Plant Pathology (2007) **56**, 934–942 Doi: 10.1111/j.1365-3059.2007.01680.x

Associations between fungal and abiotic leaf spotting and the presence of *mlo* alleles in barley

J. C. Makepeace[a], S. J. P. Oxley[b], N. D. Havis[b], R. Hackett[c], J. I. Burke[c] and J. K. M. Brown[a]*

[a]*Department of Disease and Stress Biology, John Innes Centre, Colney, Norwich NR4 7UH;* [b]*SAC, West Mains Road, Edinburgh EH9 3JG, UK; and* [c]*Teagasc, Crop Research Centre, Oak Park, Carlow, Ireland*

The hypothesis that the increased use of the powdery mildew-resistance gene *mlo* has caused the increase in spotting diseases of barley over the past 20 years was tested in field trials. Near-isogenic lines with alleles of the *Mlo* gene for susceptibility or resistance to mildew in two parental backgrounds were trialled at four sites in Scotland and two in Ireland that were prone to spotting diseases, over 3 consecutive years. Mildew was controlled by sprays with quinoxyfen. Disease levels were low in the trials, the two most important diseases being scald caused by *Rhynchosporium secalis* and ramularia leaf spot caused by *Ramularia collo-cygni*. There were high levels of abiotic spotting. Lines with mutant *mlo* alleles consistently developed less *Rh. secalis* and *Ra. collo-cygni*, but more abiotic spots. This study indicates that the *mlo* mildew-resistance gene has not alone been responsible for the rise in spotting diseases over the past 20 years. Possible reasons for the rise are discussed, including the interaction of the *mlo* gene with the environment.

Keywords: barley powdery mildew resistance, barley scald, *Hordeum vulgare*, *Ramularia collo-cygni*, ramularia leaf spot of barley, *Rhynchosporium secalis*

Introduction

In northern Europe in recent decades, there has been an increase in spotting diseases of spring barley caused by fungi. Data on disease trends in England and Wales have come from annual surveys by ADAS and the Central Science Laboratory (CSL), who surveyed spring barley between 1967 and 1980 and winter barley since then. Powdery mildew, caused by *Blumeria graminis*, was by far the most important disease of spring barley up to 1980 (data not shown). The subsequent decline in the importance of mildew on winter barley (Fig. 1) may be attributed to the use of *mlo* in spring barley, which acts as a source of inoculum for subsequent crops of winter barley, and to successful breeding for partial resistance to mildew in winter barley. Spotting pathogens, however, have become more significant on winter barley, particularly *Rhynchosporium secalis*, which causes scald or leaf blotch (Shipton *et al*., 1974; Beer, 1991), and *Pyrenophora teres*, which causes net blotch (Shipton *et al*., 1973) (Fig. 1). In a smaller dataset from Northern Ireland, powdery mildew was the major disease between 1976 and 1987, but scald became dominant by 2000 (Mercer & Ruddock, 2004). Data for Scotland, based on disease surveillance in commercial crops, show more severe incidences of scald in the late 1990s and early 2000s than in earlier years (Anonymous, 2006c).

Ramularia collo-cygni, which causes ramularia leaf spot (RLS), has recently become a serious pathogen, but there are few quantitative reports of its importance. However, since the disease was first recognized as a threat to barley production in the late 1980s, it has spread to become an important disease in most northern European countries (Pinnschmidt & Hovmoller, 2003).

It is very widely believed among barley workers that the level of necrotic spotting not accounted for by *Rh. secalis* or *P. teres* has increased greatly in the last 20 years (Oxley *et al*., 2002; Wu & von Tiedemann, 2002). There are few quantitative data on necrotic spotting of abiotic origin, although a high incidence (51% of surveyed fields affected) was recorded on spring barley in Northern Ireland in 2000, despite having only been recorded once before (in 1987) and even then with low mean severity (5%) (Mercer & Ruddock, 2004).

The *mlo* powdery mildew-resistance gene was discovered in 1942 in an Ethiopian barley line, Grannenlose Zweizeilige. It was named *mlo-11*, and along with other *mlo* alleles, is a non-functional allele of the *Mlo* gene (Jørgensen, 1992). The wild-type allele (Mlo^+) suppresses defence functions and attenuates the cell death response

*E-mail: james.brown@bbsrc.ac.uk

Accepted 16 April 2007

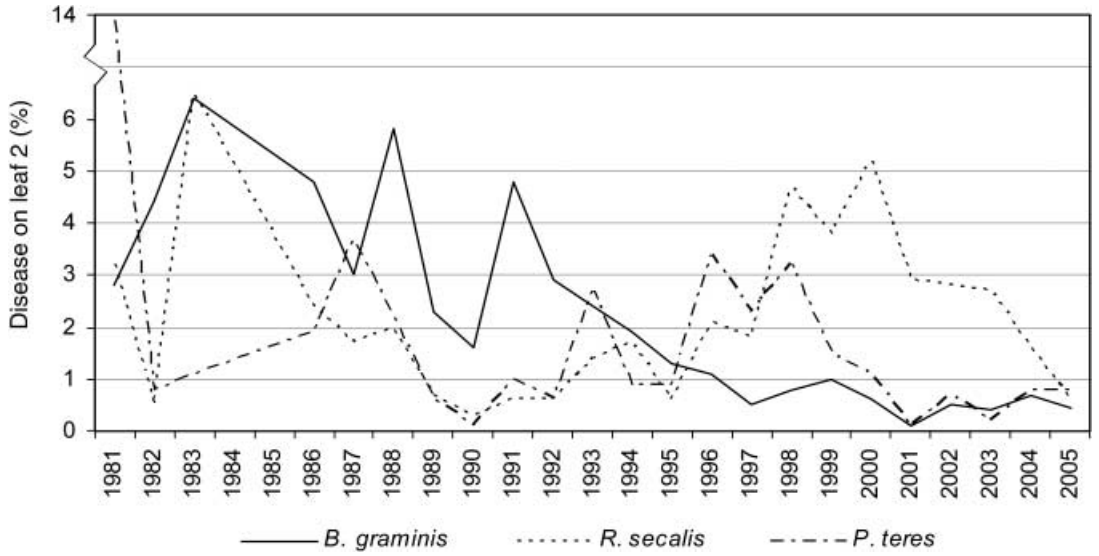

Figure 1 Mean severity of *Blumeria graminis*, *Rhynchosporium secalis* and *Pyrenophora teres* on second leaves of winter barley in the UK between 1981 and 1991. Sources of data: 1981–1991, Polley *et al.* (1993); 1992, Clarke (1992); 1993–1996, Hardwick *et al.* (1997); 1997–2001, Hardwick *et al.* (2001); and 2002–2005, Anonymous (2006a).

