to in this article is a set of 158 $F_{6:7}$ RILs developed from a B73 × Mo17 cross by selfing directly from the F_2 generation by C. Stuber and colleagues (here referred to as the "Stuber population") (Carson 1998). This population was not assessed in the present study, but previous data derived from it were reanalyzed (see below).

Field trials: All experiments were performed at the North Carolina State University Central Crops Research Station located at Clayton, North Carolina. SLB resistance was evaluated in four environments in this study. Two experiments in separate fields were rated in each of 2 years, 2005 and 2006. One experiment in each year was artificially inoculated with SLB, and the other was infected solely by natural inoculum. Henceforth these four combinations of treatment and year are referred to as inoc2005, uninoc2005, inoc2006, and uninoc2006, respectively. Although 298 lines were used in all, due to seed shortage and other factors, all lines were not represented in each environment; 229, 287, 277, and 199 lines were rated, respectively, in inoc2005, uninoc2005, inoc2006, and uninoc2006.

Each experiment consisted of two replicates of the IBM population plus parental lines (B73 and Mo17) in complete randomized blocks. With the exception of uninoc2006, experimental units in each case consisted of single-row plots arranged in randomized complete blocks with two replications. Plots were 2 m in length with a 0.6-m alley at the end of each plot. Interrow spacing was 0.97 m. Twelve seeds per plot were planted in each plot and rows were not thinned, except in the case of uninoc2005, where some thinning was done. Two plots of SLB-susceptible inbred border were planted on all sides of the experiment. Overhead irrigation was applied as needed to ensure satisfactory plant growth. Standard fertilizer and herbicide regimes for central North Carolina were used. For uninoc2006, plot length was 1 m and the plants were thinned to about six plants per plot. Other details were the same as above.

For the Carson *et al.* (2004) study, the Stuber population was inoculated with *C. heterostrophus* race O, isolate 2-16Bm, in two randomized complete blocks over 2 years in Clayton, North Carolina. Inoculation techniques used were the same as in the present study.

Fungal growth and inoculation: Techniques used for inoculum preparation were identical to those reported previously (Carson *et al.* 2004). In the artificially inoculated experiments, experimental and border plots were inoculated at the four- to six-leaf stage by placing ~20 grains of a sorghum seed culture of *C. heterostrophus* race O, isolate 2-16Bm (Carson 1998) in the leaf whorl of every plant in every plot. After inoculation, the field was irrigated to wet the sorghum seed and allow commencement of fungal growth. Uninoculated experiments were separated from inoculated experiments by at least 550 m.

Ratings: Entries in each environment were rated on a plot basis. In the artificially inoculated experiments, the first rating was taken ~4 days before anthesis. In 2005, three ratings were made at 12-day intervals. In 2006, four ratings were made at 7-day intervals. Plots were rated on a 1–9 scale, in increments of 0.5, with 1 being a symptomless plant and 9 being a completely dead plant. Thus, a 1-unit difference in rating represented an ~12.5% difference in disease severity. Days to anthesis (DTA) were determined as the number of days after planting when half the plants in the row were shedding pollen and were recorded in the two artificially inoculated environments.

For the naturally inoculated experiments, the first rating was taken 3–4 weeks after anthesis. In 2005, three ratings were taken at 7-day intervals. In 2006 only one rating was possible as the plants started to senesce shortly afterward, making further ratings impossible. The plants were likewise rated on a 1–9 scale.

Weighted mean disease (WMD) rating values were calculated for each replication in each environment. The average value of two consecutive ratings was obtained and multiplied by the number of days between the ratings. Values were then summed over all intervals and then divided by the number of days of evaluation to determine the weighted average. WMD is functionally equivalent to an area under disease progress curve (AUDPC) rating (Wilcoxson *et al.* 1974).

Statistical analyses: Due to seed shortage and poor seed germination or growth, ~5% of the lines grown in each environment were scored only in one replicate, rather than in two. To account for these missing data for subsequent analyses, least-squares means were calculated using the PROC GLM procedure of SAS (SAS Institute, Cary, NC) to obtain average ratings over the two replications for each line for each environment.

All phenotypic correlation calculations were made using the PROC CORR procedure of SAS. Mixed-models analyses for southern leaf blight and days to anthesis were conducted using Proc Mixed in SAS Version 9.1. For SLB, inoculation treatment was considered a fixed effect, and all other factors (year, replication, line) and their interactions with each other and with treatment were considered random effects. For DTA, data were collected only in inoculated plots, so the model did not include treatment or any interactions with treatment, and all effects were considered random. The main effect of treatment on SLB was tested with a type III *F*-test. The main effects of random factors were tested with likelihood-ratio tests, as follows. For each random component, a reduced model was tested that lacked the factor being tested. The difference

between the −2 residual log likelihood of the reduced and full models represented the likelihood-ratio test, which is approximately distributed as a chi-square distribution with 1 d.f. (Littell *et al.* 1996). The *P*-values for these tests were adjusted by dividing the chi-square statistic *P*-value by two to better approximate its value for testing the null hypothesis of no variance due to the tested factor (Self and Liang 1987). Heritability was estimated for each trait using the PROC MIXED procedure of SAS, as described previously (Holland *et al.* 2003).

