Published May, 1998

# Mapping of Disease Resistance Loci in Barley on the Basis of Visual Assessment of Naturally Occurring Symptoms

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## ABSTRACT

**Using field-scored data of disease severity under natural infestation, we mapped loci affecting resistance to powdery mildew (*Blumeria graminis* DC f. sp. *hordei* Èm. Marchal), leaf rust (*Puccinia hordei* Otth.), stem rust (*Puccinia graminis* f. sp. *tritici* Eriks. & E. Henn.), scald [*Rhynchosporium secalis* (Oudem.) J.J. Davis], and net blotch (*Pyrenophora teres* Drechs.). The mapping population included parents and doubled-haploid progeny of the two-row barley cross Harrington/TR306. Resistance was affected by two to five loci, explaining 8 to 45% of the phenotypic variance, per disease. All chromosomes, except chromosome 5 (1H), contained regions with at least one disease resistance locus. One region on chromosome 4 (4H) contributed to resistance to stem rust, scald, and net blotch. This region has previously been reported to affect days to heading and maturity. Two known resistance genes in the population, *Rpg1* and *Mlg*, were mapped to within 3 centimorgans (cM) of their previously estimated genomic locations by simple interval mapping of the field-scored data. This indicates that the genomic positions of disease resistance genes can be estimated accurately with simple interval mapping, even on the basis of field-scored data.**

Genetic resistance is an ecologically and economically sound approach to disease control in crops and is a common and important objective of barley (*Hordeum vulgare* L.) breeding. Breeders and pathologists select plants or lines with complete or partial disease resistance. They commonly make selections based on the results of artificially inoculated trials, on visual assessments of naturally occurring disease symptoms, or both. Plants or lines may be qualitatively classified as either resistant or susceptible. The examination of disease response may also involve the quantitative assessment of continuous variation in plant response to disease infestation.

Both qualitative and quantitative data may be used to map resistance loci relative to molecular and/or morphological markers in plant genomes. With qualitative data, classical linkage mapping may be used to map genes with major effects on disease resistance. With quantitative data, quantitative trait locus (QTL) analysis may be used to detect chromosome regions that contribute to disease resistance.

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Published in Crop Sci. 38:843–850 (1998).

Numerous disease resistance genes with major effects have been mapped in the barley genome by classical linkage analysis. For example, Abbott et al. (1995) and Graner and Tekauz (1996) mapped scald resistance genes and Jin et al. (1993) and Borovkova et al. (1997) mapped leaf rust resistance genes using this approach. QTL analysis has also been used to study resistance to several barley diseases, including powdery mildew and scald (Thomas et al., 1995; Backes et al., 1995), stripe rust (*Puccinia striformis* Westend.) (Hayes et al., 1996), and both net and spot blotch [*Cochiobolus sativus* (Ito and Kurib.) Drechs. ex Dastur.] (Steffenson et al., 1996).

A set of doubled haploid (DH) lines derived from the cross between Harrington and TR306 barley (the H/T population) was used to construct a marker map (Kasha et al., 1995). That map includes three disease resistance loci (d*Mlg*, d*Rpg1*, and d*run8*) which were mapped on the basis of a qualitative assessment of the disease reaction after inoculation with specific races of the powdery mildew, stem rust, and loose smut [*Ustilago tritici* (Pers.) Rostr.] pathogens. On the basis of additional inoculated trials, Falak (1994, unpublished data) concluded that there were two loci affecting resistance to powdery mildew in the H/T population: *Mlg* and one which was designated *Ml(TR)*. Both classical linkage analysis and QTL analysis (unpublished) confirmed the previously mapped position of *Mlg* and placed *Ml(TR)* near the RFLP marker MWG632 on chromsome 7(5H). Sato et al. (1996) inoculated seedlings of the H/T population with four isolates of *Pyrenophora teres* and mapped several QTL affecting net blotch resistance.

To map QTL affecting agronomic performance (Tinker et al., 1996) and grain and malt quality (Mather et al., 1997), the H/T population was grown in many field environments in North America. In some of these environments, the severity of one or more naturally occurring diseases was visually assessed. Here, we report on the use of the resulting data to map loci conditioning resistance to powdery mildew, leaf rust, stem rust, scald, and net blotch.

## MATERIALS AND METHODS

A population of 150 $F_1$-derived DH progeny from the H/T cross was previously used to map over 200 segregating RFLP and other marker loci (Kasha et al., 1995). For some of the DH lines, there were suspected errors in data acquisition, so subsets of DH lines were used in data analyses (146 for marker mapping, 145 for QTL analysis). Analysis of QTL was performed with a base map that consisted of 127 markers (Tinker et al., 1996).