(Büschges *et al.*, 1997). Loss of *Mlo* function results in durable resistance to powdery mildew through earlier initiation of papilla and a greater rate of production of larger papillae than in wild-type barley (Skou, 1982; Bayles *et al.*, 1990; Yokoyama *et al.*, 1991).

A side effect of uncontrolled cell death in plants with *mlo* mutant alleles is an increase in abiotic spot formation, mainly on the adaxial surface of the upper two leaves, beginning after emergence of the second leaf (Oxley *et al.*, 2002), which results in reduced yield (Schwarzbach, 1976; Bjørnstad & Aastveit, 1990; Kjær *et al.*, 1990). However, a plant's genetic background also influences the spotting phenotype (Schwarzbach, 1976), so breeders have been able to produce high-yielding *mlo* cultivars which retain resistance to *B. graminis*, presumably by re-assorting 'background' genes which modify the effects of *mlo* (Schwarzbach, 1976; Bjørnstad & Aastveit, 1990; Kjær *et al.*, 1990; Thomas *et al.*, 1998; Hackett *et al.*, 2001; Brown, 2002). However the observed increase in abiotic spots described above is likely to be at least partly caused by the presence of *mlo* causing small necrotic lesions on leaves (Wu & von Tiedemann, 2004).

The resistance allele of *Mlo* most widely used in barley breeding is *mlo-11*. The first *mlo-11* cultivar, Atem, was released in the Netherlands in 1979, since when many *mlo-11* cultivars have become commercially significant. Of the alleles produced by mutagenesis, *mlo-9* is the only one used in spring barley cultivars. The first cultivar with *mlo-9* was Alexis, released in 1986 (Jørgensen, 1992). Since the release of Atem the popularity of *mlo* cultivars has increased, such that they now constitute over half the spring barley production area in the UK and most of northern continental Europe (Schwarzbach, 2006).

In laboratory experiments, the presence of mutant *mlo* alleles in backcross lines increased the susceptibility of spring barley to two facultative pathogens, *Magnaporthe grisea* (Jarosch *et al.*, 1999) and *Cochliobolus sativus* (anamorph, *Bipolaris sorokiniana*) (Kumar *et al.*, 2001), which cause spotting diseases. In the interaction with *M. grisea*, which causes rice blast, *mlo* mutant lines showed more symptoms, produced fewer effective papillae and fewer hypersensitive responses than wild-type *Mlo* lines. These two diseases are not important in northern Europe as they naturally occur in tropical climates, but the research raised the question of whether the widespread use of the *mlo* gene has been one of the causes of the recent increase in spotting diseases of barley in northern Europe.

The aim of the research reported in this paper was to test if the presence of the *mlo* gene in barley cultivars might increase the severity of spotting diseases which are important in crops in northern Europe, in particular those caused by the pathogens *Rh. secalis* and *Ra. collo-cygni*. In turn, the experiments tested whether the increased use of *mlo* in breeding might account, at least in part, for the rise of spotting diseases of barley.

Materials and methods

Plant material

Barley cv. Ingrid and four near-isogenic lines were used to study alleles *mlo-1*, *mlo-3*, *mlo-5* and *mlo-9* (Freialdenhoven *et al.*, 1996). The cultivar Pallas and a near-isogenic line, P22, containing allele *mlo-5* (Kølster *et al.*, 1986), allowed comparison of this allele in two different genetic backgrounds. The Ingrid lines were provided by P. Schulze-Lefert (Max Planck Institute for Plant Breeding Research, Germany) and the Pallas lines by L. Munk (KVL University, Denmark).

In all trials, no artificial inoculation was done and the lines only received natural inoculum. In 2004 and 2005, cv. Optic was included in the trials as a control cultivar susceptible to *Rh. secalis*, to indicate whether local conditions were conducive to this disease. Optic has a resistance rating score of 4 (moderately susceptible) for response to *Rh. secalis* in the UK Recommended List of barley cultivars (Anonymous, 2006b).

Trial locations

Six trial sites were chosen which ranged in severity for the target diseases: Bush (Midlothian, Scotland), Coldstream (Berwickshire, Scotland), Lockerbie (Dumfriesshire, Scotland), Perth (Perthshire, Scotland), Carlow (Co. Carlow, Ireland) and Kildalton (Co. Kilkenny, Ireland). Data were obtained from Bush, Lockerbie and Carlow in 2003, 2004 and 2005, Perth and Kildalton in 2004 and 2005 and Coldstream in 2003 and 2004.

Trial design

Trials were set out as randomized blocks. In 2003, insufficient seed was available for equal replication of lines at each site, but each line was trialled in one to four plots per site. Seed was harvested in 2003 and in subsequent years, each line was grown in four plots per site. Trials were managed according to normal agronomic practice for spring barley at each site, with the exception of fungicide applications. The sowing rate was 250–360 seeds m^{-2} and plot sizes were $3{\cdot}5 \times 2$ m to 20×2 m, depending on the site.

Fungicide treatments

In 2003 a split-plot design was used to assess two fungicide regimes. In regime A, Fortress® (0·1 L ha^{-1}) was applied at growth stages (Tottman & Makepeace, 1979) 25–30 and 45–49. In regime B, Fortress® (0·1 L ha^{-1}), Unix® (0·5 L ha^{-1}) and Acanto® (0·5 L ha^{-1}) were applied at growth stage 25–30 and Fortress® 0·1 (L ha^{-1}) at growth stage 45–49.

