Cultivar mixture effects on disease and yield remain despite diversity in wheat height and earliness

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Main effects of disease resistance and yield potential of component cultivars in diverse mixtures are little affected by diversity in plant height and flowering date

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Running head: Disease in diverse wheat mixtures

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Abstract

Cultivar mixtures can stabilize yield and reduce pathogen spread in plant populations. A field experiment was performed to determine whether (i) a large difference between the cultivars in the mixture (e.g. plant height or earliness) would have an impact on mixture performance and whether (ii) such differences would modify the classical rules for mixture design. Mixtures were constituted from cultivars with diversity for many traits, including plant height, flowering date, disease resistance and yield potential. The field experiment was conducted in three years testing each year 72 to 90 mixtures of two, four or eight cultivars, and their corresponding pure stands. Disease severity and yield of cultivar mixtures were strongly related to the mean values of the component cultivars in pure stands. Despite the considerable diversity of the mixtures tested, the classic rules (*e.g.* proportion of susceptible cultivars) already tested in mixtures with similar height and earliness were effective for decreasing disease severity. Agronomic heterogeneity for traits such as plant height, yield potential or earliness of the cultivars in mixtures did not have a negative impact on disease severity and yield relative to pure stands. Increasing the number of cultivars in the mixture from two to eight had no impact on the mean disease severity and yield of the mixtures, but reduced the variability of disease severity and yield in the mixture relative to pure stands. These results suggest that it may be possible to increase within-field wheat diversity by combining more contrasted cultivars in mixtures than was previously thought.

Introduction

Cultivar mixtures have long been used to manage plant diseases, particularly in cereals (Finckh et al 2000; Borg et al., 2017), with some major successes. Mixtures have sometimes been chosen as a default solution in the past, when other disease management tools were ineffective, unavailable or not desirable (*e.g.* no fungicide or resistance genes available). Long-term success was limited in such cases. For example, barley mixtures were abandoned in former East Germany (RDA) after German reunification, because West European maltsters preferred to buy grain from single-cultivar crops, even though this required fungicide use to manage barley powdery mildew (Wolfe, 1992). In Denmark, mixture use declined because the *mlo* gene conferring resistance to barley powdery mildew was durably effective (Jørgensen, 1992). However, agriculture is currently facing new challenges that have revived interest in the use of cultivar mixtures (Barot et al., 2017). In addition to the continuous need to increase food production, there is now a demand to decrease agricultural inputs in general and fungicides in particular, as illustrated by the Ecophyto plans in France (https://agriculture.gouv.fr/ecophyto). Agricultural practices favoring diversity within cropping systems are becoming an attractive option for enhancing natural interactions and increasing agroecosystem resilience, particularly in a context of lower inputs and unpredictable climate variability.

Cultivar mixtures have been shown to have effects on diseases, such as barley powdery mildew (caused by *Blumeria graminis* f.sp. *hordei*), wheat yellow rust (caused by *Puccinia striiformis* f.sp. *tritici*, Wolfe, 1985; Mundt, 2002) and *Septoria tritici* blotch (caused by *Zymoseptoria tritici*: Cowger and Mundt, 2002; Gigot et al., 2012; Vidal et al., 2017). This cropping practice has also been shown to increase yield stability (Kiær et al., 2009; Creissen et al., 2016). Cultivar mixtures have the potential to reduce disease progression, but results can be highly variable and an appropriate design is crucial to reach the expected disease level and yield performances. Extensive studies of a range of design criteria for mixtures have been performed, as reviewed by Newton (2009) and Barot et al. (2017).

A first wave of studies highlighted the need for diversity within mixtures. For example, it was shown that the effect of the mixture can be enhanced by growing more than two cultivars together (Mille et al., 2006; Newton et al., 2008). In situations in which disease control is the main objective of mixture use, contrasting levels of disease resistance are often considered a key criterion (Gigot et al., 2014). Functional diversity can be modulated by choosing cultivars with complementary characteristics (*e.g.* one susceptible cultivar mixed with three resistant cultivars) or by choosing different proportions of the various cultivars in the mixture (*e.g.* 25% of a susceptible cultivar and 75% of a resistant cultivar). In general, higher proportions of resistant cultivars are expected to provide higher levels of disease protection (Cox et al., 2004; Dai et al., 2012). The susceptible cultivar can be replaced by a moderately susceptible or resistant cultivar carrying resistance QTLs. This makes it possible to reduce the proportion of the highly resistant cultivar required for effective protection in the mixture (Sapoukhina et al., 2013).

However, diversity in cultivar mixtures is limited by practical constraints, and agronomic homogeneity is often recommended for criteria such as plant height and flowering date. For example, in Denmark, barley mixtures are allowed only if they satisfy the following criteria: (i) less than five days difference in ripening date, (ii) less than 15 cm difference in height, (iii) the yield of each cultivar must be at least 95% the yield of the mixture and (iv) the mean disease susceptibility of component cultivars must be below a threshold established by experts (Labarthe et al., 2018). Reviews on mixtures classically recommend limiting the agronomic differences between cultivars (Wolfe, 1985). Similarly, wheat multiline development has aimed to maximize agronomic homogeneity while providing diverse mechanisms of resistance to yellow and brown rusts (Mundt, 2002).

Ecological principles contrast with agronomic concerns, in that they suggest that the stability of an ecosystem increases with its diversity (Barot et al., 2017). Indeed, increasing diversity levels could enhance positive interactions, ecological services and yield (Reiss and Drinkwater, 2018). In wheat, plant height and flowering date affect disease progression by determining the likelihood of a plant escaping the disease by spatial or temporal displacement. For example, growing taller plants can affect vertically spreading diseases such as Septoria leaf blotch. Plants with different flowering times can reduce the effect of occurrence of rain in the case of epidemics caused by *Fusarium* spp.. Finally, enhanced disease control has been observed in experiments involving mixtures with contrasting plant heights and/or flowering dates (Zhu et al., 2005; Li et al., 2012; Vidal et al., 2017).

Despite the relevance of these results, too few experimental data are currently available to draw firm conclusions on the balance between the positive (possible enhancement of disease control) and negative (agronomic management of heterogeneous mixtures) impacts of such phenotypic contrasts. The objective of this field experimentation was to assess the possible impact of increasing diversity on disease severity and yield in cultivar mixtures.

Materials and methods

We designed a range of field experiments for assessing the impact of cultivar diversity within mixtures on yield and disease severity, over three cropping seasons. Full details can be found elsewhere (Dubs et al. 2018). Two experiments were performed in 2014. We first phenotyped 58 cultivars, for plant height, flowering date and disease, in particular (E1-2014). In parallel, 20 cultivars were chosen to constitute 90 cultivar mixtures, for which yield and disease severity were recorded (E2-2014). Finally, in 2015, 16 cultivars were chosen based on the traits measured in E1 to constitute 72 cultivar mixtures (E3-2015). This E3-2015 experiment was repeated in 2016 (E3-2016).

Field experiments

The experiments were conducted over three consecutive wheat-growing seasons: 2013-2014 (hereafter called 2014), 2014-2015 (hereafter called 2015) and 2015-2016 (hereafter called 2016) .Fields were located at the INRAE Versailles Research Station in France (20 km west of Paris, 48°48'26''N 2°05'13''E, elevation 114 m). The specific features of each experiment are listed in Table 1.

Table 1 around here

In the phenotyping experiment (E1-2014), each cultivar was grown in a pure stand on a single plot. The three experiments including mixtures (E2-2014, E3-2015 and E3-2016) followed a two-block randomized design with two replicates for pure stands (one in each block or subfield, Figure S7) and one replicate for each cultivar mixture (randomly distributed in one of the subfields). Plot size depended on the experiment (Table 1).

The cultivar mixtures were sown with equal proportions of each cultivar (in terms of seed number), at the same density as pure stands. Cross-contamination between plots was limited by sowing triticale borders (×*Triticosecale* Wittm. ex A. Camus) of at least 3 m in width (250 seeds/m²). In all experiments, wheat crops were sown at a density of 180 seeds per m². We chose to use a sowing rate lower than that generally used in agronomic practice, to limit the airborne progression of disease. The microplots were sown after a previous crop of maize, rather late in the season, to limit autumn disease contamination (November 26th 2013, November 5th 2014 and October 21st 2015, respectively). The field trial was managed as with an integrated crop protection system, with relatively low input levels and a target yield of 6 t/ha in E2-2014 and 7 t/ha in E3-2015 and E3-2016 (slightly lower than the mean national wheat yield of 7.5 t/ha in France in 2015). We ensured that nitrogen was not a limiting factor for plant growth, by determining the appropriate total dose of ammonium nitrate fertilizer to be applied by the balance-sheet method (as in Gigot et al., 2012 and Vidal et al., 2017). The total dose of nitrogen was 120 kgN/ha in 2014, 160 kgN/ha in 2015 and 170 kgN/ha in 2016. This dose was applied in two installments in 2014: 70% at tillering and 30% when the second node became detectable. In 2015 and 2016, there were three installments: one quarter of the total dose at tillering, half at the beginning of stem elongation and one quarter when the second node became detectable. No fungicides or growth regulators were applied. One herbicide treatment was applied each year, in early spring, at GS31 (50 g/ha Harmony extra®, 250 g/ha Archipel® and 1 l/ha adjuvant Actirob 842 g/l esterified rapeseed oil base).

