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Early assessment of adult plant reaction of wheat (*Triticum aestivum* L) to powdery mildew (*Erysiphe graminis* f sp *tritici*) at the five-leaf seedling stage

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Summary — An original procedure leading to the early prediction of adult plant reaction to powdery mildew infection at a seedling stage was defined. Seedlings of eight wheat lines were inoculated at the two- and five-leaf stages with various inocula (either clones or synthetic mildew populations) overcoming their major specific resistance genes. The mean mildew score (MMS) and the mean time for first sporulation (MTFS) proved to be strongly correlated. For each line tested, the mildew reaction of adult plants was closely predicted by that of vernalized, five-leaf seedlings. Using inoculations on five-leaf vernalized seedlings, we can predict the adult stage mildew reaction of a line, where the specific resistance should be overcome. Using this method, it is possible to study the genotypes, the specific resistance of which are still effective in the field, thus preventing assessment of their possible non-specific adult plant resistance in the field.

***Triticum aestivum* = common wheat / *Erysiphe graminis* f sp *tritici* = wheat powdery mildew / adult plant resistance / early prediction**

Résumé — Prédiction précoce du comportement adulte du blé (*Triticum aestivum* L) vis-à-vis de l'oïdium (*Erysiphe graminis* f sp *tritici*) chez de jeunes plantes au stade cinq-feuilles. *Une procédure originale permettant de prévoir de façon précoce, chez de jeunes plantes de blé, la réaction au stade adulte vis-à-vis de l'infection par l'oïdium, a été définie. Les jeunes plantes de neuf lignées de blé ont été inoculées au stade deux-feuilles et au stade cinq-feuilles par des inocula divers – soit des clones, soit des populations synthétiques d'oïdium – contournant les gènes de résistance spécifique de ces lignées. Une forte corrélation a été établie entre la note moyenne d'attaque et le temps moyen d'apparition des premières sporulations. Pour chacune des lignées étudiées, le comportement adulte vis-à-vis de l'oïdium a pu être prédit avec précision par celui des jeunes plantes vernalisées, inoculées au stade cinq-feuilles. Cette procédure permet de prédire, dès le stade jeune plante, quel sera le comportement d'une lignée au stade adulte, si ses gènes de résistance spécifiques devaient être contournés. Cette méthode rend possible l'étude de génotypes dont les gènes de résistance spécifique sont toujours efficaces au champ, interdisant ainsi de ce fait l'évaluation de la résistance adulte non spécifique de ces génotypes.*

Triticum aestivum = *blé* / Erysiphe graminis *f sp* tritici = *oïdium / résistance adulte / prédiction précoce*

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INTRODUCTION

Powdery mildew of wheat (*Triticum aestivum* L), caused by *Erysiphe graminis* DC ex Merat f sp *tritici* Em Marchal (Syn *Blumeria graminis* (DC) EO Speer f sp *tritici* Em Marchal), occurs annually in all regions of France and has been increasingly damaging in the last few years. So far, breeding programs for powdery mildew resistance in wheat have been mainly focused on the use of major, race-specific resistance genes providing complete protection, but generally of short-lasting durability (Bennett, 1984). Concurrently, the use of a restricted number of major resistance genes, such as *Pm2*, *Pm4b*, *Pm5*, *Pm6*, *Pm8* and *Mli*, led to a progressive decrease of the available genetic diversity (Heun and Fischbeck, 1987a, b; Lutz et al, 1992, 1995; Zeller et al, 1993). However, the adult plant reaction to mildew infection does not only depend on the presence of major race-specific resistance genes, since quantitative resistance could be detected in cultivars carrying no identified qualitative resistance, or in which the qualitative resistance has been broken down (Bennett, 1981a).

The adult plant resistance, sometimes called 'slow mildewing' (Roberts and Caldwell, 1970) or partial resistance (Asher and Thomas, 1984), can delay the progress of the disease and the spore production of the pathogen (Gustafson and Shaner, 1982). Investigations on adult plant partial resistance to wheat powdery mildew has been mainly based on the accurate evaluation of some components of fungal development: latent period, pustule production (number of pustules per cm^2 of leaf area), intensity of sporulation (number of spores produced per lesion), pustule size, or sporulation index (0–3 conidial chain density scale) (Nass et al, 1981; Gustafsson and Shanner, 1982; Asher and Thomas, 1984; Sharma and Basandrai, 1991). Although a good evaluation of the components of the adult plant resistance using these parameters is feasible, they cannot be easily used by breeders for the screening of numerous genotypes. The field assessment of adult plant resistance is thus usually based on a visual rating of disease severity, after natural infection in the field by local mildew populations (Bennett, 1981b; Knudsen et al, 1986; Hautea et al, 1987; Griffey et al, 1993; Miedaner et al, 1993).