The Windows QTL cartographer version 2.5 software package was used to detect QTL. Composite-interval mapping (CIM) was performed to identify SLB and anthesis QTL in each environment separately and for overall SLB. Least-squares means of WMD scores were calculated for each line over the four environments to get a "SLBOverall" rating. For the analysis, 20 control markers were identified using forward and backward regression, and a window size of 10 cM was used, with a walk speed of 0.5 cM to identify QTL. The appropriate likelihood ratio for a 5% significance level of identified QTL was determined through permutation testing. Genotypic data for 1345 markers spaced over the genome were used for the analysis. The input files used for the analyses were based on publicly available files (http://www.maizegdb.org/qtl-data.php, verified 2/14/2007) on the MaizeGDB web site (Lawrence *et al.* 2005). Map distances were based on the IBM2 map (http://www.maizegdb.org/map.php, verified 2/14/2007). AUDPC data from a previous study using a different RIL population (Carson *et al.* 2004) were reanalyzed using CIM and the same Windows QTL cartographer version 2.5 software. This analysis was performed in exactly the same way as above except for the fact that 10 instead of 20 control markers were used because of the reduced number of QTL detected.

Multiple-interval mapping (MIM) was used to investigate epistatic interactions among main-effect QTL. Initial MIM models were constructed with QTL identified that were significant at least at the 0.01 significance level in CIM. Testing of MIM models was completed in an interactive, stepwise fashion, searching for new QTL to add to the current model, and testing their significance after each search cycle. New models were accepted if they decreased the Bayesian information criterion (BIC) (Piepho and Gauch 2001). The BIC favors models with higher likelihoods, but includes a penalty for each additional parameter added to the model, to help prevent overfitting the models. After no additional QTL could be added to a model according to the BIC, each pair of QTL in the model was tested for epistatic interactions. Epistatic interactions were chosen if they decreased the BIC. While deriving the model using multiple-interval mapping, we were mindful not to "overfit" the model such that the proportion of total variation due to QTL exceeded the entry mean heritability. If this occurred, QTL were dropped in a backward regression fashion to obtain the best model according to the BIC that did not explain a greater proportion of the phenotypic variation than the entry mean heritability.

The units of distance in the IBM population are not, strictly speaking, centimorgans (cM). "IBM centimorgans" (IcM) are therefore used as a measure of genetic distance for this study. At small-interval distances, 1 cM = ~4 IcM, but the relationship between cM and IcM is not linear (see Lee *et al.* 2002; Winkler *et al.* 2003; Falque 2005). Rather than convert IBM map positions to "true cM," we report QTL positions according to the widely used IBM2 map (http://www.maizegdb.org/map.php, verified 2/14/2007) so that they can be easily matched with publicly available molecular markers. Furthermore, to aid comparisons between the IBM and the Stuber population maps, after data analysis, QTL results were projected on map positions of common markers on the IBM map.

LOD curves near QTL are difficult to interpret in CIM because flanking markers are dropped as cofactors within the "window size" used, resulting in discrete jumps in the LOD curve that primarily represent changes in the model cofactors rather than differences in QTL effects at the position being tested. Therefore, we developed an alternative method to estimate 2-LOD support intervals for QTL positions using MIM, where the background QTL were maintained constant. To accomplish this, MIM models were created to fit the three major QTL positions in bins 1.09–1.10, 2.04, and 3.04 to the SLBOverall and combined AUDPC from Carson *et al.* (2004) (CarsonAUDPC) data sets in the MIM analysis function of QTL Cartographer. Then, the position of one of the individual QTL was shifted by 1-IcM intervals in either direction, maintaining the positions of the other two QTL, until the flanking positions at which the log likelihood dropped by two points were observed. The process was repeated for each QTL.

RESULTS

Disease and anthesis ratings: Average WMD scores followed an approximately normal distribution. Some transgressive segregation was observed (Figure 1). Phenotypic data were analyzed using mixed-models analyses for southern leaf blight and days to anthesis (see Table 1). The treatment (*i.e.*, inoculated *vs.* uninoculated) was not significant for WMD; however, there was a significant year-by-treatment-by-line interaction. For this reason the WMD QTL analyses from the four individual environments are reported (uninoc2005, uninoc2006, inoc2005, and inoc2006). Pairwise Pearson correlation coefficients of WMD scores between replications were high in each environment ($r > 0.7$; data not shown), and the correlation coefficients of WMD scores averaged over replications were high among environments ($r = 0.67$–0.77, $P < 0.0001$ in each case; Table 2). In addition, the estimated variance component due to lines was large, while the year-by-line and treatment-by-line interactions were not significant (Table 1). For these reasons, as well as analyzing WMD data from individual environments, it was considered justified to use average WMD ratings over the four environments for CIM and MIM analyses.

The entry mean heritability for disease resistance over the four environments was 0.81 (standard error 0.02). Although the inoculated environments (inoc2005 and inoc2006) had a much higher disease pressure and were consequently rated starting much earlier in the season than the uninoculated environments (uninoc2005 and uninoc2006), the correlation coefficients between the two uninoculated environments (0.73) and between the two inoculated environments (0.75) were not significantly different from the correlation coefficients found between inoculated and uninoculated environments (Table 2).

For DTA, the variance component ascribed to error was much larger than that ascribed to line effects (Table 1) and there was a significant year-by-line interaction. The Pearson correlation coefficient between the average

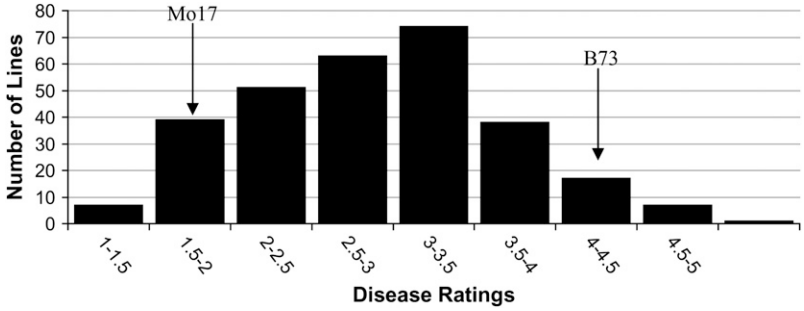

FIGURE 1.—The distribution of weighted mean disease scores for resistance to southern leaf blight of maize (average over four environments), caused by *Cochliobolus heterostrophus* race O, rated on a 1–9 scale, where 1 represented a symptomless plant and 9 represented a dead plant, in the IBM maize RIL population. The rating for each line was calculated as a least-squares mean of the ratings over the four environments rated. The positions of the average scores of the parental types, B73 and Mo17, are indicated.

