European Journal of Plant Pathology (2006) 114:163–173
DOI 10.1007/s10658-005-4036-0

Assessing four-way mixtures of winter wheat cultivars from the performances of their two-way and individual components

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Accepted 4 October 2005

Key words: Triticum aestivum, cultivar mixtures, grain quality, grain protein content, grain yield, functional diversity, genetic diversity, mixing ability, septoria leaf blotch, *Mycosphaerella graminicola*

Abstract

Seven four-way bread wheat mixtures were compared to their components (individual cultivars and two-way mixtures) for septoria tritici blotch severity, grain protein content, and grain yield. Four-way mixtures and two-way mixtures performed better than the average of individual pure line components. Disease severity and protein content were most influenced by mixtures, with mixture efficiencies being greater in the four-way than in the two-way mixtures. Performances of four-way mixtures in terms of diseased leaf area reduction, grain yield, 1000-grain weight, and grain protein content were better predicted by performances of two-way mixtures than by those of pure lines. Our results suggest that two-way mixtures should be screened to remove unfavourable cultivar pairs and those binary mixtures that show complementarity should be selected in order to construct four-way mixtures.

Introduction

The large-scale use of monocultures of single plant genotypes, and thus, of single resistance genes generates strong selective pressure for pathogen pathotypes that may overcome these resistances (Wolfe et al., 1992; Wolfe and McDermott, 1994; Bayles et al., 2000; Hovmøller, 2001; McDonald and Linde, 2002). As a consequence, cultivar resistances have to be renewed constantly. This problem can efficiently be addressed by introducing functional diversity within agroecosystems (Mundt, 2002). One way to introduce diversity in agroecosystems is through the cultivation of monospecific mixtures, which results in diversifying resistance genes within a crop stand. The main mechanisms by which disease is reduced in cultivar mixtures (CMs) are dilution of pathogen propagules and barrier effects due to resistant plants interspersed among susceptible ones (Wolfe, 1985). In addition to this, resistance induced by avirulent pathogens can also reduce infection by virulent pathotypes (Finckh et al., 2000).

Cultivar mixtures may also offer the advantage of different components complementing one another in their adaptation to yield limiting factors and environmental variation. This leads to increased yield (Finckh and Wolfe, 1997; Wolfe, 2000), and should lead to increased overall performance of the more diverse crop stand.

Different mixtures vary in the degree to which they reduce disease or generate increased yield, making them equal to, better, or worse, than the mean of the individual cultivar components grown in pure stands (Finckh and Mundt, 1992). Mixing ability has been studied with cultivars presenting several contrasting characteristics, such as different resistance genes, quality, plant height, and earliness (e.g. Knott and Mundt, 1990). Such work has usually been carried out after artificial inoculation. General and specific mixing abilities have been analysed for two-cultivar mixtures compared to pure stands (Gallandt et al., 2001), although in practice cultivar mixtures are composed of more than two components, usually four to five components. Knott and Mundt (1990) suggested the possible use of mixing ability estimates derived from two-way mixtures to predict the performance of complex mixtures with more than two components. A model was developed by Gardner and Eberhart (1966), to predict the mixing abilities of complex mixtures on the basis of results of two-component mixtures and experimental data on wheat plots inoculated with *Puccinia striiformis*. This model showed that similar predictions of percent diseased leaf area and yield of diseased stands were obtained using mixing abilities and the mean of the pure stands, because the competitive effect of the cultivars was low compared to the additive effect (Lopez and Mundt, 2000).

Few studies have focused on harvested grain quality. Complementarity among varieties can occur for harvested product quality, as demonstrated with the protein content of wheat (Sarandón and Sarandón, 1995). In the absence of nitrogen fertiliser application, a 1:2 wheat cultivar mixture of a low-yielding, but high quality cultivar with a high-yielding, but lower quality cultivar had a yield performance similar to the high-yielding cultivar alone, and the mixture had a protein content equal to its high quality, low-yielding, component. Newton et al. (1998) showed that the malting quality of barley cultivar mixtures was equal to that of the pure stands in 14 out of the 15 cultivar mixtures tested, and was better than the pure stands in one three-way cultivar mixture.

Cultivar mixtures currently rely on mixing commercial cultivars selected for pure stand cultivation, with no consideration of their use in mixtures. When cultivars are combined in one mixture, agronomical characteristics such as harvest maturity and quality should be as similar as possible while other traits such as disease resistance should be diverse. The overall performance of a mixture cannot be derived from the simple accumulation of performances of the component cultivars, nor can individual contributions to the performance of the mixture be deduced accurately from measurements in pure stands (Finckh and Mundt, 1992).

