European Journal of Plant Pathology **104:** 925–931, 1998.

Diversity and complexity of *Erysiphe graminis* f.sp. *hordei* collected from barley cultivar mixtures or barley plots treated with a resistance elicitor

Adrian C. Newton[1], Christine A. Hackett[2] and David C. Guy[1]
[1] *Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK;* [2] *Biomathematics and Statistics Scotland, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK*

Accepted 9 October 1998

Key words: barley, resistance elicitor, cultivar mixtures, complexity, diversity, RAPD, virulence

Abstract

Powdery mildew populations were analysed to determine the effects of a resistance elicitor and cultivar mixtures on genetic complexity and diversity. Isolations were made from a range of spring barley monocultures and mixtures in a field trial, and characterised for virulence and RAPD profile. In a second trial, isolates were taken from a single mixture from untreated and resistance elicitor-treated areas and from the components of the mixture in monoculture. The mildew population was not only highly heterogeneous for virulence characteristics, but also proved heterogeneous within pathotypes for molecular markers, indicating the major impact of sexual recombination on population structure and the lack of clonal dominance. Various diversity measurements were compared and the value of dissimilarity measurement for revealing genetic distance within a population was highlighted. There was a trend towards increasing complexity as the season progressed, but there was no consistent relationship between cultivar or mixture, disease control treatment, fertiliser treatment, replicate or position in trial, and pathogen genotype. Whilst the resistance elicitor did reduce mildew by 78% in the first trial, and there was no interaction with fertiliser level in its expression, control was substantially less in the second trial. There were no differences between mildew isolates from elicitor and control treatments. It was felt that more effective and consistent resistance elicitors need to be developed before it can be stated that they are unlikely to be eroded by selecting resistant or adapted mildew genotypes.

Introduction

Disease control strategies in barley, particularly against mildew, are continually eroded in their effectiveness as they are overcome by adaptations in the pathogen. This includes both application of crop protectants and 'ecological' approaches such as the use of cultivar mixtures, which select more complex powdery mildew races than the component monocultures (Chin and Wolfe, 1984; Dileone and Mundt, 1994; Huang et al., 1994, 1995), although these races do not persist from year to year (Mundt, 1994; Wolfe and Finckh, 1996). Knowledge of the genetic structure of mildew populations and how it changes in response to control measures will help to devise strategies for minimising pathogen adaptation.

The success of mixtures of cultivars expressing major genes for resistance is dependent on a genetic structure in which a substantial proportion of the pathogen population does not express virulence matching all of the host resistance genes. Only then can the three factors causing the mixture effect, the physical barrier between susceptible plants, dilution of the susceptible plants and induced resistance (Chin and Wolfe, 1984), be expressed. Even then, mixtures will tend to select for complex isolates which overcome all the resistance genes (Wolfe et al., 1992). If the population comprises few genotypes or only simple genotypes, then, provided these do not include virulence towards all the resistance components in the mixture, disease reduction is likely to be substantial. However, if the population is highly heterogeneous and complex

isolates occur, some disease reduction may occur early in the epidemic but the mixture effect is likely to be eroded as complex pathotypes virulent on all components are selected (Newton, 1997).

Another disease control strategy is to use resistance elicitors, which are being developed by several agrochemical companies and research groups (Lyon et al., 1995; Lyon and Newton, 1997). These are mostly synthetic chemicals, but there are also extracts from plant, bacterial and fungal biomass. The elicitors act by triggering the plants' defence mechanisms enabling them to express resistance better upon actual challenge by a pathogen. It is assumed that they act non-specifically against all pathotypes, but it is also known that they cause differential expression of resistance in different cultivars of barley (Reglinski et al., 1994; A.C. Newton, unpublished data).

