The causal agent of northern leaf blight (NLB) is *Exserohilum turcicum* (Pass) K.J. Leonard and E.G. Suggs [teliomorph: *Setosphaeria turcica* (Luttrell) Leonard and Suggs]. It is found in most maize (*Zea mays* L.) growing areas that have high humidity combined with moderate temperatures and is a significant problem in the northeastern United States, in sub-Saharan Africa, and in areas of China, Latin America, and India (Adipala et al., 1995; Dingerdissen et al., 1996). The genetics of NLB resistance have been extensively studied (reviewed in Welz and Geiger, 2000 and in Wisser et al., 2006). Northern leaf blight is unusual among necrotrophic diseases in that several dominant or partially dominant qualitative genes have been described that confer race-specific resistance to it, including *Ht1* (Hooker, 1963), *Ht2* (Hooker, 1977), *Ht3* (Hooker, 1981), *Htn1* (also known as *HtN*; Gevers, 1975), and *HtP* (Ogliari et al., 2005). This anomaly might be due to the fact that NLB is arguably a hemi-biotrophic rather than a pure necrotrophic pathogen.

Use of a Maize Advanced Intercross Line for Mapping of QTL for Northern Leaf Blight Resistance and Multiple Disease Resistance

Peter J. Balint-Kurti,* Junyun Yang, George Van Esbroeck, Janelle Jung, and Margaret E. Smith

**ABSTRACT**

Northern leaf blight [NLB; caused by *Exserohilum turcicum* (Pass) K.J. Leonard and E.G. Suggs] is an important fungal disease of maize (*Zea mays* L.) in the United States and worldwide. The IBM population, an advanced intercross recombinant inbred line population derived from a cross between the lines Mo17 and B73, was evaluated in three environments (Aurora, NY, in 2006 and 2007 and Clayton, NC in 2007) for two traits related to NLB resistance, weighted mean disease (WMD) and incubation period (IP), and for days to anthesis (DTA). Two WMD quantitative trait loci (QTL) in bins 2.00/2.01 and 4.08 were detected from the overall analysis; of these, only the QTL in bin 4.08 was detected in all three environments analyzed separately. Likewise, only one IP QTL, in bin 2.02, was detected in all three environments and from the overall analysis. Several environment-specific QTL for each trait were also detected. Several DTA QTL were detected with the strongest effect detected in bin 8.05. Correlations between disease resistance traits and days to anthesis were uniformly low. The results from this study were compared to those of previous studies that used the IBM population to identify QTL for two other maize foliar diseases, southern leaf blight (causal agent *Cochliobolus heterostrophus* (Drechs.) Drechs. [anamorph = *Bipolaris maydis* (Nisikado and Miyake) Shoemaker; synonym = *Helminthosporium maydis* (Nisikado and Miyake)]) and gray leaf spot (causal agent *Cercospora zeae-maydis* (Tehon and E.Y. Daniels)). Although we did not find QTL conferring resistance to all three diseases, significant correlations between resistances to these diseases in the IBM population were identified, implying the existence of loci (and possibly genes) affecting resistance to all three diseases.

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**Abbreviations:** AIL, advanced intercross line; AUDPC, area under disease progress curve; BLUP, best linear unbiased predictors; DLA, diseased leaf area; DTA, days to anthesis; GLS, gray leaf spot; IBM, intermated B73 × Mo17 population; Imu, IBM map units; IP, incubation period; LOD, log of odds; NLB, northern leaf blight; QTL, quantitative trait locus/loci; RIL, recombinant inbred line; SLB, southern leaf blight; WMD, weighted mean disease.

The causal agent of northern leaf blight (NLB) is *Exserohilum turcicum* (Pass) K.J. Leonard and E.G. Suggs [teliomorph: *Setosphaeria turcica* (Luttrell) Leonard and Suggs]. It is found in most maize (*Zea mays* L.) growing areas that have high humidity combined with moderate temperatures and is a significant problem in the northeastern United States, in sub-Saharan Africa, and in areas of China, Latin America, and India (Adipala et al., 1995; Dingerdissen et al., 1996). The genetics of NLB resistance have been extensively studied (reviewed in Welz and Geiger, 2000 and in Wisser et al., 2006). Northern leaf blight is unusual among necrotrophic diseases in that several dominant or partially dominant qualitative genes have been described that confer race-specific resistance to it, including *Ht1* (Hooker, 1963), *Ht2* (Hooker, 1977), *Ht3* (Hooker, 1981), *Htn1* (also known as *HtN*; Gevers, 1975), and *HtP* (Ogliari et al., 2005). This anomaly might be due to the fact that NLB is arguably a hemi-biotrophic rather than a pure necrotrophic pathogen.