### Field Experiments

Trials were planted in 30 environments (17 locations in 1992, and/or 1993) and local agronomic and variety testing

**Abbreviations:** QTL, quantitative trait locus or loci; QTL×E, QTL × environment; DH, doubled haploid; H/T population, the Harrington/TR306 barley population.

methods were used. One randomized complete block of parents and DH progeny was grown in 1992, and two such blocks in 1993. In 12 of those environments (Table 1), cooperating researchers assessed and recorded disease severity for one or more of the following naturally occurring diseases: powdery mildew, leaf rust, stem rust, scald, and net blotch. All diseases except leaf rust were scored on a whole-canopy plot basis with ordinal rating scales related to percent disease severity. Most data were in 0-to-9 or 1-to-9 rating scales, with the lowest rating corresponding to the absence of symptoms. For leaf rust, disease severity was assessed on 10 to 15 leaves per plot (flag leaves in 1992 and penultimate leaves in 1993) and averaged. Prior to QTL analyses, ratings from all environments and for all diseases were converted into percent disease severity per plot.

### Statistical Analysis

Analysis of QTL, performed with the software package MQTL (Tinker and Mather, 1995b), consisted of four steps: (i) performing interval mapping to find evidence of QTL, (ii) estimating thresholds for inferring QTL presence, (iii) inferring the presence of QTL and estimating their positions, and (iv) estimating the additive effects at putative QTL. Genome-wide QTL searches were performed by simple interval mapping (Tinker and Mather, 1995a), testing for QTL main effects and QTL×E interactions. QTL were declared at positions where peaks were significant for QTL main effects and/or QTL×E interaction. Significance thresholds were estimated separately for each disease by 5000 permutations (Churchill and Doerge, 1994) of the original percent disease severity data, maintaining the genome-wise Type I error rate below 5%. Such tests of significance require no assumptions of distribution and provide good control of Type I error for simple interval mapping, even with differing environmental variance.

Main effects and QTL×E interactions were estimated in multi-locus linear models. Each estimated main effect corresponded to the average difference between homozygous classes for a given QTL. We estimated reduction in variance ($R^2$) relative to a model that included only the environmental main effects for three models: (i) QTL main effects, (ii) QTL main effects and QTL×E interactions, and (iii) effects for 51 markers distributed approximately evenly throughout the genome (Tinker et al., 1996), estimated separately by environment (but with no terms for specific QTL or for QTL×QTL interaction). In the absence of epistasis, a model containing markers representing all regions of the genome should explain most of the genetic variance. The percentage of variance explained by such a model can be considered an estimate of heritability (Tinker et al., 1996). For agronomic traits in Harrington/TR306, Tinker et al. (1996) reported that heritabilities estimated in this manner were similar to those obtained from analyses of variance of data from replicated field experiments.

Following the detection of QTL, we tested for epistasis in multi-QTL linear models using a comparison-wise significance level of 0.01. Allelic classification at the marker, or marker interval, of detected QTL were employed as dependent variables for linear models of percentage disease severity (independent variable) on QTL and QTL interaction(s) (dependent variables). Where QTL interactions were significant, we inferred the presence of epistasis.

At some environments the lines could be readily classified into distinct resistant (no disease symptoms) and susceptible (some disease symptoms) classes. Where there were such evident phenotypic classes, or where the data classified parents into resistant or susceptible groups, we conducted $\chi^2$ goodness-of-fit tests for 1:1, 3:1 and 7:1 phenotypic ratios; corresponding to expectations for one-, two- and three-gene models, respectively. Where possible, heritability ($h^2$) was estimated by analysis of variance as the proportion of phenotypic variance attributable to the DH lines.

**Table 1. West to east listing of environments where disease severity was recorded for the Harrington/TR306 barley cross.**

| Code† | Location | Latitude | Longitude |
|---|---|---|---|
| WA93 | Pullman, WA | 46°46′ N | 117°09′ W |
| AB92c | Edmonton, AB | 53°34′ N | 113°25′ W |
| MB92, MB93 | Brandon, MB | 49°50′ N | 99°57′ W |
| ON92a, ON93a | Ailsa Craig, ON | 43°08′ N | 81°34′ W |
| ON92b, ON93b | Elora, ON | 43°42′ N | 80°26′ W |
| ON92c, ON93c‡ | Elora, ON | 43°42′ N | 80°26′ W |
| PE92, PE93 | Charlottetown, PE | 46°14′ N | 63°09′ W |

† For consistency with Tinker et al. (1996) and Mather et al. (1997), environments are coded as follows: uppercase letters identify the Canadian province or U.S. state, numerals identify the year (1992 or 1993), and lowercase suffixes distinguish multiple sites within the same Canadian province.

‡ Two separate trials were grown at the same location (ONb and ONc).

## RESULTS AND DISCUSSION

### Powdery Mildew

In the two environments where powdery mildew severity was recorded, the mean disease severity ($\hat{\mu}$) was only 4% and neither parent exhibited any symptoms (Table 2). Nevertheless, QTL analysis revealed significant peaks on chromosomes 4 (4H) and 7 (5H) (Fig. 1; Table 3), both corresponding to loci that Falak (1994, unpublished data) detected on the basis of inoculated

