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Genetics of responses to morpholine-type fungicides and of avirulences in *Erysiphe graminis* f. sp. *hordei*

James K. M. Brown, Stéphanie Le Boulaire and Neal Evans*
Cereals Research Department, John Innes Centre, Colney Lane, Norwich, NR4 7UH, UK (Fax: 603 502241);
**Present address: Plant Science Department, Scottish Agricultural College, Auchincruive, Ayr, KA6 5HW, UK*

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Abstract

The genetics of the responses of the barley powdery mildew pathogen, *Erysiphe graminis* f.sp. *hordei*, to three morpholine-type fungicides were studied. Resistances to a phenylpropylamine fungicide, fenpropidin, and to a morpholine, fenpropimorph, co-segregated in crosses of a sensitive isolate, DH14, with each of two resistant ones, CC151 and CC152. In the cross CC151 × DH14, the results were consistent with resistance to both fungicides being controlled by a single gene, at a locus named *Fen1*. In the other cross, CC152 × DH14, the genetics of resistance were more complicated; the data were consistent with the segregation of two complementary, unlinked genes which each conferred resistance to both fungicides. Fenpropidin-resistant progeny of CC151 × DH14 were significantly more resistant to fenpropimorph than were fenpropidin-resistant progeny of CC152 × DH14, although the resistant progeny of the two crosses did not differ significantly in their level of fenpropidin resistance. Fenpropidin-resistant progeny of CC151 × DH14 were significantly more resistant to another morpholine, tridemorph, than were fenpropidin-sensitive progeny, but this was not the case for CC152 × DH14. Resistance to triadimenol, a C14 demethylation-inhibitor (DMI) fungicide, segregated in both crosses. Triadimenol resistance appeared to be controlled by one gene in each cross and was not associated with morpholine resistance. CC151 × DH14 also segregated for eight avirulence genes. Two of these matched the *Mla6* resistance, while one gene matched a previously unknown resistance in a Pallas near-isogenic line, P17, which also carries a known resistance gene, *Mlk*. *Fen1* was not significantly linked to the triadimenol resistance gene, *Tdl(a)*, or to any of the eight avirulence genes. $Avr_{a6}1$, Avr_{a12}, Avr_{La}, Avr_{P17} and *Tdl(a)* were linked, as were Avr_{a10} and Avr_k.

Abbreviations: ED_{50} – median effective dose; Fpd – fenpropidin; Fpm – fenpropimorph; PCA – principal components analysis; Tdm – tridemorph.

Introduction

Two morpholine fungicides, fenpropimorph (Fpm) and tridemorph (Tdm), and a phenylpropylamine, fenpropidin (Fpd), have been widely used to control powdery mildew on cereals in Britain since the early 1980's (Polley and Thomas, 1991; Polley et al., 1993). Three phenotypes of *Erysiphe graminis* f.sp. *hordei* (*Egh*), the causal agent of barley mildew, classified by their responses to these fungicides, were identified in the UK in 1988 and 1990. One type, S, was sensitive to all three fungicides. A second type, R, was more resistant to Fpd and Fpm than was the S type, but was no more resistant to Tdm. The third type, RM, was still more resistant to Fpm than type R was, and was also more resistant to Tdm, but was no more resistant to Fpd than the R type (Brown et al., 1991; Brown and Evans, 1992; Brown, 1994). It should be noted that the resistances of the R and RM classes to these fungicides were still low enough for mildew to be controlled initially by a spray at the recommended rate of application, although the duration of effective control may have been reduced somewhat.

In samples from the airborne spore population in 1988, all isolates of *Egh* from England were type S, whereas those from Scotland were either S or R, except in Moray, around Elgin in the north-east, where RM isolates were also found (Brown et al., 1991). In 1990, all isolates sampled from four field trials in Scotland were of either the R or the RM types, while those sampled from a trial at Levington in eastern England were either R or S (Brown and Evans, 1992). In the trial at Levington in 1990, application of either Fpd or Fpm selected isolates that were resistant to both of these fungicides. Neither of these two fungicides selected strongly for resistance to Tdm, nor was Fpd/Fpm resistance selected by application of Tdm (Brown and Evans, 1992). The distribution of race-specific avirulences in morpholine-resistant isolates suggested that the R phenotype was carried by many different clones of *Egh* while RM isolates may have been a single clone (Brown et al., 1991; Brown and Evans, 1992).

From the pattern of cross-resistance observed in these population genetic studies, it was predicted that resistances to Fpd and to Fpm are controlled by the same gene or genes. It was also predicted that Fpd/Fpm-resistance is controlled by one or very few genes, such that variation at one locus causes a substantial difference in responses to both Fpd and Fpm. Furthermore, it was predicted that, in type R isolates, the Fpd/Fpm resistance gene or genes would have little effect on the response to Tdm, while in type RM, there would either be a second gene which enhanced resistance to both Fpm and Tdm, or the Fpd/Fpm resistance gene would affect resistance to Tdm.

The work described here was done to test these hypotheses about the genetics of responses to morpholine-type fungicides in *Egh*. The crosses analysed also allowed investigation of the genetics of responses to a sterol demethylation-inhibitor (DMI), conazole fungicide, triadimenol, and of avirulences, developing previous work on the genetics of this fungus.

Materials and methods

Isolates and crosses. Three *Egh* isolates were used in the genetic analyses described here. A class S isolate, DH14, was a gift from Dr D. W. Hollomon, Long Ashton Research Station (Hollomon, 1981). An RM isolate, CC151, and an R isolate, CC152, were collected in Moray and Kincardineshire (south of Aberdeen), Scotland, respectively, in 1988. Crosses of CC151 × DH14 and CC152 × DH14 were made on plants of barley cv. Proctor, and ascospore progeny obtained, by the method of Brown et al. (1992). Forty progeny of CC151 × DH14 and 65 of CC152 × DH14 were studied in the research described here. Several other crosses between sensitive and resistant isolates were attempted, but without success.

Tests with morpholine-type fungicides. To test the responses of isolates to Fpd, Fpm and Tdm, these fungicides, formulated as Patrol (Zeneca, Farnham), Corbel (BASF, Hadleigh) and Calixin (BASF) respectively, were applied to barley seedlings using a hand-held sprayer. Detached leaves were inoculated with conidiospores and the number of colonies visible after seven days were counted. A detailed description of the methods was given previously (Brown and Evans, 1992).

