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Levels of resistance of Erysiphe graminis f.sp. hordei to the systemic fungicide triadimenol

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Abstract

A random sample of conidiospores of *Erysiphe graminis* f.sp. *hordei* was obtained from the atmosphere above Cambridge, England, by incubating them on barley seedlings exposed on three dates in 1985. The asexual progeny of each spore was tested for its response to a range of doses of a systemic triazole fungicide, triademenol. Principal components analysis of the data revealed that the majority of isolates had a distinct level of response to triadimenol, being resistant to triadimenol at the rate apoplied to seed commercially. The remaining isolates appeared either to be sensitive to the lowest dose of triadimenol used, or to have one of two intermediate levels of resistance. There was a significant increase in the frequency of isolates with higher levels of resistance to triadimenol during 1985. This is likely to have been a response to the continuing widespread use of demethylation inhibitor (DMI) fungicides by British farmers.

Additional keywords: barley powdery mildew, principal components analysis, triazole, demethylation inhibitor.

Introduction

The systemic triazole fungicide triadimenol (1-(4-chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)-2-ol) has been widely used to control powdery mildew of barley, caused by *Erysiphe graminis* DC. f.sp. *hordei* Marchal. Triazoles are members of the demethylation inhibitor (DMI) group of compounds, which inhibit the C-14 demethylation step in the synthesis of ergosterol (Dekker, 1985). Triadimenol was introduced to the U.K. in 1980, as a seed treatment to control barley mildew, and increased rapidly in use. Since 1977, other DMIs have been used to control barley mildew, either as sprays or seed treatments, and as single-compound formulations or mixed with other fungicides with different modes of action (Gilmour, 1984; Heaney et al., 1984, 1986; Cook and Yarham, 1985; Noon et al., 1986).

Resistance to DMIs in *E. graminis* appeared in Britain in 1981 (Fletcher and Wolfe, 1981; Wolfe and Fletcher, 1981) and increased in frequency in the following years (Heaney et al., 1984, 1986; Wolfe, 1984; Wolfe et al., 1988). Skylakakis (1985) distinguished continuous and discontinuous distributions of the level of fungicide resistance in pathogen populations. Most previous reports of DMI resistance in *E.*

graminis have described levels of resistance as being continuously distributed, both in isolates derived from single colonies or spores (Butters et al., 1984; Andrivon and De Vallavieille-Pope, 1987; Limpert, 1987), and in 'bulk' isolates sampled from a plant or a field, possibly containing several clones (Heaney et al., 1984, 1986; De Waard et al., 1986; Fletcher et al., 1987; Enisz, 1988). Wolfe et al. (1986), however, reported four discrete levels of resistance to triadimenol among single colony isolates of *E. graminis* f.sp. *hordei*.

The work described in this paper was undertaken to describe the various levels of resistance to triadimenol in a population of *E. graminis* f.sp. *hordei*, and to discover how the frequencies of these levels change under selection by the use of DMI fungicides.

Materials and methods

Mildew samples. Trays of seedlings of spring barley cv. Golden Promise, which is susceptible to all known clones of *E. graminis* f.sp. *hordei* in Britain, were exposed for one week on the roof of a tall building in the centre of Cambridge, at least 2 km from the nearest field of barley, on three dates in 1985. They were then kept at 13 °C for one week. Small pieces of leaf, each bearing a single colony of powdery mildew and sampled at random, were cut from the seedlings and placed on 4.5 g/l agar containing 100 mg/l benzimidazole (Sigma Chemical Co. Ltd., Poole). Three days later, spores from each colony were tapped onto 2.5 cm detached leaf sections of cv. Golden Promise. Isolates were maintained thereafter on detached leaves, on agar containing 150 mg/l benzimidazole. 88 isolates were collected from 26 April - 3 May, 99 from 12-19 July and 100 from 4-11 October.

Resistance assays. Seeds of cv. Golden Promise were treated with triadimenol, formulated as Baytan (Bayer AG, Leverkusen) at 0.0, 0.1, 0.3, 0.5, 1.0, 1.5 (commercial rate) or 2.5 g Baytan/kg seed (25% triadimenol). The fungicide was applied to the seeds in a polymer medium by Nickersons Seed Specialists Ltd., Rothwell, using a commercial process. Seedlings were grown under spore-free conditions in a glasshouse, maintained as close as possible to 12 °C, and given 16 hours/day of light from sodium lamps.

