Plant Pathology (1999) **48**, 807–816

Host frequency and density effects on powdery mildew and yield in mixtures of barley cultivars

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The effects of frequency and density of susceptible plants on barley powdery mildew epidemics were studied in a combined set of addition and replacement series of field trials. In the addition series, plant densities in pure stands of three cultivars, Rambo, Rodos and Grosso (susceptible, moderately resistant and immune, respectively) were varied six-fold. In the replacement series, the three possible two-way mixtures were analysed at different frequencies but at a density corresponding to the maximum pure stand density. Disease and yield were assessed on a per-plant basis. In the pure stands, tillering reduced the range of densities from six-fold to between three- and four-fold, while in the mixtures, frequencies changed only slightly over time, indicating that competitive interactions among the cultivars were roughly equal. Yield per plant decreased logarithmically with increasing density as expected. However, yield per seed head was not correlated with the final number of heads per plot, indicating low competition among heads even at the highest density. Disease in susceptible pure stands increased strongly with decreasing density in 1994 and to a lesser degree in 1995. These differences could have been caused by differences in plant nutritional status and consequent epidemiological effects. Disease reduction on the susceptible cultivars in mixtures varied between 33% and 71% among years. Depending on the length and strength of the epidemic, the effects of host density and frequency on disease severity varied substantially among years.

Keywords: barley, cultivar mixtures, density effects, disease control, frequency effects, powdery mildew

Introduction

Mixtures of crop cultivars or breeding lines carrying different resistance genes are being used in many areas of the world to control airborne diseases, such as rusts and powdery mildews, and also soil-borne diseases and abiotic stresses. For example, in the north-western United States, more than 100 000 ha of wheat cultivar mixtures are currently grown for protection against yield losses from soil-borne diseases and to increase yield stability in general (C.C. Mundt, Oregon State University, personal communication). In eastern Germany, fungicide use was reduced by 80% by growing barley cultivar mixtures to control powdery mildew caused by *Erysiphe graminis* f. sp. *hordei* on more than 300 000 ha (Wolfe, 1992). Since 1981, the area sown with barley cultivar mixtures in Poland has increased steadily to an estimated 100 000 ha in 1997 (Gacek, unpublished). Mixtures are also successful for trees: a large proportion (>350 000 ha) of the coffee production in Colombia is based on line mixtures to control coffee rust caused by *Hemileia vastatrix* (Browning, 1997; Finckh & Wolfe, 1998; Moreno-Ruiz & Castillo-Zapata, 1990).

Several mechanisms may contribute to changes in disease incidence or severity in host populations that are diverse for resistance (Mundt & Browning, 1985; Finckh & Wolfe, 1997). Hypotheses include: increased distance between susceptible plants (Burdon & Chilvers, 1982); barrier effects of resistant plants (Trenbath, 1977); competitive interactions among host plants that may affect plant susceptibility (Finckh & Mundt, 1992b; Akanda & Mundt, 1996); and pathogens nonvirulent on a host genotype that may induce resistance reactions against virulent races (Chin *et al*., 1984; Lannou *et al*., 1995; Calonnec *et al*., 1996).

Besides beneficial effects in controlling diseases, including nontarget diseases, mixtures have been shown to stabilize and sometimes increase yields (Dubin & Wolfe, 1994; Mundt *et al*., 1995; Finckh *et al*., 1997), reduce weeds (Liebman & Dyck, 1993; Liebman & Gallandt, 1997) and insects (Letourneau, 1997) and buffer the crop against other stresses such as cold and drought (Finckh & Wolfe, 1998).

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Accepted 29 June 1999.

Mixture effects on disease and yield are usually studied by comparing the disease severity and yield of different mixture components grown in mixed or pure stands. Experiments are conducted as replacement series in which overall plant density is constant but the proportion of the genotypes in the mixture is varied (Fig. 1). Although this is of practical relevance for growers, comparisons are difficult because many parameters change at different rates between pure stands and the mixture:

1 While the density of the stand overall is kept constant, the density of a given mixture component varies and this may have a marked effect on disease severity on this component (Leonard, 1969; Burdon & Chilvers, 1976; Fried *et al.*, 1979) in addition to the barrier effects of the other mixture component(s).

2 Intra- and intergenotypic competition in mixtures change with the frequencies of mixture components, and competitive interactions among components can lead to altered susceptibility and yield (Finckh & Mundt, 1992b).

3 Especially in cereals, initial differences in establishment and plant density are altered by tillering so that the proportions (in terms of biomass and leaf area) of different genotypes may change greatly over time, leading to large selection effects on plants and thus on disease (Finckh & Mundt, 1992a, 1996).

The use of several pure stands at a range of densities (addition series, i.e. differences in treatments are achieved by adding plants), in combination with a replacement series (i.e. differences in treatments are achieved by replacing plants of one genotype with plants of another) may provide insights into these interactions (Fig. 1). For example, the experimental plots in Fig. 1 allow separation of density and frequency effects. Comparison of treatments a and b allows determination of intragenotypic competition and density effects on disease. Contrasting treatments a, and b with c clarifies intergenotypic competition and epidemiological mixture effects (spatial or barrier effects).