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After leaf penetration, initial growth of *E. turicicum* is mostly intracellular (Knox-Davies, 1974) in the mesophyll and does not typically cause cell death for at least a week. It is also noteworthy that, compared to most major resistance genes, the *Ht* genes seem to have unusually high environmental dependence, particularly with regard to light and temperature (Leath et al., 1987, 1990; Thakur et al., 1989a, 1989b) and they tend to confer delayed lesion development or sporulation phenotypes rather than complete resistance. Partial resistance to NLB in contrast appears to be relatively stable over a wide range of temperature and light conditions (Carson and Van Dyke, 1994).

Quantitative trait loci (QTL) for NLB resistance have been identified from several populations (Brewster et al., 1992; Dingerdissen et al., 1996; Schechet et al., 1999; Welz and Geiger, 2000; Welz et al., 1999a, 1999b; Wisser et al., 2008) and are distributed throughout the genome (Wisser et al., 2006). In these cases, as with most QTL mapping using biparentally derived populations, the QTL were defined relatively imprecisely with the support or confidence interval for a QTL position spanning 10 to 30 cM or 1 to 3% of the genome. 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 200 or fewer lines). Another contributing factor is the difficulty of scoring the phenotype accurately. Scoring is done subjectively and the phenotype is somewhat environmentally sensitive (Carson and Van Dyke, 1994), consequently partial resistance to NLB tends to be moderately heritable. Freymark et al. (1993, 1994) estimated it at 62%.

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 IBM (Intermated B73 × Mo17) population is an AIL maize population developed by four generations of random mating following the formation of the F2 generation and before the development of inbred lines (Lee et al., 2002). The increased opportunity for recombination has had the effect of expanding the relatively small size of many mapping populations (often 200 or fewer lines). Another contributing factor is the difficulty of scoring the phenotype accurately. Scoring is done subjectively and the phenotype is somewhat environmentally sensitive (Carson and Van Dyke, 1994), consequently partial resistance to NLB tends to be moderately heritable. Freymark et al. (1993, 1994) estimated it at 62%.

Field Trials
Trials were planted in a randomized complete block design in three different environments. In AU06, the experiment included three replicates, each of which was considered a block. In AU07 and CL07, the trial consisted of two complete blocks.

In AU06, each inbred was planted in a single row plot per replicate, with 20 seeds planted (10 hills of two plants each) per 4.5-m row at 0.75-m row spacing. The rows were not thinned.

In AU07 and CL07, the trial consisted of two complete blocks. In AU07, the techniques used for inoculation were essentially the same as those reported previously (Carson et al., 2004).
Experimental and border plots were inoculated at the four- to six-leaf stage by placing ~20 grains of a sorghum [Sorghum bicolor (L.) Moench] seed culture of *E. tuncicum* (a mixture or race 0, race 1, race 2,3 and race 2,3,N) in the leaf whorl of every plant in every plot (including border plots).

**Rating and Disease Assessment**

Days to anthesis (DTA) was rated as the number of days after planting when 50% of the plants in the row were shedding pollen. For disease-related traits, incubation period (IP) was recorded when 50% of the inoculated plants in a row showed small, gray-green, water-soaked lesions. In CL07, latent period rather than IP was scored. Latent period is the time from inoculation to the formation of necrotic lesions (Carson, 1995). The two traits are highly related with a reported correlation coefficient of 0.99 (Carson and Van Dyke, 1994) and have been treated as the same trait for the purposes of the analyses presented here. Disease was scored as percentage of necrotic leaf area (diseased leaf area [DLA]). Three ratings were taken in AU07 and four ratings were taken in the other trials. The initial rating in each case was taken about 2 wk after the peak of flowering and ratings were taken at approximately 1-wk intervals. Once plants had senesced to such an extent that they were no longer reliably scorable, ratings were stopped.