DTA score for each line in the two years was low, though statistically significant ($r = 0.36$, $P < 0.0001$). Likewise, within environments the between-replications correlations were low ($r = 0.2$–0.5). DTA data therefore were not combined over the two environments measured, as too much of the variation appeared to be due to error and to year-by-line interactions. The correlations between the overall averages of WMD and DTA were not significant (data not shown).

QTL analyses: CIM identified QTL for WMD and DTA in all the environments in which they were rated (see Table 3 for complete details). For WMD, four QTL were detected in all four environments, one on chromosome 1 (bin 1.10), two on chromosome 3 (both in bin 3.04), and one on chromosome 8 (bin 8.02–8.03). These QTL explained the largest portion of phenotypic variation in most of the environments. Other, lower-effect QTL, in most cases with R^2-values <0.05, were detected in one or two environments, but were not consistently detected over all the environments (Table 3).

WMD scores averaged over the four environments were also analyzed using CIM to detect QTL (here called SLBOverall QTL). As expected, the major SLBOverall QTL were again in bins 1.10, 3.04 (two QTL), and 8.02–8.03. Twelve SLBOverall QTL were detected in all (Table 3). The only other SLBOverall QTL with an R^2-value >0.05 was in bin 1.06. In several cases, including bin 1.06, SLBOverall QTL were detected in regions for which no QTL had been detected in any of the environments when analyzed individually. MIM was also used to analyze the SLBOverall values. The QTL detected were very similar to those detected using CIM (data not shown). MIM also allowed the detection of two epistatic interactions of minor effect, between the QTL in bin 1.10 and bin 8.02–8.03 ($R^2 = 0.006$) and between the QTL in bin 7.03 and bin 8.02–8.03 ($R^2 = 0.008$).

Most of the larger-effect SLBOverall QTL had resistance alleles derived from Mo17, the more resistant parent (Figure 1). However, 6 of the 12 resistance alleles for SLBOverall QTL were derived from B73, including

TABLE 1

***F*-test of fixed-effect and variance component estimates (and their standard errors) of random effects in mixed-models analysis of southern leaf blight disease resistance (WMD) scores and time to anthesis scores from the maize IBM population, a population consisting of 298 maize recombinant inbred lines from a B73 × Mo17 cross, scored in 2 years at Clayton, North Carolina**

	Southern leaf blight WMD			
Fixed factor	*F*-test	*P*-value	Days to anthesis[a]	
Treatment (inoculated *vs.* uninoculated)	0.57	NS		
	Southern leaf blight WMD		Days to anthesis[a]	
Random factor	Variance component estimate (standard error)	*P*-value	Variance component estimate (standard error)	*P*-value
Year	0.29 (0.49)	NS	2.41 (4.87)	NS
Year × treatment	0.03 (0.17)	NS	NA	
Rep(year × treatment)[b]	0.16 (0.11)	<0.0001	1.90 (1.91)	<0.0001
Line	0.59 (0.06)	<0.0001	1.98 (0.40)	<0.0001
Year × line	0	NS	1.09 (0.37)	0.004
Treatment × line	0	NS	NA	
Year × treatment × line	0.16 (0.02)	<0.0001	NA	
Error	0.18 (0.01)		4.75 (0.30)	

[a] Days to anthesis were measured only in inoculated plots, so treatment main effect and all interactions with treatment are not included in the model for days to anthesis.

[b] Replication [Rep(year)] effect for days to anthesis.

TABLE 2

Pearson correlation coefficients between WMD southern leaf blight disease ratings (scored on a 1–9 scale) for the maize IBM population obtained in four environments (inoc2005, uninoc2005, inoc2006, and uninoc2006) for WMD

	inoc2005	uninoc2005	inoc2006
uninoc2005	0.76		
inoc2006	0.75	0.77	
uninoc2006	0.67	0.73	0.73

All correlations were significant at $P < 0.0001$.

a QTL in bin 3.04 at position 217–258 IcM. No common QTL were found between DTA QTL identified from the 2005 and from the 2006 data (Table 3). No common QTL positions were found between DTA and WMD QTL.

Direct comparison between a conventional and an advanced intercross population: A previous study (Carson *et al.* 2004) had identified QTL for resistance to SLB in a conventional B73 × Mo17-derived 158-member RIL population (*i.e.*, the same cross from which the IBM population was derived). This population is here referred to as the Stuber population. The CarsonAUDPC data were reanalyzed using CIM on the basis of a map with 234 marker loci, to make the analysis process equivalent to that used for analyzing the data from the IBM population. The three main QTL reported by Carson *et al.* (2004) were again identified in almost identical positions and with similar effects on chromosomes 1 (bin 1.09–1.10), 2 (bin 2.04), and 3 (bin 3.04) (Table 3; CarsonAUDPC column). However, an additional QTL was identified on chromosome 1 by this data reanalysis, in bin 1.07. Other, smaller-effect QTL on chromosomes 1, 4, 7, and 10 that were detected in the Carson *et al.* (2004) study were not detected in this reanalysis of the data.