This paper describes the outcome of a combined replacement and addition series experiment with three spring barley (*Hordeum vulgare*) cultivars in two-way mixtures at different proportions and grown as pure stands at different densities under natural infection with powdery mildew. The main objectives were, first, to determine how severity of powdery mildew changes at decreasing plant density in pure stands. and secondly to determine how these changes relate to disease severity on a particular cultivar when grown in different mixtures.

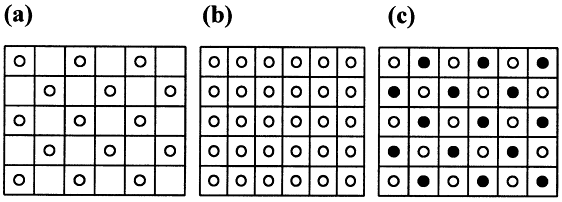

Figure 1 Replacement and addition series combination: (a) pure stand at 50% density; (b) pure stand at 100% density; (c) mixture at 100% density. (b) and (c) represent additions to (a) while at the same time in (c) half of the plants from (b) are replaced.

Materials and methods

The experiment was conducted in 1994–96 at the Experimental Plant Breeding Station in Bakow, near Kluczbork, in south-western Poland, using the spring barley cultivars, Grosso (barley mildew resistance gene *mlo*, immune in the field), Rambo (*Mla9,* low field resistance), and Rodos (*Mla3*, *Mlg*, good field resistance).

Plots (84 × 84 cm) were divided into a 12 × 12 grid and hand sown on 22, 24, and 26 April, respectively, in 1994, 95 and 96. Pure stands of each cultivar were sown at six densities: 144, 120, 96, 72, 48, and 24 seeds per plot, resulting in plant densities ranging from 203 to 34 plants m^{-2}. Missing plants were replaced with new seedlings on 10 and 12 May, 1994 and 1995, respectively (no replacements needed in 1996). Mixtures were grown only at the highest density (144 plants/plot). In 1995 and 1996, the three possible two-way mixtures were each grown as a replacement series in the ratios of 5 : 1, 2 : 1, 1 : 1, 1 : 2 and 1 : 5 of the two components. In 1994, mixtures with 1 : 6 and 2 : 6 Grosso with Rambo or Rodos were omitted because of space limitations. The 1 : 1 : 1 three-way mixture was included in all three years. Mixtures were grown according to a predetermined randomized pattern, thus allowing for the identification of the individual cultivars throughout the experiment. Plots were hand-weeded as necessary and fertilized in the same way as the adjacent fields in each year (36 kg N ha^{-1} before sowing each year, and 34, 32 and 45 kg N ha^{-1} at the end of tillering in 1994, 95 and 96, respectively. P and K were applied before sowing at 63, 46 and 90 kg P ha^{-1} and 90, 60 and 110 kg K ha^{-1} in the three respective years.

The experimental design was a randomized complete block with three replications. Plots within blocks were separated by spring wheat plots of equal size and a 2-m wide swath of a wheat–oat mixture separated the blocks.

Natural infections of powdery mildew occurred each year and mildew severity was assessed on a per-plant basis twice in 1994 (14–16 June and 4–6 July) and 1995 (24–29 June and 5–7 July). In each plot five plants per cultivar were assessed. Because of the late onset of disease, only one assessment was possible in 1996 (3–5 July). Mildew severity was assessed on a 1–9 logit scale (Table 1), where 1 represented highly resistant to immune. For the data analysis, each field observation F was transformed into proportion of diseased leaf area (x) by the following equation:

$$\ln(x/[1-x]) = -5{\cdot}09 + 0{\cdot}94 \times (10 - F) \qquad (1)$$

Table 1 Mildew severity classes and their corresponding range and mean disease severity (in percentage diseased leaf area). For derivation of equation 1, the order of the scores was reversed with 9 representing the highest and 1 the lowest disease severity. This allowed for direct logit transformation and derivation of the mean severity as listed here

Field score (*F*)	Severity (%)	Mean severity (*x*) (%)
9	0–3	1·6
8	3–7	3·9
7	7–15	9·5
6	15–31	21·2
5	31–52	40·9
4	52–73	64·0
3	73–87	82·0
2	87–95	92·1
1	95–100	96·8

Five plants per plot of each cultivar were hand-harvested and plant height, number of head-bearing tillers, number of seeds per head and seed weight were determined.

For the data analysis, the relative disease and yield on each cultivar in mixtures was calculated separately and the overall relative disease and yield in mixtures. Relative disease severity on each cultivar in mixtures was calculated with the following equation:

$$\text{Relative disease} = S_{iX}/S_{iP} \qquad (2)$$

where S_{iX} is the disease severity on cultivar *i* when grown in mixtures and S_{iP} the disease severity of cultivar *i* when grown in the highest density pure stand (the same density at which the mixture was sown). Relative yields were determined in the same way.