The cultivars included in the mixtures (E2 and E3) were chosen from a panel of 58 cultivars of four types: (i) elite cultivars (ELT: modern commercial cultivars registered for use in conventional agriculture), (ii) organic cultivars (ORG: modern commercial cultivars registered for use in organic farming), (iii) MAGIC lines (MGC: inbred lines developed from multiparental and highly recombinant INRA populations, each line was hereafter considered as a cultivar) and (iv) landraces (LDR: old traditional cultivars that evolved over decades and adapted locally under the unconscious selection of farmers, commonly cultivated until 1930). The whole panel of cultivars was screened for morphological, phenological and physiological traits (see summary of diversity assessment for root, architecture, yield and disease resistance traits in Dubs et al., 2018). Groups of cultivars had different numbers of cultivars and more or less heterogeneous characteristics, so that individual cultivars and their specific properties were considered in analyses rather than groups of cultivars and their mean properties. Disease resistance was assessed in 2014 (E1-2014). Plant height and flowering date were measured in a separate experiment in the Paris region (see Dubs et al. 2018 for details). Cultivar characteristics are summarized in Table S1. In E2-2014, cultivars were chosen so as to ensure an equal representation of the four types of cultivar (ELT, ORG, MGC, LDR), whereas, in E3-2015/2016, the choice was based on functional groups established on the basis of the phenotyping performed in 2014 (Dubs et al., 2018). This second design criterion resulted in the inclusion of a smaller number of MAGIC lines in mixtures, with six such lines included in E2-2014 but only two namely a highly resistant and a highly susceptible line in E3-2015/2016 (Table 1).

Many combinations of the considered cultivars were possible. For example, with 16 pure lines (E3-2015/2016), there were 120 possible two-cultivar mixtures (2CMs), 1820 possible four-cultivar mixtures (4CMs) and 12870 possible eight-cultivar mixtures (8CMs). It was therefore necessary to select particular mixtures for implementation in the field. The mixtures had two, four or eight component cultivars, selected so as to ensure that all cultivars were included in the same number of mixtures and to maximize the range of functional diversity from very homogeneous to highly heterogeneous mixtures (Table 1), as described by Dubs et al. (2018). We chose to increase the number of mixtures to cover a wider diversity, rather than repeating a smaller number of mixtures. Each mixture was therefore considered as a statistical individual, rather than an experimental treatment.

Disease and yield assessment

In all field experiments, disease levels were assessed three times during the cropping season. Only assessments in which scoring was considered adequate (well-developed symptoms and limited senescence) were taken into account for the analyses. In E1-2014, severity measurements were carried out at plot scale (rather than leaf scale) for the 58 pure stands. In the three years of experimentation on cultivar mixtures (E2 and E3), yellow rust and *Septoria tritici* blotch disease severity were visually assessed, on the three upper leaves of single stems, at GS59 (heading stage), based on the percentage of the total leaf area covered by sporulating lesions. The number of main stems scored per plot was proportional to the number of cultivars included in the mixture, to generate data with similar sampling rates for a similar number of plants of each cultivar, whether in pure stands or mixtures. Thus, eight stems were scored for PS (pure stands), four groups of four adjacent main stems for 2CM (i.e. 16 main stems), four groups of eight adjacent main stems for 4CM (i.e. 32 main stems), and four groups of 16 adjacent main stems for 8CM (i.e. 64 main stems). Main stems were chosen in the plots so as to avoid bias, with the same number of plants assessed in each of the two dedicated sowing strips for disease assessment.

In E3-2016, the trial was also scored for two other diseases that were present in the field: *Fusarium* head blight (FHB) and barley yellow dwarf virus (BYD). FHB severity was recorded at GS59 (heading stage), as the percentage of diseased spikelets per spike for each type of mixture. As for yellow rust scoring, the number of spikes collected per plot was proportional to the number of cultivars present in the mixture: 8 spikes for PS, 16 spikes for 2CM, 32 spikes for 4CM and 64 spikes for 8CM. The severity of barley yellow dwarf virus infections was assessed by eye, for each plot, at GS37 (stem elongation stage), as the percentage of the plot area displaying discoloration (stunting chlorosis and reddening of leaves).

All plots were harvested in early August, with a MB Hege 140 combine harvester with a cut-width of 1.75 m (Hege Maschinen GmbH, Waldenburg, Germany). Grain yield estimates are expressed as mean grain weight measured at 15% humidity over the central section of the plot, 1.75 m x plot length (10 m in E2, 8 m in E3) per plot, in tons per hectare. Mixture effects were assessed by comparing disease severity or yield in the mixture with the mean value of the corresponding pure stands:

$$ME_Y = \frac{Y_m - Y_p}{Y_p} \quad (1)$$

with ME_Y the mixture effect for variable Y, Y_m the variable measured in the mixture, Y_p the mean value of the variable of each component cultivar grown in pure stands. Mixture effects were defined as the decrease in disease severity (a positive ME value indicating a decrease in disease severity) or increase in yield (a positive ME indicating a yield increase) in mixtures relative to the mean value for the corresponding pure stands.

The three years of experimentation differed in terms of disease pressure and yield potential, as detailed in Table 2. Yellow rust pressure was exceptionally high in 2014. The Magic lines were chosen as a special genetic group from recombinant populations but their high susceptibility to YR was not known before the first year of experimentation. The very high yellow rust severity observed in 2014 accounts for the replacement of some cultivars (in particular, susceptible MAGIC lines) in the following two years of the experiment. The 2015 cropping season was considered a typical epidemiological year in terms of yellow rust disease pressure, with a relatively dry summer. Finally, 2016 was an atypically unfavorable year for the crop, resulting in very low yields in France (Ben-Ari *et al.*, 2018). In 2016, there was a very high and early general disease pressure, with the presence of uncommon diseases, such as barley yellow dwarf virus, in our experiment. Figure S8 compares yield and disease severity for each mixture of E3, in 2015 and 2016.

Table 2 around here.

Attribution of disease resistance classes

Yellow (stripe) rust caused by *Puccinia striiformis* f.sp. *tritici* was the main disease during the three years of the experiment, particularly in 2014 and 2015, when other diseases were absent or of very low severity. Each cultivar was assigned a yellow rust severity class based on expert judgement and qualitative analyses of experimental measurements of disease severity in pure stands. The severity scores obtained for each cultivar in pure stands in the different experiments, and the associated resistance class, are detailed in Table S1. We obtained a large range of severity scores over the various years of the experiment. It was therefore possible to provide detailed classes for cultivar resistance, ranging from highly resistant to highly susceptible, and to consider moderately resistant and susceptible cultivars.

Yellow rust severity differed significantly between cultivars from different resistance classes ($p<0.05$) in E2-2014, E3-2015 and E3-2016. Highly susceptible cultivars (HS, mean severity of 74.6% in 2014, 52.8% in 2015 and 21.3% in 2016) systematically displayed disease of above-mean severity. Moderately susceptible cultivars (MS, mean severity of 43.6% in E2-2014, 19.4% in E3-2015 and 10.3% in E3-2016) displayed variable behavior in the years 2015 and 2016. Resistant cultivars (moderately or highly resistant) systematically had a disease severity below the mean value. Resistant cultivars were subdivided into two classes that had small but significant differences in terms of mean disease severity: moderately resistant (MR, mean severity of 16.3% in 2014, 5.3% in 2015 and 4.5% in 2016) and highly resistant (HR, mean severity of 5.4% in 2014, 0.2% in 2015 and 1.6% in 2016). In E2-2014 there were as many susceptible cultivars (including 4 HS cultivars) as resistant cultivars, whereas, in E3, there were nine resistant cultivars and seven susceptible cultivars (including 3 HS cultivars).

In 2016, disease resistance classes were assigned for the four diseases observed in the E3 experiment, as detailed in Table S2. The other diseases were of lower severity than yellow rust, with a much narrower range of severity scores, such that only resistant and susceptible classes could be established. Classes were defined according to whether the disease severity of the pure stands was higher (susceptible) or lower (resistant) than the mean disease severity for pure stands.

Statistical analysis

Statistical analyses were carried out with R statistical software (R Core Team, 2018). The Shapiro-Wilk normality test (‘shapiro.test’ function) was used to test the normality of the variables. The Bartlett test (‘bartlett.test’ function from the ‘stats’ package) was used to test for the equality of variance across groups (homoscedacity). Means were compared by Student’s *t*-tests (‘TukeyHSD’ function) for normally distributed variables that passed the homoscedacity test, and by Kruskal-Wallis tests (‘kruskal’ function from the ‘agricolae’ library) for non-normally distributed variables or those that did not pass the homoscedacity test. Linear models were fitted with the ‘lm’ function. Correlation tests were performed with the ‘cor.test’ function, using the Pearson method for normally distributed variables and the Kendall method for non-normally distributed variables.