Fortress® (active ingredient 500 g quinoxyfen L^{-1}) protected against powdery mildew. This was necessary for comparison of the resistant *mlo* lines with the susceptible *Mlo*$^+$ lines, because mildew lesions can mask other diseases, making recording of the target diseases inaccurate. The aim of applying Unix® (active ingredient 750 g cyprodinil kg^{-1}) and Acanto® (active ingredient 250 g picoxystrobin L^{-1}) was to prevent spotting pathogens such as *Rh. secalis* from establishing too early and destroying green-leaf area before flag leaf emergence. However, in 2003 low levels of disease were recorded in all trials, so in subsequent years only regime A was applied, to encourage higher levels of spotting diseases.

Data collection

Ten single tillers were taken randomly from the centre of each plot and a visual assessment was made of the percentage area of the flag and second leaf (F-1) leaves covered by symptoms of scald, RLS, mildew, abiotic spots and chlorotic flecks. *Rhynchosporium secalis* was identified by its typical grey oval lesions with dark edges. *Ramularia collo-cygni* was identified by chlorotic halos surrounding the typical rectangular lesions or by sporulating lesions on the leaf underside (Oxley *et al*., 2002). Abiotic spots were similar in size to those of *Ra. collo-cygni*, but were not surrounded by chlorotic halos and were often found on the parts of the leaf most exposed to sunlight. Single scores for flag and F-1 leaves were recorded for each plot at each site. The Irish trials were scored during grain filling prior to crop senescence, when the maximum possible disease symptoms for that year were present. The Scottish trials were scored twice, (except for the Borders site in 2003 which was scored three times), first at the start of flowering when the flag leaf was fully emerged and again when the maximum disease symptoms were present, as in the Irish trials.

Data analysis

The values of each trait as percentage leaf area affected from each plot, from all sites and years, were transformed to logits to normalize statistical error. Disease levels were generally low and were too low at some sites for comparisons between lines to be reliable. For *Rh. secalis*, *Ra. collo-cygni* and abiotic spots only sites with significant levels of disease severity were analysed; these were chosen as sites in which at least half the plots had scores of 1% or more on the flag leaf of *Mlo*$^+$ lines.

Statistical analysis was performed using the programme GENSTAT^{TM} for Windows, 8th edition (VSN International Ltd). Multiple regression with stepwise addition or subtraction of terms was carried out using the STEP directive in GENSTAT to define the most suitable model to use in general linear modelling (GLM), namely that with the minimum residual mean squares. The chosen model was then refitted with the selected variables in a logical order. Predicted means and their standard errors for factors and factor combinations were calculated following analysis by GLM.

Results

Rhynchosporium secalis

Levels of scald were low throughout the trials and only three trials achieved significant levels of disease: Carlow in 2003 and Carlow and Kildalton in 2005. No Scottish site had sufficient levels of disease to be included in the analysis.

In 2004 and 2005, a strong relationship between levels of *Rh. secalis* and *Ra. collo-cygni* and proximity to plots of cv. Optic was detected. Trial plots next to two plots of Optic were much more heavily diseased by both pathogens than any other plots. These plots were therefore removed from the analysis and a 'NearOptic' factor was included in the models to investigate the effect of being next to one plot of Optic or to none.

Table 1 General linear modelling of *Rhynchosporium secalis* symptoms on barley at three sites in Ireland: Carlow in 2003 and Carlow and Kildalton in 2005

Term	d.f.[a]	m.s.[a]	v.r.[a]	*F.* pr.[a]
Trial	2	9·48	7·97	0·02 (P)[a]
Treatment	1	2·28	0·96	0·33 (P)
NearOptic	1	7·05	2·96	0·09 (P)
Resistance	1	17·66	7·43	0·01 (P)
Trial.Resistance	2	3·20	1·35	0·26 (P)
Trial.Plot	81	2·38	2·04	<0·001(R)[a]
Residual (within Plot)	85	1·17		

[a]d.f., degrees of freedom; m.s., mean squares; v.r., variance ratio; *F.* pr., *F*-test probability associated with variance ratio; (P) *F*-test compared to Plot, because these terms are confounded with plots; (R) *F*-test compared to Residual.

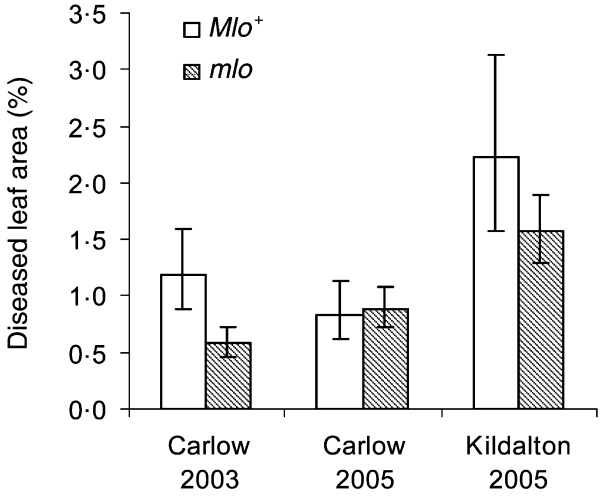

Figure 2 *Rhynchosporium secalis* percentage diseased leaf area on flag and F-1 leaves of *Mlo*$^+$ and *mlo* barley backcross lines at trial sites with significant symptom development. Bars: ±1 standard error.