**Table 2. Disease severity means for Harrington and TR306, and means, distributions and heritabilities ($h^2$)† of double-haploid lines from the Harrington/TR306 barley population, presented separately by environment.**

| | Doubled-haploid lines | | | | | Mean of Parents | |
|---|---|---|---|---|---|---|---|
| Environments | $\hat{\mu}$ | $\hat{\sigma}$‡ | Minimum | Maximum | $h^2$ | Harrington | TR306 |
| | % | | | | | | |
| **Powdery mildew severity** | | | | | | | |
| ON92a | 4 | 9 | 0 | 44 | | 0 | 0 |
| ON92b | 4 | 10 | 0 | 56 | | 0 | 0 |
| **Leaf rust severity** | | | | | | | |
| ON92c | 60 | 9 | 32 | 82 | | 67 | 39 |
| ON93c | 70 | 9 | 56 | 83 | 6 | 78 | 64 |
| **Stem rust severity** | | | | | | | |
| WA93 | 33 | 29 | 3 | 75 | 34 | 39 | 23 |
| ON92a | 16 | 17 | 0 | 56 | | 17 | 17 |
| ON93a | 36 | 18 | 6 | 78 | 47 | 56 | 33 |
| **Scald severity** | | | | | | | |
| AB92c | 1 | 3 | 0 | 23 | | 0 | 0 |
| ON92b | 6 | 13 | 0 | 56 | | 0 | 11 |
| PE92 | 18 | 18 | 0 | 63 | | 38 | 13 |
| PE93 | 6 | 8 | 0 | 25 | 20 | 6 | 13 |
| **Net blotch severity** | | | | | | | |
| AB92c | 31 | 28 | 3 | 75 | | 75 | 23 |
| MB92 | 81 | 23 | 25 | 100 | | 100 | 50 |
| MB93 | 89 | 20 | 38 | 100 | 60 | 100 | 50 |
| ON92a | 51 | 18 | 11 | 89 | | 78 | 33 |
| ON93a | 70 | 12 | 44 | 89 | 37 | 78 | 53 |
| PE92 | 58 | 15 | 25 | 88 | | 88 | 63 |
| PE93 | 77 | 12 | 50 | 100 | 63 | 88 | 69 |

† Estimated as the percentage of phenotypic variance attributable to DH lines in environments with more than one block.

‡ Phenotypic standard deviation. For environments with two blocks, $\hat{\sigma}$ was calculated after fitting a block effect.