The CarsonAUDPC QTL identified from the reanalysis of the data were compared with the QTL identified from the SLBOverall data, with respect to position, effect, and support interval length. The three highest-effect CarsonAUDPC QTL, in bins 1.09–1.10, 2.04, and 3.04, essentially colocalized with SLBOverall QTL (Table 3, Figure 2); however, the 2-LOD confidence intervals for these regions (*i.e.*, the intervals defining the region within two log-of-odds levels of the peak value) were between 2.5 and 50 times larger for the CarsonAUDPC QTL than for the SLBOverall QTL. The broad CarsonAUDPC QTL in bin 3.04 was resolved into two QTL of opposing effect using the SLBOverall data (Figure 2, Table 3).

There were several notable differences between the positions and effects of the CarsonAUDPC and SLBOverall QTL: No CarsonAUDPC QTL was identified around bin 8.02–8.03, in contrast to the relatively large-effect SLBOverall QTL ($R^2 = 0.09$) identified in this position. No SLBOverall QTL mapped to the same location as the CarsonAUDPC QTL in bin 1.07, although there was an SLBOverall QTL in bin 1.06 with the same effect sign. Twelve SLBOverall QTL were detected, compared to 4 CarsonAUDPC QTL.

As another way of comparing confidence intervals, MIM models were created to fit the SLBOverall and CarsonAUDPC data, and the 2-LOD intervals of the three common QTL positions in bins 1.09–1.10, 2.04, and 3.04 were determined by gradually varying the positions of the individual QTL and observing the point at which the log-likelihood ratio dropped by two points. Broadly similar results were obtained, with the intervals defined in the SLBOverall model being between 2 and 30 times smaller than the intervals defined in the CarsonAUDPC model (data not shown).

DISCUSSION

In this study, the IBM population, an AIL maize mapping population, was used to map QTL for resistance to SLB and for flowering time. The IBM population is unique among publicly available maize mapping populations for two main reasons: Four generations of intermating at the F_2 stage have increased the observed numbers of recombination events between tightly linked markers approximately fourfold (Lee *et al.* 2002) and the map marker density is unprecedented, with >2000 markers having been placed on the map (Coe *et al.* 2002). The IBM population also has a relatively large population size, with ~300 lines being available. The authors are aware of only one published mapping study using the full IBM population (Hazen *et al.* 2003), in which QTL for cell wall composition were mapped to intervals spanning ~10 cM. Another published study used 94 lines of the IBM population to map QTL controlling the thermal properties of maize starch (Scott and Duvick 2005).

In this study, the IBM population was scored for SLB resistance in four separate environments, two of which were inoculated to produce very high disease pressure and two of which relied on the natural development of the disease in field conditions. Due to the higher disease pressure, the inoculated trials were scored ~4 weeks earlier in the season and therefore at quite a different physiological state than the uninoculated trials and over a much longer time period (~5 weeks compared to 1–2 weeks). The fact that statistical analysis did not detect a treatment effect (Table 1) indicates that, *over the period during which ratings were taken*, the average rating values were not significantly different between treatments. The strong phenotypic correlations observed among all disease-pressure environments (Table 2), the lack of a line-by-treatment interaction (Table 1), and the fact that several of the larger-effect QTL were consistently detected across the inoculated and uninoculated environments (Table 3), all suggest that most of the SLB

TABLE 3

Chromosomal location in IBM map units (IcM) and parameters associated with major quantitative trait loci (QTL) for weighted mean disease (WMD) ratings for southern leaf blight (SLB) of maize, caused by *Cochliobolus heterostrophus* race O, and for days to anthesis (DTA), in a B73 × Mo17 advanced intercross recombinant inbred line population comprising 298 lines

Bin, parameters[a]	Flanking markers[b]	inoc2005	inoc2006	uninoc2005	uninoc20006	SLBOverall	CarsonAUDPC[g]	DTA2005	DTA2006
1.01	umc1566–umc94a		20–40[c]						
a			−0.19[d]						
LOD			4.8[e]						
R^2			0.04[f]						
1.03	umc1397–bnlg1203					237–257			
a						0.15			
LOD						5.0			
R^2						0.04			
1.05	bnlg2295–mmp61				*402–409*	400–411			
a					*−0.17*	−0.16			
LOD					*5.6*	5.9			
R^2					*0.05*	0.04			
1.06	umc2151–mmp123					568–583			
a						0.15			
LOD						3.7			
R^2						0.02			
1.06	uaz147b–asg62					*601–607*			
a						*−0.21*			
LOD						*7.7*			
R^2						*0.05*			
1.07	bnlg1556–umc1245						*660–721*		
a							*−46.2*[h]		
LOD							*3.2*		
R^2							*0.07*		
1.10	npi282b–mmp87	*925–943*	*937–942*	*935–942*	*932–942*	*928–941*	*864–939*[i]		
a		*−0.27*	*−0.35*	*−0.29*	*−0.2*	*−0.29*	*−68.7*		
LOD		*8.8*	*13.5*	*8.9*	*5.7*	*17.7*	*6.72*		
R^2		*0.08*	*0.13*	*0.09*	*0.06*	*0.12*	*0.14*		
2.03	mmp42–bnlg381				*225–243*				
a					*−0.19*				
LOD					*6.6*				
R^2					*0.06*				
2.04	prp2–bnlg108	252–277				285–303	*257–306*		
a		−0.18				−0.16	*−67.8*		
LOD		4.4				5.2	*7.4*		
R^2		0.04				0.03	*0.15*		
2.08	mmp116–umc1947				495–516				
a					0.16				
LOD					4.2				
R^2					0.04				