It is not known whether the differential resistance elicitation directly or indirectly results in selection in the pathogen population. It has been argued that no 'tolerance' or 'resistance' to resistance elicitors will develop, because they affect the whole resistance induction process rather than simply a specific receptor (Lyon and Newton, 1997). However, this hypothesis is untested. There is, therefore, a need to determine whether major shifts in the population structure, particularly selection for any adapted clones, occur in elicitor-treated plots. In mixtures, an increase in the number of complex pathotypes has been found which was inversely related to the number of other pathotypes in the mixture (Dileone and Mundt, 1994). In these situations, the frequency of either clones resistant to elicitor induction or of complex, clonally related races, is expected to increase. In this paper we survey the degree of diversity and complexity in field populations of mildew in spring barley monocultures and mixtures and determine (i) whether these populations were dominated by some clones, (ii) how stable the populations are, and (iii) whether there is any strong selection on midew populations by resistance elicitors or cultivar mixtures. This basic knowledge is necessary to enable appropriate strategies for detecting even small shifts in population structure to be devised.

Materials and methods

Barley genotypes

Twenty genotypes or mixtures of spring barley were grown in field trials in two successive years to investigate the effects of fertiliser level, fungicide and resistance elicitors on mixture efficacy, and to investigate the response of the mildew population. High fertiliser levels increase mildew infection and thus affect mixture efficacy (Newton et al., 1996). In the first trial (trial 1), the cultivars and the probable mildew resistance genes (in parentheses) they carry (Brown and Jorgensen, 1991) were: Doublet (*Mla7, MlLa*), Tweed (*Mla9, Mlg, Ml*(*CP*)), Natasha (*Mla12, Ml*(*Ab*)), the three 2-component and the 3-component equal proportion mixtures of these; Triumph (*Mla7, Ml*(*Ab*), *Ml* (*Tr3*)), Dallas (resistance genes not known), and the 1 : 1, 1 : 3 and 3 : 1 proportion mixtures of these; Atem (*MlLa, mlo*), Golden Promise (*Mla8*), the two breeding lines B87/106/4 (*Mla13*) and 22049CoI123 (*Mla12, Mlg, MlLa*) (WTB Thomas, personal communication), the three Pallas near-isogenic lines P23 (*MlLa*), P06 (*Mla7, Ml*(*Mu2*)) and P03 (*Mla6*) (Kølster et al., 1986), and the primitive line Gloire Du Velay (polygenic resistance) (Jones and Davies, 1985). In the second trial (trial 2), the cultivars were the same two series of mixtures as in the first trial, plus a third series: Prisma (*Mla12, Mlg, Ml*(*CP*), *Ml*(*Ab*)), Brewster (*Mla1, Ml*(*Ab*)), Cooper (*Mla1, MlLa*) (Slater and Clarkson, 1997), Camargue (*Mla13, Ml*(*Ab*)) (Brown and Jorgensen, 1991) and all four 3-component mixtures of these in equal proportion.

Field trials

Trials were carried out using plots measuring 1.9 × 1.22 m (excluding gaps) of each cultivar sown at the Scottish Crop Research Institute in 1994 and 1995 in a three-replicate split–split plot design using disease control as the main plot. In 1995, plot numbers were doubled by using paired beds, effectively creating almost square plots and minimising edge effects. Seed was treated with carboxin + thiabendazole + imazalil (Cerevax Extra) to control seed-borne diseases. Fertiliser levels n1 and n2 were 40 and 120 kgN ha^{-1} (P and K both 20 and 60 kg ha^{-1}) respectively. The disease-control treatments consisted of: (f0) unsprayed control, (f1) a single spray of fenpropimorph (Corbel) + carbendazim (Bavistin) when mildew first started to appear on 'Golden Promise' (GS20), followed by a single spray of propiconazole + tridemorph (Tilt Turbo) once mildew started to reappear (GS30-39), all applied at full recommended field rates, and (f2) two sprays of a yeast-derived resistance elicitor

(Reglinski et al., 1994) applied at 0.10 kg ha^{-1} in 0.025% (v/v) alkyl phenol ethylene condensate (Agral) at the same time as the fungicides. Mildew levels were assessed visually on a 1–9 scale (Newton and Hackett, 1994) four times in 1994 and six times in 1995 during the season and the area under the disease progress curve (AUDPC) was calculated.