Relative disease and yields for the mixtures were determined by dividing the disease severity or yield per plot by the mean of the pure stands, weighted by the initial proportions of the mixture components:

$$\begin{aligned}&\text{Mixture relative disease}\\&\quad = [(S_{iX} \times f_{iX}) + (S_{jX} \times f_{jX})]/[(S_{iP} \times f_{iP})\\&\qquad + (S_{jP} \times f_{jP})] \qquad (3)\end{aligned}$$

where *i* and *j* represent two cultivars and *f* is the initial proportion of a given cultivar. Mixture relative yields were determined in the same way.

Data from each year were analysed with SAS (SAS, 1988) and Excel 5·0 using analysis of variance, LSDs, linear contrasts and simple linear regression.

Results

Effects of density on the performance of pure stands

Seed head production and seed yield per plant decreased with higher initial plant densities for all three cultivars in a logarithmic fashion (Fig. 2a, Table 2), indicating density-dependence among plants. Although initial density varied six-fold, the density of head-bearing tillers per plot at harvest varied on average only 2·4-, 2·2-, and 2·7-fold, respectively, for Grosso, Rambo and Rodos. Yields varied considerably during the three years, with a mean yield per plant over all pure stands of 7·7 g, 5·2 g, and 5·2 g, respectively, in 1994, 95, and 96.

Yield per head was not affected by the final densities in the plots, indicating that competition among head-bearing tillers was low even at the highest density (Fig. 2b, Table 2). Consequently, the correlation between the number of seed heads per plot and yield was linear, with high r^2-values (Table 2), and within year yields per plot varied between 2·1-, 1·8- and 2·4-fold, respectively, for Grosso, Rambo and Rodos. There was no significant effect of density on plant height.

Competitive interactions in mixtures

Overall yields were substantially higher in 1994 than in 1995 and 1996 (Table 3).

Differences in mixture yields and the weighted means of the appropriate pure stands were statistically significant in only one of 44 cases (Table 3). Generally, the cultivars used did not respond favourably to mixing, often resulting in relative yields in mixtures lower than the weighted mean of the pure stands. There was a strong effect of the year in which the experiment was conducted, with 75% of the mixtures yielding more than expected in 1995 and 67% and 75% less than expected, respectively, in 1994 and 1996 (Table 3). Among cultivars, shifts from the initial to the harvested proportions did not exceed 5% (i.e. a cultivar at an initial proportion of 0·67 in a mixture might have been harvested at 0·62; data not shown). However, none of the cultivars outperformed either of the others consistently over years (Table 3b) and there were no obvious effects of the frequency at which a cultivar was present in the mixtures on its relative yield (data not shown).

Disease severity in pure stands

No disease developed beyond a few colonies on Grosso. The highest severities on Rodos were, respectively, 24% in 1994 and 4 and 5% in 1995 and 1996. Only Rambo developed significant disease in all three years (Table 4, Fig. 3). Disease severity increased over time in 1994 but it decreased in 1995. By the end of the season, disease severity was negatively correlated with density in 1994 and 1995 (Fig. 3). This effect was not apparent in 1996, when the epidemic lasted for only a short time. Care must be taken to consider the number of tillers harvested rather than the number of seeds sown as a basis for the correlations because of variation in tiller numbers.

Disease severity in mixtures

Disease was reduced in mixtures, as compared with the weighted mean of the maximum density pure stands, in almost all mixtures grown in the three years (Table 4), mainly because of reductions on the susceptible cultivar Rambo. Disease severity on Rambo in the mixtures was