In a previous study, Doussinault (1994) reported that the reaction of young vernalized seedlings to an inoculation with a synthetic mildew population, was closely related to the adult plant reaction, assessed under field conditions. The purpose of the present study was to define a procedure for the prediction of the adult plant mildew reaction at the seedling stage. First, a few parameters, such as testing inoculum or growing conditions for seedlings, were investigated for determining a simplified test procedure *(Experiment I)*. Second, this procedure was applied to eight genotypes in order to validate it *(Experiment II)*.

MATERIALS AND METHODS

Plant material

Three genotypes were chosen for *Experiment I*. The low lines, RE714 and RE001, obtained at the Station d'amélioration des plantes de Rennes, have shown a high level of mildew resistance at the adult stage (unpublished results). While RE714 was previously shown to possess two race-specific resistance genes to powdery mildew, *Pm4b* and *Mlre* (Robe and Doussinault, 1995), RE001 does not carry any gene. 'Hardi' was chosen as a susceptible control at both seedling and adult stages. For *Experiment II*, eight genotypes were selected, including Hardi and RE001, as controls, and six cultivars ('Apollo', 'Rendez-vous', 'Mercia', 'Maris Huntsman', 'Soissons', and 'Axona'), previously studied in field conditions for their adult stage mildew reaction (unpublished results).

Seedling stage studies

In *Experiment I*, three different growing conditions were applied to the three varieties, in order to predict the adult stage mildew reaction. First, seven plants were sown in 5 cm pots filled with a loam–peat–sand mixture with three replicates per genotype x growing condition. After emergence, the three following growing conditions were applied separately:

– NV250: not vernalized and 250 degrees days (°C day). Plants were grown over 16 days in a growth chamber under mildew proof conditions (16 °C, 16 h daylength, 203 $\mu E/m^2s$), till they reached the two-leaf stage at a thermal time of about 250 °C day. The same conditions were also used to grow a set of 29 differential lines, used to characterise the virulence spectrum of powdery mildew inoculum.

– NV500: not vernalised and 500 °C day. Emerging plants were grown at 16 °C (as described above) over 31 days, till they reached the five-leaf stage at a thermal time of about 500 °C day.

– V500, vernalized and 500 °C day. Emerging plants were first vernalized over 49 days in a mildew-proof chamber (8 °C, 8 h daylength), then acclimatized for 7 days at 16 °C. The five-leaf stage was also reached at a thermal time of about 500 °C day.

In *Experiment II*, only the NV250 and V500 conditions were used with two replicates per genotype x growing conditions combination.

Adult stage studies

All the genotypes studied in the *Experiments I* and *II* were sown in the field in October, 1994, using a hillplot design with about 30 seeds in an area of about 0.1 m^2 and a 0.50 m distance between the hillplots. The trial, sown in four replicates, was totally surrounded by hillplots of the highly susceptible cultivar 'Barbee'.

Fungal material and inoculation procedure

Experiment I

Three distinct inocula were retained. The two clones 93-25 and 93-1 were collected in France in 1993 and were derived from single-pustule progenies. Both are virulent to RE714, RE001, and 'Hardi', but 93-1 is more agressive than 93-25. Before the tests, the isolates 93-25 and 93-1 were first produced onto RE714, then multiplied on 'Barbee' seedlings. The third inoculum was a synthetic mildew population, designed to mimic field contamination conditions. This population was produced onto 13 differential genotypes including the three genotypes studied (table I). One day before the test, all contaminated leaves were shaken to remove old conidia. All the genotypes showed the same level of mildew development. Only one well-sporulating leaf was sampled for each of the ten differential cultivars, but five leaves were sampled for genotypes RE714, RE001, and 'Hardi', to ensure a high level of attack of these genotypes during the test.

Experiment II

Since eight lines had to be tested, only one clone of mildew among clones CA, K and F, already isolated in the laboratory, and a synthetic mildew population were used for each genotype. These clones were chosen for their ability to infect the tested genotypes (table II). A restricted set of ten differential genotypes was used to produce the synthetic mildew population for this second experiment (table I). Leaves were sampled from these genotypes in equal proportions.

For both experiments, fungal spores were uniformly dispersed onto plant material using a settling tower. Characterisation of the testing isolates was carried out on detached leaves of the differential genotypes (table II) as described previously (Robe and Doussinault, 1995). For each inoculation, all pots were randomized at the bottom of the tower and one Petri dish containing detached leaves of differential genotypes was also included. Four glass slides were placed in the settling tower and a homogeneous 500–700 spores/cm^2 inoculation was checked by direct counting of conidia. After inoculation, plant material was incubated at 16 °C (16 h daylength, 140 µE/m^2).