Results

Disease severity and yield in mixtures and pure stands

Cultivar mixture disease severity and yield were strongly related to the mean performances of the component cultivars grown in pure stands (Figure 1). The three experimental years differed in yield potential, with mean yields of 3.11 t/ha in E2-2014, 5.95 t/ha in E3-2015 and 1.80 t/ha in E3-2016.

The difference in yield between a cultivar mixture and the mean of its components in pure stands ranged from -1.7 t/ha to +1.3 t/ha in E2-2014 (55/90 mixtures had higher yields than the corresponding pure stands), from -1.1 t/ha to +1.2 t/ha in E3-2015 (28/72 mixtures had higher yields) and from -0.4 to t/ha +0.6 t/ha in E3-2016 (40/72 mixtures had higher yields). The difference in yellow rust severity between a cultivar mixture and the mean value for its components in pure stands ranged from -12.5% to +29.2% in E2-2014 (31/90 mixtures had lower disease severity than the pure stands), from -30.7% to +9.4% in E3-2015 (39/72 mixtures had lower disease severity) and from -17.1% to 12.5% in E3-2016 (37/72 mixtures had lower disease severity). The mean difference in yield and disease severity between cultivar mixtures and the mean of pure stands was small and not significantly different from zero according to Student's *t*-tests (and Wilcoxon tests for disease in E3-2016), for all the years considered. It was therefore possible to anticipate the performances of cultivar mixtures (disease severity and yield) from the characteristics of the component cultivars in pure stands, at least approximately. However, some mixtures displayed both positive (higher yield and/or lower disease severity) and negative (lower yield and/or higher disease severity) mixture effects on performance. Thus, cultivar mixtures did not automatically increase yield or decrease disease, but some of the mixtures tested had very interesting properties (*e.g.* yield increase of more than 1 t/ha). The characteristics of the three best and three worst mixtures in terms of yield increase (Table S3) and disease reduction (Table S4), for each year, are detailed in the supplementary tables. The relationships between mixture characteristics, yield and disease are explored further below.

Figure 1 around here

Tables 3 and 4 summarize the correlations of mixture characteristics with yield and disease severity, respectively. The principal correlations observed were those with the mean yield and disease severity of the component cultivars in pure stands. Yield was strongly correlated with the severity of yellow rust, the main disease in these experiments. This correlation was particularly strong in E2-2014 (-0.45, p=7.0 x 10^{-6}), in which disease pressure was particularly high, and was weaker in E3-2015 (-0.29, p=4.1 x 10^{-4}) and E3-2016 (-0.35, p=2.8 x 10^{-3}). Significant correlations were observed between yellow rust severity in mixtures and the contrast in resistance levels of the mixture components in all three experiments. A significant correlation between mixture yellow rust severity and the proportion of susceptible cultivars was identified in E3-2015 and E3-2016 but not in E2-2014.

The difference in height between the cultivars in the mixture had no significant impact on yield (Table 3) or disease severity (Table 4). However, mixture yield was negatively correlated with the mean plant height of the mixtures in E3 experiments (2015: -0. 42, p-value=2.6 x 10^{-7}; 2016: -0.36 p-value=1.8 x 10^{-3}) but not in E2-2014. This is illustrated in Figure S1 and Figure S2. On the other hand, the difference in flowering date among the cultivars of the mixture had an impact on two variables: (i) yield in E3-2015 (negative correlation of 0.17, p-value = 0.04) and (ii) yellow rust severity in E2-2014 (positive correlation of 0.23, p-value = 0.03). This correlation was much smaller and not significant in E2-2014 and E3-2016.

Table 3 around here

Table 4 around here

Number of cultivars in the mixture and variability of mixture disease severity and yield

The number of cultivars in the mixture (from 2 to 8) had no impact on the mean disease severity and yield of the mixtures (Table 3 and 4). However, increasing the number of cultivars within a mixture reduced the variability of yield and disease severity (Figure 2). Mean disease severity and mean yield varied considerably between years (Table 2), but similar patterns were observed for E2-2014, E3-2015 and E3-2016.

Figure 2 around here

The data for experiment E3 (mean of 2015 and 2016) are presented in Figure 3. The decrease in variability was particularly striking for yellow rust severity, which was the main disease during the experiment. Considering the means of the two years, an inverse correlation between yellow rust severity and yield was observed (-0.72 for all plots considered together, including pure stands), suggesting that disease interfered with yield formation in this experiment. In 2015, the correlation between yellow rust severity and yield was

-0.50 for the eight-cultivar mixtures compared and -0.78 for pure stands. Pure stands performed both the best and worst in terms of yield and disease resistance. Overall, cultivar mixtures did not increase mean yield or decrease disease severity, but they did greatly decrease the variation in yield and disease severity.

Figure 3 around here

In E3-2016, a number of diseases were observed in the field. The mean characteristics of pure stands and mixtures with different numbers of component cultivars were similar to those for the previous two years (Figure S3). However, variability among plots decreased with increasing number of cultivars in the mixture. Some pure stands were quite susceptible to one or two diseases in particular. Overall, cultivar mixtures were less susceptible to disease, and this was particularly true for eight-cultivar mixtures. Each mixture seemed to have a different disease profile and this effect was amplified by decreasing the number of cultivars in the mixtures. The severities of FHB and STB severity were positively correlated (0.25), whereas inverse correlations were observed between yellow rust and STB severities (0.25) and between BYD and FHB severities (0.25).

Cultivar mixture yield and disease severity were modulated by the proportions and resistance levels of the component cultivars included in the mixture

Yield was strongly related to yellow rust severity in this experiment, in all three years considered (Figure 4, Table 3). The composition of the mixtures, in terms of the disease resistance of the component cultivars, had various impacts on the disease severity and yield of the mixtures. The proportion of susceptible cultivars affected yellow rust severity and yield. The proportion of susceptible cultivars in the mixture had a visible impact for all the diseases considered. Disease severity increased with the proportion of susceptible cultivars in the mixture (Figure 5, Table 3).

Figure 4 around here

Figure 5 around here

The difference in yellow rust severity between the cultivars included in a mixture had a significant impact on the mixture effect on disease severity (Figure S5, E2: cor=-0.41, *p-value* =6.9 x 10^{-5}; E3: cor = 0.48, *p-value*=2.5 x 10^{-5}). However, this mixture effect decreased with increasing contrast in E2-2014, whereas it increased with increasing contrast in E3 (2015-2016). In E2-2014, positive mixture effects were observed in situations in which resistance differences between cultivars were small, mostly with a high proportion of resistant cultivars. Mixtures including similar proportions of resistant and susceptible cultivars had a high resistance contrast and null to negative mixture effects. The high disease pressure probably increased the severity of disease on moderately resistant cultivars grown with highly susceptible cultivars. By contrast, in experiment E3 (2015-2016), a greater difference in disease resistance between the cultivars in a mixture increased the mixture effect on disease severity. This was the case for all proportions of susceptible cultivars, and the effect increased with the proportion of susceptible cultivars. This suggests that the protective effect sought in mixture design was greater in this experiment, due to a lower disease pressure and a better balance in the range of resistance levels of the cultivars used in the mixtures.

Mixture effect increased with mean yellow rust severity of the cultivars in the mixture in E3 (2015-2016), but not in E2-2014 (Figure S6). This relationship was particularly marked for E3 mixtures in which at least 50% of the cultivars were susceptible to yellow rust. For mixtures with high proportions of resistant cultivars, which had the lowest mean disease severity, mixture effects were mostly negative, meaning that disease severity in the mixture was slightly higher than the mean value for the individual cultivars in in pure stands, although the overall level of resistance remained high. The best mixture effects for yellow rust severity were observed with 50 to 75% susceptible cultivars in the mixture, but such mixtures had a high level of disease severity, as illustrated in Figure 4. Few mixtures with high proportions of susceptible cultivars were included in the experiments but those tested gave highly variable results concerning disease severity in the E2 experiment.

In the case of yellow rust, the level of resistance to disease in pure stands varied considerably, making it possible to define more detailed resistance classes, as shown in Table S1. Table 5 summarizes the relationships between the proportion of cultivars of different resistance classes and disease severity in mixtures. Detailed data were provided in Figure 3. The proportion of highly susceptible cultivars was strongly related to disease severity. For example, increasing the proportion of highly susceptible cultivars by 10% increased disease severity by absolute values of 5.2% in E2-2014, 1.9% in E3-2015 and 1.3% in E3-2016. The proportion of moderately susceptible cultivars had a small but significant impact only in E3-2015. Though the differences between moderately and highly resistant cultivars were small, they performed differently in mixture, justifying the distinction between these two classes. Increasing the proportion of moderately resistant cultivars by 10% decreased disease severity by 2.0% in E2-2014 and had no significant impact in E3-2015 and E3-2016. Finally, the proportion of highly resistant cultivars had a large impact on disease severity, although not as significant as that of the highly susceptible cultivars. Thus, increasing the proportion of highly resistant cultivars by 10% decreased disease severity in cultivar mixtures by 3.6% in E2-2014, 1.1% in E3-2015 and 0.6% in E3-2016.