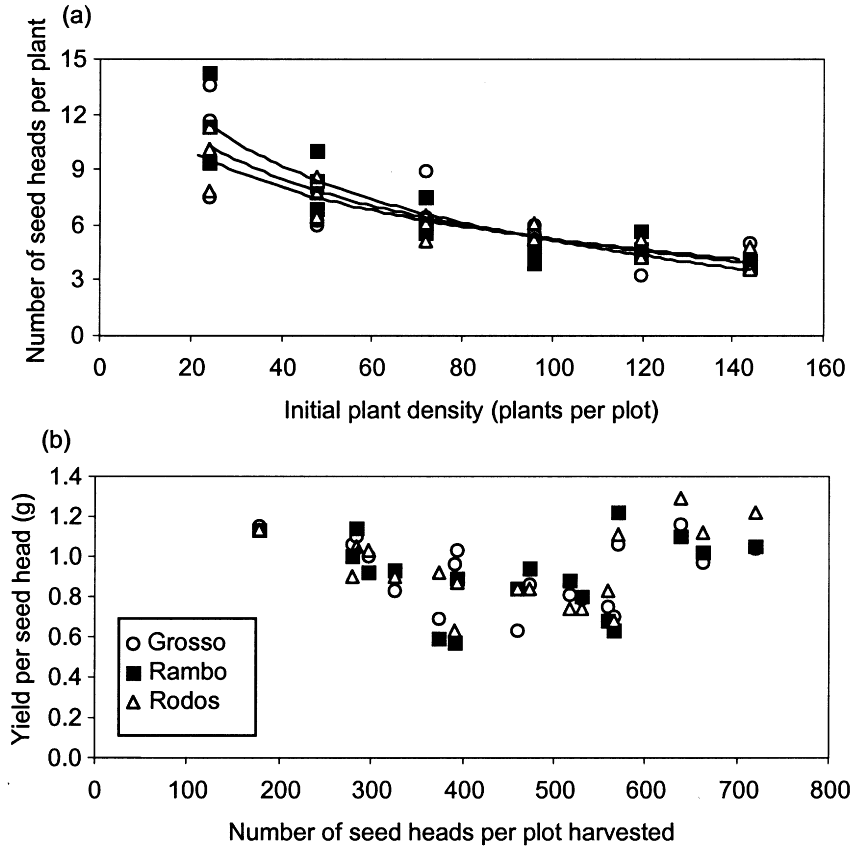

Figure 2 Effects of initial plant density on (a) the number of seed heads per plant and (b) the effect of the harvested density on the yield per seed head of three cultivars, (○) Grosso, (■) Rambo and (△) Rodos, averaged over three years. See Table 2 for r^2 values and function types.

somewhat frequency-dependent in all three years (Table 5). The correlation was more evident when Rambo was mixed with Grosso than when it was mixed with Rodos but it became statistically significant only if the data on relative disease severity were pooled over all three years for the analysis (Table 5). The different effects of Rodos and Grosso on the correlations could not be related to differences in competitive interactions.

Effects of frequency and density on disease

In the pure stands, the disease severity on Rambo increased with a decrease in absolute plant and tiller density. However, in mixtures, disease severity on Rambo decreased with a relative decrease in number of plants and tillers per plot (a decrease in frequency of Rambo within mixtures sown at the same density).

Using the data on disease in pure stands (changes in density) and mixtures (changes in frequency), changes in disease severity could be partitioned into density and frequency effects. A complicating factor was that, while the initial and harvested densities in mixtures were almost constant, the harvested densities in the pure stands varied greatly from the initial densities because of tillering. In addition to an increase in density, the differences in density at the end of the

Table 2 Correlation coefficients (r^2) for the relationships between initial plant density and harvested density and between these and yield per plot, per plant, and per head, for three spring barley cultivars grown at six different densities over three years in Poland

	Cultivar			
Correlation	Grosso	Rambo	Rodos	Function[a]
Initial density vs. harvested density	0·66	0·69	0·84	logarithmic
Initial density vs. yield/plot	0·37	0·26	0·38	logarithmic
Initial density vs. yield/plant	0·66	0·79	0·73	logarithmic
Harvested density vs. yield/plot	0·76	0·49	0·62	linear
Harvested density vs. yield/seed head	0·03	0·06	0·01	NS

[a] Logarithmic: $y = m\ln(x) + b$; linear, $y = mx + b$; NS: correlation not significant, all other correlations significant at $P < 0{\cdot}05$.

Table 3 Yield (g/m^2) of the pure stands at 100% density, the yield relative to the weighted means of the pure stands at 100% density of two-and three-way mixtures of three spring barley cultivars mixed at different ratios in three years, and the mean relative performance of each cultivar over all mixtures

Cultivar/ratio	1994	1995	1996
Pure stands			
Grosso	1058	559	599
Rambo	857	501	662
Rodos	1193	516	534
Grosso/Rambo			
5:1	1·05	1·11	0·78
2:1	0·91	1·09	0·74
1:1	1·16	0·98	0·98
1:2		1·03	0·96
1:5		0·90	0·75
Grosso/Rodos			
5:1	0·85	1·01	0·95
2:1	0·85	1·09	0·91
1:1	0·94	0·99	0·90
1:2		1·07	1·05
1:5		1·05	1·06
Rambo/Rodos			
5:1	0·81	0·94	1·06
2:1	0·93	1·11	1·05
1:1	1·08	1·02	0·85
1:2	0·91	1·07	0·96
1:5	1·05	1·23	0·79
Grosso/Rambo/Rodos			
1:1:1	0·89	1·01	0·66*
Mean	0·95	1·04	0·90
Mean relative yield of the cultivars in mixtures[a]			
Grosso	0·97	0·97	0·87
Rambo	1·08	1·07	0·85
Rodos	0·84	1·08	0·96

*Yield was significantly different from the mean of the pure stands at $P<0·05$; linear contrast.

[a] The mean relative yield on a per cultivar basis was calculated analogous to equation 2.

season were always smaller than at the beginning of the season.