**Statistical Analyses**

For individual environments, weighted mean disease (WMD) rating values were calculated using DLA ratings taken in each replication in each environment. To do this, the average value of two consecutive DLA 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. Weighted mean disease rating is functionally equivalent to an area under disease progress curve (AUDPC) rating and has been called a “Standardized AUDPC” rating in other publications (Campbell and Madden, 1990; Shaner and Finney, 1977). To account for the rare (<4%) occasions when a line was represented in only one replication within an environment, least squares means were calculated using the PROC GLM procedure of SAS (SAS Institute, Cary, NC) and have been treated as the same trait for the purposes of the analyses presented here. Disease was scored as percentage of necrotic leaf area (diseased leaf area [DLA]).

Three ratings were taken in AU07 and four ratings were taken in the other trials. The initial rating in each case was taken about 2 wk after the peak of flowering and ratings were taken at approximately 1-wk intervals. Once plants had senesced to such an extent that they were no longer reliably scorable, ratings were stopped.

**RESULTS AND DISCUSSION**

**Disease and Anthesis Ratings**

For each trait substantial transgressive segregation was observed (Fig. 1). In most cases the distribution was approximately normal with the parental lines. Somewhat surprisingly, for DTA, and to some extent for WMD, the values for the parents, B73 and Mo17, did not fall quite into the middle part of the distribution (Fig. 1). It may be that the B73 and Mo17 sources used in these trials differed somewhat from the lines used in the original cross to create the IBM population. It is also noteworthy that B73 flowered slightly after Mo17 in these trials, whereas the opposite was true in another recent study (Balint-Kurti et al., 2008). The differences here are relatively small and are likely due to experimental error.

Disease pressure was lower in CL07 than in AU06 or AU07 as illustrated by the fact that in CL07 the distribution for WMD was shifted toward the left (i.e., toward lower disease levels) in this environment compared to the other environments (Fig. 1). The lower disease pressure is likely the reason that the variation in disease resistance also was lower in CL07 than in the other environments. Periods of high humidity and somewhat cooler temperatures (15–25°C) are important for NLB development (Levy, 1983; Levy and Pataky, 1992; White, 1999). While both environments had adequate levels of humidity, Clayton, NC, is at the extreme southern edge of the range for NLB and the disease rarely occurs naturally, due to the high temperatures during the growing season (the average maximum temperature during July is 32°C). In Aurora, NY, the cooler temperatures (average maximum for July is 25°C) are near the optimum conditions for NLB. In addition, quite different inoculation methods were also used for the individual environment QTL analyses.
used for the two environments with “wet” inoculum (conidia harvested from plates) being used in Aurora and “dry” inoculum (dried infested sorghum seeds) in Clayton. Finally, different isolates were inoculated in the two environments (see Materials and Methods). Considering all these factors together, it is not surprising that the disease pressure varied between the two environments.

Corresponding-trait correlations among lines between environments for the three phenotypes rated (WMD, IP, and days to anthesis [DTA]) were all moderate (Pearson correlation coefficients 0.49–0.67) and highly significant (Table 1). Correlations between the two phenotypes related to disease resistance, WMD and IP, were likewise moderate and highly significant both within and between environments (Table 1, Pearson correlation coefficients −0.34 to −0.58). This agrees with previous reports that suggest IP and latent period are reasonable early indicators of adult NLB resistance (Smith and Kinsey, 1993; Welz et al., 1999a). Negative correlations were observed between IP and WMD, which is the expected sign of the coefficient; longer IPs are indicative of greater resistance, while higher WMDs are indicative of greater susceptibility. Correlations between DTA and the disease resistance phenotypes were uniformly low with none stronger than −0.22 (Table 1). Schechert et al. (1999) reported similarly low correlations in a population derived from a cross between two tropical maize lines, Lo951 and CML202.