Isolates were tested in 17 batches for response to triadimenol. April/May isolates were tested in batches 4, 5, 6, 13 and 14, July isolates in batches 1, 2, 8, 9 and 11; and October isolates in batches 3, 7, 10 and 12. Batches 15, 16 and 17 included isolates sampled at all three times. Each batch included control isolates, one sensitive to triadimenol (SC1L) and one resistant (SC66L). Sections, 2.5 cm long, were cut from the first leaf of each seedling, and placed on 4.5 g/l agar containing 100 μg/l benzimidazole in a clear polystyrene box, adaxial surface upwards. Either three or four replicates of each dose were used to test each isolate. Leaf sections were inoculated as described by Brown and Wolfe (1990), and then kept at 15 °C with 8 hours/day of low intensity light supplied by fluorescent tubes. Visible colonies were counted 7 days later, using a 2 × magnifying lens.

Statistical analysis. Data on the number of colonies formed by isolates at each dose were analysed by principal components analysis (PCA; Brown, 1991), using the

Genstat 5 package (Numerical Algorithms Group, Oxford). For each dose i, the variable analysed (y_i) was the mean number of colonies, divided by the sum of such means over all doses. Matrices of correlations of y_i were analysed.

The plot of the first two principal components (PCs), Z_1 and Z_2, can be interpreted by examining the loadings of y_i at each dose on Z_1 and Z_2, which indicate the basis on which the clusters are separated. When batches 5, 6 and 10 are examined, for example, the positive loading of y_i at the 0.0 g/kg dose on Z_1 contrasts with negative loadings at the 0.5, 1.0, 1.5 and 2.5 doses (Table 2). Isolates which formed few colonies on leaves treated with the higher doses therefore have high values of Z_1 while those which formed many colonies have low (negative) values of Z_1 (see Table 1).

Median effective doses (ED_{50}s) and fiducial limits were calculated by Wadley's method of probit analysis (Finney, 1971), using the Maximum Likelihood Program (Numerical Algorithms Group, Oxford) to fit probits of colony numbers to $\log_{10}$ of the dose. Wadley's method estimates probits when only surviving individuals, but not those that have died, can be enumerated.

Results

Detection of clusters of isolates. PCA was used to discover whether or not responses to triadimenol of different isolates fell into distinct categories. In all batches, Z_1 and Z_2 together accounted for most (76% to 95%) of the total variation in the data. Z_1 alone always accounted for a large part of the variation, from 49.6% to 76.0% of the total. This indicates that plots of $\{Z_1, Z_2\}$ display most of the observed variation.

Plots of $\{Z_1, Z_2\}$ for batches 5, 6 and 10 are shown in Figure 1 as examples. (These batches are presented because they contained at least one test isolate which fell into the low resistance group [see below]; several other batches did not.) In all batches, all isolates fell into one of two clusters on the basis of Z_1 values. One cluster, characterised by low (negative) values of Z_1, comprised isolates with relatively little response to triadimenol; these are described as the high resistance (H) group (data for batch 6 are given in Table 1 as an example; data for batch 5 are in Table 1 of Brown [1991]). The other cluster included isolates which had high Z_1 values and formed few or no colonies on leaves treated with the higher doses of triadimenol.

Isolates with high Z_1 values fell into three subsidiary clusters on the basis of their Z_2 values. Three isolates formed no colonies at all on treated leaves, and were classified as sensitive (group S), similar to control isolate SC1L. Twelve isolates had a low level of resistance, similar to that of SC66L (group L). The remaining isolates had a level of resistance intermediate between that of SC66L and group H, and thus have medium resistance (group M).

There were low (negative) factor loadings on Z_1 of y_i at the highest three or four doses, and a high (positive) loading of y_i at the untreated level in all batches (5, 6 and 10 given as examples in Table 2). The pattern of loadings on Z_2 was less consistent, but loadings of scores at the 0.1 and 0.3 g/kg doses were generally high (positive or negative – the sign makes no difference to the interpretation of the plot of $\{Z_1, Z_2\}$). This pattern is reflected in Figure 1, in which group H is separated from the other groups on Z_1 because M, L and S isolates produced few or no colonies on leaves treated with the highest doses of triadimenol. M, L and S isolates are separated mainly on Z_2 because differences between colony numbers were greatest at the lower doses.