Disease assessment

Seedling stage studies

Two characters were assessed separately in *Experiment I*. The mean time for first sporulation

Table I. Individual contribution of 13 plant genotypes to the dosage of the two synthetic mildew populations.

Plant genotype	*Major resistance gene(s)*	*Number of detached leaves*	
		Experiment I	Experiment II
'Asosan/8CC' [a]	*Pm3a*	1	1
'Syros'	*Pm3d = Mlk*	1	1
'Wembley'	Unknown	1	1
'Cocker 983'	*Pm6+ ?*	1	1
'Amigo'	*Pm17*	1	1
'Normandie'	*Pm1*, *Pm2*, *Pm9*	1	1
'Rendez vous'	*Pm2*, *Pm4b*, *Pm6*	1	1
'Apollo'	*Pm2*, *Pm4b*, *Pm8*	1	1
'Axona'	*Pm2*, *Pm3d*, *Mld*	1	1
'Barbee'	None	1	1
RE714	*Pm4b*, *Mlre*	5	–
RE001	None	5	–
'Hardi'	None	5	–

[a] Backcrossed eight times to 'Chancellor'.

Table II. Powdery mildew score (MMS) of a wide set of differential genotypes after inoculation with clones 93-25, 93-1, CA, K and F, and characterisation of two synthetic and one natural field mildew populations (seedlings stage studies NV250).

Cultivar/line	*Major resistance gene(s)*	*Clones of powdery mildew*					*Synthesized mildew population*		*Field mildew population EVF*
		Exp I		Exp II			Exp I	Exp II	
		93-25	*93-1*	*CA*	*K*	*F*			
'Axminster/8CC' [a]	*Pm1*	8	9	1	1	7	9	8	68
'Ulka/8CC' [a]	*Pm2*	7	9	9	9	9	9	9	92
'Asosan/ECC [a]	*Pm3a*	1	2	0	0	8	8	8	1
'Chul/8CC' [a]	*Pm3b*	0	2	1	1	1	8	7	3
'Sonora/8CC' [a]	*Pm3c*	9	9	8	3	3	9	8	44
Syros	*Pm3d = Mlk*	0	0	0	8	8	1	4	1
Michigan amber	*Pm3f = MlA*	9	9	–	–	–	9	–	44
'Khapli/8CC' [a]	*Pm4a*	9	9	8	9	9	9	9	47
'Roazon'	*Pm4b*	9	9	7	8	9	9	9	76
'Hope'	*Pm5*	9	9	9	4	7	9	9	45
'TP114/2 Starke' [b]	*Pm6*	1	8	3	9	1	7	9	73
'Transec'	*Pm7*	1	9	7	9	6	9	9	71
'Clement'	*Pm8*	9	2	0	7	0	8	5	77
'Wembley'	Unknown + *Pm12*	0	0	2	0	0	0	0	0
'Amigo'	*Pm17*	0	2	3	1	1	5	3	1
'Courtot'	*Mlar*	7	9	9	9	9	9	9	75
'Aquila'	*Mli*	1	9	9	9	7	9	9	73
'Talent'	*Tal*	7	9	9	1	2	8	5	33
'Maris Huntsman'	*Pm2, Pm6*	–	–	0	9	0	–	8	59
'Mercia'	*Mli, Tal*	1	9	6	0	0	8	4	39
'Maris Dove'	*Mld, Pm2*	0	0	1	0	8	3	5	4
'Cocker 983'	*Pm6 + ?*	0	7	0	0	0	5	3	36
'Normandie'	*Pm9, Pm*1, *Pm2*	7	9	0	0	0	8	7	15
'Rendez vous'	*Pm2, Pm4b, Pm6*	0	9	0	9	0	8	8	48
'Apollo'	*Pm2, Pm4b, Pm8*	7	9	0	9	0	8	5	71
'Axona'	*Pm2, Pm3d, Mld*	0	0	0	0	7	0	4	0
'Soisson'	*Mlar + ?*	–	–	9	3	8	–	8	85
'Barbee'	None	9	9	9	9	9	9	9	100
RE714	*Pm4b, Mlre*	8	9	1	0	0	8	1	40
RE001	None	9	9	8	7	5	9	5	59
'Hardi'	None	9	9	9	9	9	9	7	90

EVF estimated virulence frequencies, –: not tested. [a] Backcrossed eight times to 'Chancellor'. [b] Backcrossed twice to 'Starke'.

(MTFS) is the number of days from inoculation to observe the first distinguishable sporulated pustule. The MTFS was visually estimated on the first replicate by a daily observation of the seven plant, from the first to the fourteenth day after inoculation. For each of the seven plants, the MTFS was determined when distinct individual sporulating pustules became visible. The mean mildew score (MMS) is an evaluation of disease severity using a 0–9 notation scale based on number of pustules and intensity of sporulation. The MMS was determined 9 days after inoculation on the 21 plants of the same genotype (seven plants x three replicates). The disease reaction of detached leaves from differential genotypes (table II) was assessed using the same 0–9 scale.