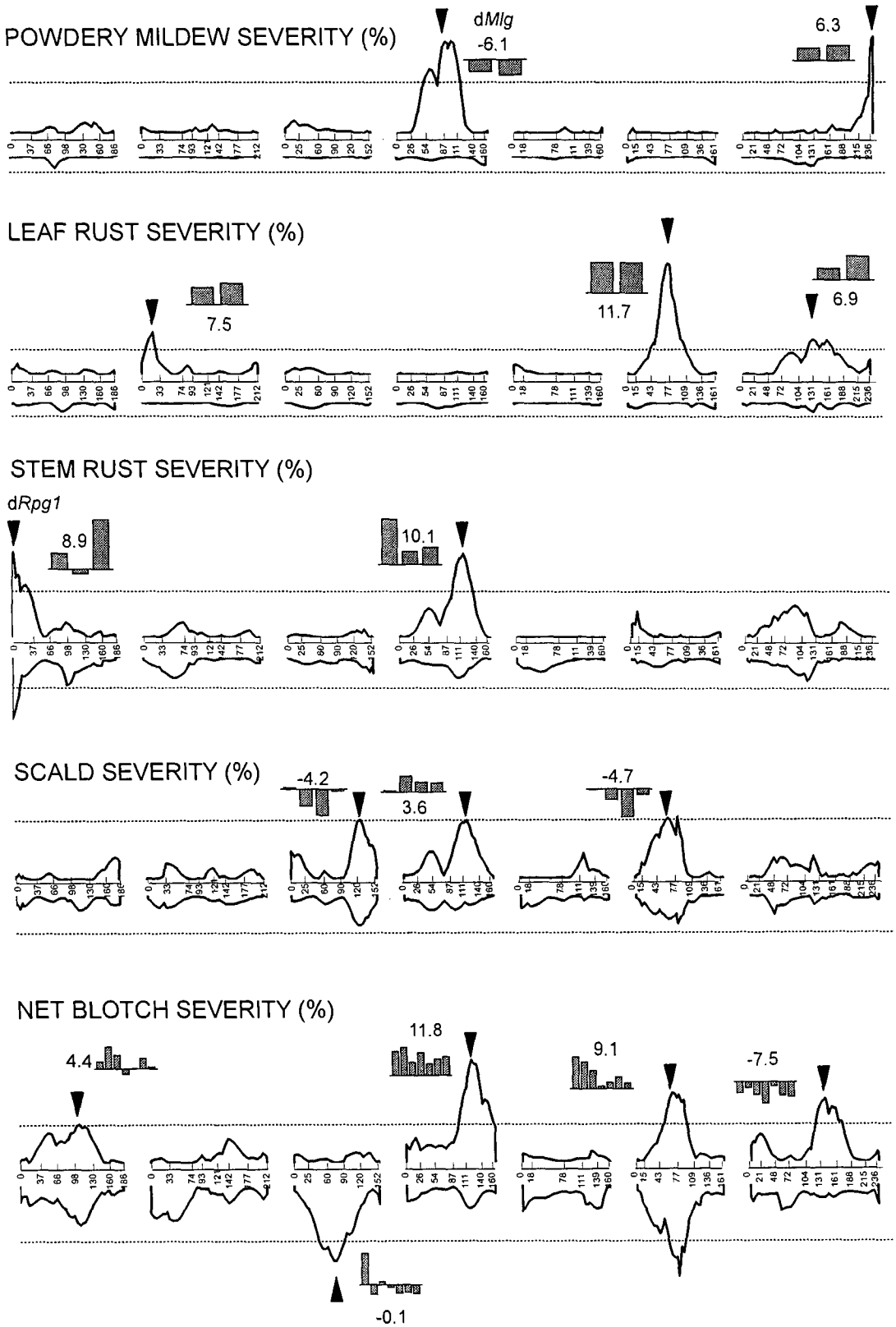


**Fig. 1. Scans of a test statistic for simple interval mapping for QTL main effects (above axes) and QTL by environment interactions (below axes). Scans are shown for disease severity (%) for five diseases as indicated. Barley chromosomes 1 (7H) to 7 (5H) are shown left to right, each oriented with the plus arm on the left. Horizontal scales show approximate centimorgan positions of background markers. Horizontal dashed lines show significance thresholds, estimated from 5000 permutations of the data to maintain the experiment-wise type-I error rate below 5%. Positions of QTL are shown by darkened triangles located at the peaks of the scans. Estimated QTL main effects (the average effect of substituting two Harrington alleles for two TR306 alleles) are shown as numbers beside the darkened triangles. Bar charts beside each QTL depict the relative magnitudes of estimated QTL effects for each environment (arranged west to east).**

**Table 3. Genomic positions of QTL for five barley diseases, detected through simple interval mapping of disease severity data within the Harrington/TR306 barley population.†**

| Disease | Chromosome number | QTL position‡ | Marker or marker interval |
|---|---|---|---|
| Powdery mildew | 4 (4H) | 87.2 | d*Mlg* |
| Powdery mildew | 7 (5H) | 235.7 | MWG632 |
| Leaf rust | 2 (2H) | 20.8 | ABG058-WG516 |
| Leaf rust | 6 (6H) | 73.9 | WG223 |
| Leaf rust | 7 (5H) | 131.1 | MWG914 |
| Stem rust | 1 (7H) | 0 | d*Rpg1* |
| Stem rust | 4 (4H) | 115.8 | ABG472-MWG655C |
| Scald | 3 (3H) | 124.8 | MWG2040 |
| Scald | 4 (4H) | 115.8 | ABG472-MWG655C |
| Scald | 6 (6H) | 60.2 | MWG916-WG223 |
| Net blotch | 1 (7H) | 107.7 | Vatp57A-MWG571D |
| Net blotch | 3 (3H) | 69.7 | *Ugp1*-ABG607 |
| Net blotch | 4 (4H) | 120.8 | ABG472-MWG655C |
| Net blotch | 6 (6H) | 70.2 | MWG916-WG223 |
| Net blotch | 7 (5H) | 136.1 | MWG914-*Ugp3* |

† The linkage map of the Harrington/TR306 population used in this study was reported by Tinker et al. (1996).
‡ Centimorgan (cM) positions are relative to the most distal marker on the 'plus' arm of the chromosome.

trials in the same population. The major peak on chromosome 4 (4H) coincided with the d*Mlg* locus, previously mapped (Kasha et al., 1995) on the basis of a single-pustule isolate inoculation on greenhouse-grown seedlings. The allele for resistance at this locus came from Harrington. The QTL analysis of our field data showed two additional significant peaks on either side of the d*Mlg* locus. It is possible that these peaks represent linked resistance genes, but including them in regression models did not result in any appreciable increase in the proportion of phenotypic variance explained.

The QTL detected on chromosome 7 (5H) corresponded to the position for which Falak (1994, unpublished data) proposed the gene designation *Ml(TR)*. Saghai Maroof et al. (1994) also reported a minor QTL effect in this region. The allele for powdery mildew resistance at this locus came from TR306.

Our results are similar to those reported by Backes et al. (1996) in a comparable study. They found that QTL for powdery mildew resistance, detected on the basis of ratings scored under natural field epidemics, mapped to the same chromosome region as QTL which were detected on the basis of symptom severity of detached primary leaves inoculated with a specific powdery mildew isolate.

Interaction between the two QTL was not significant when tested in linear models for data collected at ON92a. At ON92b, interaction between the two QTL was significant ($\alpha = 0.01$). Lines with resistance alleles at either one of the two QTL had mean severity levels of less than 2%, while lines with susceptibility alleles at both QTL had a mean severity level of 14% (Table 4). This indicates duplicate epistasis; the presence of either resistance allele was adequate to prevent symptoms under low natural disease pressure.

It is interesting that using only simple rating scale data from uninoculated field experiments, we were able to confirm the same two loci that had previously been mapped on the basis of data collected in the inoculated experiments conducted by Falak (1994). In fact, the data from either one of our two field locations were sufficient to detect and map *Mlg* and *Ml(TR)* (Fig. 2). This is particularly surprising for the ON92a data, for which the mean disease severity of the lines whose marker genotypes indicate that they carry the *Mlg* and/or *Ml(TR)* resistance alleles was 3%, compared with the same 3% for those lines with neither resistance allele. Such results indicate both the power and accuracy of QTL analysis, even when field-scored rating data with seemingly little underlying classification structure is used.