Mildew sampling

In the first trial leaves with light mildew infection were sampled on each of two occasions 36 days apart from all 60 of the n2 plots (20 genotypes in three replicates) which were not fungicide treated, yielding a total of 120 samples. In trial 2, Doublet, Tweed, Natasha and the 3-component mixture were sampled, again taking colonies from lightly infected leaves. In this case, samples were taken from each of the 4 monocultures or mixture from untreated and elicitor treated plots at both n1 and n2 fertiliser treatments, i.e. 16 combinations. For each of the 16 combinations, each of the paired plots from replicate 1 and one of the paired plots from replicate 3 were sampled on two occasions 32 days apart, giving a total of 96 samples. Leaves were excised and placed on 0.5% (w/v) agar containing 120 ppm (w/v) benzimidazole in sealed plastic boxes (Stewart Plastics, Croydon, UK) and incubated overnight at 15 °C. Spores from single colonies were picked off with a paint brush and deposited onto fresh detached leaves of Golden Promise on benzimidazole agar. Further increase of spores was by subculture onto fresh detached leaves of Golden Promise and spores were either harvested and stored frozen at −20 °C in sealed 1.5 ml Eppendorf tubes for RAPD characterisation or used immediately to inoculate differential cultivars for virulence characterisation.

Virulence testing

Fifteen differential cultivars were used comprising the 13 Pallas (P) near-isogenic lines (Kølster et al., 1986), P01 (*Mla1*), P02 (*Mla3*), P03 (*Mla6*), P06 (*Mla7*, *Ml*(*Mu2*)), P10 (*Mla12*, *Ml*(*Em2*)), P11 (*Mla13*, *Ml*(*Ru3*)), P14 (*Mlra*), P15 (*Ml*(*Ru2*)), P17 (*Mlk*), P21 (*Mlg*, *Ml*(*CP*)), P22 (*mlo5*), P23 (*MlLa*), P24 (*Mlh*), and the two cultivars Triumph (*Mla7* + *Ml*(*Ab*) + *Ml*(*Tr3*)) and Golden Promise (*Mla8*) (Brown and Jorgensen, 1991). Seedlings were grown in John Innes no. 2 compost in a growth cabinet at 17 °C for 10 days before a 3.0 cm segment of the first leaf was excised and placed on benzimidazole agar in sealed plastic boxes as above. The leaf segments were randomised and 6 replicates of the differential series were inoculated for each of the 216 mildew single-colony isolates. Plastic boxes with inoculated segments were incubated at 15 °C in a continuously lighted incubator (120 μEm^{-2} s^{-2}). Infection types were scored on a 0–4 scale with 0, 1 and 2 being considered avirulent (no, rare and sparce infection respectively), and 3 and 4 virulent (moderate and dense infection). Allowance was made for the poor resistance expression of particular differentials, notably those expressing *MlLa*, in designating presence or absence of virulence in isolates.

RAPD characterisation

Approximately 3 mg of frozen mildew conidia were ground using a Treff homogeniser (Switzerland) with a few grains of sterile sand in an Eppendorf tube containing 75 μl of extraction buffer (Kolar et al., 1988). A further 425 μl of extraction buffer was added and homogenates were heated for 20 min at 65 °C, then extracted twice with 100 μl phenol/chloroform/isoamyl alcohol (25 : 24 : 1 v/v/v) and the DNA precipitated with ethanol/sodium acetate at −20 °C. The pellet was dried and resuspended in 100 μl of RNAase/TE buffer (Maniatis et al., 1982). Reaction mixtures comprised (final concentrations in parentheses) 2.0 μl DNA (0.2 ng/μl), 0.5 μl Taq polymerase (2.5 units) (Gibco BRL), 0.75 μl 1.0 M $MgCl_2$, 2.5 μl Operon primer (200 μM), 2.5 μl dNTPs (100 μM), 2.5 μl 10 × PCR buffer (Gibco BRL), and 14.25 μl distilled water. Six Operon Biotechnologies primers were used: S07 (TCCGATGCTG), S09 (TCCTGGTCCC), G15 (ACTGGGACTC), H16 (TCTCAGCTGG), and G02 (GGCACTGAGG). These were chosen from a larger initial screen as primers which gave clear, highly reproducible, polymorphic banding patterns. Amplification conditions were 94 °C for 10 s, then 36 °C for 60 s, and 72 °C for 60 s for 45 cycles. Bands were visualised in 1.5% (w/v) agarose gels in TBE buffer (Maniatis et al., 1982) stained with ethidium bromide and visualised on a 312 nm ultraviolet light transilluminator. Presence or absence of banding were recorded for 7, 4, 5, 5 and 4 bands for each primer, respectively, these representing clear, highly reproducible bands, giving data on 25 random alleles for all 40 isolates from replicate 1 in trial 1 and for all 96 isolates from trial 2.