In the present study, we compared pure stands, two-way mixtures and four-way mixtures of winter bread wheat for their capacity to cope with disease as well as for yield and quality. The purpose was to select cultivar mixtures for field production, by taking into account the criteria imposed by producers, including the milling industry. Therefore, only possible combinations usable on a large scale were tested. We did not test all possible two-way mixtures corresponding to the pure stands but only the two-way mixtures corresponding to four-way mixtures of possible interest to the industry.

Materials and methods

Overview

In order to ensure results relevant to the selection of cultivar mixtures for large scale farming practice, a field experiment was conducted for two consecutive years to compare commercial bread wheat cultivars currently in use for their mixing abilities in two-way and four-way mixtures. Mixtures were assessed for disease severity, grain yield, and grain quality (nitrogen content). The mixtures were chosen to allow control of all foliar diseases, but no totally resistant cultivars were available against septoria tritici blotch, caused by *Mycosphaerella graminicola* (anamorph: *Septoria tritici)*, which was the main foliar disease of winter wheat *(Triticum aestivum)* in the northern half of France, where the study was conducted. The relative efficiency of the mixture in terms of disease severity, yield, and grain quality was expressed as a percentage of the average of the mixture components when grown separately. The relative efficiency of the four-way mixture was also expressed as a percentage of the average of the two-way mixtures composing the mixture.

Field experiments

Experiments were conducted during two cropping seasons on fields located at the Versailles INRA

Research Station, 20 km west of Paris, where epidemics occur regularly. We used commercial bread winter wheat cultivars chosen for high baking scores and resistance to major wheat pathogens (Table 1). With 12 pure lines, there were 66 possible two-cultivar mixtures (2CMs) and 495 possible four-cultivar mixtures (4CMs). It was therefore necessary to select a subset of 4CMs and the 2CMs that represent them. The seven 4-cultivar mixtures were selected on the basis of acceptability for the miller quality criteria, level of disease resistance, and compatible agronomic characteristics. The 4-cultivar mixtures were ABMY (cvs Apache, Baroudeur, Malacca, Camp-Rémy); ACOV (cvs Apache, Charger, Soissons, Virtuose); CPRV (cvs Charger, Paindor, Renan, Virtuose); AMSV (cvs Apache, Malacca, Somme, Virtuose); MRSY (cvs Malacca, Renan, Somme, Camp-Rémy); AOSV (cvs Apache, Soissons, Somme, Virtuose) and ALMT (cvs Apache, Aligre, Malacca, Texel) (Table 2). Each experiment included seven 4-cultivar mixtures (4CM), 12 pure stands (PS) corresponding to each 4-cultivar mixture, and all possible two-way combinations (2CM) corresponding to each 4-cultivar mixture, i.e. 31 two-way combinations (Table 3).

The experiment plan was a latinised alpha design with four replicates (Whitaker et al., 1997) in each year. Such designs are complete block designs with sub-blocks within each complete block, and they allow to control efficiently two potential orthogonal heterogeneity gradients. Plots were 8 m long containing 12 rows spaced 20.8 cm apart. Half of each plot (1.25 × 8 m) was used for disease assessment, and the other half was left for yield assessment.

The mixtures were sown with equal proportions of seed number of each cultivar, at a density of 180 seeds per m^2. Sowing rate was low to limit airborne disease progress, according to an integrated protection crop management system. The trials were sown rather late in the season (at 20 October) to limit autumn disease contamination. Ammonium nitrate fertiliser was applied according to the balance-sheet method to ensure that nitrogen was not a limiting factor for plant growth (Rémy and Hébert, 1977). Other chemical treatments included herbicides (80 g ha^{-1} Lexus Millenium, including 33.3% flupyrsulfuron and 16.7% metsulfuron-methyl) applied in early spring, and fungicides against eyespot (1 l ha^{-1} Unix including 75% cyprodinil) applied at GS31 according to Zadoks et al. (1974). No growth regulators were used.