The factors fitted were Trial (each trial at each site in each year was considered separately), Treatment (fungicide regime A or B), NearOptic (plots next to one Optic plot or none), LeafLayer (flag or F-1), Resistance (*Mlo*$^+$ or *mlo*), line (Ingrid or Pallas), allele (different *Mlo* alleles: *mlo-1*, *mlo-3*, *mlo-5* or *mlo-9*) and Plot. The maximal model to be fitted was:

Trial + Treatment + NearOptic + LeafLayer ∗ Line ∗ (Resistance/(Trial + Allele))

where ∗ indicates crossing (for example, the main effects of LeafLayer and Line, as well as the LeafLayer. Line interaction effect, were considered), a slash (/) indicates nesting (for example, Resistance and the effect of Allele within the Resistance factor). Stepwise regression was carried out by first fitting the factor Trial, then iteratively examining all currently available submodels of the maximal model. The model finally selected was:

Trial + Treatment + NearOptic + Resistance/Trial + Trial.Plot

The term Trial.Plot was included in the model after the stepwise regression because there was more than one observation per plot (separate scores for flag and F-1 leaves), causing several terms, including Resistance and NearOptic, to be confounded with Plot (Table 1).

Significantly more *Rh. secalis* was recorded on *Mlo* wild-type lines than on lines with *mlo* mutations (Resistance term in Table 1; Table 2). This effect was consistent across sites (Trial.Resistance term in Table 1), even though there were large differences between mean levels of scald at the three sites (Fig. 2). Disease levels were higher on *Mlo*$^+$ plots than *mlo* plots in the Carlow 2003 and Kildalton 2005 trials, but not the Carlow 2005 trial (Fig. 2). Plots next to one plot of cv. Optic had mean disease levels 0·9% higher than those not next to an Optic plot, but this effect was not significant. No significant differences were detected between Ingrid and Pallas lines, between different *mlo* alleles or between flag and F-1 leaves.

Ramularia collo-cygni

Levels of ramularia leaf spot were low throughout the trials, with only six sites achieving significant levels of disease, namely Borders, Perth and Bush in 2004 and Bush, Kildalton and Lockerbie in 2005. Where two scores were taken, only the late scores were included in the analysis, because the early scores were much lower.

The factors fitted in the model were the same as those described for *Rh. secalis* (Trial, Near Optic, LeafLayer,

Table 2 Percentage of symptoms caused by *Rhynchosporium secalis*, *Ramularia collo-cygni* and abiotic spotting on flag and F-1 leaves of *mlo* and *Mlo*$^+$ barley backcross lines at trial sites with significant symptom developmen

	Mlo$^+$		*mlo*		
	Mean %	C.I.[a]	Mean %	C.I.	Significance of difference between means
Rhynchosporium secalis	1·2	0·7–1·6%	0·9	0·7–1·1%	$0·01 > P > 0·001$
Ramularia collo-cygni	4·2	3·6–4·8%	2·6	2·4–2·9%	$P < 0·001$
Abiotic spots	2·5	2·4–2·6%	6·0	5·9–6·2%	$P < 0·001$

[a]C.I. = 95% confidence interval.

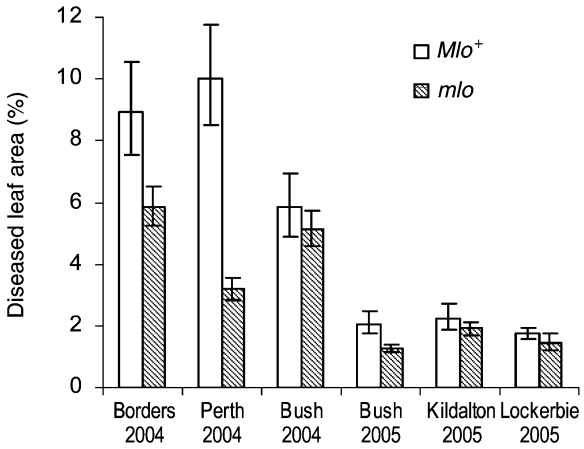

Figure 3 Adjusted means of *Ramularia collo-cygni* symptoms on flag and F-1 leaves of *Mlo*$^+$ and *mlo* barley lines at the six sites. Bars: ±1 standard error.

Table 3 General linear modelling of *Ramularia collo-cygni* symptoms on barley at six sites

Term	d.f.[a]	m.s.[a]	v.r.[a]	*F.* pr.[a]
Trial	5	27·35	35·39	<0·001 (P)[a]
LeafLayer	1	11·24	24·83	<0·001 (R)[a]
NearOptic	1	1·59	2·06	0·15 (P)
Resistance	1	13·60	17·60	<0·001 (P)
Trial.Resistance	5	2·04	2·64	0·03 (P)
Trial.Plot	151	0·77	1·71	<0·001 (R)
Residual (within Plot)	161	0·45		

[a]d.f., degrees of freedom; m.s., mean squares; v.r., variance ratio; *F.* pr., *F*-test probability associated with variance ratio; (P) *F*-test compared to Plot, because these terms are confounded within plots; (R) *F*-test compared to Residual.

Resistance, Line, Allele and Plot). The maximal model to be fitted was:

Trial + NearOptic + LeafLayer * Line * (Resistance/(Trial + Allele))

Stepwise regression was performed as for *Rh. secalis*. Two large residuals were detected and removed from the dataset, one from an F-1 leaf of Ingrid *mlo-5* and one from F-1 leaf of Pallas *Mlo*$^+$, both with no RLS symptoms (Table 3).

There were significant differences in disease levels between sites, but significantly more RLS was recorded on *Mlo*$^+$ lines than on lines with *mlo* mutations (Table 2). This effect was consistent across the six sites, but was more pronounced when more disease was present, for example in the Borders 2004 and Perth 2004 trials (Fig. 3). There was more RLS on F-1 (mean 3·4%) than on flag leaves (mean 2·3%) at four of the six sites, and overall this effect was significant, ($P < 0·001$). Plots next to one plot of cv. Optic had mean disease levels 0·18% higher than those next to no Optic plot, but this effect was not significant. No significant differences were detected between the Ingrid and Pallas lines or between different *mlo* alleles.