The heritabilities on a family mean basis of IP, WMD, and DTA were 0.53 (standard error [SE] 0.03), 0.63 (SE 0.03), and 0.63 (SE 0.03), respectively. On a plot basis the heritabilities were 0.33 (SE 0.03), 0.39 (SE 0.03), and 0.45 (SE 0.03), respectively. In our previous studies using the IBM to study resistance to SLB and GLS, heritabilities on a family mean basis were estimated to be 0.81 (SE 0.02) and 0.77 (SE 0.02), respectively (Balint-Kurti et al., 2007, 2008). This reflects our general experience with these three diseases in which SLB resistance is the most heritable and NLB resistance is the least. However, it should also be noted that in this NLB study, two quite different environments (Clayton, NC, and Aurora, NY) were used, whereas in the GLS and SLB studies all the trials were done in different years in a single location. This

Figure 1. The distribution of the three measured traits that were measured in the intermated B73 × Mo17 (IBM) population in this study (days to anthesis [DTA], weighted mean disease [WMD], and incubation period [IP]) in the three environments used in this study (Aurora, NY, 2007 [AU07]; Aurora, NY, 2008 [AU08]; and Clayton, NC, 2007 [CL07]). The values for B73 (white arrow) and Mo17 (black arrow) are indicated.
would have also been a factor in decreasing the heritability observed in this NLB study.

Line and line × environment effects were the main significant contributors to phenotypic variance in WMD and IP, while only line effects were significant for DTA (Table 2). The variance components attributable to environment were large in every case, but the standard errors were also large and the effects were consequently not significant. The environmental variance for WMD was particularly large. This can also be seen by observing the distributions shown in Fig. 1. As mentioned above, it is likely that this variation was due in part to the lower disease pressure in CL07 compared to AU06 and AU07. Also, the use of different inoculation techniques and the fact that each environment was rated by a different individual may have been important contributing factors.

QTL Analysis
Since substantial environmental variation was observed, we chose to analyze each environment separately for the disease resistance traits as well as analyzing the BLUP values for each line to identify “overall” QTL. Most of the QTL identified were of moderate effect ($R^2 < 10\%$) and were environment-specific (Table 3). A WMD QTL in bin 4.08 was identified in all three environments as well as from the over-environment BLUP data. An environment-specific NLB resistance QTL was previously identified in a population derived from the maize lines Lo951 × CML202 (Welz et al., 1999a). Another WMD QTL in bin 2.00/01 was identified from the BLUP data and two of the three individual environments. To our knowledge, no NLB QTL have previously been reported in bins 2.00 or 2.01 (Wisser et al., 2006). Environment-specific NLB resistance QTL have been noted in a number of previous studies (Brown et al., 2001; Jiang et al., 1999; Welz et al., 1999a).

Despite the highly significant correlations between the WMD and IP traits in the IBM population, we did not detect any significant QTL that had a consistent effect on both traits. In AU07 we did detect a QTL for both IP and WMD in bin 2.01 with the resistance being derived from B73 in each case. There were also colocalizing IP QTL peaks at the major WMD QTL in bins 2.00/01 and 4.08. However, these peaks did not rise to the level of significance (LOD of 2.1 and 2.0, respectively) that would allow us to formally declare them QTL. Similarly, two WMD QTL peaks colocalized with the IP QTL in bins 4.05 and 6.05 but did not rise to the threshold level for significance (LODs of 2.5 and 2.1, respectively). For each of these loci, the allele conferring resistance as measured by these two traits was derived from the same parent. Taken together, all these data suggest that QTL in bins 2.00/01, 4.05, 4.08, and 6.05 likely have pleiotropic effects on both IP and WMD.

Only one IP QTL, in bin 2.02, was identified in all three environments and in the across-environment analysis (Table 3). However, even this QTL did not precisely colocalize in each case. A QTL affecting DTA BLUP values.

Table 1. Pearson correlation coefficients between weighted mean disease (WMD) northern leaf blight ratings, incubation period (IP), and days to anthesis (DTA) for the maize IBM population obtained in three environments (Aurora 2006 [AU06], Aurora 2007 [AU07], and Clayton 2007 [CL07]).

<table>
<thead>
<tr>
<th></th>
<th>CL07WMD</th>
<th>CL07IP</th>
<th>CL07DTA</th>
<th>AU07WMD</th>
<th>AU07IP</th>
<th>AU07DTA</th>
<th>AU06WMD</th>
<th>AU06IP</th>
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<td>CL07DTA</td>
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<td></td>
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<td>AU07IP</td>
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<td>-0.03**</td>
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<td>AU07DTA</td>
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<td></td>
<td></td>
<td>0.67†</td>
<td></td>
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<td>AU06WMD</td>
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<td></td>
<td>0.63†</td>
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<tr>
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<td>0.67†</td>
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<tr>
<td>AU06DTA</td>
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<td></td>
<td></td>
<td></td>
<td>0.59†</td>
<td></td>
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</table>

*Significant at $P < 0.05$.
**Significant at $P < 0.01$.
†Significant at $P < 0.0001$.