Assessment of disease severity, yield and harvest quality

At GS69, percent diseased leaf area covered with *M. graminicola* lesions (DLA) was assessed visually on the leaves of 10 main stems per plot for PS, 10 groups of two adjacent main stems for 2CM, and 10 groups of four adjacent main stems for 4CM, in order to generate data that involve a similar number of plants of each cultivar whether in pure stands or mixtures. Main stems were chosen in the plots with an effort to avoid bias. Plots were harvested with a plot combine (Hege 1.25 m large, Hege Maschinen GmbH, Waldenburg, Germany). Grain weight per plot (grain yield expressed at 15% humidity) and 1,000 grain weight were recorded. A 50 g grain sample was ground with a Laboratory Mill 120 (Perten Instruments AB, Huddinge, Sweden). Near-infrared spectroscopy (Inframatic 8100, Perten Instruments AB, Huddinge, Sweden) was used to estimate protein content and the Zeleny index of each grain sample. Zeleny index is an assessment of gluten quantity and quality and indicates baking quality. Zeleny index has values between 0–70, <18 indicating insufficient quality, 18–28 normal quality, 28–38 superior quality, and >38 high quality.

Statistical analysis

Data of DLA, yield, protein content, protein yield (product of yield and protein), and Zeleny index were analysed using the MIXED (linear mixed model) procedure of the Statistical Analysis System (SAS Institute, Cary, NC, 1990), assuming the model:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + g_{ij} + \gamma_{jk} + d_{jkl} + \varepsilon_{ijkl},$$

where Y_{ijkl} is the response of genotype i (a pure stand or a mixture) in year j and in block k and sub-block l of the year j experiment, μ is the overall mean, α_i is the main effect of genotype i, β_j is the main effect of year j, g_{ij} is the random interaction between genotype i and year j, γ_{jk} is the effect of the complete block k of year j, d_{jkl} is the random

Table 1. Characteristics of the winter bread wheat cultivars

Cultivar code	Cultivar	Agronomic characteritics[a]			Baking quality[b]		Disease resistances[c]						
		Earliness (GS 30)	Plant height	Earliness (GS 51)	Dough strength	Baking class[b]	*Puccinia striiformis*	*Puccinia triticina*	*Blumeria graminis*	*Pseudocercosporella herpotrichoides*	*Fusarium roseum*	*Stagonopora nodorum*	*Mycospharella graminicola*
A	Apache	2	3	7	6	SQ	7	9	6	3	5	4	u[d]
B	Baroudeur	3	4	7	6	SQ	5	6	7	2	5	3	u
C	Charger	1	3	6	5	SQ	8	9	8	2	2	4	u
L	Aligre	2	4	5	6	SQ	6	6	6	4	3	4	8
M	Malacca	3	3	5	5	SQ	9	9	8	2	4	3	6
0	Soissons	4	3	7	7	SQ	1	7	7	2	6	5	5
P	Paindor	1	2	1	6	SQ	4	7	8	3	4	3	5
R	Renan	1	4	6	8	HQ	8	8	6	5	6	4	7
S	Somme	3	3	5	6	SQ	6	8	7	2	3	4	6
T	Texel	2	4	7	6	NQ	3	4	7	3	4	4	u
V	Virtuose	1	3	6	u	NQ	8	8	7	5	6	3	u
Y	Camp-Rémy	3	5	6	6	SQ	2	7	4	2	5	4	3

[a]Assessment of earliness rated from 1 (late) to 9 (early) and height from 1 (very short) to 9 (very tall) (Anon., 1999).
[b]Baking class: NQ = normal quality, SQ = superior quality, HQ = high quality (Anon., 1999).
[c]Assessment of disease resistance from 0 (low level) to 9 (high level) (Anon., 1999).
[d]u: unknown.

Table 2. Components of the 4-cultivar mixtures used in the experiments

Mixture code[a]	Cultivar code[b]											
	A	B	C	L	M	O	P	R	S	T	V	Y
a	A	B	-	-	M	-	-	-	-	-	-	Y
b	A	-	C	-	-	O	-	-	-	-	V	-
c	-	-	C	-	-	-	P	R	-	-	V	-
d	A	-	-	-	M	-	-	-	S	-	V	-
e	-	-	-	-	M	-	-	R	S	-	-	Y
f	A	-	-	-	-	O	-	-	S	-	V	-
g	A	-	-	L	M	-	-	-	-	T	-	-

[a]4-cultivar mixture a: ABMY; b: ACOV; c: CPRV; d: AMSV; e: MRSY; f: AOSV; g: ALMT.
[b]See Table 1 for cultivar names.