A direct comparison of the disease severity observed in mixtures containing a given number of tillers of a cultivar with that in a pure stand with the same number of tillers was thus impossible. Therefore, the expected disease severities for pure stands at given harvesting densities were calculated based on the regressions in Fig. 3, where these regressions were significant (i.e. Rambo in 1994 and 1995 and Rodos in 1994).

The change in disease severity caused by decreasing density was calculated by subtracting the severity in pure stands at the various densities from that at the maximum density. The contribution of frequency to changes in disease was calculated by subtracting the disease severity on a component in a mixture from the severity observed in the maximum density pure stand. As the disease severity on Rambo was affected similarly when mixed with either Grosso or Rodos, the data for the two types of mixtures are discussed together in the following and pooled in Fig. 4.

The contributions of initial density and frequency to the changes in disease severity on Rambo differed greatly among years (Fig. 4). For example, in 1994, Rambo in the 17% density pure stands had an average of 43% diseased leaf area (DLA), reducing, significantly, to only 22% DLA at the maximum pure stand density. In contrast, when sown at a frequency of 17% (i.e. 1:5 ratios) in mixtures, the mean disease severity was only 8%. Thus, decreasing the initial density of Rambo from 100% to 17% in pure stands almost doubled disease while reducing the proportion or frequency of Rambo in mixtures from 100% to 17% decreased disease by almost two thirds from 22% to 8%. On average, in pure stands in 1994, mean disease relative to the highest density pure stands was 1·65 (absolute increase in DLA = 14%) and 1·89 (absolute increase in DLA = 6·3%), respectively, for Rambo and Rodos. In contrast, relative disease on Rambo and Rodos in mixtures was 0·59 (absolute decrease in DLA = 10%) and 0·67 (absolute decrease in DLA = 2·3%).

However, in 1995, initial density affected disease severity on Rambo (the only susceptible cultivar that year) only slightly, but the trend was still discernible (Fig. 3). The mean relative disease severity on Rambo in mixtures was 0·29 (absolute reduction in DLA = 10%).

In 1996, the effects of reducing density were not uniform, with significantly reduced disease severities at initial densities of 83 and 67% while, at the lower planting densities, disease severities were similar to those in the maximum density pure stands (Fig. 3). This was mainly an effect of extremely variable tillering. In 1994 and 1995, tillering increased the densities of the lowest density stands much more than of the highest density stands; the order of initial and harvested densities among treatments was either unchanged or changed only by one rank place at the higher densities (Fig. 3). In 1996, however, tillering resulted in a considerable change of ranking among treatments from initial to harvested densities (Fig. 3).

In 1996, as in 1994 and 1995, reducing the frequency of Rambo in the mixtures reduced DLA considerably to a mean relative disease of 0·33 (absolute decrease in DLA = 19%).

Discussion

As expected, the number of tillers per plant and thus the yield per plant were density-dependent. The maximum number of head-bearing tillers m^{-2} was 1020, 802 and 895, in 1994, 1995 and 1996, respectively, with an overall mean of 860 tillers m^{-2} across all cultivars and years. These numbers are similar to those in a study by Gallagher *et al.* (1975), who counted a maximum of 927 tillers m^{-2} in a crop of the barley cultivar Proctor in the UK. Grosso had the most tillers in 1994 and 1995, but the fewest in 1996. The plants adjusted

Table 4 Percentage disease severity in pure stands of three cultivars at 144 plants per plot and disease relative to the weighted mean of the respective pure stands in two-way mixtures of different ratios and in the three-way mixture in three years

Mixture/ratio	1994 (1)[a]	1994 (2)[a]	1995 (1)[a]	1995 (2)[a]	1996
Pure stands (%)					
Grosso	2·7	2·7	1·6	2·1	1·6
Rambo	11·0	21·7	26·1	13·6	29·0
Rodos	5·3	7·0	3·1	3·0	4·4
Grosso/Rambo					
5 : 1	0·68	0·65	0·31	0·44	0·35**
2 : 1	0·68	0·81	0·30**	0·44*	0·35**
1 : 1	1·28	0·97	0·23**	0·47*	0·33**
1 : 2	–	–	0·27**	0·35**	0·30**
1 : 5	–	–	0·36**	0·35**	0·55**
Grosso/Rodos					
5 : 1	0·62	0·58	0·82	0·97	1·07
2 : 1	0·68	0·58	0·74	0·81	0·94
1 : 1	0·62	0·52	0·75	0·77	0·60*
1 : 2	–	–	0·84	0·82	0·75
1 : 5	–	–	0·67	0·73	0·71
Rambo/Rodos					
5 : 1	0·70	0·52*	0·34**	0·34**	0·33**
2 : 1	0·87	0·62	0·38**	0·48**	0·59*
1 : 1	1·03	0·82	0·30**	0·41**	0·46**
1 : 2	0·58	0·49*	0·33**	0·49*	0·52**
1 : 5	0·86	0·71	0·55	0·68	0·51**
Rambo/Rodos/Grosso					
1 : 1 : 1	0·65	0·59	0·31**	0·37*	0·36**
Mean[b]	0·77	0·69	0·47**	0·56**	0·54**

[a] (1) (2) first and second assessment; for dates see *Materials and methods*.