Sprays were applied at a volume rate of 240 l ha^{-1}. All tests included one set of plants sprayed with water only. Patrol was applied at seven doses, from 2 to 500 $\mu l\ l^{-1}$, with a ratio between successive doses of 2.510. Corbel was applied at 11 doses, over the same range of concentrations but with a dose ratio of 1.737. Calixin was applied at eight doses, from 50 to 1000 $\mu l\ l^{-1}$, with a dose ratio of 1.534. The number and spacing of doses were chosen such that all three parents would be resistant to the lowest dose and sensitive to the highest dose, and that the resistant and sensitive parents could be reliably distinguished by the set of doses used.

Analysis of responses to morpholine-type fungicides. The choice of a range of doses that spanned the interval between fully toxic and fully ineffective meant that responses to fungicides could usually be quantified, because meaningful estimates of median effective doses (ED_{50}) could usually be obtained. This was done by Wadley's method (Finney, 1971), using the MLP package (Numerical Algorithms Group [NAG], Oxford). However, in a few cases, the ED_{50}s were clearly inconsistent with the actual numbers of colonies formed at each dose, while fiducial limits often could not be obtained. These two problems are common in studies of *E. graminis*, and reasons why such difficulties are likely to arise in biotrophic pathogens have been proposed (Brown, 1991). Due to the inadequate accuracy of ED_{50}s for displaying differences between individual isolates, variation in the data was displayed by principal components analysis (PCA), using the Genstat 5 statistical package (NAG). The application of PCA to studies of responses to fungicides has been

Table 1. Barley varieties used to detect race-specific avirulences in progeny of *Erysiphe graminis* f.sp. *hordei* isolates CC151 × DH14

Variety	Resistance gene(s)[1]
Golden Promise[2]	*Mla8*
P03	*Mla6* + *Mla14*
P09	*Mla10*
P10	*Mla12*
P17	*Mlk* + *Ml(P17)*[3]
Hordeum 1063	*Mlk*
P23	*MlLa*
P24	*Mlh*

[1] References: Golden Promise, Newton (1989); Hordeum 1063, Wiberg (1974); others, Kølster et al. (1986).
[2] Susceptible control; both parents are virulent.
[3] Reported in this paper.

described in detail previously (Brown, 1991; Brown and Wolfe, 1991; Brown et al., 1991; Brown et al., 1992), and the calculations carried out are given in the footnotes to Table 2. In this context, PCA is essentially a means of classifying isolates into groups which have broadly similar responses to a fungicide; it does not, in itself, attempt to quantify the responses.

Tests of other characters. To test responses to triadimenol, this fungicide was applied as Baytan powder seed treatment (Bayer, Leverkusen) to barley cv. Golden Promise, at seven rates between 3.1 mg kg^{-1} and 2 g kg^{-1} (dose ratio = 2.0), with an untreated control. Ethirimol was applied as Milstem liquid seed treatment (Zeneca) at five rates between 0.131 and 1.33 ml kg^{-1} (dose ratio = 1.79), with an untreated control. Assays of responses to triadimenol and ethirimol were done as described by Brown and Wolfe (1991). Race-specific avirulences were tested on detached leaves of the differential set of varieties listed in Table 1, by the method of Brown and Wolfe (1990).

Estimation of linkage. Genetic analysis was done using standard methods. In cases involving avirulence towards the *Mla6* resistance, in which there was incomplete classification of progeny because three genotypes had the same avirulent phenotype, the recombination fraction between $Avr_{a6}1$ and other genes was estimated by equation (1) of Brown and Simpson (1994). Recombination fractions (r) were converted to map distances (m) in cM by Kosambi's (1944) function, $m = 25 \log_e\{(1+2r)/(1-2r)\}$.

Results

Responses to morpholine-type fungicides: CC151 × DH14. Forty progeny of CC151 × DH14 were tested for their responses to morpholine-type fungicides. Twenty-five progeny of this cross were tested in Batch 1 for their responses to Fpd and Fpm, while the other 15 were tested in Batch 2, along with 15 progeny of CC152 × DH14. All batches of tests included the three parent isolates. Tests of responses to Fpd and Fpm were done on separate days.

All progeny of CC151 × DH14 were clearly distinguishable as being either resistant to Fpd, similar to CC151, or sensitive, similar to DH14. PCAs are shown for Batch 1 (Table 2, Figure 1a) and Batch 2 (Table 2, Figure 1b). In both cases, there was a clear difference between the PC1 values of the resistant and sensitive groups of isolates. The high proportion of the total variation associated with the first principal component, PC1, and the contrast of the loadings on PC1 of data from low and high doses, mean that the PC1 scores can be interpreted as a measure of resistance. Since scores at higher doses have a positive loading on PC1 (Table 2), resistant isolates have a higher value of PC1 than do more sensitive isolates. This is also clear from the position of the parental isolates in Figs. 1a and 1b, since the S-type parent, DH14, has a negative score for PC1, while the R- and RM-type parents, CC152 and CC151, have positive scores. The ratio of the mean ED_{50}s of Fpd for the resistant and sensitive groups was 9.2, and the means of $\log(ED_{50})$ of the two groups differed significantly (Table 3).

Twenty-two progeny of CC151 × DH14 were Fpd-resistant and 18 were sensitive. This segregation is not significantly different from a Mendelian 1:1 ratio ($\chi^2 = 0.40$), and is therefore consistent with responses to Fpd being controlled by a single gene in this cross. This putative gene is designated *Fen1*, and the alleles in CC151 and DH14 are named $fen1R_{CC151}$ and $fen1S_{DH14}$ respectively, following the nomenclature of fungicide-response genes used by Brown et al. (1992).

All Fpd-resistant progeny were more resistant to Fpm than were all Fpd-sensitive progeny (Table 2; Figure 1c [Batch 1]; Figure 1d [Batch 2]). As with Fpd, the PC1 of both sets of Fpm data can be interpreted as a measure of resistance, because of the high proportion of the variation accounted for by PC1 and because of the contrast of loadings at high and low doses. Again, resistant isolates had high PC1 scores. The ED_{50}s of Fpm for the Fpd-resistant and sensitive groups were significantly different, with a ratio of 4.6 (Table 3).