Table 3. Components of the 2-cultivar mixtures used in the experiments

	Cultivar code[a]											
Cultivar code[a]	A	B	C	L	M	O	P	R	S	T	V	Y
A	-	-	-	LA	-	-	-	-	-	-	VA	YA
B	AB	-	-	-	MB	-	-	-	-	-	-	YB
C	AC	-	-	-	-	OC	PC	-	-	-	VC	-
M	AM	-	-	LM	-	-	-	RM	-	-	VM	YM
O	AO	-	-	-	-	-	-	-	-	-	VO	-
R	-	-	CR	-	-	-	PR	-	-	-	VR	YR
S	AS	-	-	-	MS	OS	-	RS	-	-	VS	YS
T	AT	-	-	LT	MT	-	-	-	-	-	-	-
V	-	-	-	-	-	-	PV	-	-	-	-	-

[a]See Table 1 for cultivar names.

effect of the sub-block l of year j, and ε_{ijkl} is the random plot error. For DLA, a square root transformation was used to improve the homogeneity of variances. Estimates of the mixture effect corresponding to the difference between the cultivar mixture and the mean of the PS or the mean of the six corresponding 2CMs were calculated. Mixture efficiency (ME) was calculated for all the measured criteria as the relative difference between the least-squares mean of a cultivar mixture and (i) the average least-squares mean of the component PS (4CM-PS)/PS, (2CM-PS)/PS or (ii) the average least-square mean of the six component 2CMs (4CM–2CM)/2CM.

Results

Throughout the two years of the experiment, septoria tritici blotch severity was relatively low, ranging from 1.8 to 13.1% based on the average of the three top leaves of the most susceptible cv. Somme and the most resistant cv. Aligre grown in pure stands, respectively. Average grain yield ranged from 7.15 t ha^{-1} to 9.68 t ha^{-1} for the pure stands, with Paindor the lowest yielding pure cultivar and Apache the highest, reflecting the potential yields of these cultivars (data not shown).

The analysis of the entire experiment (Table 4) shows that mixtures (both 4CM and 2CM compared to mean PS) had significant and effects on DLA and protein content. For all other criteria than diseased leaf area and protein content, mixture efficiencies of both 4CM and 2CM were always favourable, although non significant. 4CM mixture efficiencies were also always higher than 2CM mixture efficiencies, but the differences between 4CM and 2CM were not significant for any trait.

Similar results were observed on the seven four-cultivar mixtures considered independently (Figure 1). For all the criteria, the regression line of 2CM was intermediate between the regression lines of 4CM and PS, but closer to the 4CM

Table 4. Mixture efficiency (ME) and estimate of mixture effect of 4-cultivar mixtures (4CM) and 2-cultivar mixtures (2CM) compared to the mean of the pure stands (PS) or the mean of the corresponding 2CM

	4CM / PS			2CM / PS			4CM / 2CM		
	ME[a] (%)	estimate[b]	*Pr* > *t*[c]	ME (%)	estimate	*Pr* > *t*	ME (%)	estimate	*Pr* > *t*
percent diseased leaf area	32.8	−2.54	0.006 **	26.0	−1.81	0.009 **	6.8	−0.53	0.360
grain yield (kg ha^{-1})	0.9	75	0.640	0.6	13	0.901	0.3	51	0.720
1000 grain weight (g)	1.8	0.86	0.261	0.9	0.32	0.536	0.9	0.41	0.544
protein content (%)	2.9	0.34	0.009 **	1.1	0.17	0.041 *	1.8	0.13	0.226
grain protein yield (kg ha^{-1})	3.2	31.8	0.094	2.3	20.0	0.120	0.9	8.58	0.608
Zeleny index	3.4	1.0	0.392	1.0	0.6	0.453	2.4	0.3	0.783

[a]ME (%): relative difference between observed values on cultivar mixtures and the mean of the pure stands (4CM-PS) / PS, (2CM-PS) / PS or the mean of the six binary mixtures (4CM–2CM) / 2CM.
[b]Estimate: difference between estimated values for the cultivar mixture and the mean of the pure stands or the mean of the six binary cultivar mixtures.
[c](*Pr* > *t*): probability level; *, ** significant at $P < 0.05$, 0.001, respectively.

regression line. Differences between each 4CM and the mean of its PS components (data not shown) allowed to classify cultivar mixtures with respect to mixture efficiency. For diseased leaf area, the best 4CMs with that respect were AMSV, AOSV and ACOV. Among these 4CMs,