For *Experiment II*, the MMS was assessed by a single score assessed simultaneously on the seven plants of each of the two replicates.

Adult stage studies

For both *Experiments I* and *II*, field assessment of adult plant mildew reaction was effected at the end of May. The global disease level at the adult plant stage was assessed using a 0–9 scale, based on the sporulation intensity, the surface of leaves covered by sporulation, and the progression of symptoms along the stem axis. This scale can be separated into three classes; resistant (0–3), intermediate (4–6) and sus-

ceptible (7–9). This scoring scale is commonly used in our breeding programs.

The naturally occurring field mildew population was checked for virulence spectrum and frequencies. A set of differential lines at the seedling stage (table II) was maintained for 8 h inside the field trial, then incubated in the laboratory at 16 °C (16 h daylength, 140 μE/m²s). The assessment of virulence frequencies was carried out by counting pustules on the two sides of the primary leaves of a five sampled plants of each differential line. The estimated virulence frequencies (EVF) were finally expressed by the number of pustules of each line in percentage of the number of pustules on the most severely attacked genotype 'Barbee'.

RESULTS

Wheat powdery mildew score of differential genotypes to isolates 93-25, 93-1, CA, K and F as well as the mildew scores of the two synthetic mildew populations, are given in table II. The EFV on the field mildew population is also given.

Experiment I

Seedling stage studies

The synthetic mildew population showed a large virulence spectrum since only the two genotypes 'Wembley' *(Pm12)*, and 'Axona' (*Pm2*, *Pm3d*, *Mld*), did not display any symptoms, although they had been used to produce the synthetic population. RE714, RE001, and 'Hardi', were strongly attacked by both the clones and the synthetic population.

Highly significant interactions between genotype and growing condition factors were observed for MTFS and MMS (table III). In order to study the effect of inoculum on MTFS and MMS, genotype and growing condition factors were decomposed and the means for inoculum effect in every genotype x growing condition combination were compared (table IV).

Analyses of variance performed with every cultivar for NV250 condition and with 'Hardi' for every growing confirmed the validity of the choice of NV250 and 'Hardi' as susceptibility controls (table IV).

When inoculating seedlings of RE714, highly significant differences were found between inocula, for both characters, and growing conditions NV500 and V500. RE001 exhibited a strong resistance reaction, with numerous chlorotic spots, when tested at the five-leaf stage (conditions NV500 and V500). Contrary to RE714, it was not possible to evaluate the MTFS, except for the condition NV500 towards the clone 93-1.

Analyses of variance of the effect of the two growing conditions NV500 and V500 and of

Table III. Analyses of variance for the effect of inoculum (I), genotype (G), growing conditions (GC), and their interactions on the MTFS and the MMS in *Experiment I*.

Source	*Mean time for first sporulation*				*Mean mildew score*			
	df	*SS*	F	P	df	*SS*	F	P
Total	125	335.00			566	7 141.30		
Main effects								
Inoculum	2	19.82	40.29	< 0.0001	2	203.57	160.44	< 0.0001
Genotype	1	144.64	587.90	< 0.0001	2	2 588.22	2 039.92	< 0.0001
Growing conditions	2	67.11	136.39	< 0.0001	2	2 384.22	1 879.14	< 0.0001
Interactions								
I x G	2	10.71	21.77	< 0.0001	4	202.05	79.62	< 0.0001
I x GC	4	6.08	6.18	0.0002	4	153.57	60.52	< 0.0001
G x GC	2	55.43	112.65	< 0.0001	4	1 154.13	454.82	< 0.0001
I x G x GC	4	5.28	5.37	0.0006	8	112.97	22.26	< 0.0001
Residuals	108	26.57			540	342.57		

Analyses performed with genotypes RE714 and Hardi (MTFS) and with genotypes RE714, RE001, and Hardi (MMS). *df* degrees of freedom; SS sums of squares; *F* Fisher's *F* ratio; *P* probability associated with *F*.

Table IV. Newman and Keuls multiple range tests on MTFS and MMS for inoculum effect, in each genotype x growing condition.