Table 4 General linear modelling of abiotic spot symptoms on barley at all sites

Term	d.f.[a]	m.s.[a]	v.r.[a]	*F.* pr.[a]
Trial	14	78·22	131·13	<0·001 (P)[a]
Month	2	18·07	30·28	<0·001 (P)
Trial.Month	9	12·94	21·70	<0·001 (P)
Treatment	1	0·55	0·93	0·34 (P)
NearOptic	2	1·19	1·99	0·14 (P)
LeafLayer	1	344·64	1128·44	<0·001 (R)[a]
Line	1	2·42	4·05	0·05 (P)
LeafLayer.Line	1	6·09	19·95	<0·001 (R)
Resistance	1	279·17	468·01	<0·001 (P)
Resistance.Allele	3	3·92	6·57	<0·001 (P)
Trial.Resistance	14	4·05	6·79	<0·001 (P)
Month.Resistance	2	6·82	11·44	<0·001 (P)
LeafLayer.Resistance	1	8·33	27·29	<0·001 (R)
Line.Resistance	1	4·52	7·59	0·01 (P)
Trial.Month.Resistance	9	3·53	5·93	<0·001 (P)
Trial.LeafLayer.Resistance	28	2·45	8·04	<0·001 (R)
Month.LeafLayer.Resistance	4	2·43	7·95	<0·001 (R)
Trial.Line.Resistance	28	2·07	3·48	<0·001 (P)
Month.Line.Resistance	4	3·51	5·89	<0·001 (P)
Trial.Month.LeafLayer.Resistance	18	0·72	2·36	0·001 (R)
Trial.Month.Line.Resistance	18	0·50	0·85	0·65 (P)
Trial.Plot	350	0·60	1·95	<0·001 (R)
Residual (within Plot)	966	0·31		

[a]d.f., degrees of freedom; m.s., mean squares; v.r., variance ratio; *F.* pr., *F*-test probability associated with variance ratio; (P) *F*-test compared to Plot, because these terms are confounded within plots; (R) *F*-test compared to Residual.

Abiotic spots

Levels of abiotic spotting were high throughout the trials, so all trials were included within the analysis. No relationship was found between levels of abiotic spotting and proximity to plots of Optic, so all plots were included in the analysis. The factors fitted were the same as described for *Rh. secalis*, with one addition: Month (early or late scores). The maximal model to be fitted was:

Trial * Month + Treatment + NearOptic + LeafLayer * Line + Resistance/Allele + Resistance.
(Trial * Month * (LeafLayer + Line))

In stepwise regression, performed as described above, all factors contributed to the model so the maximal model was fitted (Table 4). Fifteen large residuals were detected and removed from the dataset as they all clearly deviated from expected values within a normal distribution. (However, inclusion of these points would not greatly affect the conclusions as reported here.) As many factors were associated with significant differences, only the largest or most interesting effects are mentioned here.

There were significant differences in levels of abiotic spots between trials, with the lowest recorded at Carlow in 2004 and the highest at Perth in 2004 (1·2% and 20·9%, respectively; Trial term in Table 4). Across all sites and lines, more abiotic spotting was consistently recorded on lines with *mlo* alleles than on lines with the *Mlo*$^+$ allele, (Resistance term in Table 4; means in Table 2). More abiotic spots were recorded on F-1 leaves than flag leaves (7·9% and 3·1%, respectively, $P < 0{\cdot}001$) and this was consistent across the Ingrid and Pallas lines and lines with *Mlo*$^+$ or *mlo* alleles. Early scores were significantly lower than late scores (4·6% and 6·5%, respectively; Month term in Table 4, $P < 0{\cdot}001$), but this result was not consistent across sites (note the large Trial.Month term in Table 4). Pallas lines had significantly more abiotic spotting than Ingrid lines, but the difference was small (5·6% and 4·9%, respectively, $P = 0{\cdot}05$). Of the mutant alleles, lines with *mlo-1* had the lowest levels of abiotic spotting and lines with *mlo-3* the highest (5·1% and 8·1%, respectively; Resistance.Allele term in Table 4, $P < 0{\cdot}001$). Fungicide regime had no effect on level of abiotic spotting (Treatment term in Table 4).

Other traits

Mildew levels were low in all trials because of the fungicide regimes, and chlorotic flecks were identified as the initial symptoms of abiotic spots, so these results were not analysed.

Discussion

Significantly higher severities of disease caused by *Rh. secalis* and *Ra. collo-cygni* were found on *Mlo*$^+$ wild-type lines than on mutant *mlo* lines. These results are consistent with those of a genetic experiment in which the confidence interval for a quantitative trait locus (QTL) for resistance to *Rh. secalis*, *Rrsq2*, mapped in a cross of two barley cultivars, was found to flank the *mlo* locus (Shtaya *et al.*, 2006). Barley lines with *mlo* alleles therefore appear to have a resistance mechanism against *Rh. secalis* and *Ra. collo-cygni*.

The implication of these results is that under the environmental conditions experienced during these trials, the mutant *mlo* gene in spring barley cultivars cannot be solely responsible for the rise in these two leaf spotting pathogens. These results contrast with those described for *M. grisea* (Jarosch *et al.*, 1999) and *C. sativus* (Kumar *et al.*, 2001), in which lines with *mlo* mutant alleles were more susceptible to these pathogens. One explanation for this difference are the environmental conditions experienced during these experiments. The temperature conditions, 26°C continuously for 24 h after inoculation, in which *mlo* backcross lines became susceptible to *M. grisea*, were above the range normally experienced by barley in the field, especially at night. This suggests that loss of *mlo* resistance could depend on temperature or other environmental conditions. It has been shown that heat induces susceptibility of *mlo* lines to *B. graminis* in detached leaf tests (Schwarzbach, 2001). However, the optimum temperature for *Rh. secalis* to develop is 15–25°C (Ryan & Clare, 1975), with constant humidity. If field temperatures rose to the level where *mlo* lines became susceptible (i.e. above 26°C), *Rh. secalis* would not be viable. Although no reports exist on the conditions required for *Ra. collo-cygni* to infect, they may be similar to those for *Rh. secalis* because the two pathogens are reported from the same geographical areas.