Table 5 around here

Discussion

We tested diverse cultivar mixtures, some of which included highly contrasted phenotypes (Table 1). Despite this diversity, we observed that mixture disease severity and yields were strongly related to the mean performances of the component cultivars grown in pure stands (Figure 1). This suggests that, whatever the rules used to design mixtures, the mean disease severity and yield of pure stands can be used as a first indicator, to anticipate the behavior of the mixture, and that the mixture can then be refined on the basis of more precise knowledge of the mechanisms affecting disease severity and yield in mixtures. However, the predictability of mean disease severity and yield may conceal other effects on one of the component cultivars not considered in this study. For example, large decreases in disease severity have been reported for susceptible plants grown in mixtures (Gigot et al., 2012; Vidal et al., 2017), but these decreases might be concealed by calculations of the mean disease severity for a mixture of several cultivars including resistant ones, as in our study. Some of the properties of mixtures may reflect changes in cultivar proportions relative to those at sowing. Indeed, the proportion of cultivars within mixtures may change considerably between sowing and harvest (Finckh and Mundt, 1992, 1993, 1996; Finckh et al., 2000; Belhaj Fraj et al., 2003; Vidal et al., 2017). This effect may be enhanced if one cultivar is particularly competitive due to its greater height or earlier stem elongation. Such mechanisms may have occurred in the mixtures studied here, but they had no significant impact on mixture disease severity and yield. The mean effect of mixtures was relatively small, but this effect was also highly variable, ranging from -30.7% to +29.2% for yellow rust severity and from -1.7 t/ha to +1.3 t/ha for yield. In E2-2014, many mixtures had more disease than the mean of pure stands (Figure 1d). This could be explained by the presence of many susceptible cultivars in this experiment (Table 1 and S2), which was not the case in 2015 and 2016 when other cultivars were included. In a meta-analysis comparing 386 mixtures, Borg et al. (2018) reported mixture effects on wheat yield ranging from −40% to +60%, with larger mixture effects under conditions of high disease pressure. We observed mixture effects ranging from -33% to +27% for yield, the largest effects (either positive or negative) being observed under high disease pressure (E2-2014). In another meta-analysis comparing 161 bread wheat cultivar mixtures, Huang et al. (2012) reported decreases in yellow rust severity of 22.5% to 33.4%, with a mean decrease of 28.0% and larger mixture effects under high disease pressure. The variability of cultivar mixture effects could potentially be reduced by an appropriate design, including a large number of cultivars. The effects of some key mixture characteristics are described below.

Agronomic homogeneity is a classic recommendation for the design of cultivar mixtures. It simplifies crop practices and it is often claimed that a contrast in competitive ability would have a major impact on the least competitive cultivar, resulting in a poor performance of the mixture. Agronomic homogeneity has direct practical benefits, particularly for harvest scheduling, to our knowledge, no systematic negative impact of heterogeneity has ever been demonstrated experimentally. On the other hand, several studies have reported positive effects of mixing cultivars of cereal species with different heights or flowering dates. These effects concerned airborne diseases, yield, grain quality, lodging, water use efficiency and weed suppression, as reviewed in Vidal et al. (2017). For example, mixtures of a tall traditional susceptible glutinous rice cultivar with a shorter modern resistant cultivar have been reported to display good control of panicle blast disease, which is caused by *Magnaporthe grisea* (wind-dispersed), due to a decrease in canopy humidity levels (Zhu et al., 2005). A certain degree of difference between components may be acceptable in mixtures if it confers properties of interest on the mixture. In our experiment, we mixed different types of cultivars (elite cultivars, organic agriculture cultivars, MAGIC lines from a multiparent population and landraces), thereby considerably extending the concept of a "mixture" beyond the more widely accepted mixtures of several elite cultivars with different resistance genes or a susceptible cultivar of superior agronomic characteristics with several resistant cultivars of lower yield potential. Agronomic contrast in height or earliness had little or no deleterious effect on mixture yield (Table 3) and disease severity (Table 4).

We investigated the impact of the number of cultivars in the mixture. In ecology, the number of species, or species richness, is recognized to be a key criterion with potential impacts on ecosystem performance in assessments of ecosystem diversity (Gaba et al., 2015; Yang et al., 2019). Sapoukhina et al. (2013) reported that the use of three-component mixtures made it possible to design a broader range of effective control strategies than the use of two-component mixtures. The number of cultivars in mixtures has been shown to be positively correlated with overyielding (Mundt et al., 1995; Newton et al., 2008; Kiær et al., 2009; Reiss and Drinkwater, 2018). We observed no significant impact of the number of component cultivars on mean mixture disease severity and yield. However, the variability of mixture performances strongly decreased with increasing number of cultivars (Figure 2). This pattern was reported in previous experimental studies and clearly emerged from meta-analysis results (Huang et al., 2012; Borg et al., 2017; Reiss and Drinkwater, 2018). The main advantage of increasing the number of cultivars seems to be a greater flexibility in mixture design, with the possibility of including cultivars with a wider range of desirable properties, such as resistance to various different diseases, resistance to abiotic stresses and good grain quality.

The proportion of susceptible cultivars in the mixture was strongly related to disease severity on the mixture (Figure 5). This finding confirmed that the proportion of susceptible cultivars has a crucial impact on disease protection, as already demonstrated in previous studies (Cox et al., 2004; Dai et al., 2012). This protection of a susceptible cultivar by resistant ones can be optimized in mixtures with a proportion of one third to one quarter susceptible plants (Gigot et al., 2012; Gigot et al., 2014). The proportion of highly susceptible cultivars had the largest and most significant impact on yellow rust severity (Table 5). The proportions of moderately susceptible cultivars had little impact on disease severity in the mixture, consistent with the results of the modeling study by Sapoukhina et al. (2013).

When cultivar mixtures are used for disease management, it is generally recommended to associate cultivars with contrasting levels of disease resistance (Jeger et al., 1981; Mundt et al., 1995; Gigot et al., 2014). In this experiment, the contrast in disease resistance between the cultivars in a mixture had a significant impact on the effect of the mixture on yellow rust severity (Table 4, Figure S5). However, this impact was negative for E2-2014, under conditions of exceptionally high yellow rust pressure, but positive in E3-2015/2016.

The extent and direction of this mixture effect varied with disease pressure, consistent with previous observations (Cowger et al., 2002; Gigot et al., 2012). In E2-2014, yellow rust pressure was high and mixture efficacy was low. However, the highly susceptible elite cultivar included in some mixtures was protected in those mixtures. In E3-2015/2016, the mixture effect increased with the mean severity of disease on the component cultivars in pure stands (Table3, Figure S6), consistent with previous findings (Jeger *et al.*, 1981;

Mundt *et al.*, 1995). When disease pressure was very high, with high levels of disease on both cultivar mixtures and component cultivars (up to 40-50% disease severity in E2), even positive mixture effects were insufficient for adequate disease protection. Similarly, when disease pressure was very low or if the mixture components were all resistant or moderately resistant, no benefit was expected from the cultivar mixtures, in terms of a decrease in disease levels in a particular season. Benefits were observed mostly in cases of moderate disease pressure, with a susceptible cultivar included in the mixture.

To conclude, cultivar diversity for various traits, including plant height and flowering date, may have a positive impact (possible positive interactions such as compensation, facilitation or complementarity), or a negative impact (possible negative interactions, such as competition) on the performance of cultivar mixtures. We found that, despite contrasts between cultivars, mixture disease severity and yield were strongly related to the mean values for these variables obtained for pure stands of the component cultivars of the mixture. Some mixtures had very interesting properties, with yield increases of up to 1.3 t/ha relative to pure stands, and decreases in disease severity by up to 30%. For the large diversity of mixtures considered here, the variability of disease severity and yield decreased with increasing number of cultivars in the mixture. Adding more cultivars did not reduce disease or increase yield significantly, but gave more flexibility to mixture design, with the possibility of including cultivars with interesting characteristics and/or modifying the proportion of cultivars with an interesting trait (e.g. resistance to a particular disease). Despite the agronomic contrasts within the mixtures tested, we found that variations in yield and disease severity were related to known design criteria mostly established in experiments on agronomically homogeneous mixtures. In particular, the proportion of susceptible cultivars affected disease severity, yield and mixture effects. Disease pressure also had an impact on mixture effects. Our results confirm the potential of cultivar mixtures for use in disease management and the robustness of the established rules. Mixtures may also provide other ecosystem services (e.g. weed reduction, resistance to water stress or lodging, grain quality) and such properties might require the mixing of cultivars with contrasting characteristics. Our results suggest that such contrasts would not modify the established rules and that the mean yield and disease severity values for cultivars in pure stands would remain a good indicator of the likely performance of mixtures.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Barot, S., Allard, V., Cantarel, A., Enjalbert, J., Gauffreteau, A., Goldringer, I., Lata, J.-C., Le Roux, X., Niboyet, A., Porcher, E., 2017. Designing mixtures of cultivars for multifunctional agriculture with the help of ecology. A review. *Agronomy for Sustainable Development*. 37:13. https://doi.org/10.1007/s13593-017-0418-x

Belhaj Fraj M., Falentin-Guyomarc'h H., Monod H., de Vallavieille-Pope C., 2003. The use of microsatellite markers to determine the relative proportions of grain produced by cultivars and the frequency of hybridisation in bread wheat mixtures. *Plant Breeding* **122**, 385-391.