Genotype *Inoculum*	*Growing conditions*					
	NV250		*NV500*		*V500*	
	MTFS	*MMS*	*MTFS*	*MMS*	*MTFS*	*MMS*
RE714						
Pop	6.85 [a]	8.76 [a]	10.28 [a]	0.95 [a]	10.71 [a]	1.00 [a]
93-25	6.43 [a]	8.86 [a]	8.86 [b]	4.05 [b]	9.57 [b]	3.38 [b]
93-1	6.28 [a]	8.95 [a]	7.28 [c]	6.28 [c]	9.28 [b]	3.24 [b]
Mean	6.52	8.86	8.80	3.76	9.85	2.54
'Hardi'						
Pop	6.28 [a]	9.00 [a]	6.43 [a]	9.00 [a]	6.57 [a]	8.76 [a]
93-25	6.14 [a]	9.00 [a]	6.00 [a]	8.90 [a]	6.28 [a]	8.76 [a]
93-1	6.14 [a]	9.00 [a]	6.14 [a]	8.95 [a]	6.28 [a]	8.86 [a]
Mean	6.19	9.00	6.19	8.95	6.38	8.79
RE001						
Pop	6.14 [a]	8.76 [a]	–	1.14 [b]	–	0.90 [a]
93-25	6.28 [a]	8.23 [a]	–	0.09 [a]	–	0.45 [a]
93-1	6.28 [a]	8.90 [a]	7.40	4.86 [c]	> 14.00	2.00 [b]
Mean	6.23	8.63	–	2.03	–	1.10

–: MTFS could not be determined since plants remained symptomless until the end of the experiment (14 days after inoculation).

inoculum on both MTFS and MMS characters revealed a highly significant interaction between inoculum and growing condition factors (data not shown). As a consequence, *F*-ratios for inoculum and growing conditions were not calculated with the residual mean square, but with the interaction mean square, in order to determine the statistical significance of these factors in front of interaction (table V). For RE714 and RE001, the mean squares of inoculum and growing condition effects were not significantly different from the interaction mean square. Examination of the mean values of MTFS and MMS (table IV) indicates that most of the interaction between inoculum and growing condition factors was provided by the combination NV500 x clone 93-1. Generally, the MTFS decreased and the MMS increased from V500 to NV500.

For RE714, a great similarity was observed between the results of MTFS and MMS, which was confirmed by the high value of the linear correlation coefficient between these variables (0.99) (fig 1). Such a relationship may suggest that these two characters share at least a part of their genetic determinism.

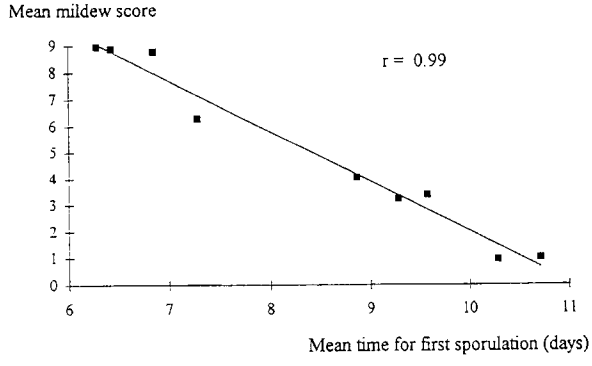

Fig 1. Linear regression analysis between MTFS and MMS for genotype RE714 for means on the three growing conditions x three inocula.

Adult plant stage

The natural field population also displayed a large virulence spectrum, since only the seedlings of Wembley and Axona did not show any symptoms (table II). Seedlings of Hardi, RE714, and RE001 were widely attacked by the field mildew population, making it possible to perform the field evaluation of their non-specific adult-plant resistance to mildew.

Table V. Analyses of variance for the effect of MTFS and MMS for the effect of the two growing conditions NV500 and V500, for genotypes RE714 and RE001.

Source	*Mean time for first sporulation*				*Mean mildew score*			
	df	*SS*	F	P	df	*SS*	F	P
RE714	41	63.33			125	640.13		
Inoculum	2	34.62	7.06 [a]	0.1241	2	320.97	5.82 [a]	0.1466
Growing conditions	1	11.52	4.70 [a]	0.1625	1	47.05	1.71 [a]	0.3215
I x GC	2	4.90	7.19 [a]	0.0024	2	55.16	15.25 [a]	< 0.0001
Residuals	36	12.29			120			
RE001								
Total	–	–			125	403.75		
Inoculum	–	–	–		2	226.55	3.72 [a]	0.2119
Growing conditions	–	–	–		1	26.07	0.86 [a]	0.4614
I x GC	–	–	–		2	60.92	42.30 [a]	< 0.0001
Residuals	–	–			120	86.40		

[a] *F*-ratios are based on the (I x GC) interaction mean square and the residual mean square. df degrees of freedom; SS sums of squares; *F* Fisher's F ratio; *P* probability associated with *F*.

In figure 2, adult plants of 'Hardi' appear very susceptible with a score of 8, while the other two genotypes RE714 and RE001 show resistance (scores of 2 and 1.5, respectively). The MMS in the condition V500 appeared to be closely related to that of the adult plant. NV500 showed more variation, due to the inoculum effect, and did not fit as well with the adult stage behaviour.