The results presented in this paper contrast with recent research showing cultivars with mildew-resistance genes including *mlo* to be more susceptible to *Ra. collo-cygni* (Pinnschmidt *et al.*, 2006; L. Reitan, Graminor AS, Verdal, Norway, personal communication). One explanation for this difference is the plant material used in these experiments. The Ingrid and Pallas backcross lines only differ at the *Mlo* locus, allowing comparison of *Mlo*$^+$ and *mlo* mutant alleles without the influence of background genes, whereas the cultivars tested in the earlier experiments have a variety of background genes which may influence their response to pathogens. It is possible that breeders have inadvertently selected for susceptibility to spotting pathogens such as *Ra. collo-cygni*, whilst breeding successfully for mildew resistance. Finally, interactions between different diseases may influence results of field trials. It has been observed that lines attacked heavily by mildew do not show RLS symptoms, possibly because a heavy attack by mildew induces host resistance to other diseases (L. Reitan, personal communication). Lines with *mlo* alleles are not successfully infected with mildew under normal conditions and basal resistance mechanisms may remain switched off. In the experiments described in this paper quinoxyfen was used to control mildew levels, so no heavy infections occurred on lines with wild-type *Mlo*$^+$ alleles, possibly explaining why the *Mlo*$^+$ lines were more susceptible to leaf spotting diseases.

Significantly fewer abiotic spots were found on *Mlo*$^+$ lines than on *mlo* lines. This was expected as the backcross lines were designed in order to examine the impact of the *Mlo* gene alone. There were no significant differences between the Ingrid and Pallas lines, showing that the background genes had little impact on these results. A greater level of abiotic spots in *mlo* lines has been widely reported (Schwarzbach, 1976; Kølster *et al.*, 1986; Bjørnstad & Aastveit, 1990; Kjær *et al.*, 1990). Greater abiotic spotting was detected on F-1 leaves than on flag leaves, although *mlo*-related spotting is usually characteristic of the upper leaves which have been exposed to sunlight (Wu & von Tiedemann, 2002, 2004) and is characteristic of the lower leaves because they are older, meaning that there has been more time for symptoms to develop. This result raised the question of whether abiotic spots were identified correctly, because in the early stages of disease the symptoms of different diseases and of abiotic spotting may appear similar, especially to an inexperienced observer. However, the NearOptic term was not significant, which is important because plots close to the susceptible cv. Optic had more disease caused by both *Ra. collo-cygni* and *Rh. secalis*. As

abiotic symptoms are not transmissible and because no link was found between abiotic spotting and proximity to plots of Optic, it is very likely that symptoms of abiotic spotting were correctly identified as such. Lower leaves are older, therefore symptoms have had more time to develop, and at one stage they were at the top of the canopy, possibly explaining why more abiotic symptoms were found on the F-1 leaves.

There were low levels of scald in all years, with significant levels of disease only found in three of the Irish trials. A possible explanation is that *Rh. secalis* requires sustained periods of leaf wetness and temperature conditions of 15–25°C to infect (Ryan & Clare, 1975); these cool humid conditions are more often observed in Ireland than Scotland.

Ramularia collo-cygni is becoming an increasingly important pathogen of barley. This study found more *Ra. collo-cygni* than *Rh. secalis*. The identification of *Ra. collo-cygni* is difficult and it is possible that the pathogen has caused symptoms on barley crops for longer than it has been identified: with increased awareness of this pathogen more positive identifications are occurring. Difficulties in distinguishing the symptoms produced by *P. teres* from several other pathogens including *Ra. collo-cygni* have been documented (Sachs *et al*., 1998). A reliable method for distinguishing RLS symptoms from others was developed in 2001, based on the production of a red pigment by *Ra. collo-cygni*-infected tissue on agar under acidic conditions (Tschope & Sachs, 2001). Other methods include spore identification, while a PCR diagnostic was developed recently (Havis *et al*., 2006). These methods should enable accurate positive identifications to be made in future, although they are not yet sufficiently rapid for routine use in quantitative scoring of field trials.

This study indicates that the *mlo* mildew-resistance gene has not been solely responsible for the rise in spotting diseases. Disease trends can be strongly influenced by cultivar choice. Cultivars differ in their susceptibility to diseases, but the choice of which cultivar to grow depends strongly on market demand. For example, cv. Optic has been the dominant spring barley cultivar in the UK since 1997 (Home Grown Cereals Authority (HGCA), London, UK, Seedstat data, personal communication) and is moderately susceptible to *Rh. secalis* and *Ra. collo-cygni* (Oxley, 2006), but continues to be sown because of its good malting characteristics. It is unlikely the use of *mlo* would have greatly reduced the fungicide applications used and thus caused an increase in spotting diseases, because non-*mlo* varieties were commercially dominant until the mid-1990s (HGCA, Seedstat data, personal communication) and several of the *mlo* lines which were grown in significant quantities in the mid- to late 1990s, e.g. cvs Derkado and Chariot, were rated as susceptible to *Rh. secalis* (Anonymous, 1994).

Necrotic spot production is influenced by fungicide applications. Azole and strobilurin fungicides were found to reduce abiotic spot production (Wu & von Tiedemann, 2002). However, Corbel®, a morpholine fungicide (active ingredient fenpropimorph) can cause a loss in green leaf area, especially if applied after the boot stage (Oxley *et al*., 2002). Some commercial fungicides may therefore have a negative influence, causing an increase in susceptibility to spotting diseases.

Environmental changes may also alter plant fungal relationships. Depletion of the ozone layer has led to an increase in the levels of UVB radiation reaching the Earth's surface (Moseley & Mackie, 1997). *Ramularia collo-cygni* produced toxins which induced the formation of reactive oxygen species (ROS) within the host through light-dependent photodynamic reactions, producing spotting symptoms (Heiser *et al*., 2003, 2004; Miethbauer *et al*., 2003, 2006). Increased UVB in these cases may have increased the effectiveness of the toxins produced by *Ra. collo-cygni*, resulting in greater damage by the pathogen. Subtle rises in temperature may also change finely balanced plant-fungal relationships. The temperature dependency of the *mlo* gene has already been mentioned, but temperature also affects resistance to other pathogens. For example, the rust-resistance genes *Lr20* and *Sr15* lose effectiveness as temperature increases (Ramage & Sutherland, 1995).