Ben-Ari, T., Boé, J., Ciais, P., Lecerf, R., Van der Velde, M., Makowski, D., 2018. Causes and implications of the unforeseen 2016 extreme yield loss in the breadbasket of France. *Nature Communications* **9**, 1627. https://doi.org/10.1038/s41467-018-04087-x

Borg, J., Kiær, L.P., Lecarpentier, C., Goldringer, I., Gauffreteau, A., Saint-Jean, S., Barot, S., Enjalbert, J., 2017. Unfolding the potential of wheat cultivar mixtures: A meta-analysis perspective and identification of knowledge gaps. *Field Crops Research* **221**, 298-313. https://doi.org/10.1016/j.fcr.2017.09.006

Cowger, C., Mundt, C.C., 2002. Effects of wheat cultivar mixtures on epidemic progression of *Septoria tritici* blotch and pathogenicity of *Mycosphaerella graminicola*. *Phytopathology* **92**, 617–623. https://doi.org/10.1094/PHYTO.2002.92.6.617

Cox, C.M., Garrett, K.A., Bowden, R.L., Fritz, A.K., Dendy, S.P., Heer, W.F., 2004. Cultivar mixtures for the simultaneous management of multiple diseases: tan spot and leaf rust of wheat. *Phytopathology* **94**, 961–969. https://doi.org/10.1094/PHYTO.2004.94.9.961

Creissen, H.E., Jorgensen, T.H., Brown, J.K.M., 2016. Impact of disease on diversity and productivity of plant populations. *Functional Ecology*. **30**, 649–657. https://doi.org/10.1111/1365-2435.12552

Dai, J., Wiersma, J.J., Holen, D.L., 2012. Performance of hard red spring wheat cultivar mixtures. *Agronomy Journal*. **104**, 17. https://doi.org/10.2134/agronj2011.0123

Dubs, F., Le Roux, X., Allard, V., Andrieu, B., Barot, S., et al. 2018. An experimental design to test the effect of wheat variety mixtures on biodiversity and ecosystem services. https://hal.archives-ouvertes.fr/hal-01843564

Finckh M.R., Mundt C.C. 1992. Stripe rust, yield, and plant competition in wheat cultivar mixtures. *Phytopathology* **82**, 905–913

Finckh, M. R., and Mundt, C. C. (1993). Effects of stripe rust on the evolution of genetically diverse wheat populations. *Theor. Appl. Genet* **85**, 809-821.

Finckh, M. R., and Mundt, C. C. (1996). Temporal dynamics of plant competition in genetically diverse wheat populations in the presence and absence of stripe rust. *J. Appl. Ecol*. 33, 1041-1052.

Finckh, M. R., Gacek, E. S., Goyeau, H., Lannou, C., Merz, U., Mundt, C. C., Munk, L., Nadziak, J., Newton, A. C., de Vallavieille-Pope, C., and Wolfe, M. S. (2000). Cereal variety and species mixtures in practice, with emphasis on disease resistance. *Agronomie* **20**, 813-837.Gaba, S., Lescourret, F., Boudsocq, S., Enjalbert, J., Hinsinger, P., Journet, E.-P., Navas, M.-L., Wery, J., Louarn, G., Malézieux, E., Pelzer, E., Prudent, M., Ozier-Lafontaine, H., 2015. Multiple cropping systems as drivers for providing multiple ecosystem services: from concepts to design. *Agronomy for Sustainable Development*. **35**, 607–623. https://doi.org/10.1007/s13593-014-0272-z

Gigot, C., de Vallavieille-Pope, C., Huber, L., Saint-Jean, S., 2014. Using virtual 3-D plant architecture to assess fungal pathogen splash dispersal in heterogeneous canopies: a case study with cultivar mixtures and a non-specialized disease causal agent. *Annals of Botany*. **114**, 863–876. https://doi.org/10.1093/aob/mcu098

Gigot, C., Saint-Jean, S., Huber, L., Maumené, C., Leconte, M., Kerhornou, B., de Vallavieille-Pope, C., 2012. Protective effects of a wheat cultivar mixture against splash-dispersed *Septoria tritici* blotch epidemics. *Plant Pathology*. **62**, 1011–1019. https://doi.org/10.1111/ppa.12012

Huang, C., Sun, Z., Wang, H., Luo, Y., Ma, Z., 2012. Effects of wheat cultivar mixtures on stripe rust: A meta-analysis on field trials. *Crop Protection*. **33**, 52–58. https://doi.org/10.1016/j.cropro.2011.11.020

Jeger, M.J., Griffiths, E., Jones, D.G., 1981. Disease progress of non-specialised fungal pathogens in intraspecific mixed stands of cereal cultivars. I. Models. *Annals of Applied Biology* **98**, 187–198.

Jørgensen, J.H., 1992. Discovery, characterization and exploitation of Mlo powdery mildew resistance in barley, in: Johnson, R., Jellis, G.J. (Eds.), *Breeding for Disease Resistance*, *Developments in Plant Pathology*. Springer Netherlands, Dordrecht, pp. 141–152. https://doi.org/10.1007/978-94-017-0954-5_12

Kiaer, L., Skovgaard, I., Ostergard, H., 2009. Grain yield increase in cereal variety mixtures: A meta-analysis of field trials. *Field Crops Research*. **114**, 361–373.

Kiær, L., Skovgaard, I., Østergård, H., 2012. Effects of inter-varietal diversity, biotic stresses and environmental productivity on grain yield of spring barley variety mixtures. *Euphytica* 1–16. https://doi.org/10.1007/s10681-012-0640-1

Labarthe, P., Coléno, F., Fugeray-Scarbel, A., Hannachi, M., Lemarié, S., 2018. Diffusion des mélanges variétaux pour la production de blé : une comparaison entre France et Danemark. *Notes Études Socio-Économiques* 77–103.

Li, N., Jia, S.-F., Wang, X.-N., Duan, X.-Y., Zhou, Y.-L., Wang, Z.-H., Lu, G.-D., 2012. The effect of wheat mixtures on the powdery mildew disease and some yield components. *Journal of Integrated Agriculture*. **11**, 611–620. https://doi.org/10.1016/S2095-3119(12)60048-3

Mikaberidze, A., McDonald, B.A., Bonhoeffer, S., 2015. Developing smarter host mixtures to control plant disease. *Plant Pathology*. **64**, 996–1004. https://doi.org/10.1111/ppa.12321

Mille B., Belhaj Fraj M., Monod H., de Vallavieille-Pope C., 2006. Assessing four-way mixtures of winter wheat cultivars from the performances of their two-way and individual components. *European Journal of Plant Pathology* **114**, 163-173.

Mundt, C. C. 1994. Use of host genetic diversity to control cereal diseases: implications for rice blast. Pages 293-308 in: *Rice Blast Disease*, Zeigler, R. S., Leong, S. A., and Teng, P. S., eds. CAB International, London.

Mundt, C., 2002. Use of multiline cultivars and cultivar mixtures for disease management. *Annual Review of Phytopathology* **40**, 381–410. https://doi.org/10.1146/annurev.phyto.40.011402.113723

Mundt, C.C., Brophy, L.S., Schmitt, M.S., 1995. Choosing crop cultivars and cultivar mixtures under low versus high disease pressure: A case study with wheat. *Crop Protection* **14**, 509–515.

Mundt, C., Leonard, K., 1986. Effect of host genotype unit area on development of focal epidemics of bean rust and common maize rust in mixtures of resistant and susceptible plants. *Phytopathology* **76**, 895–900. https://doi.org/10.1094/Phyto-76-895

Mundt, C.C., Brophy, L.S., Schmitt, M.S., 1995. Disease severity and yield of pure-line wheat cultivars and mixtures in the presence of eyespot, yellow rust, and their combination. *Plant Pathology*. **44**, 173–182. https://doi.org/10.1111/j.1365-3059.1995.tb02726.x

Newton, A.C., 2009. Plant disease control through the use of variety mixtures, in: Walters, D. (Ed.), *Disease Control in Crops: Biological and Environmentally Friendly Approaches.* Wiley-Blackwell, Oxford, UK, pp. 162–171.

Newton, A.C., Hackett, C.A., Swanston, J.S., 2008. Analysing the contribution of component cultivars and cultivar combinations to malting quality, yield and disease in complex mixtures. *Journal of the Science of Food and Agriculture*. **88**, 2142–2152. https://doi.org/10.1002/jsfa.3326

R Core Team, 2018. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Reiss, E.R., Drinkwater, L.E., 2018. Cultivar mixtures: a meta-analysis of the effect of intraspecific diversity on crop yield. *Ecological Applications*. **28**, 62–77. https://doi.org/10.1002/eap.1629

Sapoukhina, N., Paillard, S., Dedryver, F., de Vallavieille-Pope, C., 2013. Quantitative plant resistance in cultivar mixtures: wheat yellow rust as a modeling case study. *New Phytologist*. **200**, 888–897. https://doi.org/10.1111/nph.12413

Vidal, T., Boixel, A.-L., Durand, B., de Vallavieille-Pope, C., Huber, L., Saint-Jean, S., 2017. Reduction of fungal disease spread in cultivar mixtures: Impact of canopy architecture on rain-splash dispersal and on crop microclimate. *Agriculture and Forest Meteorology*. **246**, 154–161. https://doi.org/10.1016/j.agrformet.2017.06.014

Wolfe, Ms., 1985. The current status and prospects of multiline cultivars and variety mixtures for disease resistance. *Annual Review of Phytopathology*. **23**, 251–273.