Data analysis

The virulence and RAPD data were analysed for each trial and sampling date separately then compared in various combinations. Values for the proportion of the most common type, the proportion of unique types and the richness (the number of different types divided by the total number of individuals in a sample; Müller et al., 1996) were calculated. In addition, another measure of richness, the Gleason index, which is claimed to be less sensitive to sample size (Groth and Roelfs, 1987; Sanders, 1968), was calculated. The Simpson index of diversity (Simpson, 1949), which combines a measure of evenness of distribution with richness (Groth and Roelfs, 1987), was calculated with a standard error estimated from 500 bootstrap samples. The genetic distance, D_{rs}, between isolates r and s was estimated by calculating the mean and standard error of the sample dissimilarity (Müller et al., 1996), an estimate of the proportion, rather than the number, of loci for which there were differences between isolates:

$$D_{rs} = \frac{1}{p}\sum_{i=1}^{p} |x_{ri} - x_{si}|$$

where x_{ri} and x_{si} are the presence/absence scores for isolates r and s for the ith differential or RAPD band ($i = 1 \ldots p$).

A generalised linear model with binomial errors was used to examine the pattern of virulences to look for significant associations with cultivar, position in the field and sampling date.

Results

Mixture and elicitor performance for mildew control

The fungicide gave almost complete protection for the whole season in both trials. In trial 1 the resistance elicitor achieved a 78% ($p < 0.05$) reduction in the AUDPC compared with the control, but the 24% reduction achieved in trial 2 was not significant at the 5% level, indicating a considerable environmental effect on elicitor performance. Under high (n2) fertiliser conditions, mildew infection was over six times heavier in trial 1 and three times heavier in trial 2 than in the respective low fertiliser treatments. There was no interaction between the reduction in disease caused by the resistance elicitor and fertiliser level.

In trial 1 there was no significant reduction of mildew in the mixtures compared with the mean of their respective monocultures, but in trial 2 all mixtures gave significant mildew reductions. There was no significant effect of fertiliser, fungicide or resistance elicitor on mixture efficacy.

Virulence testing

Several isolates were lost during multiplication or failed to give clear virulence data and are therefore excluded from the analysis. Table 1 presents the number of pathotypes successfully observed, the proportion of the most common type, the proportion of unique pathotypes, the richness, Simpson index of diversity with standard error, the Gleason index with standard error and theoretical maximum (in parentheses), and the mean and standard error of the sample dissimilarity. Virulence on all differential cultivars except P22 (*mlo5*) occurred at high frequencies and most combinations were recorded. Isolates from the two sample dates did not differ significantly for any measure of diversity in either trial.

In trial 1 there were 46 pathotypes among the 94 isolates, and 22.5% of the isolates were unique combinations. The average virulence complexity, i.e. the number of virulence factors expressed by each isolate, increased significantly ($p < 0.001$) from 9.2 (se 0.18) to 10.1 (se .016) between the two sampling

Table 1. Diversity analysis of virulence and RAPD data from isolates of *Erysiphe graminis* f.sp. *hordei* sampled at two times in two years

Trial/ sample	No. obs.	No. types	% most common type	% unique types	Indices of diversity			
					Richness	Simpson's index	Gleason index	Dissimilarity
Virulence:								
Trial 1	94	46	6.4	22.5	0.49	0.979 ± 0.003	9.94 ± 0.84 (20.5)	0.219 ± 0.002
Trial 2	96	32	22.9	19.8	0.33	0.916 ± 0.015	6.8 ± 0.75 (20.8)	0.144 ± 0.001
RAPDs:								
Trial 1	32	32	3.1	100.0	1.00	1.000 ± 0.000	8.9 ± 0 (8.9)	0.257 ± 0.004
Trial 2	96	70	10.4	68.8	0.73	0.991 ± 0.001	16.7 ± 0.64 (20.8)	0.199 ± 0.001

dates. The individual virulences on P03 (*Mla6*) and P23 (*MlLa*) were present significantly more often on the second sampling date ($p < 0.05$). There was also some indication of a spatial element to the distribution of virulence on P23, with more on bed 4 than on beds 12 and 14. There were no significant associations with the cultivar or mixture grown.