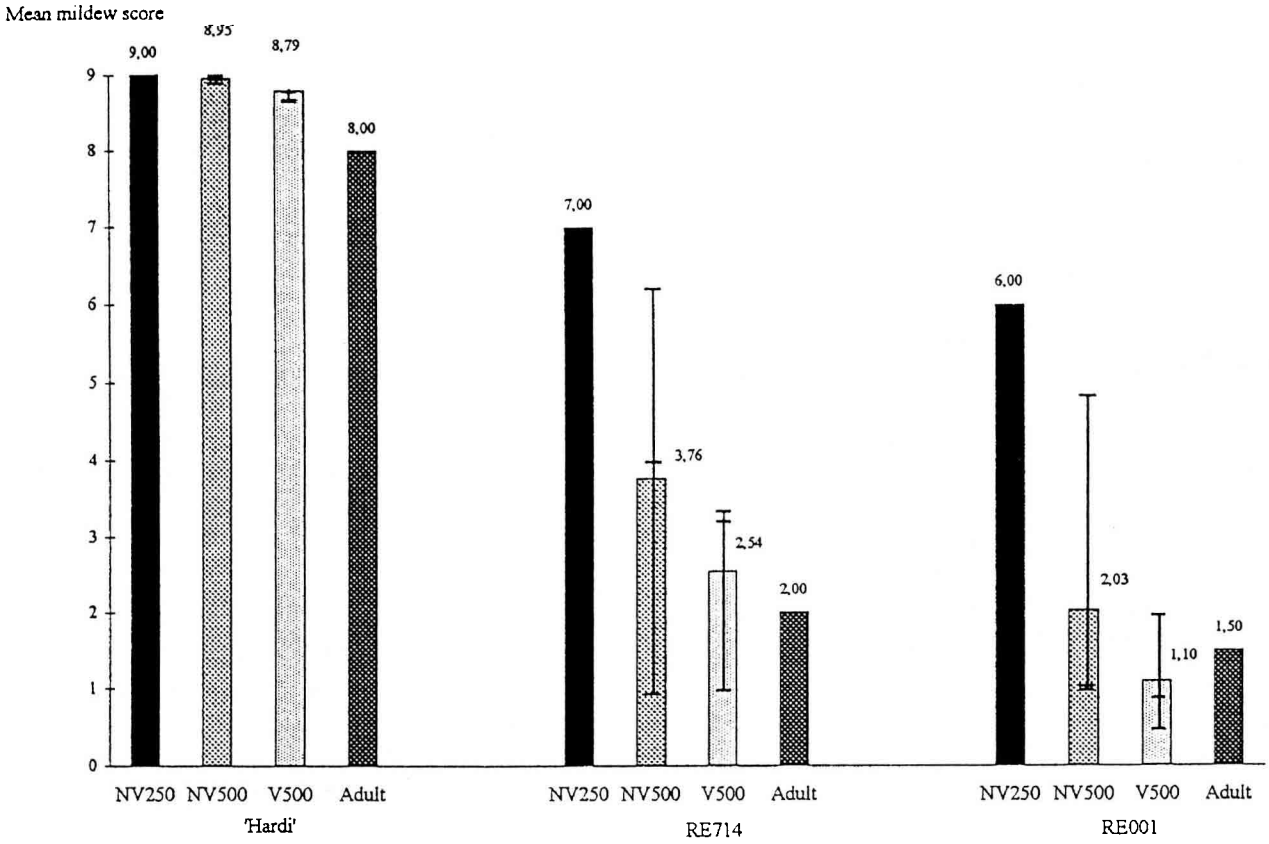

Fig 2. Comparison of MMS of five-leaf seedlings (NV500 and V500) and adult plant mildew reaction. Each MMS value represents the mean value of three different inocula. Variations between the three inoculations are represented by the vertical line.

Experiment II

When inoculated with clones CA, K or F, seedlings of the eight genotypes studied (fig 3) were susceptible at the seedling stage (NV250), except 'Soissons' whose intermediate score of 3.50 surprisingly departed from the detached leaf susceptible score of 8 (table II). When inoculating with the synthetic mildew population (growing condition NV250), a stronger variation was observed, with an MMS on detached leaves ranging from 4.25 ('Axona') to 7.5 ('Maris Hunstman'). The field mildew population was virulent to all genotypes studied except 'Axona', which was fully resistant under field conditions (table II).