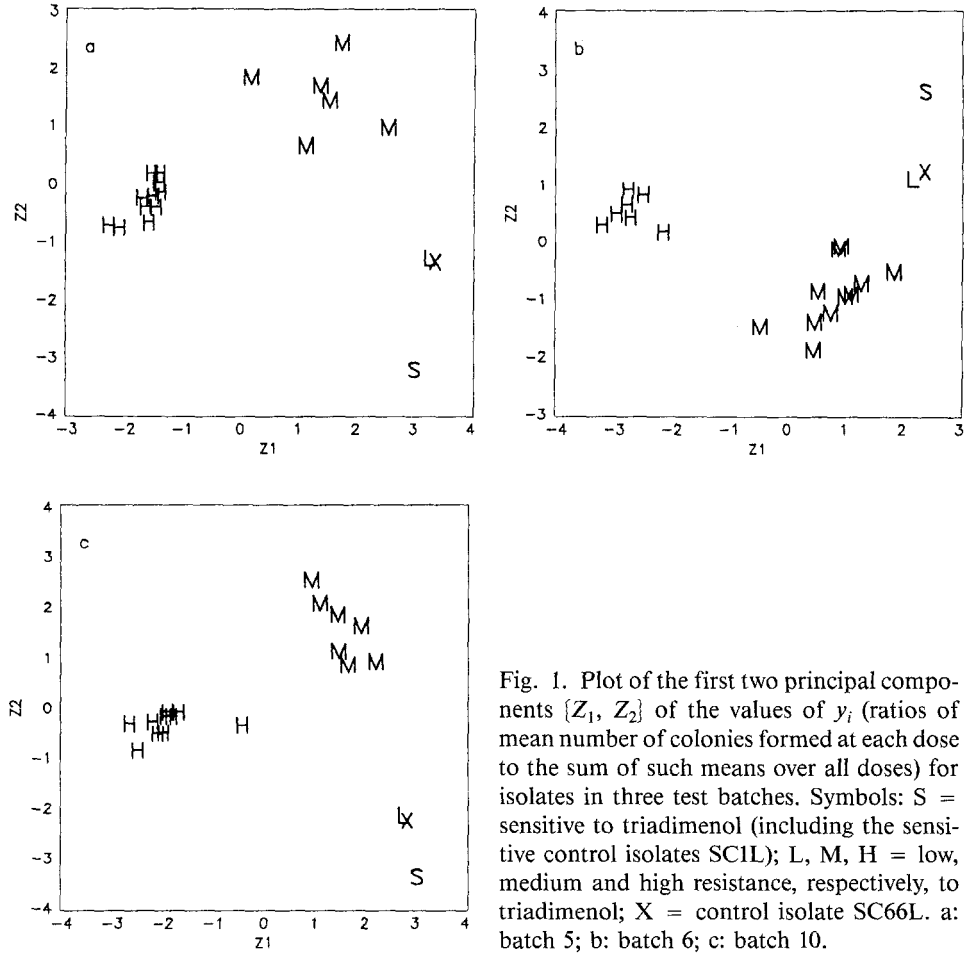

Fig. 1. Plot of the first two principal components $\{Z_1, Z_2\}$ of the values of y_i (ratios of mean number of colonies formed at each dose to the sum of such means over all doses) for isolates in three test batches. Symbols: S = sensitive to triadimenol (including the sensitive control isolates SC1L); L, M, H = low, medium and high resistance, respectively, to triadimenol; X = control isolate SC66L. a: batch 5; b: batch 6; c: batch 10.

Of the 287 isolates tested, 279 (97.2%) fell clearly into one of the four groups. Of the remainder, four survived for a second test to be carried out, in which all were placed in group H. Since a second test was definitive, the four isolates that could not be re-tested were placed in the group to which they were nearest in the PC plot for their batch, for the purpose of further analysis. These included one M isolate from April/May and one July and two October isolates in group H.

A check batch of isolates was tested, to discover if the three levels of resistance into which isolates were grouped in the separate batches were the same three levels in all cases. At least one isolate from the H, M and L groups in each batch (some of which had been tested in more than one batch), SC1L and SC66L were tested. The plot of $\{Z_1, Z_2\}$ (Figure 2a) shows that the H isolates formed a distinct, coherent cluster (Tables 2 and 3). The distinction between isolates previously classified as M, L or S was less clear. When the data were analysed with the H isolates omitted, the three remaining groups were distinguished by Z_1, although the clusters were not clearly isolated (Figure 2b).

Table 1. Mean numbers of colonies formed at each dose by the isolates tested in batch 6, scores on the first and second principal components $\{Z_1, Z_2\}$ and the classification of isolates as having high (H), medium (M) or low (L) resistance to triadimenol, or as sensitive (S).