In trial 2 there were 32 pathotypes present. All indices of diversity were lower than in trial 1, and a single virulence combination occurred in 23% of the isolates. The lower dissimilarity value indicated that isolates were more closely related. The individual virulences on P06 (*Mla7* + *Ml*(*Mu2*)) and P24 (*Mlh*) were present significantly more often on the second sampling date ($p < 0.05$) and the overall virulence complexity increased from 11.08 (se 0.244) to 11.52 (se 0.236), although this was not significant at the 5% level. The virulence combination which was most common in sample 1 (10 isolates) was one of the two most common in sample 2. One of the two most common combinations in sample 2 was only present once in sample 1. Again, there were no significant interactions with the cultivar or mixture from which mildew isolates were obtained.

RAPD characterisations and comparison with virulence data

RAPD profiles were highly reproducible in replicate amplifications from separate DNA preparations, and all bands recorded were polymorphic. In both trials, the RAPD profiles showed greater diversity than the pathotypes (Table 1). In trial 1, all isolates were different, and in trial 2, 68.8% were unique compared with values of 22.5% and 19.9%, respectively, for virulence. The greater genetic distance was particularly noticeable in the increase in dissimilarity values compared with those for virulence. There were no relationships between isolates and cultivar or mixture, position in the trial or sample time. There was also little relationship between virulence combination and RAPD profile in either trial. Only in trial 2, sample 2, were three pairs of isolates of the same virulence and RAPD profile found, but these were spatially dispersed. Thus, there was no evidence for clonal clusters.

Discussion

No evidence of the dominance of any clone was found in either virulence or RAPD data. The few isolates which shared the same RAPD and virulence characters were spatially dispersed suggesting no selective advantage at their point of origin. The local clusters observed occasionally were never consistent over the two samples in either trial for virulence type or RAPD profile, and there was no consistent pattern to these clusters. The population was clearly very heterogeneous, particularly in trial 1 where half the population consisted of unique genotypes on the basis of virulence data alone, and the entire sub-set characterised for RAPD profile were unique genotypes. Two virulences did increase between sampling dates in trial 1, one of which seemed to be a small cluster in bed 4. However, this was not simply due to a single clonal effect as the isolates concerned were heterogeneous for RAPD profiles (data not shown). A similar increase in certain virulences was found at the second sampling date in trial 2 but no spatial pattern was found in this observation. The population clearly changed rapidly and had a tendency towards increased complexity with time, which has been reported previously for mixtures (Dileone and Mundt, 1994; Huang et al., 1994, 1995; Wolfe, 1984). Whilst not all samples were taken from mixtures, the nature of a small-plot multiple-cultivar trial such as this is by its very nature similar to a mixture.

In contrast to virulence, RAPD markers are under different selection pressure, presumed to be far less. In this study, all measures revealed greater diversity for RAPD markers than for virulence, possibly reflecting the selection imposed by host resistance genes on the virulence markers and the ability of mildew to generate variation. This contrast is found also in pathogens such as the cereal rusts where the sexual cycle is of less or no importance (Chen et al., 1993, 1995; Jennings et al., 1997).

For a resistance elicitor not yet formulated for field application or optimised against mildew, a 78% reduction in the AUDPC for mildew compared with the control in trial 1 was good performance compared with previous trials (Reglinski et al., 1994), but the reduction of only 24% in trial 2 was poor. There was no evidence of selection by either the elicitor treatment, fertiliser level or specific mixtures for any genotypes of mildew at this scale. However, before it can be stated that resistance elicitors do not exert selection on the mildew population, a larger scale field trial using elicitor treatments which exert much greater selection pressure, i.e., reduce mildew infection by, say, over 95%, is needed to determine whether there is directional selection pressure. Unfortunately, samples were only taken from trial

2. However, it is unlikely that an effect would have been detected even in trial 1 where much greater control was achieved, and there are no resistance elicitors currently available which consistently achieve greater disease control on spring barley.