(*continued*)

TABLE 3

(Continued)

Bin, parameters[a]	Flanking markers[b]	inoc2005	inoc2006	uninoc2005	uninoc20006	SLBOverall	CarsonAUDPC[g]	DTA2005	DTA2006
3.04	npi446–umc2000	*164–167*	*163–166*	*167–173*	*169–176*	*164–166*	*159–259*		
a		*−0.52*	*−0.47*	*−0.29*	*−0.25*	*−0.33*	*−53.9*		
LOD		*25.3*	*25*	*8.9*	*8.4*	*21*	*4.25*		
R^2		*0.28*	*0.21*	*0.08*	*0.09*	*0.13*	*0.09*		
3.04	mmp69–umc1920	*218–260*	243–263	*237–268*	258–280	*217–258*	*159–259*		
a		*0.23*	0.20	*0.22*	0.15	*0.20*	*−53.9*		
LOD		*5.7*	4.9	*5.6*	4.0	*7.4*	*4.25*		
R^2		*0.06*	0.03	*0.05*	0.04	*0.05*	*0.09*		
3.06	psr754a–csu1183				*464–479*				
a					*−0.16*				
LOD					*4.2*				
R^2					*0.05*				
3.09	umc2152–umc1813								741–748
a									0.51
LOD									3.6
R^2									0.04
4.02–4.03	umc1943–umc1926					123–137			
a						0.16			
LOD						5.5			
R^2						0.04			
4.05	umc1511–umc1142		297–302						
a			0.17						
LOD			4.2						
R^2			0.03						
4.07	umc66–mmp115	399–417							
a		0.16							
LOD		3.7							
R^2		0.03							
4.09	lim446–php10025				567–577				
a					−0.19				
LOD					4.6				
R^2					0.04				
5.00	ufg36–umc1445				0–37				
a					0.16				
LOD					4.5				
R^2					0.04				
6.02	y1ssr–umc1656		128–152			124–128			
a			0.17			0.12			
LOD			4.5			3.7			
R^2			0.03			0.02			
7.03	npi394–umc56			388–397		340–356			
a				0.18		0.17			

(*continued*)

TABLE 3

(Continued)

Bin, parameters[a]	Flanking markers[b]	inoc2005	inoc2006	uninoc2005	uninoc20006	SLBOverall	CarsonAUDPC[g]	DTA2005	DTA2006
LOD				4.4		6.6			
R^2				0.03		0.04			
8.02–8.03	umc1913–csu329	*175–179*	*163–176*	*163–185*	*165–179*	*165–175*			
a		*−0.18*	*−0.25*	*−0.2*	*−0.25*	*−0.25*			
LOD		*4.2*	*8.0*	*4.7*	*8.7*	*12.5*			
R^2		*0.04*	*0.07*	*0.05*	*0.10*	*0.09*			
8.05	ufg74–umc1316								*366–381*
a									*0.77*
LOD									*6.8*
R^2									*0.08*
9.02–9.03	mmp30–umc1634			170–193					
a				−0.18					
LOD				3.9					
R^2				0.04					
9.08	mmp171a–umc1505							*591–616*	
a								*0.7*	
LOD								*6.6*	
R^2								*0.15*	
10.05	umc1506–umc1477				*348–363*				
a					*−0.18*				
LOD					*6.3*				
R^2					*0.06*				

QTL for WMD in four environments are shown (inoc2005, inoc2006, uninoc2005, and uninoc2006), together with WMD QTL for overall values averaged over the four environments (SLBOverall). QTL associated with days from planting to anthesis for the inoc2005 and inoc2006 environments are also shown. In each case composite-interval mapping (CIM) was used to identify QTL. All ratings were made on a 1–9 scale, where 1 represented a symptomless plant and 9 represented a dead plant. SLB QTL identified in a separate B73 × Mo17 population consisting of 158 recombinant inbred lines are also shown (CarsonAUDPC). In this case average area under disease progress curve (AUDPC) data from a previous publication (Carson *et al.* 2004) were reanalyzed using CIM.

[a] Chromosome bin location of QTL peak on one of the 10 chromosomes of the maize genome. Bins divide the genetic map into 100 approximately equal segments. The segments are designated with the chromosome number followed by a two digit decimal (*e.g.*, 1.00, 1.01, 1.02, and so on).

[b] Markers that flank the 2-LOD confidence interval. In the case where multiple QTL in different environments were detected in the same bin, the flanking markers for the SLBOverall QTL are shown. Otherwise the markers flanking the QTL encompassing the largest genetic region are shown.

[c] The positions that define the 2-LOD interval around the position of peak likelihood for the QTL. All values are in IBM map units (IcM) and are based on the IBM2 map (http://www.maizegdb.org/map.php).

[d] The additive effect of the QTL. For disease ratings, with the exception of CarsonAUDPC, this is in terms of the 1–9 scale employed. For days to anthesis this is in terms of days. For CarsonAUDPC it is in terms of AUDPC values. A positive number indicates that the allele for resistance (or late anthesis) was derived from B73.

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

[f] R^2 estimates the proportion of phenotypic variance (percentage) explained by the detected QTL. QTL identified with an R^2-value of ≥0.05 are shown in italics.

[g] The 2-LOD interval for CarsonAUDPC is based on the IBM2 map distances, not the distances from the original B73/Mo17 RIL pop, which are much smaller. The IBM2 map distances were inferred from markers common to both maps.

[h] Note that the additive effect of the CarsonAUDPC QTL is based on AUDPC measures and cannot be directly compared to the additive effect of the other SLB resistance QTL reported here, which are based on the 1–9 scale employed.

