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Utility of defeated resistance genes to powdery mildew, Erysiphe graminis f. sp. hordei, in spring barley variety mixtures

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Abstract

In 1980 and 1981 experiments have been performed to study the utility of defeated resistance genes to powdery mildew in spring barley variety mixtures. For this purpose the epidemic development of powdery mildew in pure stands and mixtures of four spring barley varieties was monitored. In three varieties the resistance was overcome several years ago. One variety is still resistant. Changes in frequencies of corresponding virulence genes were also studied.

It was found that the variety mixtures slowed down the mildew epidemic only at the beginning of the season. Some reduction was lost later on since the pure stands reached their saturation level of infection at an earlier date than the more healthy mixtures. The level of reduction of infection depended on the composition of the mixtures and the number of components. Two-way mixtures appeared to be very risky due to the high frequency of corresponding combinations of virulence genes in the pathogen. Furthermore, two-way mixtures appeared to be unable to reduce the infection rate sufficiently when the infection pressure was high.

In 1981 the frequencies of combinations of corresponding virulence genes increased very much in the Diva-Mazurka mixture but declined in the pure stands of both varieties.

On the basis of these results it is concluded that the utility of defeated resistance genes involved in this study is very limited.

The low number and the irregular distribution of the Dutch spring barley varieties over the different resistance groups prohibit the use of variety mixtures in the Netherlands.

Additional keywords: epidemic development, virulence genes.

Introduction

In Western Europe breeding for resistance against powdery mildew, *Erysiphe graminis* f. sp. *hordei* in spring barley has not led to long-term success since the resistance of new varieties usually became less effective within a few years after their introduction, due to changes in the pathogen population. The durability of a combination of defeated resistance genes in a variety appeared to be still shorter. This resulted in a situation in which nearly all the leading varieties are rather susceptible to mildew. For this reason farmers are often forced to use fungicides to protect the barley crop against mildew.

Wolfe and Barrett (1980) report promising results in suppressing mildew infection by the use of variety mixtures. Even mixtures of susceptible varieties appeared to reduce the mildew infection level.

The reduction of the infection level in mixtures depends not only on the number and character of components but also on the frequency of complex mildew races, which combine the corresponding virulence genes. The use of variety mixtures could possibly favour the development of complex races. Wolfe and Barrett (1980) argue that the apparent advantage of complex races will be counteracted by a greater fitness of the more simple races.

During 1980 and 1981 experiments were performed to investigate mildew infection progress in pure stands and in mixtures of spring barley varieties possessing defeated resistance genes. Also the frequencies of complex and simple races were estimated.

Material and methods

In 1980, the mixtures were composed of the varieties Mazurka (resistance genes: Ml-g, Ml-k, Ml-a_7), Diva (Ml-a_{12}), Miranda (Ml-g, Ml-v) and Atem (Ml-v, ml-o).

The varieties Diva, Mazurka and Miranda are described as very susceptible, rather susceptible and slightly susceptible, successively, for powdery mildew (Anon., 1980, 1981). The resistance genes of these varieties were defeated because a high level of compatible virulence developed in the mildew population. The variety Atem is still resistant.

On 18 March 1980 mixtures and monocultures were sown in plots of 30 × 30 m, which were surrounded by strips of 30 m sown with the variety Atem. Seed mixtures contained equal numbers of seeds of the components. Per m^2 250 seeds were sown in rows. The distance between the rows was 25 cm.

The study in 1980 involved the four monocultures and the mixtures Mazurka-Diva-Miranda-Atem, Mazurka-Diva-Miranda, Mazurka-Atem, Diva-Atem, Mazurka-Miranda and Diva-Miranda without replicates. Atem was not sown as an isolated plot but evaluated in the surrounding strips.

In 1981 the investigations were restricted to the monocultures of Mazurka and Diva and their 1 : 1 mixture. The plot design was similar to that of 1980. The objects were sown on 30 March 1981 in three replicates.

In 1980 a spontaneous mildew epidemic started early in the season. On 19 May the first sampling took place and was repeated every ten days until 10 July. In each plot 50 tillers were cut at random in a diagonal way. Except the first infected leaf layer, each leaf layer was evaluated at least at two consecutive sampling dates. On the first observation day the number of pustules was counted and the percentage infected leaf area was estimated. During the second observation only the percentage infected leaf area was estimated because individual pustules could hardly be distinguished.