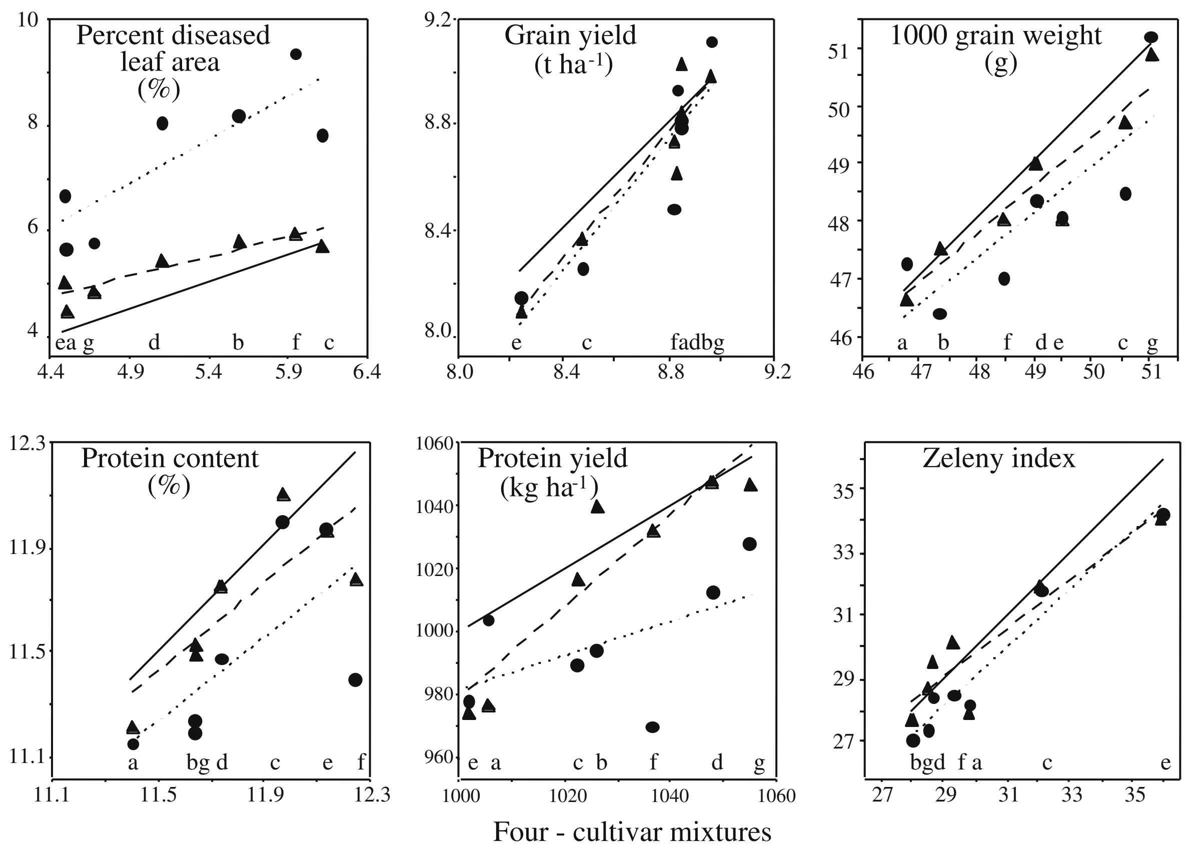

Figure 1. Observed values of percent diseased leaf area, grain yield, 1000 grain weight, protein content, protein yield and Zeleny index for the seven 4-cultivar mixtures (solid 1:1 line), mean of corresponding six 2-cultivar mixtures (triangles on Y-axis) and mean of corresponding four pure stands (circles on Y-axis). The dashed line and dotted line are the regression lines of triangles and circles, respectively. a-g: (see Table 2) 4-cultivar mixtures ABMY, ACOV, CPRV, AMSV, MRSY, AOSV and ALMT.

AMSV was the least diseased. For protein content, the best 4CMs were AOSV and ALMT, with the highest protein content produced by AOSV. AOSV produced the highest protein yield of all the 4CMs. The best mixtures had benefit compared to the mean of the pure stands for all criteria (disease level, grain yield and grain quality). These results indicated that AOSV, having significant benefit compared to the mean of the pure stands for all criteria was the best of the seven 4CMs tested.