Wolfe, M.S., 1992. Barley diseases: maintaining the value of our varieties In L. Munk (ed.): *Barley Genetics* VI. Munksgaard Int. Publishers, Ltd., Copenhagen, p.1055-1067.

Yang, Y., Tilman, D., Furey, G., and Lehman, C. (2019). Soil carbon sequestration accelerated by restoration of grassland biodiversity. *Nature Communications* **10**, 718.

Zhu, Y.-Y., Fang, H., Wang, Y.-Y., Fan, J.X., Yang, S.-S., Mew, T.W., Mundt, C.C., 2005. Panicle blast and canopy moisture in rice cultivar mixtures. *Phytopathology* **95**, 433–438. https://doi.org/10.1094/PHYTO-95-0433

Supporting information legends

Table S1: Plant height, flowering date and yellow rust severity of the bread wheat cultivars grown in pure stands over the three field experiments (E1, E2 and E3)

Table S2: Disease resistance classes for the four diseases assessed during the E3-2016 experiment on 16 bread wheat cultivars grown in pure stands

Table S3: Characteristics of the best and worst mixtures in terms of yield increase

Table S4: Characteristics of the best and worst mixtures in terms of disease reduction

Table S5: Composition of the best and worst cultivar mixtures for yield increase or disease reduction

Figure S1: Yield and agronomic characteristics of mixtures

Figure S2: Yellow rust severity, plant height and flowering date in cultivar mixtures

Figure S3: Principal component analysis of the severity of four diseases in pure stands and cultivar mixtures of bread wheat including different numbers of cultivars in the 2016 cropping season.

Figure S4: Yellow rust severity in mixtures as a function of the proportion of cultivars with different levels of resistance to disease

Figure S5: Mixture effect on wheat yellow rust severity as a function of the contrast in disease resistance level between the two most extreme cultivars included in the mixtures in the (a) E2 and (b) E3 experiments.

Figure S6: Mixture effect on wheat yellow rust severity as a function of the percentage of susceptible (HS and MS) cultivars included in the mixtures in the (a) E2 and (b) E3 experiments

Figure S7: Yield and disease severity for two years of experiment. Each point corresponds to one mixture.

Figure S8: Yellow rust severity (%) in two repetitions of pure stands.

Figure legends

Figure 1: Relationship of yield (a,b) and yellow rust severity (c,d) in cultivar mixtures to the mean value for the component cultivars in pure stands in the E2 and E3 experiments. *In graphs (a) and (c), each point corresponds to one mixture in one experiment. The experiment is indicated by the color and shape of the point: green squares, E2-2014; black circles, E3-2015; red triangles, E3-2016.*

Figure 2: Variability of yield (a) and disease severity (b) as a function of the number of components in the mixture (more than 1 cultivar) or in pure stands (1 cultivar) during the three cropping seasons. *Point shape indicate the variable. Squares: yield; circles: yellow rust; triangles:* Septoria tritici *blotch; plusses: barley yellow dwarf virus; crosses:* Fusarium *head blight. The type and color of line indicates the year: dotted green, E2-2014; continuous black, E3-2015; dashed red: E3-2016.*

Figure 3: Yield and yellow rust severity in a range of bread wheat pure stands and mixtures including 2, 4 or 8 cultivars. *Each point corresponds to mean values for the measurements in one mixture, during two cropping seasons (E3-2015 and E3-2016). Colors indicated the number of cultivars in each mixture. For each group, the center of the ellipse corresponds to the mean coordinates, the two diameters indicate the confidence intervals of the two variables, and the slope indicates the correlation between disease severity and yield. Area of ellipses: 20.9 for pure stands, 15.7 for 2-way mixtures, 7.8 for 4-way mixtures, 4.8 for 8-way mixtures.*

Figure 4: Relationship between yellow rust severity and yield in cultivar mixtures, for different proportions of susceptible cultivars, in the E2-2014 (a), E3-2015 (b) and E3-2016 (c) experiments. *Each open symbol corresponds to a single mixture. The color and shape of the symbols indicate the proportion of susceptible cultivars within the mixture. Dark green squares: less than 25% susceptible cultivars; light green circles: between 25% and 50% susceptible cultivars; orange triangles: between 50% and 75% susceptible cultivars; red diamonds: at least 75% susceptible cultivars. Closed symbols indicate the mean values for each group (corresponding to different proportions of susceptible cultivars). Bars indicate the confidence intervals. Susceptible cultivars included both moderately and highly susceptible cultivars. Gray lines indicate the relationship between yield (y) and yellow rust severity (x): (a) $y = 3.8 – 0.018 x$ ($R^2 = 19.7\%$); (b) $y = 6.2 – 0.025 x$ ($R^2=12.1\%$); (c) $y = 2.0 – 0.020 x$ ($R^2=10.8\%$). Error bars indicate the confidence intervals. Letters indicate significant differences between proportions of susceptible plants. Tukey multiple pairwise-comparisons test were used for comparing means of groups for variables that were normally distributed and had homogeneous variances. This was the case for yield in E2-2014 (Shapiro-Wilk p-value = 0.54, Bartlett test p-value = 0.09), disease in E2-2014 E2-2014 (Shapiro-Wilk p-value = 0.39, Bartlett test p-value = 0.10), disease in E3-2015 (Shapiro-Wilk p-value =*

0.73, Bartlett test p-value = 0.16) and yield in E3-2016 (Shapiro-Wilk p-value = 0.09, Bartlett test p-value = 0.26). Non parametric test (Kruskal-Wallis test) were used for yield in E3-2015 (Shapiro-Wilk p-value = 0.01, Bartlett test p-value =0.10) and disease in E3-2016 (Shapiro-Wilk p-value = 0.05, Bartlett test p-value =0.02). Upper case letters indicate differences in yield, lower case letters indicate differences in disease severity.

Figure 5: Impact of the proportion of susceptible (moderately susceptible and highly susceptible) cultivars in the cultivar mixture on the relative severity of (a) yellow rust, (b) *Septoria* leaf blotch, barley yellow dwarf virus and *Fusarium* head blight. *(a) Yellow rust severity over three cropping seasons, (b) Diseases occurring in E3-2016. Relative severity corresponds to the mean disease severity in mixtures of each class (0-25, 25-50, 50-75, 75-100% susceptible cultivars) divided by the mean severity for all mixtures for the year and disease considered. Letters indicate significant differences between proportions of susceptible cultivars in the mixture.* Septoria tritici *blotch* and *barley yellow dwarf virus data were not normally distributed, so the mean values were compared in Kruskal-Wallis tests. For all other variables, means were compared in t-tests. For yellow rust in E2-2014 and* Septoria tritici *blotch in E3-2016, no significant differences were observed.*

Table 1: Field experiment design

Experiment	E1-2014	E2-2014	E3-2015/2016
Plot area	26 m²	52.5 m²	84 m²
Plot size	5.25 x 5 m	5.25 x 10 m	10.5 x 8 m
Number of plots	58	130	104
Number of cultivars	**58**	**20**	**16**
Susceptible cultivars*	34	10	7
Highly susceptible cultivars*	15	5	3
Elite cultivars	-	8	9
Organic cultivars	-	4	4
MAGIC lines	-	6	2
Landraces	-	2	1
Number of mixtures	**0**	**90**	**72**
2-cultivar mixtures	-	30	24
4-cultivar mixtures	-	30	28
8-cultivar mixtures	-	30	20
Low heterogeneity**	-	11	6
High heterogeneity**	-	69	55

** Resistance classes were defined on the basis of yellow rust severity in pure stands for each cultivar, across different experiments.*

*** Heterogeneity was defined here according to the Danish rules, as reported by Labarthe et al. (2018). Low heterogeneity describes mixtures with flowering dates differing by less than five days and plant heights differing by less than 15 cm. High heterogeneity describes mixtures with flowering dates differing by at least five days and plant heights differing by at least 15 cm.*

Table 2: Summary statistics for the yield and disease severity of bread wheat cultivars grown in pure stands during three cropping seasons

	E2-2014		***E3-2015***		***E3*-2016**				
	Yield	***YR***	***Yield***	***YR***	***Yield***	***YRS***	***STB***	***FHB***	***BYD***
min	0.95	0.0	4.50	0.0	0.60	0.1	0.0	0	0.0
max	5.57	85.6	7.86	75.5	2.57	38.4	34.3	98.6	80.0
mean	3.11	35.5	5.95	17.2	1.80	9.9	7.2	60.0	23.2
SD	0.75	20.3	0.95	21.8	0.51	10.7	10.2	31.3	20.5

Yield os expressed in tonnes per hectare. Disease severity is expressed as a percentage (foliar area, spike, or plot area) for all diseases. YR: yellow rust, STB: Septoria tritici *blotch; FHB:* Fusarium *head blight; BYD: barley yellow dwarf virus.*