Frequencies of virulence genes were estimated by means of exposing seedlings of the varieties Mazurka, Diva and Miranda during 7 h to the mildew in the plots. After the exposure, the seedlings were returned to the glasshouse. Seven days later the number of pustules was counted. This survey was limited to the plots with monocultures of Mazurka and Diva and the mixture Mazurka-Diva-Miranda. From the pure stands the frequency of combinations of virulence genes could be calculated directly by counting the number of pustules on the seedlings of the exposed varieties. It was assumed that each mildew spore collected from a pure stand possessed virulence to that variety. Combinations of genes conferring virulence to Mazurka and Diva from the mixtures could not be determined directly. The mildew which developed on the seedlings of Mazurka and Diva had to be reinoculated separately on these two varieties afterwards. Virulence gene frequencies were estimated at the beginning of the epidemic on 23 May, but could not be estimated during July, because of poor weather conditons.

Van der Plank's apparent infection rate r was used to express the rate of development of the mildew epidemic. The value r is the regression coefficient of logit x and time; with logit $x = \ln x/(1-x)$, in which x = fraction of diseased leaf area. Results from mixtures involving the variety Atem were corrected for maximum attainable infection percentage before data were transformed into logits.

In 1981 again a spontaneous mildew epidemic developed well. The mildew infection was recorded as in 1980, but less frequently. On 22 May the number of pustules on the leaf of the first node and the two leaves below was counted per tiller. The infection percentage on the leaves of the second and third node was estimated on 22 June. The number of pustules on the flag leaf was counted on 14 July. Frequencies of virulence genes could be calculated from infection levels on seedlings of the varieties Mazurka and Diva which were exposed to the mildew population in the pure stands and the mixture on 22 May and 6 July. The simple and combined virulence against Mazurka and Diva were examined in the same way as in the year before.

Results

In 1980 the mildew infection on each tiller increased until 19 June. After that date the infection level decreased because the severely infected leaves senesced and the upper two leaves became more resistant than the lower ones. Therefore, the apparent infection rate could only be calculated over the first four observation dates. From Table 1, it appears that the mean r value of the mixtures does not differ from the expected value based on the results of the pure stands. This agrees with the mean reduction of infection in the mixtures, which changed slightly from 45% on 10 May to 38% on 19 June. The Diva-Atem mixture affected the mean reduction strongly negatively. The r value of the Diva-Atem mixture exceeded the r value determined of the Diva pure stand. After an initial reduction of the infection level of 55%, the infection level on 19 June exceeded the calculated mean of the monocultures with 5%.

Fig. 1 illustrates the expected and observed number of primary pustules on the successive leaves in the Mazurka-Atem mixture, expressed on a ln-distributed scale. The expected number is similar to the observed number of primary pustules in the pure stands of Mazurka. In the six variety mixtures and in the pure stand of Miranda the number of primary pustules increased from the second until the fourth successive leaf and decreased thereafter until the sixth successive leaf, similar to the disease curve of the Mazurka-Atem mixture in Fig. 1. In the pure stand of Mazurka and Diva the increase in number of primary pustules ended after the third successive leaf, suggesting that a further increase was impeded.

One would expect that the primary infection of newly developed leaves will be reduced more than the secondary infection within a variety mixture due, to the amount of auto infection. Table 2 gives the obtained reduction of the number of pustules at the first observation of the successive leaves. The main reduction of the mixtures in-

Table 1. The percentage leaf area covered with mildew of four spring barley varieties and six variety mixtures on 19 May and 19 June 1980, the achieved disease reduction in the mixtures and the apparent infection rate r over this period.

Variety or variety mixture	19 May 1980		19 June 1980		r value	
	observed	reduction (%)	observed	reduction (%)	observed	expected
Marzuka (U)	3.3		52		0.11	
Diva (D)	3.9		77		0.14	
Miranda (I)	1.5		25		0.11	
Atem (A)	0		0		0	
UDIA	1.1	50	16	59	0.10[1]	0.12
UDI	1.8	38	27	47	0.10	0.12
UA	0.3	82	9	65	0.12[1]	0.11
UI	1.9	21	27	31	0.10	0.11
DA	0.9	55	41	−5	0.18[1]	0.14
DI	2.1	22	35	31	0.11	0.12
Mean		45		38	0.12	0.12

[3] After correction for maximum attainable disease level.

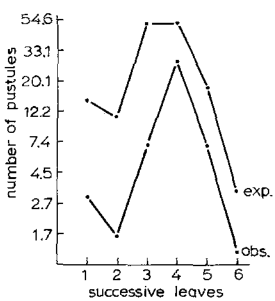

Fig. 1. The observed and expected number of primary pustules on the successively developed leaves of the Mazurka-Atem variety mixture of spring barley after correction for maximum attainable disease level.

Table 2. Number of primary pustules on the successive leaves of six variety mixtures, expressed as a percentage of the calculated mean of the pure stands.