The two extremes were AOSV and ABMY for which the corresponding six 2CMs are presented in Figure 2. For the mixture with the best performance, AOSV, all 2CMs except one had favourable mixture efficiencies. The best 2CMs were OS, AS and VS for DLA reduction, and OS, AS, AO

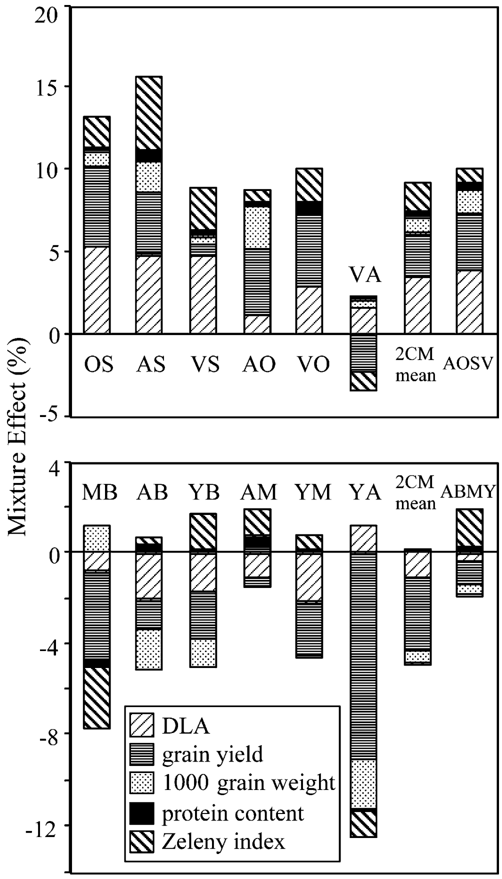

Figure 2. Estimate of mixture effect (%) of the 2-cultivar mixtures corresponding to the 4-cultivar mixture AOSV (A) and ABMY (B) and the mean of the six 2CM for percent diseased leaf area (DLA), grain yield, 1000 grain weight, protein content, and Zeleny index.

and VO for grain yield increase. For all criteria, AOSV was similar to the mean of the six 2CMs. The 2CMs provided a good estimation of the performance of the 4CM. In contrast, for the mixture with the poorest performance, ABMY, the corresponding 2CMs provided only a small reduction in DLA, and for most of the criteria, the 2CM mixture efficiencies were negative, although not significantly. Nonetheless, ABMY was still equivalent to the mean PS.

Disease severity, grain yield, 1000 grain weight, and protein content were strongly correlated between the four-way mixtures and the means of the corresponding six two-way mixtures (r = 0.93, 0.94, 0.93, and 0.81, respectively), between the four-way mixtures and the means of the four pure stands (r = 0.84, 0.90, 0.82, and 0.67, respectively) and between the two-way mixtures and the means of the pure stands (r = 0.95, 0.83, 0.87, and 0.92, respectively) (Figure 1). The higher correlations between 4CM values and their respective mean 2CM component values than with the mean PS component values indicated the importance of testing 2CMs in order to select 4CMs. The ranking of 4CMs on the basis of PS components (circles on Figure 1) was less accurate than based on 2CMs (triangles on Figure 1). Grain yield and protein content were not correlated with the reduction in disease severity (r = −0.18, and −0.49, respectively). The mixture efficiencies for grain yield and protein content were not correlated with disease severity reduction (r = 0.33, and 0.64, respectively).

Discussion

For the entire experiment, the mean effect of the mixtures for the seven 4CM mixtures were not all significant but were favourable for all criteria, with diseased leaf area reduced and yield and quality components increased. Although disease pressure was relatively low and the cultivars were chosen from those most resistant to major foliar diseases, the mixture efficiencies of 2CM mixtures and 4CM mixtures for *M. graminicola* severity remained relatively high, confirming previous studies (Jeger et al., 1981; Mundt et al., 1995a; Mille and Jouan, 1997). However in one case of a two-component mixture composed of a susceptible and a moderately resistant cultivar, no significant mixture efficiency was observed for *M. graminicola* severity (Cowger and Mundt, 2002). Some 2CM mixtures and 4CM mixtures did not show any cultivar mixture efficiency for diseased leaf area (e.g. ABMY and its binary components). This might imply that there is no complementarity between the resistance genes in the corresponding cultivars. No *M. graminicola* resistance genes are identified in these particular cultivars.

The majority of research on disease reduction in cultivar mixtures has been conducted on small grains with rusts (caused by *Puccinia* spp.) and powdery mildew (caused by *Blumeria* spp.), which are airborne obligate plant pathogens, and which interact with their hosts on a gene-for-gene basis (Wolfe, 1985; Mundt, 2002). Here we confirmed with *M. graminicola* that cultivar mixtures can provide protection against rainsplash-dispersed pathogens (Jeger et al., 1981; McDonald et al., 1988; Mundt et al., 1994; 1995a; Mille and Jouan, 1997).