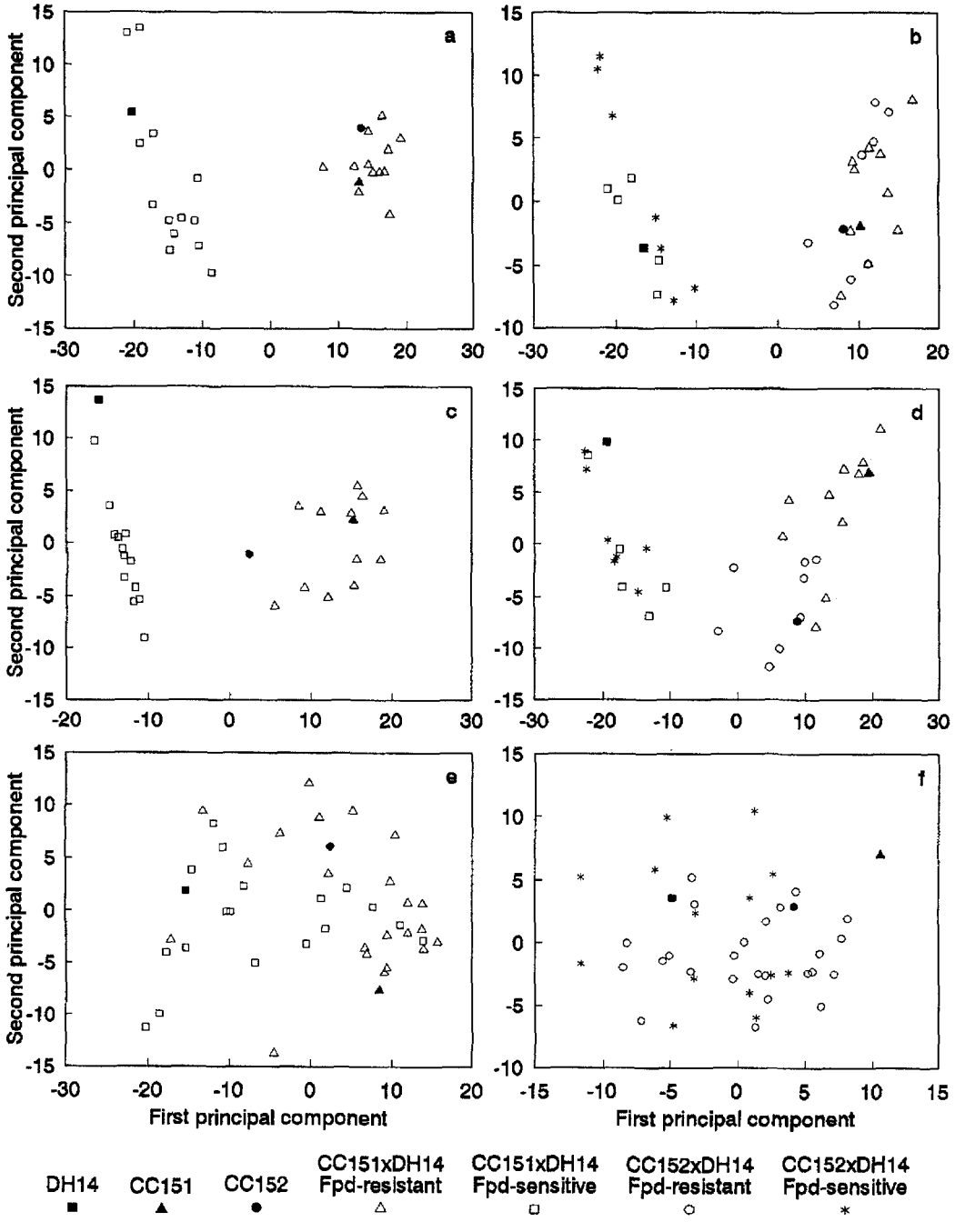

Figure 1. Plots of scores on the first two principal components of data on the responses of progeny of the *Erysiphe graminis* f.sp. *hordei* isolate DH14 crossed with CC151 or CC152, and the parent isolates, to the fungicides fenpropidin (Fpd; a, b), fenpropimorph (c, d) and tridemorph (e, f). a, c: 25 progeny of CC151 × DH14 and 3 parents. b, d: 15 progeny of CC151 × DH14, 15 progeny of CC152 × DH14 and 3 parents. e: 40 progeny of CC151 × DH14 and 3 parents. f: 39 progeny of CC152 × DH14 and 3 parents. Details of analysis in Materials and methods and in footnotes to Table 2.

Table 2. Principal components analyses (PCAs) of variation in six tests of progeny of the *Erysiphe graminis* f.sp. *hordei* isolate DH14 crossed with either CC151 or CC152

PC[1]	% of variation[2]	Dose (μl l^{-1})[3]										
Fenpropidin		2.00	5.02	12.6	31.6	79.4	199	500				
Batch 1: 3 parents, 25 progeny of CC151 × DH14												
1	74.3	−0.28	−0.23	0.06	0.28	0.78	0.42	0.00				
2	9.1	−0.06	0.38	0.83	0.30	0.14	0.22	0.00				
Batch 2: 3 parents, 15 progeny of CC151 × DH14, 15 progeny of CC152 × DH14												
1	71.4	−0.23	−0.29	0.04	0.49	0.70	0.37	0.00				
2	11.2	−0.34	0.31	−0.29	−0.58	0.13	0.59	0.00				
Fenpropimorph		2.00	3.47	6.03	10.5	18.2	31.6	54.9	95.4	166	288	500
Batch 1: 3 parents, 25 progeny of CC151 × DH14												
1	67.6	−0.19	−0.15	−0.15	−0.18	0.07	0.20	0.71	0.55	0.20	0.00	0.00
2	8.8	0.17	0.18	−0.14	0.23	−0.70	−0.51	0.06	0.27	0.22	0.00	0.00
Batch 2: 3 parents, 15 progeny of CC151 × DH14, 15 progeny of CC152 × DH14												
1	61.9	−0.17	−0.22	−0.18	−0.06	0.24	0.45	0.61	0.45	0.22	0.00	0.00
2	10.9	0.07	0.03	−0.07	−0.13	−0.61	−0.30	−0.11	0.55	0.44	0.00	0.00
Tridemorph		50.0	76.7	118	181	277	424	652	1000			
3 parents, 40 progeny of CC151 × DH14												
1	62.5	−0.32	−0.24	0.12	0.38	0.80	0.19	0.00	0.00			
2	17.3	−0.36	−0.17	0.67	0.41	−0.45	−0.16	0.00	0.00			
3 parents, 39 progeny of CC152 × DH14												
1	38.4	−0.25	−0.16	0.12	0.31	0.90	0.00	0.00	0.00			
2	21.9	−0.28	0.47	0.38	0.70	−0.28	0.00	0.00	0.00			

[1] Data for each isolate were mean colony numbers on two replicate leaf sections treated with each dose of fungicide, divided by the sum of such means over all doses (including untreated leaves) for that isolate. These means were then transformed by the angular transformation. PCA was carried out on the matrix of covariances between these data for all doses excluding the untreated leaves.