Variety mixture[1]	19.5.80 leaf 1	19.5.80 2	29.5.80 3	9.6.80 4	9.6.80 5	19.6.80 6 = flag leaf
UDIA	56	37	18	48	31	23
UDI	64	52	19	74	32	55
UA	20	15	14	55	38	35
UI	83	55	51	67	60	106
DA	45	29	81	113	57	57
DI	80	62	56	62	29	63
Mean	58	42	40	70	41	57
Reduction	42	58	60	30	59	43

[1] U = Mazurka, D = Diva, I = Miranda, A = Atem.

creased until the third leaf. On the fourth leaf the reduction decreased considerably. The Diva-Atem mixture is again a negative exception. In this mixture the decrease in reduction started already after the second leaf. Positive exceptions are the three- and four-way mixture in which the reduction of the third leaf increased much more compared to the second leaf than in two-way mixtures. The mean reduction of primary infection of the upper five leaves of the six mixtures amounted to 50%. This differs little from the mean reduction of 42% achieved on the first leaf on 19 May. Ten days after the first evaluation, each leaf layer was evaluated again. The first leaf layer was evaluated only once because of early senescense. A small decline in the reduction in the mean of the infection levels is apparent after the first evaluation (Table 3). This is mainly caused by the loss of reduction in the Diva-Atem mixture.

The frequencies of virulence genes estimated on 23 May are presented in Table 4. Combinations of genes conferring virulence to Mazurka and Diva occurred less frequently in the pure stands of these varieties than in the Mazurka-Diva-Miranda mixture. Combinations of genes virulent to Mazurka and Miranda in the Mazurka monoculture and virulent to Diva and Miranda in the Diva monoculture occurred rather frequently.

Table 5 illustrates the mean infection level of Mazurka and Diva in pure stands and in the mixture in 1981. The mixture initially reduced the infection level with 50% and one month later the reduction still amounted to 31%. The infection of the flag leaves at the end of the epidemic was reduced with only 20%.

Table 3. Mean infection level at the first and second evaluation of the upper five leaf layers of six variety mixtures in 1980, expressed as a percentage of the calculated mean of the infection level in the pure stands.

Variety mixture[1]	First evaluation	Second evaluation
UDIA	31	34
UDI	46	52
UA	31	39
UI	68	59
DA	67	92
DI	54	52
Mean infection level	50	55
Mean reduction	50	45

[1] U = Mazurka, D = Diva, I = Miranda, A = Atem.

Table 4. Frequencies of combinations of virulence genes in mildew samples from the Mazurka and Diva varieties as pure stands and from the Mazurka-Diva-Miranda mixture on 23 May 1980.

Variety or variety mixture	Virulence combinations		
	Mazurka + Diva	Mazurka + Miranda (I)	Diva + Miranda
Mazurka (U)	7	58	—
Diva (D)	8	—	36
UDI	–)	—	—

Table 5. The mildew infection in the Mazurka and Diva pure stands and in the mixture in 1981.

Variety or variety mixture	a[1] on 22.5.81	b[2] on 22.6.81	c[3] on 14.7.81
Mazurka	11	21	1.3
Diva	36	50	4.4
Mixture observed	12	25	2.3
Mixture expected	24	36	2.9
Mixture relative (%)	50	69	80
Reduction (%)	50	31	20

[1]a = mean number of pustules on the first three infected leaves.
[2]b = mean infection percentage of the 4th and 5th leaf.
[3]c = mean number of pustules on the 7th (flag) leaf.

Table 6. Mean frequencies of combinations of genes, virulent to Mazurka and Diva in the Mildew samples from these varieties grown in pure stands and as a mixture.

Variety or variety mixture	Mean frequencies on	
	22.4.81	6.7.81
Mazurka	46	31
Diva	27	13
Weighted mean[1]	31	18
Mixture	21	42

[1] According tot the infection level on 22.5.81 and 22.6.81.

The mean frequencies of virulence genes in the pure stands of Mazurka and Diva and in the mixture in 1981 are presented in Table 6. The mean frequency of double-virulent mildew in the monocultures decreased from 31 to 18%, whereas it increased in the mixture from 21 to 42%.

Discussion

The average reduction of the infection level in the mixtures amounted to 38% on 19 June 1980. The results per mixture varied from 65% reduction to a 5% higher infection level. The mean results are in good agreement with results obtained by Wolfe and Barrett (1982), whose experiments concerned six high yielding spring barley varieties. In their experiments the 15 two-way mixtures gave 40% and the 20 three-way mixtures 60% reduction in mildew infection, averaged over 1980 and 1981 and over all the possible combinations.

The relative reduction of the infection in the mixtures was not uniform over the leaf layers (Table 2). The diminished relative reduction in the number of primary pustules on the fourth leaf layer in 1980 was not caused by an acceleration of the epidemic in the mixtures but by inhibition of the increase in number of primary pustules in the pure stands of Mazurka and Diva after infection of the third leaf layer. Wolfe and Barrett (1980) also found diminishing reductions during the season. They argue that the infection of the monocultures reaches the saturation level at an earlier date than of the more healthy mixtures. There is some evidence that the number of primary pustules in the pure stands of Mazurka and Diva also reached their saturation level on the third and fourth leaf layer. The mean number of pustules on those leaf layers reached 50. Also in other experiments (unpublished) the number of primary pustules hardly exceeded 50 per fully expanded leaf. This is in agreement with results of Forche (1980).