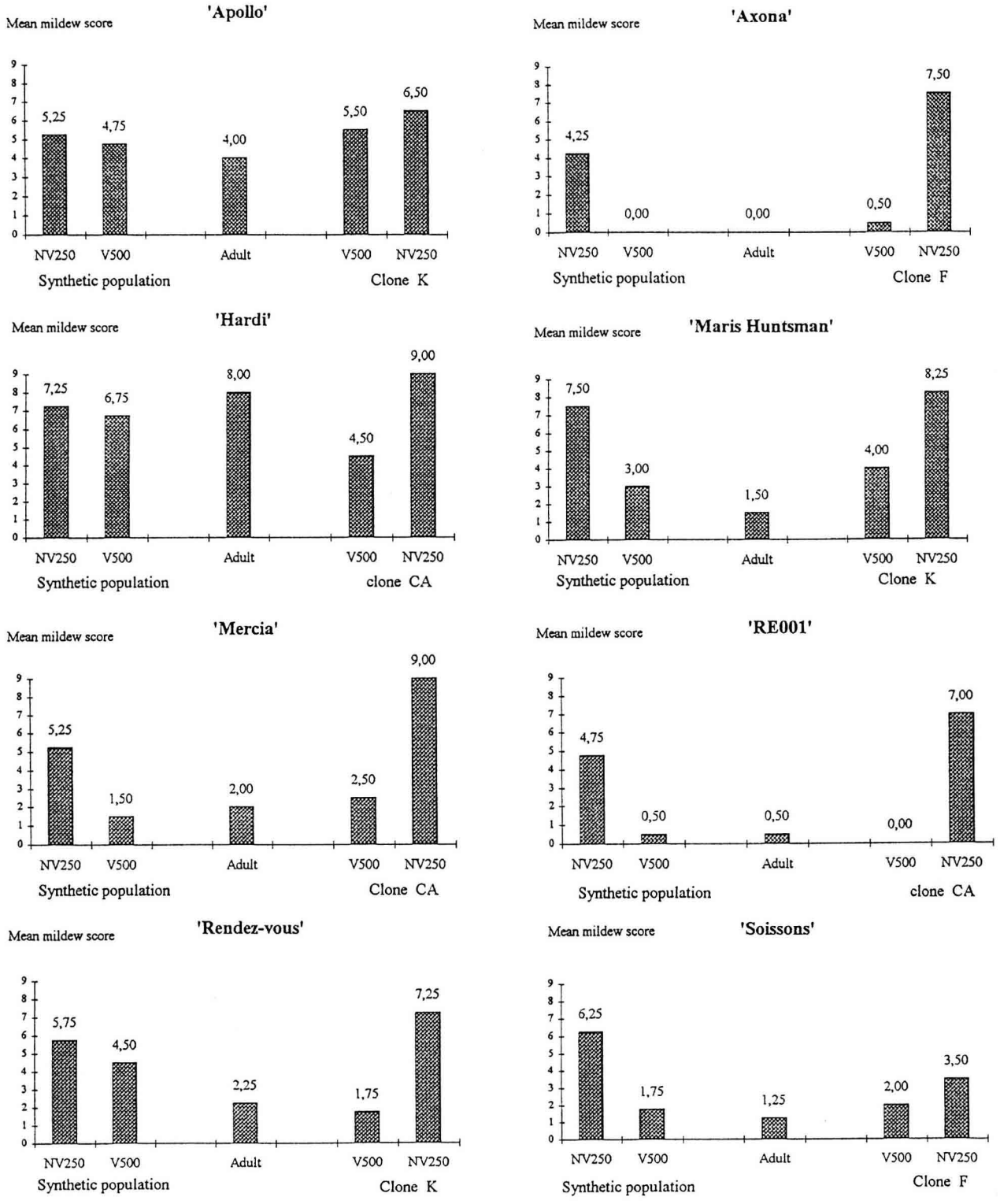

Fig 3. Comparison of the MMS of two-leaf (NV250) and five-leaf (V500) seedlings, after separate inoculation with a synthetic population and a clone, with the adult plant mildew reaction. Tests were performed on entire plants.

Using this simplified procedure, the adult stage resistance of RE001 and the adult stage susceptibility of Hardi were well predicted at the five-leaf stage (fig 3), confirming the conclusions of *Experiment I*.

The genotypes 'Maris Huntsman', 'Mercia', 'Soissons', and 'Rendez-vous' showed high levels of resistance in field conditions which were well predicted in the condition V500, with both types of inoculation. Separate inoculations with both a synthetic mildew population and a clone were necessary for a good prediction of adult plant reaction. For example, the intermediate reaction of 'Rendez-vous' at the adult age was better predicted when inoculating with the synthetic mildew population. Using this procedure, 'Apollo' was assumed to be susceptible at the adult stage which is in agreement with previous field studies (unpublished data).

The study of 'Axona' is of great interest since the specific resistance genes of this genotype have not yet been overcome in the field; it is therefore impossible to predict what would be the adult stage reaction of this cultivar, if confronted to a virulent strain. A high level of resistance of V500 seedlings was observed when inoculating 'Axona' with both inocula (fig 3). This suggests that 'Axona' possesses supplementary adult plant resistance, in addition to the specific resistance genes.