[2] Eigenvalue of the appropriate PC divided by the sum of all eigenvalues.

[3] Concentration of formulated product, applied in volume rate of 240 l ha^{-1}. Fenpropidin applied as Patrol, fenpropimorph as Corbel and tridemorph as Calixin (see Materials and methods).

These data are consistent with the *fen1*R_{CC151} allele reducing sensitivity to Fpm as well as to Fpd. If this is indeed so, the resistances to Fpd and to Fpm are pleiotropic effects of *fen1*R_{CC151}.

All progeny were tested in a single batch for their response to Tdm. The distributions of the Tdm-responses of Fpd/Fpm-resistant and sensitive isolates overlapped (Figure 1e). Again, higher values of PC1 were associated with greater resistance to Tdm (Table 2). However, the mean Tdm-resistance of Fpd/Fpm-resistant progeny was significantly higher than that of Fpd/Fpm-sensitive progeny, although the ratio between the mean ED_{50} of Tdm for Fpd/Fpm-resistant and sensitive progeny was only 1.53 (Table 3). These data are consistent with the *fen1*R_{CC151} allele conferring a small reduction in sensitivity to Tdm as well as to Fpd and Fpm. They could also be explained by other genetic models, however, such as there being a Tdm-resistance gene linked to *Fen1*.

Responses to morpholine-type fungicides: CC152 × DH14. Forty progeny of CC152 × DH14 were tested for responses to the three fungicides. The 15 isolates tested in Batch 2 segregated for Fpd-resistance. The two levels of Fpd-response were distinct (Table 2, Figure 1b), eight isolates being resistant and seven sensitive. On average, resistant progeny of CC152 × DH14 were 6.9 times less sensitive to Fpd than

Table 3. Median effective doses (ED_{50}s) of progeny of *Erysiphe graminis* f.sp. *hordei* crosses CC151 × DH14 and CC152 × DH14

Fungicide[3]	Fpd-resistant progeny[1]			Fpd-sensitive progeny[1]			t[5]	(df)[6]	Parents' mean ED_{50}s[2]		
	Mean ED_{50}^{4}	sd of log(ED50)	No. of isolates	Mean ED_{50}	sd of log(ED50)	No. of isolates			CC151	CC152	DH14
CC151 × DH14											
Fpd	0.152	0.136	22	0.0166	0.379	18	10.25	(21) ***[7]	0.112	0.107	0.0134
Fpm	0.102	0.191	22	0.0224	0.169	18	11.56	(38) ***	0.146	0.0769	0.0094
Tdm	0.264	0.164	22	0.173	0.178	18	3.32	(35) **	0.315	0.240	0.151
CC152 × DH14											
Fpd	0.144	0.258	51	0.0209	0.211	14	12.54	(25) ***			
Fpm	0.0697	0.213	33	0.0150	0.089	7	13.32	(23) ***			
Tdm	0.165	0.098	25	0.147	0.147	14	1.16	(20)			

[1] Progeny resistant of sensitive to fenpropidin (see Figs. 1a and 1b). Where a progeny set was tested in more than one batch, data were pooled across batches; there was little heterogeneity in ED_{50}s between batches of tests.
[2] Fpd, Fpm: mean of three tests, Tdm: mean of two tests.
[3] Fpd: fenpropimorph (applied as Patrol), Fpm: fenpropimorph (Corbel), Tdm: tridemorph (Calixin). Fungicides applied at volume rate of 240 l ha^{-1}.
[4] Means of $\log_{10}(ED_{50})$, back-transformed to the original scale, in ml l^{-1} formulated product.
[5] t-tests for difference of mean $\log_{10}$(ED50) of Fpd-resistant and sensitive progeny of each cross.
[6] df: effective degrees of freedom.
[7] ***: $P < 0.001$; **: $0.001 \leq P < 0.01$ (P: probability of t at least as large occurring by chance).

were sensitive progeny; this difference was significant (Table 3). There was no significant difference between the Fpd-responses of the resistant progeny of this cross and the resistant progeny of CC151 × DH14, or between those of the sensitive progeny of the two crosses.

In Batch 3, 25 progeny of CC152 × DH14 were tested, and all were resistant to Fpd (data not shown). Twenty of these isolates were hatched from cleistothecia on a single leaf section on a single day. (Note, however, that 14 of the 15 progeny tested in Batch 2 were also derived from this same set of cleistothecia; of these, seven were resistant and seven were sensitive). In case the unexpectedly high frequency of resistance in Batch 3 had been caused by an unknown factor associated with that particular set of cleistothecia, 25 more progeny from other cleistothecia were tested with Fpd (Batch 4). Eighteen of these were resistant and seven sensitive (data not shown). Excluding the 20 isolates in Batch 3 which were derived from one set of cleistothecia, a total of 31 isolates were Fpd-resistant and 14 were sensitive. This is a poor fit to a 1:1 segregation ratio ($\chi^2 = 6.42$, $P = 1.1 \times 10^{-2}$) but not significantly different from a 3:1 ratio ($\chi^2 = 0.90$, $P = 0.34$). With the 20 additional resistant isolates from Batch 3 included, the ratio of 51 resistant to 14 sensitive also fits a 3:1 ratio well ($\chi^2 = 0.42$, $P = 0.52$) but not a 1:1 ratio ($\chi^2 = 21.06$, $P = 4.5 \times 10^{-6}$). However, the proportions of resistant and sensitive progeny were not homogeneous among the three batches ($\chi^2 = 17.36$, 2 df, $P = 1.7 \times 10^{-4}$). We have no explanation for this heterogeneity.

The 3:1 segregation ratio of resistant to sensitive progeny is consistent with there being two complementary genes which control Fpd-resistance in CC152, with DH14 having the sensitivity allele of both genes. Only progeny with both sensitivity alleles would be sensitive to Fpd, while the resistance alleles of each gene would confer similar levels of resistance. However, other, more complex models are also possible. For instance, there may be a single Fpd-response gene, the segregation of which might have been distorted, perhaps by being linked to a gene which affected the survival of progeny. We cannot reach further conclusions about the genetics of Fpd-resistance in this cross without testing alternative hypotheses by means of back-crosses and sib-crosses. CC152 cannot be crossed with CC151, to test if CC152 has a resistance allele at the *Fen1* locus identified in CC151, because these two isolates have the same mating type.