The observed apparent infection rate in the mixed stands was hardly lower than expected during the period of 19 May to 19 June 1980. In experiments with multilines for mildew resistance of the wheat variety Chancellor the r value only declined significantly if the mixture consisted of at least 75% resistant plants (Fried et al., 1979). The reduction in mildew infection depended mainly on the reduction of effective initial inoculum. From Tables 1 and 2 it appears that in the mixtures the main reduction was also achieved at the beginning of the epidemic.

From the infection rates in Table 1 it is apparent that the investigated two-way mixtures are unable to suppress the mildew infection. In the Mazurka-Miranda and the Diva-Miranda mixture this failure could be due to the high frequency of combinations of corresponding virulence genes. Complex compatible races could also be responsible for some loss of disease reduction in the Diva-Mazurka mixture in 1981. In spite of the lower fitness of races with both virulence genes, which is apparent from the decrease in frequency in the pure stands in 1981, their frequency increased rapidly in the mixture.

Finally it can be concluded that the utility of the defeated resistance genes involved in this study is very limited for use in variety mixtures. This is caused by the high frequencies of complex races and the insufficiently reduced infection rate in the two-way mixtures.

For use in variety mixtures it is recommended to choose varieties with recently introduced resistance genes. The level of corresponding virulence genes in the mildew population will be low and undesirable combinations of virulence genes will be rather rare (Wolfe et al., 1981).

The durability of non-defeated resistance genes will be extended if these genes are combined in one variety. However, allelic genes cannot be combined in one genotype. In order to increase the durability of allelic resistance genes, variety mixtures are very suitable.

A well-known locus with many allelic genes is the Ml-a locus. Several genes from this locus have been introduced already but many have not yet been employed (Giese et al., 1981).

The Dutch situation. In the Netherlands 6 of the 12 recommended varieties (Anon., 1983) belong to one resistance group. The other six varieties are distributed over five different groups. In four groups the resistance genes are defeated. The varieties involved in this study comprise four groups. Considering the results of this study and the irregular distribution over the different resistance groups, the present assortment appears to be unsuitable for the use in variety mixtures. An increase in the number of varieties is not expected because of the small area (less than 30 000 ha) grown with spring barley. Within-field diversity could also be realised by the introduction of 'poly-genotype' varieties by the breeders (Groenewegen and Zadoks, 1979). In a country with a small acreage, this could be a good solution.

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Samenvatting

Bruikbaarheid van doorbroken resistentiegenen tegen meeldauw, Erysiphe graminis f. sp. hordei, in rassenmengsels van zomergerst

In 1980 en 1981 is onderzoek verricht over de bruikbaarheid van doorbroken resistentiegenen tegen meeldauw, *Erysiphe graminis* f. sp. *hordei,* in zomergerstrassenmengsels. Daartoe werd de epidemieontwikkeling in monocultures en mengsels van vier zomergerstrassen bestudeerd. Van drie rassen is de resistentie reeds verscheidene jaren doorbroken. Eén ras is nog resistent. De frequenties van compatibele meeldauwvirulentiegenen werden eveneens bepaald.

Het bleek dat in vergelijking met de monocultures in de rassenmengsels de meeldauwepidemie alleen in het begin van het seizoen werd afgeremd. Later in het seizoen ging weer enige reductie in aantasting van de mengsels verloren doordat in de monocultures de aantasting eerder het verzadigingspunt bereikte dan in de minder aangetaste mengsels. De mate waarin de aantasting gereduceerd werd was afhankelijk van de aard en het aantal componenten in het mengsel. Twee-componentenmengsels bleken vrij riskant te zijn vanwege de aanwezigheid van combinaties van corresponderende virulentiegenen in de meeldauwpopulatie. Verder bleken twee-componentenmengsels niet in staat de infectiesnelheid afdoende af te remmen wanneer de infectiedruk hoog werd.

De frequentie van combinaties van corresponderende virulentiegenen nam sterk toe in het Diva-Mazurka-mengsel doch nam af in de monocultures van deze beide rassen.

Gezien de verkregen resultaten lijkt de bruikbaarheid van de in dit onderzoek betrokken doorbroken resistentiegenen erg beperkt. Het geringe aantal zomergerstrassen op de rassenlijst en de ongelijke verdeling over de te onderscheiden resistentiegroepen belemmeren de toepassing van rassenmengsels in de Nederlandse akkerbouw.

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