Under rational use of nitrogen fertiliser, the protein content increase in both 2CM and 4CM mixtures could be due to better nitrogen uptake by the mixtures, improved exploitation of the soil and aerial space, or compensation between the cultivars for different traits. Nitrogen yield is a main factor to take into account, for a better use of nitrogen uptake. The mixtures could be interesting in conditions where protein yield is a limiting factor. However, this protein content increase was not confirmed in a two-way mixtures experiment in western United States under varying fertiliser application rates across the 33 sites, where each site was representative of prevalent practices at individual sites (Gallandt et al., 2001). Mixing ability demonstrated that six winter wheat pure lines differed in their ability to determine both grain yield and protein content in the 15 possible two-way mixtures. The correlation between the protein content of the two-way mixtures and the average protein content of the two component pure lines was high (0.88), but no significant difference was shown for protein content between pure lines and mixtures. In wheat cultivated without nitrogenous fertilisers, Sarandón and Sarandón (1995) found that a 1:2 mixture of a low-yielding high quality wheat cultivar and a high-yielding lower quality wheat cultivar, had a protein content as high as the high-yielding cultivar alone. The mixture efficiency on grain protein content disappeared under high nitrogen input.

Considering all criteria together (*M. graminicola* severity, grain yield, 1000 grain weight, protein content, grain protein yield and Zeleny index), it was possible to detect whether there was negative interaction between cultivars. In the present study, it was found that three of the 2CM mixtures had an unfavourable mixture efficiency for DLA and 28 had a favourable mixture efficiency for diseased leaf area, but only two of these favourable mixture efficiencies were statistically significant (VC and OS). Unfavourable mixture efficiencies were detected for protein content in eight 2-cultivar mixtures but none was significant. Mixture efficiencies for protein content were favourable in 23 2-cultivar mixtures, only three of these were significant (LA, AS, LT). For 1000 grain weight and Zeleny index, no significant difference was observed between 2CM and PS. For grain yield, only one 2CM (YA) had a significant mixture efficiency. Among the 31 2-cultivar mixtures tested for all the criteria, only one was significantly unfavourable compared to the mean of the pure stands; therefore only this mixture should be avoided when constituting 4-cultivar mixtures. However, several mixtures had significant favourable mixture efficiency and should be recommended.

For the mixture with the poorest performance, ABMY, the corresponding 2CM mixture efficiencies were negative for most of the criteria, although not significantly. Nonetheless, ABMY was still equivalent to the mean PS. It is suggested that the small incompatibilities between cultivars detected in 2CMs were compensated in the 4CM.

The performances of two-way mixtures were well predicted by PS for all criteria, confirming previous results on grain yield (Gallandt et al., 2001). Furthermore, significant effects for diseased leaf area and protein content were observed between 4CM and PS and between 2CM and PS, but never between 4CM and 2CM. The two-way mixtures gave a better estimate of the 4CM performance than did the PS. Studying the 2CMs provided a better sense of the overall mixture efficiency. Futhermore, we confirmed that greater mixture complexity improved mixture efficiency, e.g. three to five components were more efficient for disease reduction and yield increase than two-component mixtures (Gacek et al., 1996; Newton et al., 1997).

Mixing ability of cultivars for growing in mixtures has been determined on the basis of yield differences between five pure stands and the corresponding two-way mixtures, and between two-way mixtures and the corresponding three-way mixtures, over three years and three locations, for winter wheat (Gacek et al., 1997) and spring barley (Gacek and Nadziak, 2000). The contribution of the best and worst cultivars to the two-way mixtures, and of the pairs of cultivars to the three-component mixtures confirmed the interest of testing two-component mixtures to evaluate three-component mixtures. For example, the best yielding pure stand cultivar was not the best contributor to two-component and three-component mixtures. Furthermore, mixture efficiencies of two out of three three-component mixtures including the worst cultivar pairs were negative. These results on grain yield in wheat and barley cultivars confirmed the usefulness of testing two-way mixtures to build more complex mixtures. However, the means of the three two-way mixtures corresponding to the three-way mixtures were not calculated and no direct comparison could be made with the present study.