[b] Mean of all mixtures.

Disease in mixtures was different from the mean of the pure stands at $^{*}P<0{\cdot}05$ and $^{**}P<0{\cdot}01$; linear contrast.

to lower-than-normal densities through compensation by increased tillering, leading to similar competition among head-bearing tillers and constant yield per tiller. Populations of wheat and grasses have been shown to achieve similar densities of tillers over a wide range of initial densities (Harper, 1977; Darwinkel, 1978).

The ranges in yield variation from the lowest (34 plants m^{-2}) to the highest (203 plants m^{-2}) density (1·8- to 2·4-fold) were much larger than in a similar experiment with wheat (Darwinkel, 1978), in which an increase in density from 25 to 200 plants m^{-2} resulted in only about 30% yield increase. At a planting density of 25 wheat plants m^{-2} a mean of 15 ears developed per plant, compared with only one to three ears at densities higher than 100 plants m^{-2}. In the present experiment, at 34 barley plants m^{-2} the maximum number of head-bearing tillers per plant ranged from 9·8 to 11·6 while at 96–144 plants m^{-2} the number of heads per plant averaged 4·7–5·1. At the lowest density, plants tended to develop in a prostrate manner and produced many more infertile tillers than at higher densities (E. Gacek, personal observation), suggesting that barley needs a degree of crowding for uniform production. Tillering of wheat at low densities (5 or 25 plants m^{-2}) continued more than four weeks longer than at densities of 100–800 plants m^{-2} (Darwinkel, 1978). Similarly, it is likely that tillers that develop in low-density barley plots are of more variable age, explaining the more variable flowering at low densities observed in the present work.

The initial and harvested proportions of the cultivars changed only little, indicating that competitive interactions among the three cultivars were similar. This is in contrast to other studies with barley (Harlan & Martini, 1938; Blijenburg & Sneep, 1975), oats (Murphy *et al.*, 1982), and wheat (Finckh & Mundt, 1992a), where initial and harvested frequencies usually varied greatly.

The selected cultivars did not respond favourably to mixing, with many of the mixtures yielding less than the mean of their pure stands, although this was not significant. The unfavourable mixture response is especially surprising as disease levels in the mixtures were often reduced significantly. There was no relationship between disease reduction and relative yield. Only a few cases of poor mixture performance are reported in the literature (e.g. Klages, 1936), while data on equal or superior performance abound (Wolfe & Finckh, 1997). This is also true for mixtures in Poland. For example, over the past 10 years more than 10 multi-location mixture trials, involving overall more than 25 barley cultivars, have been conducted in Poland. In all experiments, yields of mixtures were equal to or slightly greater than the means of the pure stands (Gacek *et al.*, 1996a,b,c,d; Finckh *et al.*, 1997; Wolfe & Finckh, 1997).

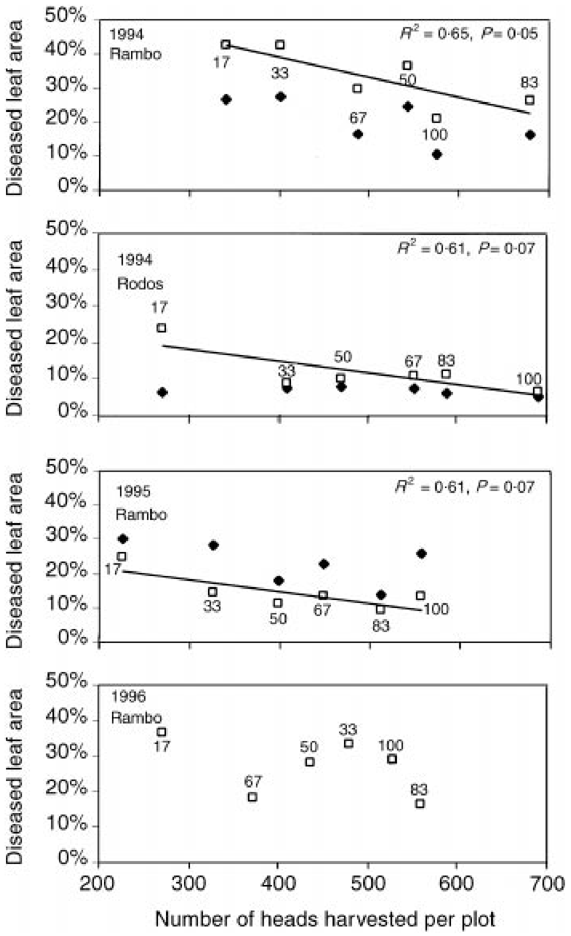

Figure 3 Effect of the number of heads per plot on the disease severity on pure stands of the cultivar Rambo in three years and of Rodos in 1994. Assessment times were: 14–16 June 1994, 24–29 June 1995 (◆) and 4–6 July 1994, 5–7 July 1995, 3–5 July 1996 (□). Numbers next to the data points represent the initial plant