Table 2. Variance component estimates and standard errors for weighted mean disease (WMD) and incubation period (IP) for northern leaf blight resistance and days to anthesis (DTA) assessed in the intermated B73 × Mo17 (IBM) population.†

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WMD</th>
<th>P value</th>
<th>DTA</th>
<th>P value</th>
<th>IP</th>
<th>P value</th>
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</thead>
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<tr>
<td>Environment</td>
<td>470.69 (472.27)†</td>
<td>NS</td>
<td>2.11 (2.49)</td>
<td>NS</td>
<td>3.65 (5.12)</td>
<td>NS</td>
</tr>
<tr>
<td>Replication within</td>
<td>2.75 (2.06)</td>
<td>NS</td>
<td>0.70 (0.52)</td>
<td>NS</td>
<td>3.10 (2.22)</td>
<td>NS</td>
</tr>
<tr>
<td>Line</td>
<td>49.32 (5.79)</td>
<td>&lt;0.0001</td>
<td>4.81 (0.50)</td>
<td>&lt;0.0001</td>
<td>8.27 (1.00)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Environment by line</td>
<td>35.80 (3.45)</td>
<td>&lt;0.0001</td>
<td>0.23 (0.20)</td>
<td>NS</td>
<td>3.83 (0.62)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Residual</td>
<td>40.07 (1.77)</td>
<td>&lt;0.0001</td>
<td>5.54 (0.24)</td>
<td>&lt;0.0001</td>
<td>12.70 (0.55)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

†WMD, weighted mean disease; DTA, days to anthesis; NS, not significant.
‡Parenthetical values are the standard error.
was also detected in this region. Maturity has been correlated with disease resistance in many cases (see Wisser et al., 2006), but in this case the phenotypic correlations between IP and DTA were quite low (Table 1). It seems unlikely, therefore, that the same gene is regulating IP and DTA, especially because the traits were measured at very different stages of development; however, it should be noted that flowering time is to some extent determined early in development (Kiniry et al., 1983; Tollenaar and Hunter, 1983). As expected, the strongest QTL for DTA-BLUP was detected in bin 8.05, which corresponds to the position of the \textit{vgt1} flowering time gene (Salvi et al., 2002). We have detected this QTL in several of our previous studies (Balint-Kurti et al., 2006, 2007, 2008).

The lack of correspondence between IP and WMD QTL was somewhat unexpected. Incubation period has previously been identified as a useful trait for the early screening of NLB resistance and it was shown to correlate

<table>
<thead>
<tr>
<th>Trait†</th>
<th>Chr.‡</th>
<th>Bin§</th>
<th>2-LOD interval¶</th>
<th>LOD#</th>
<th>R² (%)††</th>
<th>A‡‡</th>
<th>Flanking markers §§</th>
</tr>
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<td>BLUPWMD</td>
<td>2</td>
<td>2.00–2.01</td>
<td>0–21.8</td>
<td>4.1</td>
<td>6.7</td>
<td>2.28</td>
<td>isu053a-isu144a</td>
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<td>4</td>
<td>4.08</td>
<td>449.2–456.9</td>
<td>3.12</td>
<td>4.3</td>
<td>–1.23</td>
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<td>1</td>
<td>1.06</td>
<td>534–546</td>
<td>7.26</td>
<td>10.2</td>
<td>4.2</td>
<td>isu053a-isu144a</td>
</tr>
<tr>
<td>AU06WMD</td>
<td>2</td>
<td>2.00–2.01</td>
<td>0–23.8</td>
<td>3.45</td>
<td>4.6</td>
<td>2.79</td>
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<tr>
<td>AU06WMD</td>
<td>3</td>
<td>3.05</td>
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<td>3.78</td>
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<td>2.92</td>
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<td>727.2–732.5</td>
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<td>5.3</td>
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<tr>
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<td>437.5–449.4</td>
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<td>0–27.4</td>
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<td>4</td>
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<td>449.2–455.9</td>
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<td>–1.06</td>
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<td>3.9</td>
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<td>480.5–502.9</td>
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<td>3.9</td>
<td>0.39</td>
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<td>8.05</td>
<td>374.5–379.2</td>
<td>4.32</td>
<td>5.3</td>
<td>–0.47</td>
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<tr>
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†For each trait, the environment in which it was measured (BLUP, AU06, AU07 or CL07) is indicated, followed by the actual trait measure (WMD, IP, or DTA). IP, incubation period; WMD, weighted mean disease.