Table 3: Correlation between yield in cultivar mixtures and the characteristics of the component cultivars and mixtures, for three field experiments

Experiment	Explanatory variable	tau / cor	z/t	*p*-value
E2-2014	Yellow rust severity	-0.45	-4.78	7.0 x 10^{-6} *
	Mean yield of components in pure stands	0.59	6.88	8.3 x 10^{-2}
SW: 0.54	Contrast in yield potential	-0.18	-1.67	9.9 x 10^{-2}
Pearson	Mean plant height of components in pure stands	-0.11	-1.03	0.31
	Contrast in flowering date	0.09	0.82	0.42
	Number of cultivars	0.04	0.41	0.68
	Contrast in plant height	0.002	0.03	0.98
E3-2015	Mean yield of components in pure stands	0.46	5.67	1.4 x 10^{-8} *
	Mean plant height of components in pure stands	-0.42	-5.15	2.6 x 10^{-7} *
SW: 0.012	Yellow rust severity	-0.29	-3.53	4.1 x 10^{-4} *
Kendall	Contrast in flowering date	-0.17	-2.04	4.2 x 10^{-2} *
	Contrast in yield potential	0.09	1.06	0.29
	Contrast in plant height	-0.11	-1.36	0.17
	Number of cultivars	0.05	0.59	0.55
E3-2016	Mean yield of components in pure stands	0.82	12.0	< 10^{-15} *
	Mean plant height of components in pure stands	-0.36	-3.24	1.8 x 10^{-3} *
SW: 0.09	Yellow rust severity	-0.35	-3.09	2.8 x 10^{-3} *
Pearson	Contrast in yield potential	-0.12	-1.04	0.30
	Contrast in plant height	-0.08	-0.68	0.49
	Contrast in flowering date	-0.04	-0.32	0.74
	Number of cultivars	0.04	0.29	0.77

SW indicates the value of the Shapiro-Wilk normality test of yield, for each experiment. Correlation tests were performed with the Pearson method for normally distributed variables and the Kendall method for non-normally distributed variables. Stars indicate significant relations.

Table 4: Correlation between yellow rust severity in cultivar mixtures and the characteristics of component cultivars and mixtures, for three field experiments

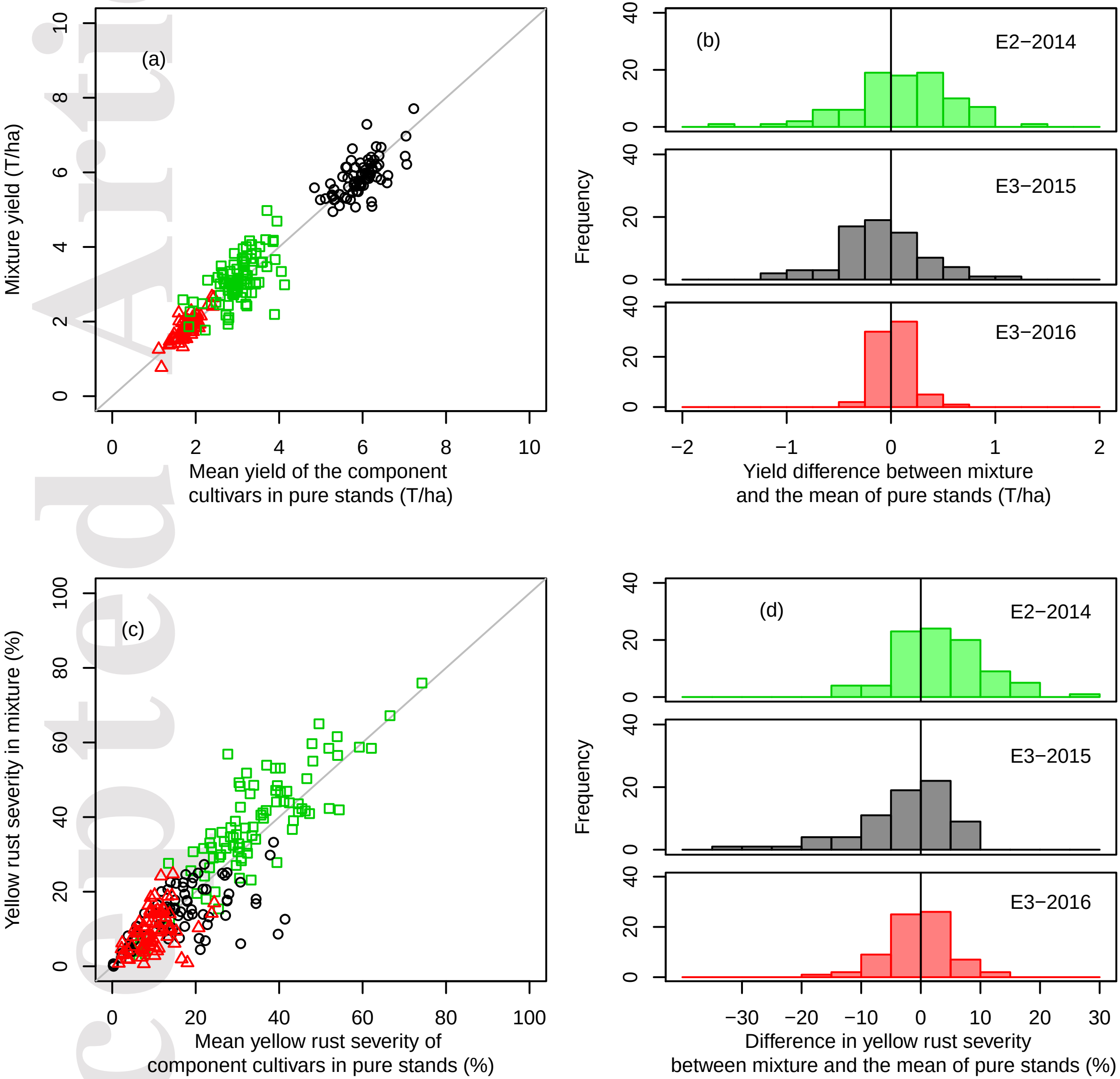
Experiment	Explanatory variable	tau / cor	z/t	*p*-value
E2-2014	Mean yellow rust severity of components in pure stands	0.87	16.44	$< 10^{-15}$ *
	Proportion of susceptible cultivars	0.74	10.40	$< 10^{-15}$ *
SW: 0.39	Contrast in disease resistance	0.49	5.34	7.1×10^{-7} *
Pearson	Contrast in flowering date	0.23	2.25	2.7×10^{-2} *
	Contrast in plant height	0.17	1.57	0.12
	Number of cultivars	0.13	1.24	0.22
E3-2015	Mean yellow rust severity of components in pure stands	0.57	5.83	1.6×10^{-7} *
SW: 0.60	Proportion of susceptible cultivars	0.56	5.70	2.6×10^{-7} *
Pearson	Contrast in disease resistance	0.29	2.57	1.2×10^{-2} *
	Contrast in plant height	0.18	1.55	0.13
	Contrast in flowering date	0.16	1.33	0.19
	Number of cultivars	0.11	0.92	0.36
E3-2016	Mean yellow rust severity of components in pure stands	0.43	3.94	1.9×10^{-4} *
SW: 0.054	Proportion of susceptible cultivars	0.33	2.88	5.2×10^{-3} *
Pearson	Contrast in disease resistance	0.24	2.08	4.1×10^{-2} *
	Contrast in plant height	-0.08	-0.64	0.52
	Number of cultivars	0.06	0.48	0.63
	Contrast in flowering date	-0.04	-0.36	0.71

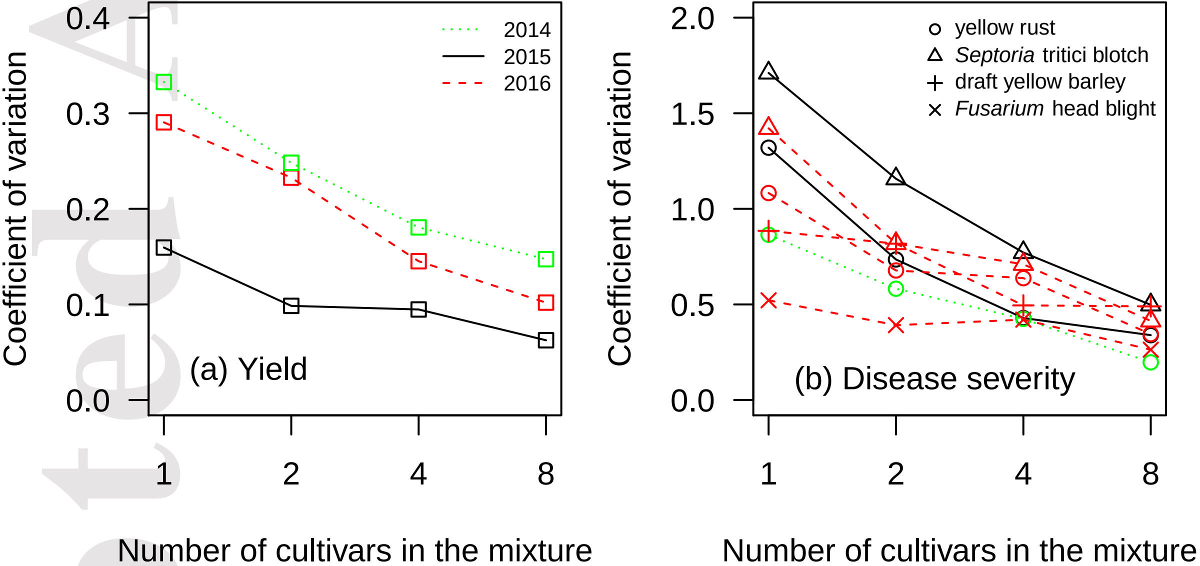
SW indicates the value of the Shapiro-Wilk normality test of yellow rust severity, for each experiment. Correlation tests were carried out with the Pearson method for normally distributed variables and the Kendall method for non-normally distributed variables. Stars indicate significant relations.