Table 5 Correlation between frequency of Rambo in mixtures and the disease severity on Rambo in three years when mixed either with Grosso or with Rodos

Year	Mixed with	R^2	*P*
1994[a]	Grosso	0·87	0·07
1994	Rodos	0·46	0·14
1995	Grosso	0·60	0·07
1995	Rodos	0·49	0·12
1996	Grosso	0·75	0·02
1996	Rodos	0·52	0·11
All years[b]	Grosso	0·50	0·001
All years[b]	Rodos	0·41	0·003

[a] In 1994, Rambo was mixed with Grosso only at frequencies of 1 : 1, 1, 1 : 2, and 1 : 5.

[b] The regressions for all three years combined were performed using the relative disease severities on Rambo (see equation 2) to eliminate additional variation attributable to year effects.

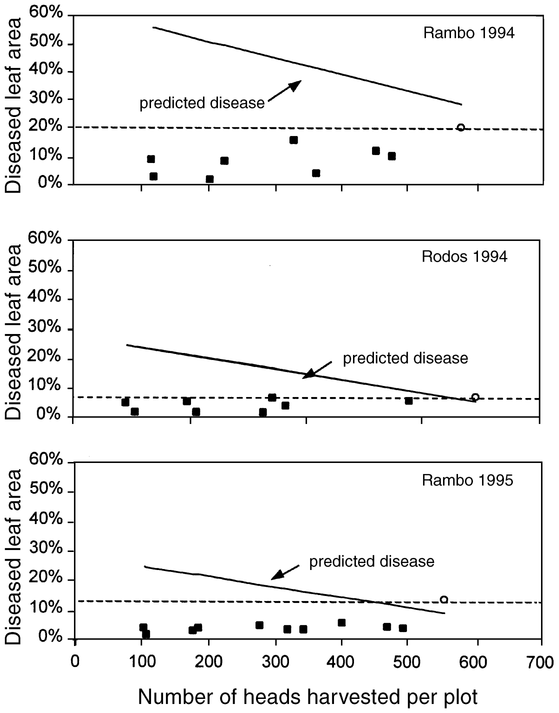

Figure 4 Mildew severity (■) on the cultivars Rambo in two years and Rodos in one year when sown in mixtures with either Grosso or Rodos, and with Rambo, respectively. The number of harvested tillers at the end of the season is shown. The solid line represents the predicted disease severity in a pure stand with the same number of tillers per plot, based on the regressions from Fig. 3. Also shown is (○) the observed disease severity and density of the 100% pure stands of Rambo and Rodos in the respective years. For convenience, a horizontal dashed line is drawn through the open circles to allow for easy visual comparison of disease severity at different frequencies with the pure stands at the same density as the mixtures.

The unexpected yield result with the mixtures in the present experiments could have arisen in part from the use of small plots, which meant that the plants used for the measurements were surrounded by ‘edge’ plants that were not affected by competition in the same way as plants in the plot centres.

The criteria used for mixing are primarily agronomic properties such as quality and even maturity, in addition to differences in resistance. For cultivars to be suitable for mixing, however, they also need to have good ecological combining ability *sensu* Allard & Adams (1969), i.e. the mixture components should respond neutrally or with increased yield to mixing without any negative effect on their companion cultivars. This will often be the case when intragenotypic competition is stronger than intergenotypic competition. In 44 out of 91 cases described here, the cultivars yielded less in mixtures than in pure stands, and the differences were statistically significant in four cases in 1996 (linear contrasts on a per-cultivar basis in each year separately. The maximum number of comparisons per data set was

11; data not shown). These results indicate that intercultivar competition was similar to or stronger than intracultivar competition.

Good ecological combining ability may be achieved by maintaining and selecting breeding lines in diverse populations. For example, bean and barley lines that had coevolved in a composite cross population for many years before final selection were much better mixers than lines that had been selected as pure lines (Allard, 1960; Allard & Adams, 1969). Breeding and selection approaches that might favour the production of lines or cultivars suitable for mixing are: composite crosses (Harlan & Martini, 1929; Jensen, 1988), topcrosses (Mahalakshmi *et al.*, 1992) and bulk selection (Finckh & Wolfe, 1997). Bulk selection to improve suitability for mixing is now practised in some public sector barley breeding programmes in Poland (Czembor & Gacek, 1996).

For realism and simplicity, natural infection was preferred here to artificial inoculation. This is especially important in mixture experiments because inoculations often produce unrealistically high levels of infection and thus reduced mixture effects. As it is impossible to predict the genetic composition of the naturally occurring inoculum, it is also impossible to be sure of the occurrence or extent of infection of a given cultivar in a given year.