DISCUSSION

Growing conditions

In the present study, we defined an alternative method for the breeder, allowing early detection and evaluation of the adult plant mildew reaction at the five-leaf stage. The inoculation of vernalized, five-leaf stage seedlings (V500) provided the best prediction of the adult reaction to mildew infection. Mildew score of the V500 seedlings proved to be very stable, depending only on the genetic structure of the testing inoculum. Conversely, the mildew reaction of NV500 seedlings is variable, and depends on both genetic structure and aggressiveness of the testing inoculum. Moreover, vernalized seedlings display less symptoms and a longer MTFS than non-vernalized ones. It can be suggested that the development of the adult plant resistance is reinforced by low temperatures during emergence of the first five leaves. Young plants of barley were shown to display less mildew symptoms when vernalized for 6 weeks (White and Jenkyn, 1995). This clear-cut difference in the mildew reaction may be due to the physiological and photosynthetic stage of tested seedlings, and might not only depend on vernalization. While the V500 seedlings were healthy, with a green colour and an erected habit, NV500 seedlings progressively yellowed and wilted, due to a low ratio of light to temperature. These seedlings suffered from insufficient lighting and presented a rapid growth. They might not have finally expressed their potential level of resistance. Several reports indicated that growing plants under low lighting conditions favoured disease development (Tvaruzek, 1991, 1994).

Choice of inoculum

Few separate inoculations with a synthetic mildew population, on the one hand, and with clones, on the other hand, improved the quality of the prediction and should be recommended. Inoculating seedlings with a compatible clone of mildew underestimates the adult-plant mildew reaction but it remains the easiest way to overcome the eventual race-specific resistance. In return, inoculation with a synthetic mildew population approaches the conditions of a natural infection in the field and leads to an over-estimation of adult plant resistance.

Great attention must be paid to the production of the inoculum. In *Experiment II*, genotypes carrying a complex combination of specific resistance genes, such as 'Axona' (*Pm2*, *Pm3d*, *Mld*), or 'Rendez-vous' (*Pm2*, *Pm4b*, *Pm6*), were only moderately attacked at the NV250 stage, as a consequence of a moderate frequency of the compatible virulence genes in the synthetic mildew population.

Moreover, the genetic background of the genotypes used to increase and select the inoculum (synthetic population or clone) has probably a great influence on the aggressiveness and the variability of the subsequent testing inoculum, modifying its ability to induce symptoms. In *Experiment I*, RE001 contributed 20% of the synthetic mildew population, resulting in a high level of disease on its NV250 seedlings. Conversely, in *Experiment II*, RE001 did not contribute to the synthetic mildew population which resulted in an intermediate reaction on NV250 seedlings, despite the absence of known resistance gene in this time. The genetic background of the geno-

type used for inoculum production probably exerted a selection of conidia with a greater fitness to this genotype.

It may be suggested that we can produce the synthetic mildew population in two steps. First, a wide set of differential genotypes should be used, in order to ensure a great variability of virulence genes. Then, this primary population should be multiplied and matured onto seedlings of genotypes studied, during two to three cycles, in order to select the best adapted compatible mildew strains.

Disease assessment and consequences for breeding

The assessment of the MMS, a visual estimate of disease severity, and of the MTFS, a broad estimate of the latent period, makes possible a rapid evaluation of the mildew reaction. A strong correlation was observed between these two parameters. Similarly, studying the components of partial resistance to powdery mildew, Asher and Thomas (1984) in barley, and Elen and Skinnes (1988) in wheat, also established a close correlation between latent period and infection frequency, and between latent period and percentage of leaf area infected. These characters were suggested to be under a common genetic control (Elen and Skinnes, 1988) and to be different components of resistance that affect fungal development (Asher and Thomas, 1984).

Asher and Thomas (1984) also reported that the components of partial resistance to powdery mildew in barley were generally correlated with each other. In return, the components of slow-mildewing can vary between the seedling and the adult stages; while Rouse et al (1980) showed that the wheat cultivar 'Knox' reduces the infection frequency, at the adult stage 'Knox' was shown to reduce the sporulation (Shaner, 1973). As pointed out by Rouse et al (1980), the use of screening methods, involving seedlings alone to estimate components of slow-mildewing, could be misleading.

The present method is not intended to accurately predict the components of mildew resistance at the adult plant stage but to reach a rapid and early screening of a large number of accessions for their potential adult plant resistance, especially when the accession possesses specific resistance factors which are not yet overcome by the natural field mildew populations. The genotypes, selected with this method will subsequently be studied in the field. Field assessment of adult plant resistance remain undoubtedly the most effective method for incorporating partial resistance in new valuable wheat cultivars when specific resistance factors are already overcome by the field mildew populations.

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