All Fpd-resistant progeny of CC152 × DH14 tested in Batch 2 were more resistant to Fpm than were all Fpd-sensitive ones (Table 2, Figure 1d). However, they were, on average, significantly less resistant to Fpm than were the Fpd-resistant progeny of CC151 × DH14. All progeny in the batch of 25 Fpd-resistant isolates were also resistant to Fpm (data not shown).

The Fpd-resistant progeny were 4.6 times less sensitive, on average, to Fpm than were Fpd-sensitive progeny (Table 3). Resistances to Fpd and to Fpm may therefore be pleiotropic effects of the resistance gene or genes which segregate in CC152 × DH14.

Thirty-nine progeny of CC152 × DH14 were tested for their responses to Tdm. Twenty-five of these isolates were Fpd-resistant and 14 were Fpd-sensitive. The isolates were taken from Batches 2 and 4, in which both resistant and sensitive progeny had been identified. The proportion of the total variation accounted for by PC1 was not as high as for the other sets of data, which indicates that the range of variation in responses to Tdm was comparatively small (Figure 1f). Although the Fpd-resistant progeny were slightly more Tdm-resistant, by a factor of 1.12, this small difference was not significant (Table 3).

Responses to other fungicides. DH14 was sensitive to triadimenol, while CC151 had medium resistance and CC152 high resistance, as defined by Brown and Wolfe (1991). In the progeny of CC151 × DH14, there were two classes of response to triadimenol, 20 isolates having medium resistance, similar to that of CC151, and 20 being sensitive (Table 4). This 1:1 segregation is clearly consistent with a single gene controlling triadimenol responses in this cross. This putative gene is named *Tdl(a)* (the naming of fungicide-response genes is discussed below). The resistance allele in CC151 is named $tdl(a)R_{CC151}$ and the sensitivity allele in DH14 is named *tdl(a)S*.

In CC152 × DH14, 25 of the 59 progeny tested were sensitive to triadimenol and 34 had resistance similar to that of CC152. This segregation is consistent with a 1:1 ratio ($\chi^2 = 1.37$, $P = 0.24$), and therefore with a single gene controlling triadimenol-responses in this cross. However, this gene is not necessarily at any of the currently known *Tdl* loci. It is notable that the 20 progeny of CC152 × DH14 tested in Batch 2, which had hatched from the same group of cleistothecia on the same day, segregated for their responses to triadimenol, even though they did not segregate for morpholine-responses. This confirms that these 20 progeny were indeed not genetically identical.

All three parental isolates were resistant to ethirimol. The progeny were therefore not tested for their responses to this fungicide.

Segregation of avirulences in CC151×DH14. In tests on 24 barley varieties, there were six differences in avirulence phenotypes between CC151 and DH14, on P03, P09, P10, P23, P24 and Hordeum 1063. The genetics of these avirulences were studied, partly to try to locate *Fen1*.

In the progeny of CC151 × DH14, the segregation ratios of avirulence and virulence corresponding to *MlLa* and to *Mlh* did not differ significantly from 1:1. Segregations of avirulence and virulence corresponding to *Mla10* and *Mla12*, and to the *Mlk* gene in Hordeum 1063, did not differ significantly from either 1:1 or 3:1 ratios at the $P > 0.05$ level, but in all three cases, they fitted the 1:1 ratio better (Table 4). These data are consistent with one avirulence gene matching each of the resistance genes *Mla10, Mla12, Mlh, Mlk* and *MlLa*. These genes have previously been named Avr_{a10}, Avr_{a12}, Avr_h, Avr_k and Avr_{La} (Brown and Simpson, 1994; Brown and Jessop, 1995).

The segregation of avirulence and virulence on P17 fitted a 3:1 segregation ratio, but not a 1:1 ratio (Table 4). P17 has *Mlk* (Kølster et al., 1986), in common with Hordeum 1063, but this segregation indicates that P17 has an additional, previously unknown resistance specificity, which is designated *Ml(P17)*. Since DH14 is virulent on Hordeum 1063 but not on P17, it presumably has the genotype Vir_k Avr_{P17} since only Vir_k is needed for virulence on a variety which only has *Mlk* (Vir_x denotes a virulence allele of an avirulence gene Avr_x; see Brown and Simpson (1994)). If this is the case, CC151 has the genotype Avr_k Vir_{P17}. Only recombinant progeny with the genotype Vir_k Vir_{P17} would be virulent on P17.

Avirulence and virulence towards *Mla6* also segregated in a 3:1 ratio. *Mla6* is associated with a second, closely-linked gene, *Mla14* (Giese et al., 1981; Mahadevappa et al., 1994). *Mla6* confers an incompatible infection type (IT) of 0, while *Mla14* confers IT 2–3. All avirulent progeny of CC151 × DH14 had IT 0. If the avirulent parent, DH14, had avirulence genes matching both *Mla6* and *Mla14*, namely Avr_{a6} and Avr_{a14}, half the progeny – those with Avr_{a6} – would have had IT 0. This is because Avr_{a6}, which would confer the lower IT, would be epistatic to Avr_{a14}, which would confer an intermediate IT. A quarter of the progeny would have the genotype Vir_{a6} Avr_{a14}, and therefore an intermediate IT, while the remaining quarter, with the genotype Vir_{a6} Vir_{a14}, would be fully virulent, with IT 4. The observed segregation of 30 avirulent, 0 intermediate and 10 virulent progeny deviates significantly from a 2:1:1 ratio ($\chi^2 = 15.00$, $P = 5.5 \times 10^{-4}$). It therefore seems that both CC151 and DH14 have Vir_{a14}, while DH14 has two avirulence genes matching the *Mla6* resistance, both of which

Table 4. Segregation of responses to triadimenol and avirulence on seven varieties in progeny of the *Erysiphe graminis* f.sp. *hordei* cross CC151 × DH14, and presumed parental genotypes