Dose of triadimenol (g Baytan/kg seed)							Principal component scores		Classification
0.0	0.1	0.3	0.5	1.0	1.5	2.5	Z_1	Z_2	
10.50	9.25	19.75	20.25	15.00	14.00	5.00	−3.31	0.31	H
26.00	27.00	33.00	32.75	23.25	24.50	18.00	−3.15	0.50	H
33.50	17.25	25.75	39.50	20.00	18.75	13.25	−2.87	0.66	H
49.00	19.00	39.25	34.75	27.00	23.50	22.50	−2.84	−0.94	H
36.50	31.00	45.75	33.75	29.25	35.00	16.50	−2.80	0.44	H
8.25	5.50	4.25	7.00	5.25	5.75	2.25	−2.57	0.85	H
19.50	15.00	17.25	16.50	12.00	11.50	4.50	−2.22	0.17	H
14.75	12.75	15.00	11.00	3.50	0.00	0.00	−0.49	−1.52	M
22.50	29.75	16.00	13.25	0.75	0.00	0.00	0.47	−1.92	M
34.75	17.00	30.50	8.00	0.25	0.25	0.00	0.49	−1.44	M
29.75	18.00	19.00	1.50	4.25	1.50	0.50	0.55	−0.89	M
19.25	11.25	12.25	4.50	0.00	0.00	0.00	0.78	−1.27	M
15.75	10.75	8.50	3.50	0.25	0.00	0.00	0.91	−1.24	M
15.75	15.75	3.75	2.50	2.75	0.00	0.00	1.03	−0.98	M
37.50	20.75	17.50	4.75	0.25	0.25	0.00	1.15	−0.94	M
11.75	7.50	3.00	2.25	0.00	0.00	0.00	1.32	−0.74	M
53.50	34.50	8.75	2.50	0.25	0.00	0.00	1.90	−0.54	M
68.25	34.25	8.00	2.75	0.25	0.00	0.00	1.98	−0.08	M
34.00	8.75	0.25	1.25	0.00	0.00	0.00	2.22	1.13	L
18.25	5.00	0.00	0.00	0.00	0.00	0.00	2.44	1.15	L (SC66L)
9.75	0.00	0.00	0.00	0.00	0.00	0.00	2.45	2.71	S (SC1L)

Differences within and between clusters. PCA is not based on an underlying model, so tests of significance of the cohesion within clusters or differences between clusters cannot be made. PCA can only discern patterns which may then be tested by a different method, if one is available. Contingency tables were used to examine variation within and between groups.

χ^2 statistics, comparing responses to the set of doses, between all isolates in each batch, between isolates within each group and between groups are given in Table 4. Data for the eight isolates which were not assigned to one of the four groups were omitted. As there were several missing values, the mean colony numbers formed by isolates at each dose were used as data. Since values of χ^2 depend on the sample size, Cramer's coefficient of contingency (C; Everitt, 1977) was used to indicate the relative strength of associations in contingency tables.

In all batches, χ^2 for the between-group contrast was a much larger fraction of χ^2 between all isolates than were within-group χ^2 values. Values of C for between-group contrasts were relatively high, from 0.051 (batch 5) to 0.16 (batch 14), indicating large differences between the responses of the four groups of isolates to triadimenol. With three notable exceptions, C was low for within-group contrasts, from 0.0040 (batch

Table 2. Factor loadings on the first two principal components, $\{Z_1, Z_2\}$, of ratios of mean numbers of colonies at each dose to the sum of such means over all doses (y_i), for each dose, and percentages of the total variation accounted for by Z_1 and Z_2. Analyses are presented for batches 5, 6 and 10, for which plots of $\{Z_1, Z_2\}$ are shown in Figure 1, and the check batch with and without high resistance (H) isolates (Figure 2).

Batch	PC	% total variation	Dose of triadimenol (g Baytan/kg seed)						
			0.0	0.1	0.3	0.5	1.0	1.5	2.5
5	1	57.9	0.444	0.269	−0.157	−0.365	−0.467	−0.443	−0.399
	2	23.7	−0.315	0.473	0.679	0.332	−0.157	−0.169	−0.231
6	1	63.3	0.408	0.163	−0.218	−0.427	−0.449	−0.442	−0.426
	2	23.5	0.394	−0.617	−0.564	−0.164	0.124	0.219	0.236
10	1	59.9	0.429	0.093	−0.133	−0.455	−0.463	−0.453	−0.405
	2	30.3	−0.329	0.647	0.636	0.061	−0.151	−0.137	−0.152
Check (all isolates)	1	70.1	0.394	0.242	−0.372	−0.415	−0.412	−0.421	−0.358
	2	14.9	−0.471	0.793	0.330	0.014	−0.077	−0.068	−0.171
Check (no H isolates)	1	47.6	0.571	−0.478	−0.472	−0.205	−0.392	−0.166	0.000
	2	17.9	0.027	−0.255	0.020	0.687	−0.293	0.613	0.000