## Leaf Rust

Symptoms were severe in the two environments where leaf rust was evaluated (Table 2). TR306 had lower severity levels than Harrington in both environments. Disease severity data could not be classified into any simple phenotypic ratios. There were significant QTL peaks on chromosome 2 (2H), 6 (6H) and 7 (5H) (Fig. 1; Table 3). We found no QTL×E interaction for leaf rust. At all three QTL, the allele for leaf rust resistance came from TR306.

The QTL peak on chromosome 7 (5H) was part of a large bimodal peak that could represent linked resistance genes. Regression models indicated that most phenotypic variance in this region was explained by the peak at 131.1 cM. Main effects of the three putative QTL explained 45% of the phenotypic variation (Table 5, Model 1).

Interaction between the QTL on chromosome 2 (2H) and 6 (6H) was significant in both environments. The presence of TR306 alleles at both QTL resulted in the lowest symptom severity in both environments (Table 4). At ON93c, there was a substantial reduction in symptom severity only when TR306 alleles were present at both QTL. On lines with only one TR306 allele, symptoms were almost as severe as on lines with Harrington alleles at both QTL. At ON92b, each of the TR306 alleles had some effect individually, but the effect of having TR306 alleles at both QTL was greater than the sum of the individual effects. Thus, the interaction

**Table 4. Mean powdery mildew and leaf rust severity for genotypic classes at environments (ON92b, ON92c, ON93c) where QTL×QTL interactions were significant ($P < 0.01$).**

| Powdery mildew | | | Leaf rust | | | |
|---|---|---|---|---|---|---|
| Allele at QTL on chromosome 4 (4H) | Allele at QTL on chromosome 7 (5H) | Disease severity (ON92b) | Allele at QTL on chromosome 2 (2H) | Allele at QTL on chromosome 6 (6H) | Disease severity (ON92c) | Disease severity (ON93c) |
| | | % | | | % | |
| Harrington | Harrington | 0 | Harrington | Harrington | 68 | 74 |
| Harrington | TR306 | 0 | Harrington | TR306 | 59 | 71 |
| TR306 | Harrington | 14 | TR306 | Harrington | 66 | 73 |
| TR306 | TR306 | 1 | TR306 | TR306 | 49 | 64 |

between these two leaf rust QTL seems to involve synergistic action of the two TR306 alleles.

Jin et al. (1993) reported linkage between the incompletely dominant leaf rust resistance gene *Rph12* and the *s* locus (short rachilla hairs), with a recombination value of 39.5 ± 2.9%. The *s* locus is a morphological marker polymorphic in the H/T population (m*Srh* in Tinker et al., 1996), mapping at 109.5 cM on chromosome 7 (5H), or 21.6 cM from the QTL we found on chromosome 7 (5H). Several other leaf rust resistance genes have been mapped to barley chromosomes, including those coinciding with the locations of QTL reported here. Tuleen and McDaniel (1971) determined that *Rph1* is on chromosome 2 (2H). Feuerstein et al. (1990) reported that *Rph11* is linked with the isozyme loci *Acp3* and *Dip2* on chromosome 6 (6H).

## Stem Rust

In the three environments where stem rust severity was evaluated, the mean disease severity was low to moderate (Table 2). Symptoms were less severe on TR306 than on Harrington (WA93, ON93a) or were equally severe on the two parents (ON92a). Significant QTL peaks were detected on chromosomes 1 (7H) and 4 (4H) (Fig. 1).

The QTL on chromosome 1 (7H) was indicated by a significant main-effect peak at the 3-cM position and a significant QTL×E peak at the 0-cM position, coinciding with the d*Rpg1* locus that previously had been mapped at 0 cM of chromosome 1 (7H) (Kasha et al., 1995). This gene (the "T" gene) effectively protected North American barley cultivars from stem rust for over 50 yr. In 1989, a new pathotype (Pgt-QCC), against which *Rpg1* shows little resistance, became established throughout the Great Plains (Steffenson, 1992). Fox and Harder (1995) reported that genes with minor effects may augment *Rpg1* resistance and the quantitative character of *Rpg1* resistance may vary with pathotype.

Effects of the *Rpg1* locus differed among environments. The allele from TR306 contributed strongly to resistance at ON93a, but had no effect at ON92a (Fig. 1). The resistance allele for the QTL on chromosome 4 (4H) was contributed by TR306. There was no significant QTL×E interaction at this peak. Tests for epistasis uncovered no significant interaction between the two reported QTL.

**Table 5. Average percentages of within-environment phenotypic variance for disease severity explained by models of QTL effects and interactions.**

| Disease | Model† 1 | 2 | 3 |
|---|---|---|---|
| | % | | |
| Powdery mildew | 20 | 20 | 47 |
| Leaf rust | 45 | 46 | 67 |
| Stem rust | 11 | 19 | 52 |
| Scald | 8 | 15 | 45 |
| Net blotch | 21 | 29 | 55 |

† Model 1, QTL main effects for chromosome positions where QTL were detected; Model 2, QTL main effects and QTL × environment interaction effects for chromosome positions where QTL were detected; Model 3, Main effects and interactions with environment for 51 marker loci distributed approximately evenly throughout the genome.