Fungicide or variety	Phenotype[1]	No. of progeny	Phenotype	No. of progeny	$\chi^{2(2)}$ 1:1	$\chi^{2(2)}$ 3:1	Allele(s) DH14	Allele(s) CC151
Triadimenol	R	20	S	20	0.00	13.33	*tdl(a)S*	$tdl(a)R_{CC151}$
P03	A[3]	30	V	10	10.00	0.00	$Avr_{a6}1$ $Avr_{a6}2$	$Vir_{a6}1$ $Vir_{a6}2$
P09	A	25	V	15	2.50	3.33	Vir_{a10}	Avr_{a10}
P10	A	25	V	15	2.50	3.33	Avr_{a12}	Vir_{a12}
P17	A	31	V	9	12.10	0.13	Vir_k Avr_{P17}	Avr_k Vir_{P17}
Hordeum 1063	A	25	V	15	2.50	3.33	Vir_k	Avr_k
P23	A	20	V	20	0.00	13.33	Avr_{La}	Vir_{La}
P24	A	23	V	16	1.26	6.70	Avr_h	Vir_h

[1] R, resistant; S, sensitive; A, avirulent; V, virulent.
[2] P (chance probability of χ^2 at least as large) < 0.05 for $\chi^2 > 3.84$; $P < 0.01$ for $\chi^2 > 6.63$; $P < 0.001$ for $\chi^2 > 10.89$.
[3] Avirulent infection types: 0 on P03, P09, P10, P23 and P24; 0–1, on P17; 1 on Hordeum 1063. Virulent infection types: 4, except on P23 (3–4).

confer IT 0 in incompatible interactions. By analogy with similar situations with the avirulences which match *Mla7* (Brown and Jessop, 1995) and *Mla13* (Caffier et al., 1996), these two genes are designated $Avr_{a6}1$ and $Avr_{a6}2$.

Linkage. Two linkage groups were identified in CC151 × DH14 (Table 5). The first group included Avr_{a10} and Avr_k, between which there was no recombination. The second group included five loci which fell into a single cluster. *Tdl(a)*, Avr_{a12}, Avr_{P17}, and one of the two avirulence genes which match *Mla6*, arbitrarily assumed to be $Avr_{a6}1$, were linked to each other. Avr_{La} was linked to Avr_{P17} and *Tdl(a)*, but was not associated significantly with $Avr_{a6}1$ or Avr_{a12}. The remaining three genes, Avr_h, *Fen1* and $Avr_{a6}2$, were not linked to any other gene. (This analysis assumes that $Avr_{a6}1$ and $Avr_{a6}2$, and Avr_k and Avr_{P17}, are unlinked.)

There were no recombinants between Avr_{P17} and *Tdl(a)*. Analysis of double-crossovers indicated that these loci lie between Avr_{a12} and Avr_{La}. Associations between phenotypes (Table 5) and analysis of double-crossovers indicated that *Tdl(a)* and Avr_{P17} probably lie between $Avr_{a6}1$ and Avr_{La}. However, whether $Avr_{a6}1$ is between Avr_{a12} and *Tdl(a)*/Avr_{P17}, or Avr_{a12} between $Avr_{a6}1$ and *Tdl(a)*/Avr_{P17}, could not be determined. Map distances are given in Table 5.

Responses to Fpd and Fpm and to triadimenol were not significantly associated in the progeny of CC152 × DH14 ($\chi^2 = 0.17$, $P = 0.68$), indicating that the triadimenol-response gene which segregated in this cross was not linked to those controlling responses to morpholines.

Discussion

Responses to morpholines. The work described here aimed to test the hypotheses that (i) in British isolates of *Egh*, responses to Fpd and to Fpm are controlled by the same genes, (ii) there are only one or a very few such genes, (iii) in type R isolates, such as CC152, the Fpd/Fpm-resistance gene or genes have little effect on the response to Tdm, and (iv) in type RM isolates, such as CC151, there are either additional genes which enhance resistance to both Fpm and Tdm, or the Fpd/Fpm-resistance genes also affect resistance to Tdm.

(i): In both crosses, resistances to Fpd and Fpm co-segregated. It is impossible to prove absolutely that the same genes control the two resistances without molecular cloning of the genes concerned. However, if separate genes control the responses to the two fungicides, they must be closely linked. This is consistent with the results of two large surveys of *Egh* in Great Britain in 1988 (Brown et al. 1991) and 1990 (Brown and Evans, 1992), in which all isolates tested were resistant to both Fpd and Fpm or to neither.

(ii): The segregation of responses to Fpd and Fpm in CC151 × DH14 was consistent with the existence of a single locus, designated *Fen1*. The segregation of responses to Fpd and Fpm in CC152 × DH14 fitted a 3:1 ratio. This ratio may be accounted for by the existence of two unlinked, complementary resistance genes in CC152. However, if there were a single resistance gene, a deviation from the expected 1:1 ratio might have been caused by the segregation of other genes which affected the fitness of sensitive progeny.

Table 5. Associations between responses to two fungicides and avirulence on seven varieties in progeny of the *Erysiphe graminis* f.sp. *hordei* cross CC151 × DH14