‡Chromosome on which the QTL is located.

§Chromosome bin location of QTL peak on 1 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). The marker order determined for the population used in this experiment largely follows the marker order shown in the standard maize genetic map (the IBM map).

¶The positions that define the two LOD interval around the position of peak likelihood for the QTL. All values are in IBM map units (Imu) and are based on the IBM2 map.

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

††\( R^2 \) estimates the proportion of phenotypic variance (%) explained by the detected QTL. QTL identified with an \( R^2 \) value of 0.05 or higher are shown in italic.

‡‡The additive effect of the QTL. For disease ratings this is in terms of the diseased leaf area scale employed. For days to anthesis this is terms of days. For WMD a positive number indicates that the allele for resistance was derived from B73. For IP a positive number indicates the allele for resistance was derived from Mo17. For DTA a positive number means the allele for late anthesis was derived from B73.

§§The closest molecular markers flanking the 2-LOD interval.
with overall field resistance in both this and other studies (Smith and Kinsey, 1993; Welz and Geiger, 2000). Incubation period and WMD QTL for NLB resistance have been shown to colocalize in other studies (Schechert et al., 1999; Welz et al., 1999a).

**QTL for Multiple Disease Resistance**

Southern leaf blight, GLS, and NLB are all foliar and (at least to some extent) necrotrophic pathogens of maize. *Cochliobolus heterostrophus* (causal agent of SLB) typically penetrates the maize leaf directly through the cuticle at junctions between epidermal cells, though stomatal penetration has been occasionally observed (Hilu and Hooker, 1964, 1965; Jennings and Ulstrup, 1957; Knox-Davies, 1974). *Cercospora zeae-maydis* (causal agent of GLS) enters the leaf through stomata (Beckman and Payne, 1982). For both pathogens, initial growth is intercellular (Beckman and Payne, 1982; Jennings and Ulstrup, 1957; Toth and Smith, 1982; Wheeler, 1977). In contrast, *E. turcicum* (causal agent of NLB) usually penetrates the epidermal cell directly and growth of *E. turcicum* is mostly intracellular (Knox-Davies, 1974). While *C. heterostrophus* and *C. zeae-maydis* parasitize the chlorenchyma and do not invade the vasculature, the hyphae of *E. turcicum* grow intracellularly in the mesophyll and invade the xylem vessels and tracheids (Jennings and Ulstrup, 1957). For *C. zeae-maydis*, and to a lesser extent *C. heterostrophus*, hyphal growth in the leaf is limited by the vascular system, resulting in the rectangular lesions that are characteristic of GLS. The typical NLB wilting lesions observed on susceptible maize lines are likely a result of xylem plugging (Jennings and Ulstrup, 1957). Wilting might also result from tissue collapse that occurs when *E. turcicum* hyphae grow out from the xylem vessels into the surrounding bundle sheath and chlorenchyma (Hilu and Hooker, 1964). *C. zeae-maydis* typically grows for 2 to 3 wk in the leaf before symptoms are observed, while the latent period (the period from inoculation to lesion development) is typically much shorter for *E. turcicum* and *C. heterostrophus* infections (usually 3–6 d).

The available information on pathogen development therefore suggests that SLB is the most necrotrophic of these diseases. Gray leaf spot and NLB, while their modes of pathogenesis are somewhat different, might both arguably be considered to be hemibiotrophic diseases due to the extended latent period in the case of GLS and due to the intracellular growth of the hyphae in living cells in the case of NLB. However, all the diseases share certain aspects of their pathogenesis strategies (e.g., they all penetrate the leaf and initially grow in living tissue) and ultimately derive their nutrition from dead tissue. One can hypothesize that an allele in maize that has an effect on one of these shared strategies might confer resistance to more than one of the diseases.