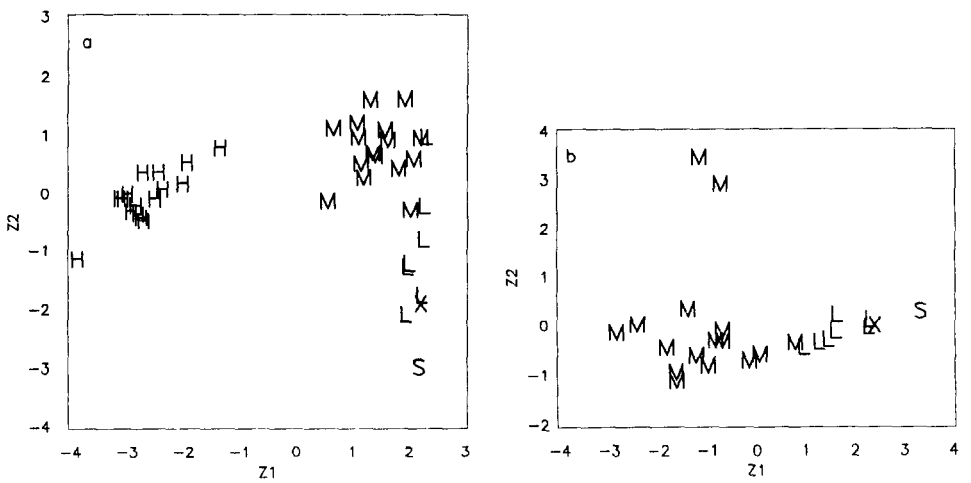

Fig. 2. Plot of the first two principal components $\{Z_1, Z_2\}$ of the values of y_i (ratios of mean number of colonies formed at each dose to the sum of such means over all doses) for isolates in the check batch of tests. Symbols: S = sensitive to triadimenol (including the sensitive control isolate SC11); L, M, H = low, medium and high resistance, respectively, to triadimenol; X = control isolate SC66L. a: all isolates; b: only isolates classified previously as M, L or S.

Table 3. Mean numbers of colonies formed at each dose by the isolates tested in the check batch, scores on the first and second principal components $\{Z_1, Z_2\}$ when data of all isolates, or with highly resistant (H) isolates omitted, were analysed, and the classification of isolates as having high, medium (M) or low (L) resistance to triadimenol, or as sensitive (S) in the original batches of tests.

Dose of triadimenol (g Baytan/kg seed)							Principal component scores – all isolates		Principal component scores – omitting H isolates		Classification in previous batch
0.0	0.1	0.3	0.5	1.0	1.5	2.5	Z_1	Z_2	Z_1	Z_2	
11.50	6.00	9.00	9.67	13.67	9.33	8.00	−3.10	−0.93			H
17.50	13.67	20.67	16.67	9.67	16.67	4.33	−3.06	−0.23			H
12.50	21.33	15.33	15.67	15.33	12.67	2.67	−2.84	0.19			H
20.67	12.00	14.00	10.33	17.67	12.33	2.67	−2.81	−0.65			H
28.00	21.33	37.00	20.67	24.00	13.00	9.33	−2.79	−0.07			H
19.50	12.67	17.00	12.00	8.00	14.00	0.00	−2.76	−0.25			H
25.00	30.67	30.00	31.00	18.00	20.33	15.33	−2.63	0.05			H
19.33	17.33	17.00	17.67	11.00	11.67	8.00	−2.45	−0.13			H
16.67	13.00	13.00	16.33	8.33	6.00	4.00	−2.36	−0.14			H
26.33	25.67	22.00	21.00	16.33	17.00	14.33	−2.29	−0.11			H
31.00	26.00	21.00	20.33	23.67	14.67	13.00	−2.26	−0.28			H
10.67	8.00	8.00	8.67	6.00	2.00	0.67	−2.23	−0.04			H
14.00	11.00	11.33	9.67	2.00	9.00	7.67	−1.98	−0.05			H
19.67	21.00	18.33	14.67	8.67	6.33	3.00	−1.96	0.43			H
24.00	21.67	28.00	18.33	17.33	12.00	1.67	−1.70	0.10			H
14.33	15.00	11.33	5.67	4.33	6.67	0.00	−1.69	0.54			H
20.67	24.00	7.00	0.00	0.33	0.33	0.00	1.17	1.62	−2.84	−0.13	M
29.67	25.33	13.00	4.00	0.33	0.00	0.00	0.47	1.06	−2.42	0.03	M
35.67	28.67	11.00	2.33	0.33	0.00	0.00	0.94	0.94	−1.82	−0.43	M
51.50	44.67	7.33	2.33	0.67	0.00	0.00	1.51	0.96	−1.63	−0.95	M
26.33	18.00	6.00	0.33	0.33	0.00	0.00	1.27	0.65	−1.63	−1.11	M
8.67	8.33	2.67	1.00	0.00	0.00	0.00	0.93	1.21	−1.39	0.34	M
48.33	33.67	11.33	1.33	0.33	0.00	0.00	1.22	0.68	−1.22	−0.61	M
12.67	7.67	2.00	1.33	0.00	0.33	0.00	1.06	0.26	−1.16	3.47	M
7.33	9.00	1.00	0.00	0.00	0.00	0.00	1.83	1.70	−0.97	−0.80	M
19.00	17.33	3.67	0.67	0.00	0.00	0.00	1.46	1.13	−0.83	−0.30	M
12.67	6.33	1.67	4.33	0.00	0.00	0.00	0.38	−0.20	−0.73	2.92	M
13.67	7.67	4.33	0.00	0.00	0.00	0.00	1.00	0.47	−0.69	−0.31	M
44.50	33.00	0.67	4.33	0.33	0.00	0.00	1.73	0.50	−0.68	−0.09	M
25.00	23.00	0.67	0.00	0.00	0.00	0.00	2.14	1.06	−0.14	−0.71	M
18.67	14.00	1.00	0.00	0.00	0.00	0.00	2.01	0.68	0.07	−0.58	M
24.67	11.33	1.33	0.00	0.00	0.00	0.00	1.95	−0.23	0.80	−0.34	M
36.33	18.67	0.00	0.00	0.00	0.00	0.00	2.21	−0.13	0.95	−0.42	L
6.00	5.67	0.00	0.00	0.00	0.00	0.00	2.24	1.10	1.27	−0.33	L
32.00	11.67	0.00	0.00	0.00	0.00	0.00	2.20	−0.74	1.46	−0.27	L
15.00	3.67	0.67	0.00	0.00	0.00	0.00	1.94	−1.20	1.61	−0.10	L
13.67	3.33	0.33	0.33	0.00	0.00	0.00	1.90	−1.26	1.63	0.25	L
22.67	4.00	0.00	0.00	0.00	0.00	0.00	2.18	−1.72	2.28	−0.02	L
7.33	0.67	0.33	0.00	0.00	0.00	0.00	1.88	−2.09	2.32	0.14	L
50.50	7.67	0.00	0.00	0.00	0.00	0.00	2.17	−1.88	2.41	0.02	L (SC66L)
24.00	0.00	0.00	0.00	0.00	0.00	0.00	2.15	−2.98	3.34	0.30	S (SC1L)