Fungicide or variety	Pathogen gene	Fungicide or variety	Pathogen gene	χ^2	P[1]	r^2	m[3]
Fenpropidin	*Fen1*	Triadimenol	*Tdl(a)*	0.00			
Fenpropidin	*Fen1*	P03	$Avr_{a6}1$ $Avr_{a6}2$	1.21			
Fenpropidin	*Fen1*	P09	Avr_{a10}	1.32			
Fenpropidin	*Fen1*	P10	Avr_{a12}	0.24			
Fenpropidin	*Fen1*	P17	Avr_k Avr_{P17}	0.00			
Fenpropidin	*Fen1*	P23	Avr_{La}	0.40			
Fenpropidin	*Fen1*	P24	Avr_h	0.16			
Fenpropidin	*Fen1*	Hordeum 1063	Avr_k	1.32			
Triadimenol	*Tdl(a)*	P03	$Avr_{a6}1$ $Avr_{a6}2$	8.53	**	10.0[4]	10.1
Triadimenol	*Tdl(a)*	P09	Avr_{a10}	0.96			
Triadimenol	*Tdl(a)*	P10	Avr_{a12}	18.03	***	17.5	18.3
Triadimenol	*Tdl(a)*	P17	Avr_k Avr_{P17}	11.61	***	0.0[5]	0.0
Triadimenol	*Tdl(a)*	P23	Avr_{La}	6.40	*	30.0	34.7
Triadimenol	*Tdl(a)*	P24	Avr_h	0.02			
Triadimenol	*Tdl(a)*	Hordeum 1063	Avr_k	0.96			
P03	$Avr_{a6}1$ $Avr_{a6}2$	P09	Avr_{a10}	2.88			
P03	$Avr_{a6}1$ $Avr_{a6}2$	P10	Avr_{a12}	6.01	*	20.7[4]	22.0
P03	$Avr_{a6}1$ $Avr_{a6}2$	P17	Avr_k Avr_{P17}	5.78	*	18.2[4,5]	19.1
P03	$Avr_{a6}1$ $Avr_{a6}2$	P23	Avr_{La}	0.00			
P03	$Avr_{a6}1$ $Avr_{a6}2$	P24	Avr_h	0.01			
P03	$Avr_{a6}1$ $Avr_{a6}2$	Hordeum 1063	Avr_k	2.88			
P09	Avr_{a10}	P10	Avr_{a12}	0.06			
P09	Avr_{a10}	P17	Avr_k Avr_{P17}	18.34	***	0.0[6]	0.0
P09	Avr_{a10}	P23	Avr_{La}	2.67			
P09	Avr_{a10}	P24	Avr_h	0.37			
P09	Avr_{a10}	Hordeum 1063	Avr_k	40.00	***	0.0[6]	0.0
P10	Avr_{a12}	P17	Avr_k Avr_{P17}	4.22	*	20.0[5]	21.2
P10	Avr_{a12}	P23	Avr_{La}	0.96			
P10	Avr_{a12}	P24	Avr_h	0.60			
P10	Avr_{a12}	Hordeum 1063	Avr_k	0.06			
P17	Avr_k Avr_{P17}	P23	Avr_{La}	7.03	**	20.0[5]	21.2
P17	Avr_k Avr_{P17}	P24	Avr_h	1.02			
P17	Avr_k Avr_{P17}	Hordeum 1063	Avr_k	19.36	***	na[7]	na
P23	Avr_{La}	P24	Avr_h	2.06			
P23	Avr_{La}	Hordeum 1063	Avr_k	2.67			
P24	Avr_h	Hordeum 1063	Avr_k	0.32			

[1] * $0.05 \geq P > 0.01$; ** $0.01 \geq P > 0.001$; *** $0.001 \geq P$ (P: chance probability of χ^2 at least as large).
[2] r, recombination fraction (%).
[3] m, map distance (cM).
[4] Linkage with $Avr_{a6}1$ matching *Mla6* in P03.
[5] Linkage with Avr_{P17} matching the *Ml(P17)* resistance in P17.
[6] Linkage with Avr_k matching *Mlk* in P17 or Hordeum 1063.
[7] na, not applicable because both varieties have *Mlk*.

These two possibilities cannot be distinguished without an extensive programme of test-crossing. The data do not indicate whether or not the resistance alleles in CC152 are at the *Fen1* locus.

(iii) and (iv): In CC151 × DH14, but not in CC152 × DH14, the Fpd/Fpm-resistant progeny were significantly more resistant to Tdm than were the sensitive progeny. Also, the resistant progeny of CC151

× DH14 were significantly more resistant to Fpm, but not to Fpd, than were the resistant progeny of CC152 × DH14. One hypothesis which accounts for these observations is that the $fen1R_{CC151}$ allele confers reduced sensitivity to Tdm, whereas the Fpd/Fpm-resistance genes in CC152 have little effect on the response to Tdm. This may mean that the morpholine-resistance genes in CC152 are not at the *Fen1* locus.

The genetics of cross-resistance suggest that, in these isolates, the Fpd/Fpm-response may be principally associated with the common factor of these two compounds, the *N*-substituent chain, rather than with the *N*-heterocycle, which differs in Fpd and Fpm but is the same in Fpm and Tdm (Baloch et al. 1984). The lack of cross-resistance between Fpd and Fpm on one hand, and Tdm on the other, reflects the modes of action of the fungicides in a number of fungi, including *Egh* (Baloch et al., 1984; Berg et al., 1984; Girling et al., 1988). All three fungicides inhibit the reduction or isomerisation of two C:C bonds in the ergosterol synthesis pathway, but Fpd and Fpm preferentially inhibit Δ^{14}-reductase, while Tdm preferentially inhibits $\Delta^8 \rightarrow \Delta^7$ isomerase. In *Ustillago maydis*, Fpm mainly inhibits Δ^{14}-reductase, but also inhibits $\Delta^8 \rightarrow \Delta^7$ isomerase to a greater extent than Fpd does (Baloch et al. 1984).

The genetics support the view that the correlation of resistances to Fpd and to Fpm in the British population of *Egh* is caused by the two resistances being pleiotropic effects, rather than by a chance association of two resistance genes in several different clones of the pathogen. It also indicates that these genes have comparatively little effect on response to Tdm. This supports the view that Tdm belongs to a different cross-resistance group of fungicides to that of Fpd and Fpm, and implies that alternating or mixing Tdm with Fpd or Fpm would lead to better control of mildew than would mixing or alternating Fpd and Fpm with each other.

Of course, an analysis of just two isolates may well not detect all the Fpd/Fpm-resistance genes which might exist in the British population of *Egh*. Nor does it indicate that mutation of other genes, giving higher levels of resistance, may not occur in the future. A fuller understanding of the genetics of morpholine-responses would therefore require analysis of more isolates.

By contrast to *Egh*, resistance to Fpm in both the barley net blotch fungus, *Pyrenophora teres* (Peever and Milgroom, 1993), and in cereal eyespot fungi, *Pseudocercosporella* spp. (Hocart and McNaughton, 1994) was continuously distributed in the progeny of crosses. This suggests that in these cases, resistance may be polygenic, and indicates that no single genetic model of morpholine-resistance may be appropriate for all fungi.

Hollomon (1994) remarked that CC151's resistance was within the range of wild-type variation in *Egh* in Scotland in 1993. However, since the mid-1980's, morpholines were used extensively to control barley mildew in north-east Scotland, where CC151 was collected in 1990. The *Egh* population was therefore subjected to strong selection by morpholines, and in 1990, no S-type isolates were found in four sites in Scotland. No isolates as resistant as CC151 or CC152 were collected before 1986 (Wolfe et al., 1987). The phenotypic variation in the 1993 sample discussed by Hollomon (1994) was therefore not truly wild-type, but reflected the earlier shift in morpholine-responses between 1986 and 1990 (Wolfe et al., 1987; Brown et al., 1991; Brown and Evans, 1992). The reason why there has not been a serious loss of control of mildew by morpholines, despite the evolution of resistance to the level of CC151 or CC152, may be because the application rate of these fungicides is comparatively high. Fpd and Fpm are toxic to S-type isolates, such as DH14, at one to two hundredths of the recommended rate (Table 3, and see Brown et al., 1991). A ten-fold fall in the sensitivity of *Egh* to morpholines might therefore still allow good control of mildew by these fungicides at the time of application, but reduce the length of time for which disease control is satisfactory.