Widespread use of the *mlo* mildew-resistance gene along with successful breeding for partial resistance in winter barley cultivars has led to a decrease in the incidence of powdery mildew. However, there has been a concomitant increase in spotting diseases. The results reported here indicate that, by itself, the *mlo* gene does not increase the susceptibility of spring barley to the spotting pathogens *Rh. secalis* and *Ra. collo-cygni*, but does increase susceptibility to abiotic spots under field conditions. However, the background genetic effect of cultivars influences the extent to which *mlo* induces abiotic spotting (Schwarzbach, 1976; Kjær *et al*., 1990; Bjørnstad & Aastveit, 1990; Thomas *et al*., 1998). The design of the experiments reported here, using pairs of near-isogenic lines, did not allow a test of the hypothesis that modification of the genetic 'background' might alter susceptibility to spotting diseases.

Many factors could hypothetically be responsible for the recent increase in spotting diseases of barley in Europe, but it does not appear that widespread use of *mlo* is one of them. Other possibilities are that environmental changes may have created conditions suitable for formerly insignificant pathogens, perhaps by altering plants' responses to attack, or that changes in farming practices are implicated.

Acknowledgements

This research was supported by a Walsh Fellowship from Teagasc, the Irish Agriculture and Food Development Authority, and by the Home-Grown Cereals Authority.

References

Anonymous, 1994. *Cereal Variety Handbook: NIAB Recommended Lists of Cereals 1994*. Cambridge, UK: NIAB.

Anonymous, 2006a. *Crop Monitor*. http://www.cropmonitor.co.uk/.

Anonymous, 2006b. *HGCA Recommended List for Spring Barley 2005*. http://www.hgca.com/document.aspx?fn=load&media_id=1478&publicationId=1254

Anonymous, 2006c. *SAC Adopt a Crop*. http://143.234.195.176/~adoptacrop/index.php.

Bayles CJ, Ghemawat MS, Aist JR, 1990. Inhibition by 2-deoxy-D-glucose of callose formation, papilla deposition, and resistance to powdery mildew in an *ml-o* barley mutant. *Physiological and Molecular Plant Pathology* **36**, 63–72.

Beer WW, 1991. Die Blattfleckenkrankheit der Gerste (*Rhynchosporium secalis*) [Leaf blotch of barley (*Rhynchosporium secalis*)]. *Zentralblatt Für Mikrobiologie* **146**, 339–58.

Bjørnstad A, Aastveit K, 1990. Pleiotropic effects on the *ml-o* mildew resistance gene in barley in different genetical backgrounds. *Euphytica* **46**, 217–26.

Brown JKM, 2002. Yield penalties of disease resistance in crops. *Current Opinion in Plant Biology* **5**, 339–44.

Büschges R, Hollricher K, Panstruga R *et al*., 1997. The barley *mlo* gene: a novel control element of plant pathogen resistance. *Cell* **88**, 695–705.

Clarke WS, 1992. Survey of winter barley disease in England and Wales 1992. In: *UK Cereal Pathogen Virulence Survey 1992 Annual Report*. Cambridge, UK: HGCA, MAFF, 7.

Freialdenhoven A, Peterhänsel C, Kurth J, Kreuzaler F, Schulze-Lefert P, 1996. Identification of genes required for the function of non-race-specific *mlo* resistance to powdery mildew in barley. *The Plant Cell* **8**, 5–14.

Hackett CA, Meyer RC, Thomas WTB, 2001. Multi-trait QTL mapping in barley using multivariate regression. *Genetical Research* **77**, 95–106.

Hardwick NV, Slough JE, Jones DR, 1997. Winter wheat and barley disease surveys 1997. In: *UK Cereal Pathogen Virulence Survey 1997 Annual Report*. Cambridge, UK: HGCA, MAFF, 6–15.

Hardwick NV, Slough JE, Jones DR, 2001. Winter cereal disease surveys 2001. In: *UK Cereal Pathogen Virulence Survey 2001 Annual Report*. Cambridge, UK: HGCA, DEFRA, 8–21.

Havis ND, Oxley SJP, Piper SR, Langrell SRH, 2006. Rapid nested PCR-based detection of *Ramularia collo-cygni* direct from barley. *FEMS Microbiology Letters* **256**, 217–23.

Heiser I, Heß M, Schmidtke KU, Vogler U, Miethbauer S, Liebermann B, 2004. Fatty acid peroxidation by rubellin B, C and D, phytotoxins produced by *Ramularia collo-cygni* (Sutton et Waller). *Physiological and Molecular Plant Pathology* **64**, 135–43.

Heiser I, Sachs E, Liebermann B, 2003. Photodynamic oxygen activation by rubellin D, a phytotoxin produced by *Ramularia collo-cygni* (Sutton et Waller). *Physiological and Molecular Plant Pathology* **62**, 29–36.

Jarosch B, Kogel KH, Schaffrath U, 1999. The ambivalence of the barley *Mlo* locus: mutations conferring resistance against powdery mildew (*Blumeria graminis* f. sp., *hordei*) enhance susceptibility to the rice blast fungus *Magnaporthe grisea*. *Molecular Plant-Microbe Interactions* **12**, 508–14.

Jørgensen JH, 1992. Discovery, characterization and exploitation of *Mlo* powdery mildew resistance in barley. *Euphytica* **63**, 141–52.

Kjær B, Jensen HP, Jensen J, Jørgensen JH, 1990. Associations between 3 *ml-o* powdery mildew resistance genes and agronomic traits in barley. *Euphytica* **46**, 185–93.