[i] This QTL spans bins 1.09 and 1.10.

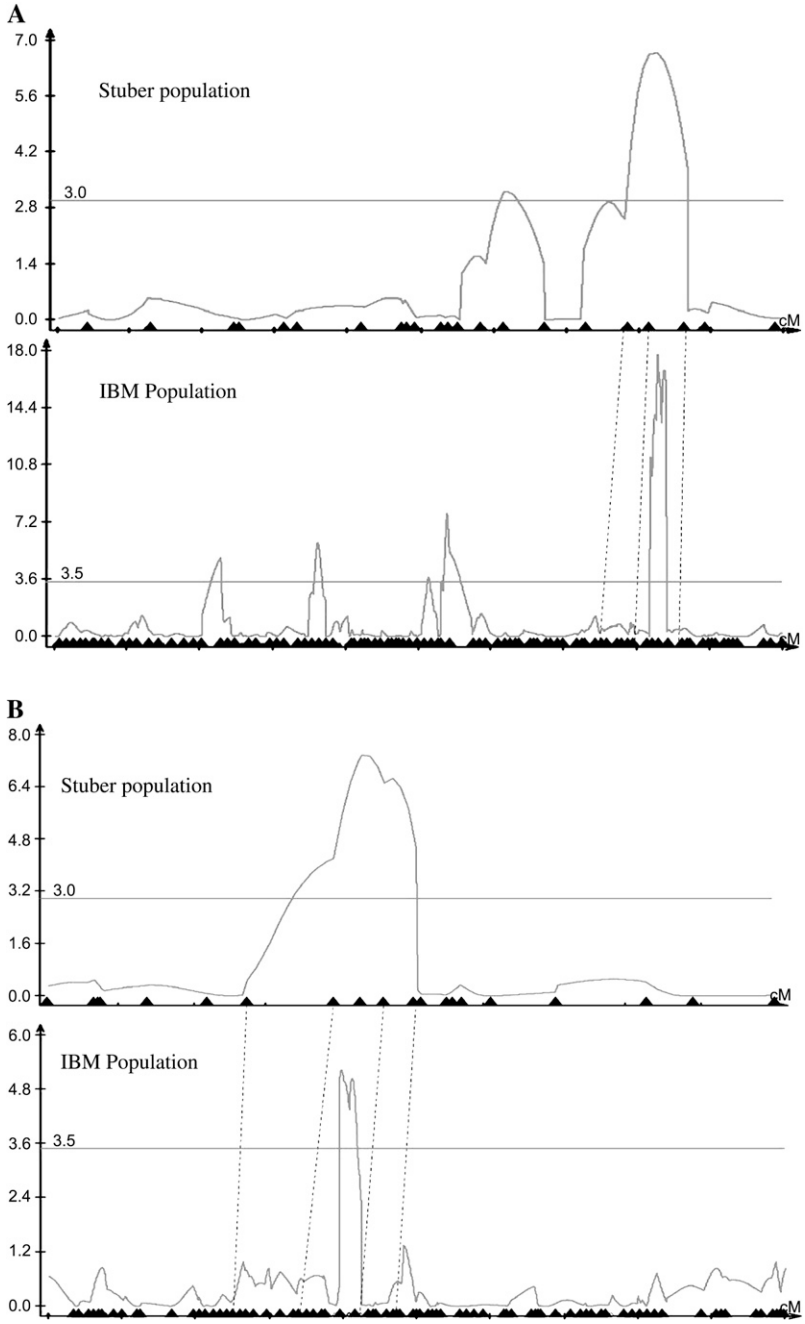

FIGURE 2.—QTL likelihood plots of SLB resistance from analysis of results averaged over two environments in a conventional 158-line RIL population (the Stuber population) derived from a B73 × Mo17 cross (top plot in each case) compared to QTL likelihood plots of SLB resistance from analysis of data averaged over four environments from the IBM population (bottom plot in each case). Analysis in each case was based on composite-interval mapping using similar parameters. The vertical axes in each graph indicate LOD scores, and the horizontal lines indicate the empirically derived LOD threshold for calling a QTL position ($P = 0.05$). The top and bottom graphs are scaled in each case so that equivalent genetic locations are vertically in line with each other. Dotted lines around the detected QTL connect the same molecular markers mapped in the two populations. Small triangles on the *x*-axes denote the position of mapped molecular markers in the two populations. One triangle may represent one or more markers in the case of very closely linked markers. (A) chromosome 1; (B) chromosome 2; (C) chromosome 3.

resistance genes segregating in the IBM population are effective over a wide range of disease pressures.

There are many examples of reports identifying QTL effective under varying levels of a particular stress (*e.g.*, LEDEAUX *et al.* 2006; VARGAS *et al.* 2006), but, to our knowledge, this is the first report directly comparing disease-resistance QTL identified under varying levels of disease pressure in the absence of other variables. Disease-resistance QTL for northern leaf blight of maize were detected at fairly consistent positions within the same population evaluated in differing environments in Iowa and Kenya (FREYMARK *et al.* 1993, 1994; DINGERDISSEN *et al.* 1996). Disease pressure was more severe in Kenya than in Iowa, implying that similar resistance mechanisms were effective under variable disease pressure also for northern leaf blight.

B73 is a relatively SLB-susceptible inbred and Mo17 is relatively resistant. In replicated studies in a population composed of 309 diverse maize inbred lines (lines described in FLINT-GARCIA *et al.* 2005 with a few additional lines), after correcting for relative maturity, Mo17 was the 7th most SLB-resistant line and B73 was the 270th (P. BALINT-KURTI, unpublished data). Nevertheless, there was evidence for transgressive segregation for SLB resistance, presumably due to the segregation of the six B73-derived resistance alleles identified.