Compared to our previous studies using the IBM population to identify QTL for resistance to SLB and GLS (Balint-Kurti et al., 2007, 2008), the NLB QTL we detected were of generally lower effect and were more environmentally sensitive. It appears that there were simply fewer large-effect QTL for NLB resistance segregating in the IBM population. In fact the IBM population was not ideally suited to the mapping of NLB QTL as the two parents, Mo17 and B73, differed little for NLB resistance (Fig. 1). Comparatively, they differ quite substantially with regard to SLB and GLS resistance (Balint-Kurti et al., 2007, 2008). For all three diseases however, Mo17 is more resistant than B73.

We had previously reported that BLUPs for SLB and GLS WMD measured in the IBM population were correlated with a correlation coefficient of 0.42 (Balint-Kurti et al., 2008). We can now add that in the IBM population (for the environments studied) BLUPs for NLB WMD are significantly correlated with the BLUPs for WMD for the other two diseases though at a lower level (Pearson correlation coefficient with SLB = 0.16, with GLS = 0.26, see Table 4). This implies that there are loci (and possibly genes) conferring multiple disease resistance to these three diseases in this population. However neither of the NLB WMD QTL we identified in this study colocalize with any of the SLB or GLS WMD QTL reported previously (Balint-Kurti et al., 2007, 2008). We previously noted that among the GLS and SLB QTL for WMD, only a single QTL (in bin 2.04) colocalized.

We conclude that, at least in the IBM population, correlations between resistances to SLB, GLS, and NLB are likely caused either by alleles that confer high levels of resistance to one disease and lower levels to others (whose effects are undetectable by QTL analysis), or by alleles that confer low—again, undetectable by QTL analysis—levels of resistance to two or more diseases. In this latter case, it could be that genes conferring resistance to the different diseases are distinct but tend to be clustered at specific loci. In a synthesis of published studies, Wisser et al. (2006) noted the statistical clustering of maize QTL for resistance to different diseases. In this case it was not possible to determine whether this was due to the pleiotropic effects of single genes or to the clustering of genes that gave resistance to single diseases.

**Table 4. Pearson correlation coefficients between weighted mean disease best linear unbiased predictors for northern leaf blight (NLB), southern leaf blight (SLB), and gray leaf spot (GLS) resistance measured in the intermated B73 × Mo17 population (IBM population). Data were derived from this and two previous studies (Balint-Kurti et al., 2007, 2008). For each pairwise comparison data was available for 286 to 289 of the 302 lines in the IBM population.**

<table>
<thead>
<tr>
<th></th>
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<th>GLS</th>
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<tr>
<td>GLS</td>
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<tr>
<td>SLB</td>
<td>0.16**</td>
<td>0.42†</td>
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</table>

*Significant at P < 0.01;
†Significant at P < 0.0001.
Our hope when we embarked on this work was (i) to determine whether there was evidence for genes or loci conferring multiple disease resistance to these three diseases, (ii) to elucidate the basis of this multiple disease resistance, and (iii) to identify some multiple disease resistance loci that might be of use to maize breeders. While some progress was made on the first two goals, no multiple disease resistance alleles conferring resistance to all three diseases were identified, though one QTL allele in bin 2.04 was found to confer resistance to both GLS and SLB (Balint-Kurti et al., 2008). If it is indeed true that multiple disease resistance tends to be based on many small-effect genes, it will be difficult to deploy marker-assisted selection to breed for this trait.

This paper represents one of the few studies in maize in which a single population has been used to assess resistance to multiple diseases. In a maize mapping population derived from a cross between a highland inbred and a lowland inbred, Jiang et al. (1999) found no positional correspondences between QTL identified for NLB, SLB, and common rust [caused by Puccinia sorghi (Schwein)]. Considering only the QTL detected in both years of their study of the cross IL731a × W6786, Brown et al. (2001) suggested that QTL for NLB, common rust, and Stewart’s wilt were unlinked. In contrast, Kerns et al. (1999) found 21 QTL and 14 QTL, associated with resistance to common rust and common smut [caused by Ustilago maydis (DC.)], respectively, nine of which colocalized. Welz et al. (1999b) mapped resistance to four diseases {head smut [caused by Sphacelotheca reilana (Kühn)]}, common smut, common rust, and NLB} in the same population. They found strong evidence for the association of loci for resistance to NLB, head smut, and common rust (but not common smut).

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References