Kølster P, Munk L, Stølen O, Løhde J, 1986. Near-isogenic barley lines with genes for resistance to powdery mildew. *Crop Science* **26**, 903–7.

Kumar J, Hückelhoven R, Beckhove U, Nagarajan S, Kogel KH, 2001. A compromised *Mlo* pathway affects the response of barley to the necrotrophic fungus *Bipolaris sorokiniana* (teleomorph: *Cochliobolus sativus*) and its toxins. *Phytopathology* **91**, 127–33.

Mercer PC, Ruddock A, 2004. Surveys of cereal diseases in Northern Ireland, 1976 to 2000. *Irish Journal of Agricultural and Food Research* **43**, 85–101.

Miethbauer S, Haase S, Schmidtke KU, Günther W, Heiser I, Liebermann B, 2006. Biosynthesis of photodynamically active rubellins and structure of new anthraquinone derivatives produced by *Ramularia collo-cygni*. *Phytochemistry* **67**, 1206–13.

Miethbauer S, Heiser I, Liebermann B, 2003. The phytopathogenic fungus *Ramularia collo-cygni* produces biologically active rubellins on infected barley leaves. *Journal of Phytopathology* **151**, 665–8.

Moseley H, Mackie RM, 1997. Ultraviolet B radiation was increased at ground level in Scotland during a period of ozone depletion. *British Journal of Dermatology* **137**, 101–2.

Oxley SJP, 2006. *Barley Disease Control*. Technical note TN583. ISSN 0142 7695 (ISBN 1 85482 840 1) http://www.sac.ac.uk/consultancy/cropclinic/cropadvice/technotes/.

Oxley SJP, Havis ND, Sutherland KG, Nuttall M, 2002. Development of a rationale to identify the causal agent of necrotic lesions in spring barley and to identify control mechanisms. London, UK: Home-Grown Cereals Authority: HGCA Project Report, 2139.

Pinnschmidt HO, Christiansen AE, Sindberg SA, 2006. Expression of resistance of barley varieties to ramularia leaf spot and the status of the disease in Denmark. In: *1st European Ramularia Workshop, March 2006, Göttingen, Germany. Book of Abstracts*.

Pinnschmidt HO, Hovmoller MS, 2003. Ramularia, en ny sygdom i byg – opsamling af nuvaerende viden. [Ramularia, a new disease of barley – a review of present knowledge.] In: *20th Danish Plant Protection Conference 'Cereal, Potatoes, Pests, Environment and Posters', 2003*. Tjele, Denmark: Det Jordbrugsvidenskabelige Fakultet: DJF-rapport nr. **89**, 313–21.

Polley RW, Thomas MR, Slough JE, Bradshaw NJ, 1993. Surveys of diseases of winter barley in England and Wales, 1981–1991. *Annals of Applied Biology* **123**, 287–307.

Ramage RA, Sutherland MW, 1995. High and low pre-inoculation temperatures decrease the effectiveness of the *Lr20* and *Sr15* rust resistance genes in wheat. *Plant Pathology* **44**, 772–8.

Ryan CC, Clare BG, 1975. Effects of light, temperature and period of leaf-surface wetness on infection of barley by *Rhynchosporium secalis*. *Physiological Plant Pathology* **6**, 93–103.

Sachs E, Amelung D, Klappach K, 1998. Die Symptome der Netzfleckenkrankheit der Gerste, hervorgerufen durch *Drechslera teres* (Sacc.) Shoem., und deren Verwechslungsmöglichkeiten. [The symptoms of net blotch on barley, caused by *Drechslera teres*, and risks for diagnostic

mistakes.] *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes* **50**, 58–63.
Schwarzbach E, 1976. The pleiotropic effects of the *ml-o* gene and their implications in breeding. In: *Barley Genetics III*. Münich, Germany: Karl Thiemig, 440–5.
Schwarzbach E, 2001. Heat induced susceptibility of *mlo*-barley to powdery mildew. *Czech Journal of Genetics and Plant Breeding* **37**, 82–7.
Schwarzbach E, 2006. *The* Mlo *Gene of Barley*. http://www.crpmb.org/mlo/.
Shipton WA, Boyd WJR, Ali SM, 1974. Scald of barley. *Review of Plant Pathology* **53**, 839–61.
Shipton WA, Khan TN, Boyd WJR, 1973. Net blotch of barley. *Review of Plant Pathology* **52**, 269–90.
Shtaya MJY, Marcel TC, Sillero JC, Niks RE, Rubiales D, 2006. Identification of QTLs for powdery mildew and scald resistance in barley. *Euphytica* **151**, 421–9.
Skou JP, 1982. Callose formation responsible for the powdery mildew resistance in barley with genes in the *ml-o* locus. *Phytopathologische Zeitschrift* **104**, 90–5.
Thomas WTB, Baird E, Fuller JD *et al*., 1998. Identification of a QTL decreasing yield in barley linked to *Mlo* powdery mildew resistance. *Molecular Breeding* **4**, 381–93.
Tottman DR, Makepeace RJ, 1979. An explanation of the decimal code for the growth stages of cereals, with illustrations. *Annals of Applied Biology* **93**, 221–34.
Tschope O, Sachs E, 2001. Die Farbstoffbildung von *Ramularia collo-cygni* Sutton & Waller als Grundlage für eine Schnelldiagnose. [The production of colour in *Ramularia collo-cygni* Sutton & Waller as a basis for a rapid diagnosis.] *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes* **53**, 161–4.
Wu YX, von Tiedemann A, 2002. Evidence for oxidative stress involved in physiological leaf spot formation in winter and spring barley. *Phytopathology* **92**, 145–55.
Wu YX, von Tiedemann A, 2004. Light-dependent oxidative stress determines physiological leaf spot formation in barley. *Phytopathology* **94**, 584–92.
Yokoyama K, Aist JR, Bayles CJ, 1991. A papilla-regulating extract that induces resistance to barley powdery mildew. *Physiological and Molecular Plant Pathology* **39**, 71–8.