## Scald

In the four environments where scald severity was evaluated, the mean disease severity was low (Table 2). Neither parent exhibited symptoms in AB92b and only TR306 exhibited symptoms in ON92b. In PE92, Harrington exhibited more severe symptoms than TR306, while the opposite occurred in PE93. There were signifi-

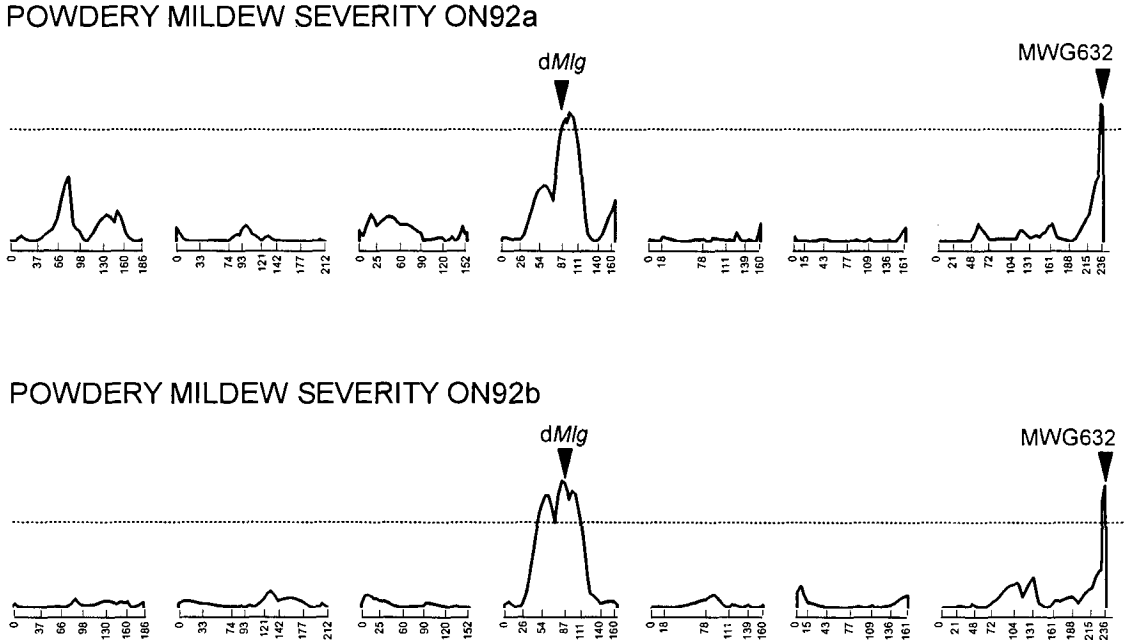


**Fig. 2. Scans of a test statistic for simple interval mapping for QTL main effects for powdery mildew severity at the two environments ON92a and ON92b. Barley chromosomes 1 (7H) to 7 (5H) are shown left to right, each oriented with the plus arm on the left. Horizontal scales show approximate centimorgan positions of background markers. Horizontal dashed lines show significance thresholds, estimated from 1000 permutations of the data to maintain the experiment-wise Type I error rate below 5%. Positions of the previously estimated positions of *Mlg* and *MI(TR)* are shown by darkened triangles.**

cant QTL peaks on chromosome 3 (3H), 4 (4H) and 6 (6H) (Fig. 1; Table 3). The peak on chromosome 3 (3H) was at 124.8 cM, on the minus arm, with the resistance allele contributed by Harrington. Other studies have reported scald resistance genes near the centromere on the same chromosome arm (Barua et al., 1993; Hayes et al., 1996; Graner and Tekauz, 1996). Thomas et al. (1995), using QTL analysis, located scald resistance loci on two regions of chromosome 3 (3H).

TR306 contributed the allele for resistance at the peak on chromosome 4 (4H). The third QTL mapped to 60.2 cM on chromosome 6 (6H) near MWG916, in the vicinity of *Rrs13* (Abbott et al., 1995), with the resistance allele contributed by Harrington. QTL main effects accounted for only 8% of phenotypic variation (Table 5), indicating much unexplained quantitative variation. Tests for epistasis uncovered no significant interaction between the three QTL in any environment.

## Net Blotch

In the seven environments where net blotch symptoms were evaluated, the mean symptom severity was moderate to severe, ranging from 31% (AB92c) to 89% (MB93) (Table 2). Main-effect QTL peaks occurred on chromosome 1 (7H), 4 (4H), 6 (6H), and 7 (5H) (Fig. 1; Table 3). Resistance alleles at the QTL on chromosome 1 (7H), 4 (4H), and 6 (6H) were contributed by TR306 and the resistance allele at the QTL on chromosome 7 (5H) was contributed by Harrington. Both QTL main effects and QTL×E interaction were significant in the region on chromosome 6 (6H). Here, the QTL contributed to resistance mainly in the three western environments (AB92c, MB92, and MB93), possibly reflecting pathotype-specific genetic resistance. The large QTL main effect on chromosome 4 (4H) was common and stable in all seven environments. A further QTL affecting net blotch resistance was detected on chromosome 3(3H). There was significant QTL×E interaction at this location, but no significant main effect (Fig. 1). At this locus, the TR306 allele contributed to resistance in the AB92c environment, with no apparent effect in other environments. Steffenson and Webster (1992) reported a number of pathotypes of the net blotch pathogen, and suggested that the severity of symptoms in the field may vary with differing combinations of resistance genes in the host and virulence genes in the pathogen.