Responses to triadimenol. The $tdl(a)R_{CC151}$ allele conferred a medium level of triadimenol-resistance as defined by Brown and Wolfe (1991), and was linked to the avirulence genes $Avr_{a6}1$, Avr_{a12}, Avr_{La} and Avr_{P17}, but not to $Avr_{a6}2$, Avr_{a10}, Avr_h or Avr_k. In another cross, CC107 × DH14, a triadimenol-response gene, *Tdl1*, of which the allele $tdl1R_{CC107}$ conferred medium resistance, was also unlinked to Avr_h; the other seven avirulence genes which segregated in CC151 × DH14 did not segregate in CC107 × DH14 (Brown and Jessop, 1995). However, in another cross, CC138 × DH14, a different gene, *Tdl2*, of which the allele $tdl2R_{CC138}$ conferred high resistance to triadimenol, was linked to Avr_h, but not to Avr_{a12} or Avr_{La}. *Tdl2* and Avr_k were unlinked in this cross, as were *Tdl(a)* and Avr_k in CC151 × DH14, while the other four avirulences which segregated in CC151 × DH14 either did not segregate in CC138 × DH14 or were not tested (Brown and Jessop, 1995). These linkages indicate

that *Tdl2* is a different locus to both *Tdl1* and *Tdl(a)*. However, whether or not *Tdl1* and *Tdl(a)* are the same locus is an open question, which could be tested by inter-crossing resistant progeny of the crosses CC107 × DH14 and CC151 × DH14.

Regarding the name of the *Tdl(a)* locus, we propose that a fungicide response locus should only be assigned a serial number (as in *Tdl1* and *Tdl2*) if it is either indistinguishable from a known locus, which already has a serial number, or differs from all known loci, in which case it should be given a new serial number. *Tdl(a)* is clearly not the same locus as *Tdl2*, but whether or not it is the same as *Tdl1* is not known. We propose the use of serial letters, in brackets, as in *Tdl(a)*, for provisionally identified loci; this will prevent confusion in the numerical series of loci.

Fen1 was not linked to *Tdl(a)*, nor were responses to Fpd and Fpm associated with responses to triadimenol in CC152 × DH14. That resistances to morpholines and triazoles are controlled by different genes is consistent with morpholines and DMIs, such as triadimenol, acting at different points in the ergosterol biosynthesis pathway (Mercer, 1991).

In *Pyrenophora teres*, genetic correlation coefficients between responses to Fpm and to four DMIs were not significantly different from zero, indicating that in this fungus, as in *Egh*, responses to these two types of fungicide are controlled by separate genes (Peever and Milgroom, 1993). In *Pseudocercosporella herpotrichoides*, a major gene, controlling a large difference in responses to DMIs, had no significant effect on the response to Fpm, but additional, minor genes for triadimenol resistance did have some effect on the Fpm-response. In an inter-specific cross of *P. herpotrichoides* × *P. anguioides*, responses to Fpm and to the DMI difenconazole were weakly correlated (Hocart and McNaughton, 1994). This indicates that no one genetic system of cross-resistance between the two groups of ergosterol biosynthesis inhibitors may apply to all fungi.

Avirulences. Two avirulence genes matched the *Mla6* resistance. A reason why neither of these genes corresponds to *Mla14*, which is closely linked to *Mla6* (Mahadevappa et al., 1994), was given in the Results section. A strict application of the gene-for-gene hypothesis would predict the existence of a third gene, also closely linked to *Mla6*, such that either $Avr_{a6}1$ or $Avr_{a6}2$ matches *Mla6*, while the other matches the putative third gene (cf. Jørgensen, 1992, regarding other *Mla* genes). However, molecular genetic experiments have shown that, in *Pseudomonas syringae*, two avirulence genes match a single resistance gene, *RPS3/RPM1*, in *Arabidopsis thaliana* (Bisgrove et al., 1994). It is therefore conceivable that both $Avr_{a6}1$ and $Avr_{a6}2$ may match *Mla6* itself.

A previously unknown resistance specificity was identified by the segregation of avirulence on P17. This variety is a near-isogenic line of cv. Pallas, with *Mlk* introgressed from Monte Cristo (Kølster et al., 1986), which was the source of *Mla9* in barley breeding. Two other sources of *Mlk* are known, Hordeum 1063, which has been used as a differential variety in population studies of *Egh*, and Lyallpur 3645, which donated both *Mlk* and *Mla7* to barley breeding. The situation with *Mlk* has parallels with the *Mla3* gene, which was introduced into breeding from two sources. One of these, Turkish, has an additional specificity which is not carried by the other source, Ricardo (Jensen et al., 1992). In a more complex situation, there are four sources of *Mla7*. Two Avr_{a7} genes match all four sources, (Brown and Jessop, 1995), while there are also additional specificities in one or two of these sources (Jensen et al., 1992; Jørgensen, 1992).

Variation in the avirulence genes that are detected by the two sources of *Mlk* could be due to variation in the exact specificity of the products of the *Mlk* genes in Hordeum 1063 and Monte Cristo, or, perhaps more likely, to the presence of a second gene in Monte Cristo, closely linked to *Mlk*. These alternative hypotheses are unlikely to be resolved without molecular cloning of the resistance and avirulence genes.

In CC151 × DH14, as in CC138 × DH14, Avr_{a12} and Avr_{La} were not associated. However, in CC151 × DH14, both of these genes were linked to *Tdl(a)* and Avr_{P17}. In CC138 × DH14, Avr_{a11} was 25.4 cM from Avr_{La}, but was not linked to Avr_{a12}. This suggests that the order of these genes is probably Avr_{a12} – *Tdl(a)*/Avr_{P17} – Avr_{La} – Avr_{a11}. $Avr_{a6}1$ is also linked to this group of genes, but its position cannot yet be determined. Avr_{a10} and Avr_k have previously been shown to be closely linked, with estimates of $m = 4.1$ cM (Brown and Jessop, 1995) and $r = 7\%$ (Jørgensen, 1988). The lack of recombination between these loci in CC151 × DH14 was probably due to the relatively small number of progeny analysed.

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