A number of different measures were used to characterise the diversity of the virulence and RAPD patterns among the isolates in each experiment to reflect different aspects. Diversity encompasses different components: richness, evenness of distribution, genetic distance between genotypes and complexity, and several indices have been devised to quantify these. Conclusions concerning the sensitivity of these indices to sample size vary in different studies (e.g., Müller et al., 1996; Andrivon and de Vallavieille-Pope, 1995; Pinon and Frey, 1997) but sample size in some of these studies varies by more than 20-fold. In this study, sample size varies over a narrow range of 32–96, but this may be too small to describe the true variability present in the population for the markers used, especially where nearly all isolates were different. The Gleason index has been reported to be less sensitive to sample size than the richness ratio (Groth and Roelfs, 1987) but in this study the problem of the theoretical maximum was a disadvantage in comparisons.

Comparison of the mildew population structure between eastern Germany, Denmark and other European regions showed that pathotypes harboured by cultivar mixtures tend to be more diverse and present in more even proportions than those present on pure stands (Müller et al., 1996). The previous study used only virulence data, and our values for the Simpson index and richness for trial 1 are comparable to theirs for Scotland but are much lower for dissimilarity (0.219 compared with 0.316 when expressed as a proportion). Our trial 2 values are more similar to the values for northern Italy although again our dissimilarity value was much lower (0.144 compared with 0.295 when expressed as a proportion). This could reflect the differences between sampling over large distances and within a single trial where we might expect more isolate relatedness if clonal multiplication were of major importance, reflecting the sensitivity of the dissimilarity value to diversity or genetic distance within a sample. However, the scale differences were too large to draw firm conclusions.

Whilst the population sampled was not very large, few associations between pathotypes and RAPD pattern were observed, and there was no correlation between any RAPD bands and individual virulence. An intensive AFLP marker search would be a better approach for obtaining markers, linked to virulence although in wheat yellow rust (*Pucinnia striiformis*) a RAPD band was associated with virulence towards a particular resistance (Chen et al., 1993). However, the study demonstrated that most isolates were unique, that any clones were small and of transient importance and that the only directional selection was towards increased isolate complexity.

Acknowledgements

We thank the Scottish Office Agriculture, Environment and Fisheries Department for financial support, and the Estate staff at SCRI for assistance with field trials.

References

Andrivon D and de Vallavieille-Pope C (1995) Race diversity and complexity in selected populations of fungal biotrophic pathogens of cereals. Phytopathology 85: 897–905

Brown JKM and Jørgensen JH (1991) A catalogue of mildew resistance genes in European barley varieties. In: JH Jørgensen. Integrated control of cereal mildews: virulence patterns and their change. pp. 263–286. Risø National Laboratory, Roskilde, Denmark

Chen XM, Line RL and Leung H (1993) Relationship between virulence variation and DNA polymorphism in *Puccinia striiformis*. Phytopathology 83: 1489–1497

Chen XM, Line RL and Leung H (1995) Virulence and polymorphic DNA relationships of *Puccinia striiformis* f.sp. *hordei* to other rusts. Phytopathology 85: 1335–1342

Chin KM and Wolfe MS (1984) Selection on *Erysiphe graminis* in pure and mixed stands of barley. Plant Pathology 33: 535–546

Dileone JA and Mundt CC (1994) Effect of wheat cultivar mixtures on populations of *Puccinia striiformis* races. Plant Pathology 43: 917–930

Groth JV and Roelfs AP (1987) The concept and measurement of phenotypic diversity in *Puccinia graminis* on wheat. Phytopathology 77: 1395–1399

Huang R, Kranz J and Welz HG (1994) Selection of pathotypes of *Erysiphe graminis* f. sp. *hordei* in pure and mixed stands of spring barley. Plant Pathology 43: 458–470