Furthermore, Mundt et al. (1994) compared four barley cultivars as pure stands and 11 possible cultivar mixtures (six two-way mixtures, four three-way mixtures, one four-way mixture). One two-way mixtures consistently increased yield and one of the two-way mixtures had consistently lower yields than the mean of the pure stands, over three years in the presence of scald and net blotch disease. It is therefore possible to identify mixtures with positive effects on disease control and yield, but not the component cultivars.

Our approach differed from that of Lopez and Mundt (2000) who calculated specific and general mixing abilities. We tested whether the performances observed in two-way mixtures can permit the construction of more complex mixtures, composed for example of three to five cultivars. We tested the four-way mixtures here. We compared two-way and four-way. In the experiments we did not test all possible two-way mixtures corresponding to all pure stands, but compared only those corresponding the four-way mixtures in order to provide four-way mixtures to be grown in commercial field.

Lopez and Mundt (2000) used percent diseased leaf area and yield under disease of five wheat cultivars inoculated with yellow rust and all possible two-way mixtures during one year across three locations, to estimate the relative contribution of each cultivar to the two-way mixture mean and to predict the means of all possible three-, four-, or five-way mixtures according to a model, including additive and competitive effects. Similar rankings of the complex mixture performances (10 three-way mixtures, five four-way mixtures and one five-way mixture) were obtained with the means of the cultivars in pure stand and the mixing ability analysis, given the small competitive effects compared to additive effects observed in this experiment. High additive and competitive effects would indicate the possibility of obtaining superior complex mixtures. In the present study, we did not test all possible two-way mixtures but only those possibly compatible, having similar agronomic characteristics (plant height and earliness), and having complementary resistances to foliar diseases and high baking quality. Our conclusions differed from the observations on wheat yellow rust experiments (Knott and Mundt, 1990; Lopez and Mundt, 2000). In the present study, the two-way mixtures provided a better estimation of the four–way mixtures than did the mean of the pure stands. For all the criteria (disease resistance, 1000 grain weight, protein content, protein yield), except the Zeleny index, the correlation coefficients between 4CM and 2CM were higher than between 4CM and PS, indicating that the two-way mixtures were better predictors of the four-way mixtures than the pure stands. This implies that the competitive effects were not negligible.

The lack of an increase in grain yield and protein content when the diseased leaf area was reduced may indicate an incompatibility between the cultivars involved. The 4CM AMSV tested in small plots in the present study has also been tested in large fields over two years. Grain yield increase was on average 0.5% in small plots and 6% in large fields (Belhaj Fraj et al., in preparation). Large fields give a more realistic estimate of grain yield mixture efficiency (Garrett and Mundt, 1999) and are therefore more appropriate for this criterion. Protein content increase was on average 2.3% in small plots and 1.8% in large fields. The disease reduction was of the same order of magnitude in both studies.

The experiment was conducted on one site during 2 years under an integrated crop protection management system. Compared to the intensive crop management system commonly used, the integrated crop protection management system combines several techniques to reduce disease levels: low sowing density, late sowing, nitrogen quantity and timing limited to the needs of the plants, choice of resistant cultivars. Leaf disease severity was low during the two years. The plots were uninoculated, and no fungicide was applied against foliar diseases. We showed under these conditions a beneficial mixture efficiency for reducing disease levels, increasing grain yield and quality. Our conclusion is that the cropping technique of using cultivar mixtures could be one element of an integrated crop protection management system, contributing to reduced disease levels and increased yield and grain quality.

Cultivar mixtures have been suggested as a means of controlling foliar diseases in small grains, but little information is available on how to choose components for use in cultivar mixtures. The present results show that it is possible to develop a general strategy for choosing cultivars for commercial cultivation as mixtures. Firstly, binary mixtures should be tested to exclude incompatible or insignificant combinations. This step is particularly important for cultivars with unknown resistance genes - a favourable mixture efficiency for disease resistance would indicate that different genes are present. The 2CM should be tested in the same environmental conditions as where the final mixtures are to be grown. Secondly, more complex mixtures can be developed on the basis of the performances of two-cultivar mixtures, which can be assembled, and tested. Without the knowledge on two-cultivar mixtures, it would not be possible to improve the choice of cultivars for complex mixtures. We proposed a practical way of selecting complex mixtures, without having to test too many combinations and without having to test combinations incompatible for agronomic traits (for example, large differences in culm height), or no resistance to foliar diseases.

Acknowledgements

The authors wish to thank Serge Savary for useful comments on the manuscript. This work was funded by the French Environment Ministry (*programme pesticides N*°568).

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