Table 4. χ^2 statistics (and degrees of freedom) for mean colony numbers, in isolate (or group) × dose contingency tables.

Batch of	Contrast									
	between all isolates		between groups		within high group		within medium group		within low group	
1	564	(95)	473	(15)	29.9	(45)	83.0	(35)		
2	834	(114)	581	(12)	64.0	(54)	250	(48)		
3	1640	(120)	1440	(18)	158	(66)	15.0	(18)	5.63	(18)
4	834	(114)	683	(18)	102	(54)	50.8	(42)		
5	632	(114)	476	(18)	108	(60)	48.8	(30)	0.594	(6)
6	720	(126)	587	(18)	29.8	(36)	133	(60)	0.816	(6)
7	215	(42)	132	(12)	87.3	(30)				
8	661	(114)	508	(12)	62.9	(60)	47.7	(36)	18.2	(6)
9	1270	(126)	1010	(18)	205	(72)	79.6	(30)	11.6	(6)
10	1050	(114)	894	(18)	101	(54)	48.9	(36)	1.62	(6)
11	290	(65)	188	(15)	81.1	(40)	12.4	(10)		
12	1170	(126)	876	(18)	119	(78)	93.9	(30)		
13	459	(66)	417	(18)	10.2	(12)	22.3	(30)	0.490	(6)
14	96.4	(36)	79.7	(6)	12.2	(12)	6.49	(18)		
15	2040	(300)	1370	(18)	466	(168)	175	(84)	8.06	(12)
16	428	(108)	261	(18)	104	(36)	28.8	(54)		
17	674	(54)	613	(18)	12.6	(12)	45.2	(24)		
Check	1130	(240)	920	(18)	102	(90)	79.0	(90)	24.3	(42)

1) to 0.028 (batch 16) for group H, 0.0084 (batch 3) to 0.030 (batch 6) for M and 0.0043 (batch 3) to 0.017 (check batch) for L. These values indicate relatively little difference in triadimenol response of isolates in the same group. The exceptional values of C were 0.21 within two L isolates in batch 8, 0.059 within two L isolates in batch 9 and 0.15 within three M isolates in batch 11. In the case of the L group in batch 8, χ^2 was significant ($P < 0.01$), which may indicate that the two isolates differ in their response to triadimenol even though both fell into the same cluster in PCA. In the latter two cases, however, χ^2 was not significant ($P > 0.05$); interpretation of these results is problematical.