The variability in disease severity on Rodos in the different years probably reflects the higher frequency of *Mla3*-virulent spores present in aerial samples collected in 1994 (31·5%) as compared with 1995 (11·8%) (Schaerer, Gacek & Wolfe, unpublished). Disease severity increased from the first to second assessment in 1994 while it decreased in 1995 (Fig. 3). This could be attributed to a slow-down in the epidemic caused by weather conditions while plants were still growing. However, even a lower percentage of diseased leaf area does not necessarily mean an absolute decrease in amount of disease.

The negative correlation of mildew severity on the pure stands of Rambo and Rodos with increasing density in the plots in 1994 and 1995 (Fig. 3) is contrary to the commonly held view that disease should decrease with reduced plant density (Burdon & Chilvers, 1982). Powdery mildew severity is usually greater under conditions of high soil fertility (Newton *et al.*, 1996) and plants in the lower density plots had a higher nutritional status, as indicated by the much higher yields per plant (1994: 14·2 and 4·2 g plant^{-1} at the lowest and highest planting density, respectively, 1995: 10·6 and 2·5 g plant^{-1}; and 1996 10·6 and 3·2 g plant^{-1}). Overall, the nutritional status and other growing conditions were best in 1994, as indicated by the highest yields in that year (Table 3). Correspondingly, the strongest correlation between density and mildew was observed in that year (Figs. 3, 4).

It is difficult to assign quantitative values to the effects of density and frequency on disease in mixtures. For a complete and unequivocal analysis, the experimental design should have included the mixtures at all of the different densities. This would allow for a response-surface analysis of the data as in ecological experiments (Law & Watkinson, 1987). However, each additional density for the mixtures would have increased the number of mixture treatments by 15, which was not possible for practical reasons. Also, pure stands at much lower densities would have been needed to achieve tiller numbers in the pure stands that correspond with the tiller numbers of the same cultivar in mixtures. Even if such low densities had been achieved, the changes in growth habit and uneven maturation in low-density plots would have made straight comparisons difficult.

Despite all these confounding factors, the data clearly show that the effects of reducing plant density and of reducing the frequency of a susceptible cultivar may differ greatly between cultivars and between years and, in the present case, may even have opposite effects. While density effects were important in 1994, they played a minor role in 1995 and it is impossible to determine their role in 1996. Frequency effects, i.e. of mixing, on the other hand, were important in all three years and reduced disease severity on the susceptible cultivars significantly.

Differences in disease pressure, plant architecture and nutritional status all affect epidemic development. In the pure stands, early in the season, disease severity was density-independent, which would be consistent with a generalized inoculation by an external spore cloud.

In 1994, the epidemic was relatively long, disease pressure was high and plant nutritional status was also high, leading to strong increases in disease with reduced density in the pure stands (see above). In the high density stands, upper leaf layers may have prevented spores from landing lower down in the canopy and reduced the spread of spores within the plots, further contributing to the reduced disease severities at higher densities. The relatively moderate disease reductions on Rambo and Rodos in the mixtures may have resulted from a high general inoculum pressure in the beginning of the epidemic followed by several mildew generations in which autoinfection was relatively important. In contrast, in 1995, disease pressure was lower and growth conditions much less favourable overall, resulting in much lower yields. Thus, differences in the nutritional status of plants at different densities may not have translated into differences in disease severity. On the other hand, in the mixtures, frequency played a major role in the system. Similarly, frequency effects were important in 1996 when the epidemic was short. The comparatively greater reductions in relative disease severity with mixing in 1995 and 1996 may be explained by the shortness of the epidemic, as it is well known that mixtures are most effective early in epidemic development (Sitch & Whittington, 1983).

The frequency-dependence of disease severity on Rambo is consistent with observations on wheat cultivar mixtures (Finckh & Mundt, 1992b; Akanda & Mundt, 1996). There, severity of stripe rust (caused by *Puccinia striiformis*) was frequency-dependent when the competitive abilities of the mixture components were similar. In the present experiments, the competitive abilities of the cultivars were also quite similar. It is interesting that the frequency-dependence was observed in both host–pathogen systems despite large differences in biology and epidemiology. Disease increase in stripe rust is largely a result of lesion expansion, with a generation time of a minimum of two weeks, while powdery mildew increases through new infections, with generation times as short as one week.

The results presented here demonstrate that frequency, as well as density, are important factors in disease restriction in genetically diverse host populations. These effects suggest a great complexity of epidemiological (distance, barrier effects) and ecological (plant nutritional status and possibly microclimate) interactions occurring in cultivar mixtures where disease and plant–plant interactions all affect one another.

Acknowledgements

This work was supported in part by grant KBN 0430-S3–93–05 from the Polish Committee for Science. We would like to thank Jacob Weiner and two anonymous reviewers for many useful comments and suggestions on the manuscript.

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