SLB, like other necrotrophic foliar diseases of maize such as gray leaf spot and anthracnose, is generally a late

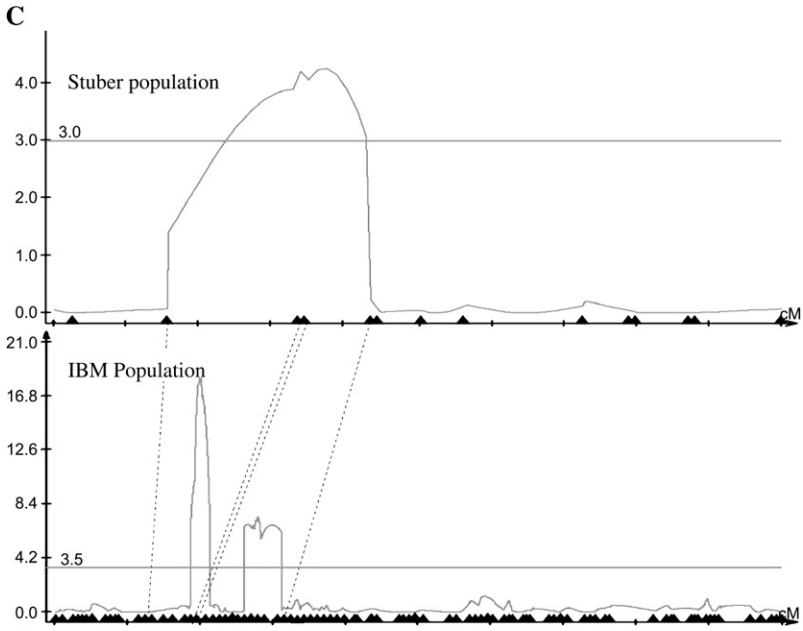

Figure 2.—(*Continued*)

season disease, with most disease development occurring postanthesis (White 1999). Thus there is concern that disease ratings might be affected by variations between lines in time to maturity. In this study, no significant correlation was observed between disease rating and time to anthesis, nor were there any shared QTL for flowering and disease traits (Table 3). This suggests that the SLB-resistance QTL detected primarily condition disease-resistance phenotypes, rather than acting through effects on maturity. It should be noted, however, that the high contribution of error effects to the overall variation for DTA means that a greater number of replications and/or environments might be required for accurate localization of DTA QTL in the IBM population.

Several mapping studies have examined both maturity-related and disease-related traits for maize necrotrophic diseases in the same populations (Bubeck *et al.* 1993; Jung *et al.* 1994; Jiang *et al.* 1999; Clements *et al.* 2000; Carson *et al.* 2004; Balint-Kurti *et al.* 2006). While none showed a strong correlation between the two traits, colocalization of disease QTL and maturity-related QTL and/or significant correlations between disease resistance and time to anthesis were observed in some of these studies, especially for studies on gray leaf spot-resistance QTL. In the diverse 309-line panel of maize germplasm mentioned above, 23% of the variance for SLB resistance was explained by variation in flowering time (P. Balint-Kurti, unpublished data). A metaanalysis of reported maize disease-resistance QTL also found some correspondence between flowering time and disease-resistance QTL (Wisser *et al.* 2006).

There was little correspondence between DTA QTL detected in the two environments tested. The low correlations between DTA data from the two environments and the relatively small line effect on variance (Table 1) were likely partly due to the fact that the two parents of the IBM population, B73 and Mo17, flower at approximately the same time and may share many of the alleles affecting the timing of flowering. The DTA QTL detected in bin 8.05 in 2006 mapped to approximately the same region as *vgt1*, a major QTL involved in floral transition (Salvi *et al.* 2002). Segregation distortion has previously been noted in the IBM population in this region (Fu *et al.* 2006).

A previous study (Carson *et al.* 2004) mapped SLB resistance QTL in Clayton, North Carolina, in a B73 × Mo17-derived RIL population developed by Stuber and others (Senior *et al.* 1996), and here called the Stuber population. Therefore, the major significant difference between this previous study and the study reported here, apart from the years in which they were conducted, was that, in the present study, an independently derived B73 × Mo17 AIL population was employed. AIL populations have been used successfully in animals and plants to localize QTL to relatively narrow chromosomal segments (*e.g.*, Hazen *et al.* 2003; Wang *et al.* 2003). Studies in mice found greater precision of QTL detection in an F_{11} AIL population compared to an F_2 population (Iraqi *et al.* 2000; Hernandez-Valladares *et al.* 2004). Fewer recombination events have been captured in the Stuber population, leading to a total map length of 1729.4 cM compared to a map length of 7060.1 IcM for the IBM population. This agrees closely with the ~4× map expansion reported for the IBM population by Lee *et al.* (2002). It should be noted that Falque *et al.* (2005) have suggested that the IBM2 map may be artificially expanded due to a low level (1–2%) of misgenotyping. There are at least two reports of formulas available to convert map units derived from an AIL population into "centimorgan equivalents" (Winkler

et al. 2003; Falke *et al.* 2006). We have not reported centimorgan equivalent conversions here as our intention is primarily to report precise locations for SLB QTL in terms of a widely used map (the IBM2 map) and to compare the results achieved with the IBM and Stuber populations. Instead, QTL intervals were compared on the basis of IcM, after projecting CarsonAUDPC QTL intervals onto the IBM2 map using the positions of common markers.