Change in frequencies of levels of response. The numbers of isolates in each sample in the four groups are given in Table 5. The overall sample × group χ^2 statistic ($\chi^2_6 = 11.33$; $P < 0.1$) was partitioned into a component due to linear regression of phenotype frequency on sampling date and a component of departure from regression (Evritt, 1977; section 3.6), with equally-spaced arbitrary scores assigned to successive levels of response and to successive dates. The regression χ^2_1 was 5.77 ($P < 0.025$), while the random departure χ^2_5 was 5.56 ($P > 0.1$). There was therefore a significant increase in the frequency of isolates with higher levels of resistance through the period of sampling.

Estimated ED_{50}s. ED_{50}s of triadimenol were calculated for each isolate. Fiducial

Table 5. The number of *Erysiphe graminis* f.sp. *hordei* isolates in each sample which were sensitive, or had low, medium or high resistance, to triadimenol.

Sample	Sensitive	Resistant			Total
		low	medium	high	
April/May	2	4	41	41	88
July	0	3	35	61	99
October	1	5	27	67	100
Total	3	12	103	169	287

Association of levels of resistance with sampling date:

Source of variation	Degrees of freedom	χ^2	P [1]
Due to linear regression of phenotype frequency on sampling date	1	5.77	0.016
Departure from regression	5	5.56	0.351
Overall	6	11.33	0.079

[1] P = probability of a greater value of χ^2 occurring by change.

limits of the ED_{50} could not be calculated for many isolates. The standard error (s.e.) of $\log_{10}(ED_{50})$ for many isolates could not be estimated, while estimates of s.e. $\{\log_{10}(ED_{50})\}$ of several others were unreasonably large. Some isolates which showed little response even to the highest dose had very low estimated ED_{50}s. These shortcomings of probit analysis, when used to estimate the response of biotrophic parasites to systemic chemicals, are discussed by Brown (1991).

To give a broad impression of the levels of resistance observed, ED_{50}s were estimated for groups of isolates in batches 5, 6 and 10 and the check batch (Table 6). In all four batches, the estimated ED_{50} of the H group was greater than the commercial rate of triadimenol (1.5 g Baytan/kg seed). Estimates of s.e. $\{\log_{10}(ED_{50})\}$, which would be meaningless for data pooled across several isolates, are omitted.

Table 6. Estimates of the median effective dose (ED_{50}) of triadimenol, as g Baytan/kg seed, of isolates in batches 5, 6, 10 and the check batch. Data were the totals of mean colony numbers formed at each dose by isolates within each group. Probits of colony number were fitted to $\log_{10}$(dose) by maximum likelihood, using Wadley's method.

Batch of isolates	Level of resistance		
	low	medium	high
5	0.083	0.40	2.6
6	0.057	0.27	2.3
10	0.021	0.31	1.8
Check	0.073	0.16	1.6

Discussion

Levels of resistance to triadimenol. Efforts to control resistance to pesticides require knowledge of how the level of resistance changes in response to pesticide use. We used PCA to classify isolates of *E. graminis* f.sp. *hordei* by their response to triadimenol. This classification was meaningful in that clusters of isolates thus produced corresponded to different levels of response (Table 1).

A cluster of isolates with high resistance (H) to triadimenol was found in all batches (Figures 1, 2a). There is good evidence for group H being relatively distinct because it was clearly separated from isolates with lower resistance on Z_1. In batches 1-17, the cluster of isolates with higher Z_1 values was divided into three subsidiary clusters, distinguished by values of Z_2, containing sensitive (S) isolates or those with low (L) or medium (M) resistance (Figure 1). However, the clear separation of the M, L and S groups was not apparent in the check batch, although the L group fell between the S isolate and group M on Z_1 in Figure 2b. A possible cause of this discrepancy is that M isolates were apparently more sensitive than they were in the original tests (Table 6). Factor loadings (Table 2) show that M, L and S isolates were effectively discriminated by the lowest two doses, rather than three, in the check batch. Poor discrimination between *E. graminis* f.sp. *hordei* isolates which were previously classifiable into separate groups has occurred on other occasions (unpublished results). This has only happened when plants were grown in a glasshouse during unusually hot weather, and has always coincided with an apparent decrease in isolates' resistance. An alternative explanation is that, although distinct medium and low levels of resistance were observed in earlier batches, isolates in each batch had *different* medium and low levels; we do not find this plausible.

Since it is not based on an underlying model, PCA cannot test the significance of variation within or between groups. Nonetheless, analysis of contingency tables indicated substantial between-group differences in response, but relatively little within-group variation (Table 4).