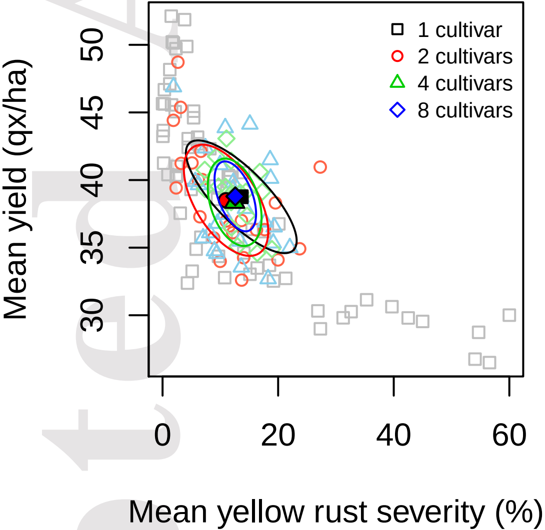
Table 5: Yellow rust severity in cultivar mixtures as a function of the proportion of cultivars with different levels of resistance to disease

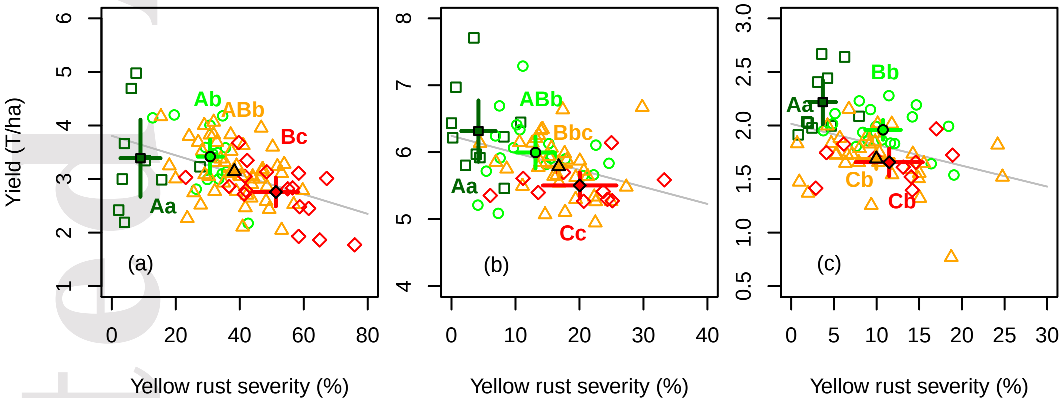
Resistance class	Experiment	Int.	Slope	R²	*p*-value **
HS	E2-2014	23.6	52.0	0.59	$< 10^{-15}$ *
	E3-2015	11.0	19.2	0.19	6.6×10^{-5} *
	E3-2016	7.1	13.0	0.15	3.1×10^{-4} *
MS	E2-2014	36.0	2.2	-0.01	0.77
	E3-2015	12.9	6.8	0.05	2.8×10^{-2} *
	E3-2016	9.2	1.1	-0.01	0.64
MR	E2-2014	40.6	-19.8	0.06	1.2×10^{-2} *
	E3-2015	16.5	-6.2	0.03	7.9×10^{-2}
	E3-2016	10.1	-1.8	-0.007	0.49
HR	E2-2014	47.3	-35.8	0.29	2.0×10^{-8} *
	E3-2015	17.5	-11.5	0.14	6.3×10^{-4} *
	E3-2016	10.9	-5.7	0.06	2.5×10^{-2} *

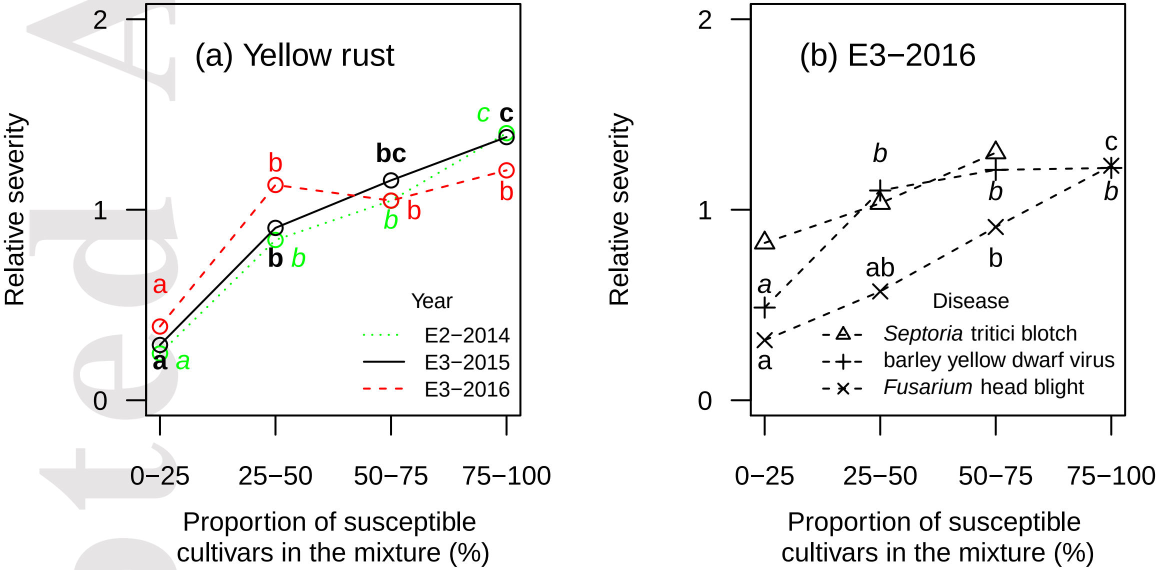
HS: highly susceptible, MS: moderately susceptible, MR: moderately resistant, HR: highly resistant. Linear models were fitted to the data. Resistance classes - HS: highly susceptible; MS: moderately susceptible; MR: moderately resistant; HR: highly resistant. Stars indicate significant relations between yellow rust severity and the proportion of cultivars belonging to different resistance classes.


(a)
Mixture yield (T/ha)
0
2
4
6
8
10
Mean yield of the component cultivars in pure stands (T/ha)
(b)
E2−2014
E3−2015
E3−2016
Frequency
0
20
40
−2
−1
0
1
2
Yield difference between mixture and the mean of pure stands (T/ha)
(c)
Yellow rust severity in mixture (%)
0
20
40
60
80
100
Mean yellow rust severity of component cultivars in pure stands (%)
(d)
E2−2014
E3−2015
E3−2016
Frequency
0
20
40
−30
−20
−10
0
10
20
30
Difference in yellow rust severity between mixture and the mean of pure stands (%)


Coefficient of variation
0.4
0.3
0.2
0.1
0.0
2014
2015
2016
(a) Yield
1
2
4
8
Number of cultivars in the mixture
Coefficient of variation
2.0
1.5
1.0
0.5
0.0
○ yellow rust
△ *Septoria* tritici blotch
+ draft yellow barley
× *Fusarium* head blight
(b) Disease severity
1
2
4
8
Number of cultivars in the mixture


1 cultivar
2 cultivars
4 cultivars
8 cultivars
Mean yield (qx/ha)
30
35
40
45
50
0
20
40
60
Mean yellow rust severity (%)


Yield (T/ha)
1
2
3
4
5
6
Ab
ABb
Bc
Aa
(a)
0
20
40
60
80
Yellow rust severity (%)
4
5
6
7
8
ABb
Bbc
Aa
Cc
(b)
0
10
20
30
40
Yellow rust severity (%)
0.5
1.0
1.5
2.0
2.5
3.0
Bb
Aa
Cb
Cb
(c)
0
5
10
15
20
25
30
Yellow rust severity (%)


(a) Yellow rust
Relative severity
0
1
2
a
a
a
b
b
b
b
bc
b
b
c
c
b
Year
E2−2014
E3−2015
E3−2016
0−25
25−50
50−75
75−100
Proportion of susceptible cultivars in the mixture (%)
(b) E3−2016
Relative severity
0
1
2
a
a
b
ab
b
b
c
b
b
Disease
Septoria tritici blotch
barley yellow dwarf virus
Fusarium head blight
0−25
25−50
50−75
75−100
Proportion of susceptible cultivars in the mixture (%)