It was expected that common QTL would be detected between the two studies and that a substantial reduction in the support interval for QTL would be achieved using the IBM mapping population. This is essentially what was observed (Figure 2, Table 3). The three largest "CarsonAUDPC" QTL detected by reanalysis of the data of Carson *et al.* (2004) precisely colocalized with SLBOverall QTL (one of the CarsonAUDPC QTL was resolved to two SLBOverall QTL). The 2-LOD intervals of the SLBOverall QTL were ~6-fold smaller for the QTL in bin 1.09–1.10 and 2.5-fold smaller for the QTL in bin 2.04. Two SLBOverall QTL of opposing effects mapped in the same place as the single CarsonAUDPC QTL in bin 3.04. The larger-effect SLBOverall QTL in this case had the same direction effect as the CarsonAUDPC QTL; *i.e.*, resistance was derived from the Mo17 allele in both cases. In the case of these two QTL, the 2-LOD interval for SLBOverall QTL was 50-fold smaller. This magnitude of increased precision is clearly a special case as in this case one QTL was resolved to two. In addition, it appears there may have been some local map expansion of the Stuber population map relative to the IBM2 map in the region of the CasonAUDPC QTL in bin 3.04 (Figure 2C), contributing to the relatively large genetic distance encompassed by the CasonAUDPC QTL in this region. It is possible that the two QTL in bin 3.04 might have also been resolved in the Stuber population if there had been greater marker density in this region. The CarsonAUDPC QTL in bin 1.07 (660–721 IcM) did not precisely colocalize with an SLBOverall QTL. However, the SLBOverall QTL in bin 1.06 (601–607 IcM) might conceivably have been caused by the same underlying locus with the differing positions caused by imprecise mapping. It should be noted that all the IBM lines were not assessed in all the environments and in one case (uninoc2006) only 199 lines were assessed. While appropriate statistical procedures were used to account for this, it is likely that if all lines had been assessed in each environment, still greater resolution may have been possible.

Laurie *et al.* (2004) created a maize AIL population for the investigation of QTL for kernel oil concentration. In this case, 10 generations of random mating were carried out at the F_1 stage (200 plants per generation) and 500 lines were subsequently derived. As in the present study, high-resolution mapping of the identified QTL was possible, with an ~2- to 3-cM resolution being achieved. We believe that the present study constitutes the first study in plants where the precision of QTL detection in an inbred AIL population and that in a conventional RIL population, derived from the same parents, are directly compared.

As mentioned above, there are three significant differences between the IBM and the Stuber populations that might contribute to the increased precision of QTL mapping in the IBM population: the larger number of lines (298 used in this study *vs.* 158), the larger number of markers mapped in the IBM population (1345 used in this study *vs.* 234 markers in the Stuber population), and the approximately fourfold map expansion of the IBM compared to the Stuber population due to increased recombination. To gain some understanding of the relative contributions of these three factors, five simulations were performed where the number of IBM lines analyzed was randomly reduced to 158 (data not shown). In these cases, the larger-effect QTL (those in bins 1.10, 2.04, 3.04, and 8.02–8.03) were consistently detected, though with lower LOD-likelihood scores, while the smaller-effect QTL in many cases were not detected. The confidence intervals for the detected QTL remained almost the same, although in some cases the actual QTL likelihood peaks were shifted by a few (5–10) IcM. Taking the QTL in bin 1.10 as an example, markers were deleted from the IBM2 map in the QTL region, leaving only the markers shared between the IBM2 and Stuber population maps. When the analysis was performed using this reduced data set, the SLBOverall QTL identified in the IBM2 population in bin 1.10 had a substantially increased 2-LOD confidence interval (903–961 IcM, data not shown), which approached the size of the 2-LOD confidence interval for the CarsonAUDPC QTL in this region. It is clear that all these three differences between the Stuber and IBM populations are important for the increased power and precision derived from the IBM population, although a more sophisticated analysis would be required to precisely partition their effects. In some cases the effects were clearly interdependent; for instance, the large number of recombination events captured in the IBM population would not be a significant advantage if there were not also a large number of markers mapped onto the population that detect these recombination events.

The high degree of consistency between environments, coupled with the previously stated advantages of the IBM population, has allowed several QTL for SLB resistance to be mapped to narrowly defined regions with a high degree of confidence. If the maize genome contains 50,000 genes (Haberer *et al.* 2005), the gene density would be on average 7 genes/IcM, although this value clearly is very variable and would likely be substantially higher at QTL loci (Wisser *et al.* 2005, 2006). Therefore, in the best-case scenario, one might expect that some of these narrowly defined QTL would encompass <100 genes. Once the maize genome sequence is

available in 2008 it could be reasonably expected that a small set of 10–25 candidate disease-resistance genes, based on known functions or homologies and expression analyses, could be identified at these loci. At present 10–20 expressed sequence tags (ESTs) mapping to each of the four major SLBOverall QTL can be identified by querying available databases (http://www.maizegdb.org/est.php and http://www.tigr.org/tigr-scripts/tgi/T_index.cgi?species=maize). As expected, many of these ESTs either do not encode proteins with a well-defined function or encode proteins not known to be relevant to disease resistance. However, an EST mapping to the 164- to 166-IcM QTL region in bin 3.04 encodes a glutathione *S*-transferase. Glutathione *S*-transferases constitute a large gene family in maize. In rice this gene family has been previously implicated in disease resistance (Wisser *et al.* 2005). In addition, a resistance gene analog has been mapped to the SLB QTL at 925–933 IcM in bin 1.10 (Quint *et al.* 2003). To date, no reports of genes controlling quantitative disease resistance in plants have been published. The work reported here constitutes a significant step toward identification of genes responsible for quantitative resistance to SLB.

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