Sato et al. (1996) mapped genes in the H/T population conferring partial resistance to net blotch, following artificial inoculation with two net- and two spot-form isolates. They reported significant LOD (log of the odds difference) scores for resistance to both net-form (K105 and WRS102) and one (WRS1566) spot-form isolate between markers MWG820 and MWG916, spanning the approximate centromere location on chromosome 6 (6H). The genome location and source of resistance alleles (TR306) coincide with one of the QTL we report here. Sato et al. (1996) also mapped a QTL conferring partial resistance to the spot-form isolate WRS1566 in the marker interval CDO504-MWG781 on the minus arm of chromosome 7 (5H). The resistance allele was contributed by TR306, whereas at the locus we detected in that region, the resistance allele came from Harrington. Sato et al. (1996) also mapped resistance loci on chromosome 1 (7H), but their positions are not close to the QTL we report here for that chromosome. Steffenson et al. (1996) used QTL analysis on inoculated lines of the Steptoe/Morex barley population and mapped adult net blotch resistance loci on all barley chromosomes except chromosome 5 (1H). On the basis of positions relative to common markers, the loci they reported do not seem to correspond with those found in our study. The results of this study indicate that net blotch resistance can be affected by many genes with minor effects.

## $\chi^2$ Goodness-of-Fit Tests

Where disease severity data could be grouped into evident phenotypic classes, we conducted $\chi^2$ goodness-of-fit tests for various genetic models. Such analyses offer a comparison between QTL mapping of field-scored disease data and traditional genetic analyses of classification data.

The ratio of lines with a powdery mildew severity level of no symptoms to those with severity levels greater than 0% was 123:22 at ON92a and 122:23 at ON92b. These observed ratios did not deviate significantly ($\alpha = 0.10$) from a 7:1 ratio expected for segregation of three resistance genes [$\chi^2 = 0.95$, $P = 0.33$ (ON92a); $\chi^2 = 1.50$, $P = 0.22$ (ON92b)]. Examination of the marker genotype data for the susceptible and resistant classes, however, gave no evidence of an additional gene. Lines within these classes had no markers which uniformly consisted of alleles from one parent.

For stem rust, at ON92a, where the two parents had similar stem rust severity (Table 2), the ratio of lines with no symptoms to those with symptoms was 65:80. This phenotypic classification fit a one-gene segregation ratio ($\chi^2 = 1.55$, $P = 0.21$), but it did not correspond to the genotypic classification at either the *Rpg1* locus or the marker interval for the QTL on chromosome 4 (4H). In WA93, mean severity on TR306, the resistant parent, was 23% (Table 2). The ratio of lines with less than 25% disease severity to those with 25% or more disease severity was 76:69, a one-gene segregation ratio ($\chi^2 = 0.34$, $P = 0.56$), but this phenotypic classification did not correspond to the genotypic classification at either the *Rpg1* locus or the marker interval for the QTL on chromosome 4 (4H). At ON93a, the ratio of lines with less than 25% disease severity to those with 25% or more disease severity was 37:108; a two-gene segregation ratio ($\chi^2 = 0.02$, $P = 0.96$). Here, all 37 lines with less than 25% disease severity carried the *Rpg1* resistance allele, but there was no discernable classification pattern for genotypic markers at the marker interval for the QTL on chromosome 4 (4H). It is possible that some lines with no symptoms may simply have escaped infection, making it difficult to detect genetic effects through conventional $\chi^2$ analyses.

For scald, segregation ratios at environments AB92c and ON92b for lines with no disease symptoms to those

with symptoms were 129:16 and 108:37, respectively. These data conform to a three-gene segregation ratio at AB92c ($\chi^2 = 0.29$, $P = 0.59$) and a two-gene ratio at ON92b ($\chi^2 = 0.02$, $P = 0.96$). These phenotypic classifications did not correspond to genotypic classification at flanking markers for the three QTL reported above. Here again, lines with no symptoms may have simply escaped infection, making it difficult to detect genetic effects through conventional $\chi^2$ analyses.

Conventional $\chi^2$ results thus corresponded rather poorly with the QTL results for powdery mildew, stem rust and scald. This is not surprising. The loci detected by QTL analysis did not confer complete resistance, and would not be expected to result in classical phenotypic ratios. Furthermore, there may have been disease escapes under low natural disease epidemics. For the type of data used here, $\chi^2$ tests provided little useful information, while QTL analysis permitted detection of both position and effect of loci affecting disease severity.