Huang R, Kranz J and Welz HG (1995) Increase of complex pathotypes of *Erysiphe graminis* f.sp.*hordei* in two-component mixtures of spring barley cultivars. Journal of Phytopathology 143: 281–286

Jennings JM, Newton AC and Buck KW (1997) Detection of polymorphism in *Puccinia hordei* using RFLP, RAPD and differential cultivars and analysis of the intergenic spacer region of rDNA. Journal of Phytopathology 145: 511–519

Jones IT and Davies JER (1985) Partial resistance to *Erysiphe graminis hordei* in old European barley varieties. Euphytica 34: 499–507

Kolar M, Punt PJ, van den Hondel CAMJJ and Schwab H (1988) Transformation of *Penicillium chrysogenum* using dominant selection markers and expression of an *Escherichia coli locZ* fusion gene. Gene 62: 127–134

Kølster P, Munk L, Stølen O and Løhde J (1986) Near isogenic barley lines with genes for resistance to powdery mildew. Crop Science 26: 903–907

Lyon GD, Reglinski T and Newton AC (1995) Novel disease control chemicals: The potential to 'immunize' plants against infection. Plant Pathology 44: 407–427

Lyon GD and Newton AC (1997) Do resistance elicitors offer new opportunities in integrated disease control strategies? Plant Pathology 46: 636–641

Maniatis T, Fritsch EF and Sambrook J (1982) Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Mundt CC (1994) Techniques for managing pathogen co-evolution with host plants to prolong resistance. pp. 193–205. In: *Proceedings of the International Rice Research Conference*, April 1992. P.S. Teng, K.L. Heong, and K. Mooody, eds. P.O. Box 933, 1099 Manila, Philippines. International Rice Research Institute

Müller K, McDermott JM, Wolfe MS and Limpert E (1996) Analysis of diversity in populations of plant pathogens: the powdery mildew pathogen across Europe. European Journal of Plant Pathology 102: 385–395

Newton AC and Hackett CA (1994) Subjective components of mildew assessment on spring barley. European Journal of Plant Pathology 100: 395–412

Newton AC, Thomas WTB and Goleniewski G (1996) Effects of nitrogen on mildew levels and yield in major gene and partial resistance spring barley cultivar mixtures. pp. 165–172. *In*: Integrated control of cereal mildews and rusts: towards coordination of research across Europe. Proc. Workshop COST 817, Zürich 5–10 Nov 1994, E. Limpert, M.R. Finckh and M.S. Wolfe, eds. European Commission, Luxembourg, 276 p

Newton AC (1997) Cultivar mixtures in intensive agriculture. *In*: Gene-for-gene relationship in plant parasite interactions. Eds: IR Crute, J Burdon and E Holub, pp. 65–80. CAB International, Wallingford, Oxford

Pinon J and Frey P (1997) Structure of *Melampsora laricipopulina* populations on wild and cultivated poplar. European Journal of Plant Pathology 103: 159–173

Reglinski T, Newton AC and Lyon GD (1994) Assessment of the ability of yeast-derived resistance elicitors to control barley powdery mildew in the field. Journal of Plant Disease and Protection 101: 1–10

Sanders HL (1968) Marine benthic diversity: A comparative study. American Naturalist 102: 243–282

Simpson EH (1949) Measurement of diversity. Nature 163: 688

Slater SE and Clarkson JDS (1998) Mildew of barley. pp. 31–39. *In*: United Kingdom Cereal Pathogen Survey Annual Report for 1996. National Institute of Agricultural Botany, Cambridge, UK

Wolfe MS (1984) Trying to understand and control powdery mildew. Plant Pathology 33: 451–466

Wolfe MS, Braendle UE, Koller B, Limpert E, McDermott JM, Müller K and Schaffner D (1992) Barley mildew in Europe: population biology and host resistance. Euphytica 63: 125–139

Wolfe MS and Finckh MR (1996) Diversity of host resistance within the crop: effects on host, pathogen and disease. In: *Resistance of crop plants*. Eds: H Hartleb, R Heitefuss and HH Hope, Gustav Fischer Verlag