We conclude that 58.9% of the isolates tested had a distinct, high level of resistance to triadimenol. There is more tentative evidence that the remaining 41.1% either had one of two lower levels of resistance, or were sensitive to the lowest dose used. The existence of different levels of response to lower doses, or of small differences in response between isolates within each major group, cannot be excluded. The significant difference between the two L isolates in batch 8 may be an instance of such minor genetic variation.

A drawback of PCA is that it does not quantify the resistance of isolates. We obtained rough estimates of ED_{50}s by pooling data across isolates within groups (Table 6). Estimation of ED_{50}s by probit analysis (or similar regression models), is a widely-used method of describing resistance to a pesticide (Finney, 1971). A major assumption of this method is that the dose applied to each leaf is absolutely known; currently available methods do not apply an exact dose to individual plants. The failure of this assumption led to our encountering the problems described in the Results section of this paper, which prevented sensible inference on differences between the ED_{50}s of isolates (Brown, 1991). We therefore ceased applying probit analysis to individual isolates.

Comparison with earlier reports. Our results agree with those of others, in showing resistance to triazoles among single colony isolates of *E. graminis* f.sp. *hordei* in northern Europe (Butters et al., 1984; Andrivon and De Vallavieille-Pope, 1987; Limpert, 1987). In our work, there appeared to be discrete levels of response, in contrast to the continuous distributions described in earlier papers. The population structure of the July 1985, studied by DNA restriction fragment length polymorphisms, was consistent with triadimenol resistance being under oligogenic, rather than polygenic, control (Brown et al., 1990).

We have considered possible causes of this contrast. Firstly, whereas our samples appeared to contain isolates with only four of many possible levels of response, there may have been a continuous range of responses in the populations sampled by Butters et al. (1984), Andrivon and De Vallavieille-Pope (1987) and Limpert (1987). In particular, we classified only 15 isolates as L or S; the distinction between groups S, L and M may have been an artefact, in that isolates with resistances intermediate between those of the L and M, or L and S, groups were insufficiently frequent to appear in our samples.

Secondly, details of the methods used by previous workers may have led to an inability to detect discrete levels of response. The methods of Butters et al. (1984), Limpert (1987) and Andrivon and De Vallavieille-Pope (1987) estimated effective doses of triadimenol, by various methods. Such estimation requires the experimenter to assume that the dose of fungicide applied to each leaf is known precisely; this assumption is clearly incorrect when systemic fungicides are applied to a biotrophic parasite via its host plant. The consequences of the failure of this assumption for the estimation of effective doses, and for inference on differences between effective doses, has been discused elsewhere (Brown, 1991). We therefore cannot exclude the possibility that the appearance of triazole resistance as a continuously distributed variable may have been, in part, the result of inappropriate methods of analysis.

Continuous distributions of resistance to DMIs found in bulk isolates of *E. graminis* (Fletcher and Wolfe, 1981; Heaney et al., 1984;Heaney et al., 1986; De Waard et al., 1986; Enisz, 1988), or in mildew on plants exposed to the aerial population of *E. graminis* spores (Fletcher and Wolfe, 1981; Fletcher et al., 1987; Wolfe and Van Kints, 1987; Wolfe et al., 1987), may be due to bulk isolates or natural populations being composed of differing proportions of clones with various discrete levels of resistance.

Selection for triazole resistance. The significant increase in the frequency of H isolates through 1985 may have been due to selection by DMI fungicides, which continued to be widely used, either on their own or in combination with hydroxypyrimidines or morpholines (Heaney et al., 1986; J.E. King and R.W. Polley, pers. comm.).

H isolates were resistant to the commercial rate of triadimenol (1.5 g Baytan/kg seed; Tables 1, 3 and 6). Such isolates may be important in infecting crops. Although ED_{50}s of L and M isolates were lower than the commercial rate, they may have epidemiological significance in allowing infection of treated crops earlier than would occur if sensitive isolates predominated in the population.

In the past, manufacturers recommended repeated applications of a single fungicide or of chemicals with the same mode of action (Anonymous, 1986a, 1986b, 1988), although this is no longer the case. Results in this paper support other observations

that the prolonged and widespread use of DMIs to control cereal mildews has selected increased resistance (Butters et al., 1984; Heaney et al., 1984, 1986; Wolfe, 1984; De Waard et al., 1986; Andrivon and De Vallavieille-Pope, 1987; Fletcher et al., 1987; Limpert, 1987; Enisz, 1988). Although no sudden loss of effectiveness of DMIs has been reported, the majority of isolates described in this paper (group H) were resistant to a dose of triadimenol similar to that applied commercially. Populations sampled five years previously did not show this level of resistance (Fletcher and Wolfe, 1981; Wolfe and Fletcher, 1981).

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