## GENERAL DISCUSSION

Three disease resistance loci were part of the original published marker map of the H/T population: d*Mlg* on chromosome 4 (4H) for powdery mildew, d*Rpg1* on chromosome 1 (7H) for stem rust, and d*run8* on chromosome 5 (1H) for loose smut resistance (Kasha et al., 1995). Here, d*Mlg* and d*Rpg1* were both mapped to within 3 cM of their previously reported genomic locations, as significant QTL. This indicates that the genomic positions of disease resistance genes can be estimated accurately with simple interval mapping, even on the basis of field-scored data.

We observed no simple monogenic control of field resistance for any of the five diseases studied. Disease severity varied quantitatively because of the presence of multiple resistance loci and substantial environmental variance. The number of QTL detected ranged from two to five per disease. For 12 of 15 QTL, the effects were reasonably constant across environments. For the remaining three loci, the effects were present in some environments and absent in others. TR306 contributed the resistance alleles at 11 of the 15 loci, including all rust resistance alleles. Both parents contributed alleles that increased resistance to powdery mildew, scald, and net blotch.

Averaged across environments, main effects of QTL accounted for proportions of phenotypic variance ranging from 8% for scald to 45% for leaf rust resistance (Table 5, Model 1). Interactions of QTL with environments accounted for an additional proportion of phenotypic variance for stem rust (8%), net blotch (8%) and scald (7%), but were not important for powdery mildew (0%) or leaf rust (1%) (Table 5, Model 2). For the five diseases, models that included 51 evenly distributed markers explained between 45 and 67% of the phenotypic variance (Table 5, Model 3). Thus the detected QTL and QTL×E interaction accounted for between 33% (scald) and 69% (leaf rust) of the apparent genetic variation (Table 5, Models 2 and 3). There may also be undetected QTL of small effect and/or interaction among QTL.

The 18 data sets of disease severity examined here included only seven from 1993, when field tests were replicated. Estimation of $h^2$ was therefore not possible for powdery mildew and was possible at only one (leaf rust, scald), two (stem rust), and three (net blotch) environments for the other diseases. In contrast to results reported by Tinker et al. (1996), where proportions of variance explained by the 51 evenly distributed markers were approximately equal to $h^2$ for many agronomic traits, the $h^2$ estimates we computed (Table 2) were generally less than the proportions of variance explained by the 51 markers. Our results, however, were based on one to three environments, while those reported by Tinker et al. (1996) were based on five to 13 environments.

Many research groups have now reported on some disease resistance QTL in barley mapping populations (e.g., Backes et al., 1995; Hayes et al., 1996; Thomas et al., 1995). The lack of common markers between studies and the differences in germplasm and environment make comparisons of common resistance QTL between studies difficult. Hayes et al. (1996) and Thomas et al. (1995) both mapped a scald resistance locus near the centromeric region of chromosome 3 (3H), not coinciding with the chromosome location we report for a scald resistance locus on that chromosome. Thomas et al. (1995) and Backes et al. (1995) all mapped a further scald resistance locus to the minus arm of chromosome 3 (3H), near a locus we report. Backes et al. (1995) mapped an additional scald resistance locus on chromosome 6 (6H), also near a locus we report. Powdery mildew QTL reported by Thomas et al. (1995) and Backes et al. (1995) do not coincide with the two QTL we report here.

We mapped QTL affecting scald, stem rust, and net blotch within the ABG472-MWG655C marker interval on chromosome 4 (4H) (110.8-124.8 cM), with resistance to all diseases conferred by TR306 alleles. TR306 alleles within this genomic region also contribute to higher soluble protein levels (Mather et al., 1997) and later days to heading and maturity (Tinker et al., 1996). On both chromosome 2 (2H) and 6 (6H), QTL detected for scald resistance were in the same region as QTL affecting heading date, with the Harrington alleles contributing to scald resistance and later heading. On chromosome 3(3H), a QTL affecting net blotch resistance was in the same region as one affecting heading date. Common genomic regions for disease resistance and late heading or maturity may reflect escape from disease caused by differential maturation.

On chromosome 1 (7H), TR306 contributed a resistance allele for stem rust (*Rpg1*) linked in coupling with a QTL allele for higher kernel weight (Tinker et al., 1996) and a resistance allele for net blotch linked in coupling with a QTL allele with favorable effects on kernel weight and test weight (Tinker et al., 1996). Similarly, on chromosome 7 (5H) the QTL for powdery mildew resistance [*Ml(TR)*] is conferred by the TR306 alleles linked in coupling for higher test weight. How-

ever, the same region contains important QTL for grain and malt quality traits, all with the favorable allele coming from Harrington (Mather et al., 1997).

While the genomic positions of QTL are presumably constant, the effects of QTL alleles may vary with environment. This becomes especially important in the study of disease resistance, as different pathotypes in different environments may affect resistance mechanisms. This study could not determine the cause of QTL×E interactions, but it demonstrates the importance of studying QTL effects in more than one environment.

## ACKNOWLEDGMENTS

In Canada, this research was funded by the Natural Sciences and Engineering Research Council of Canada. In the USA, this research was funded by the North American Barley Genome Mapping Project, with funds from USDA-CSRS, barley growers, and the malting and brewing industry. We thank the researchers of the North American Barley Genome Mapping Project for developing the Harrington/TR306 marker map.

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