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Grain and straw yield interactions in barley cultivar mixtures

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Abstract

Cultivar mixtures of winter barley and spring barley, together with their component monocultures, were grown in field trials to assess the effect of cultivar combinations on both straw and grain yield. The overall grain yields for all trials were significantly higher for the cultivar mixtures than for the corresponding component monocultures. Also, significant decreases in rhynchosporium disease severity for cultivar mixtures were recorded for most non-fungicide treatments. The size of these responses was often significantly correlated with the component number of the mixtures. The amount of straw produced in mixtures was sometimes changed significantly, but not always in a positive direction and it was only correlated with increasing mixture component number in two environments. No correlation of straw yield potential of cultivars with performance in mixtures was found. Cultivar × cultivar mixture × environment interactions appeared to affect the relative yield of grain and straw differentially and therefore it was not possible to predict the effect of mixtures on the harvest index.

Introduction

Many plant–plant interactions involving complementary traits result in greater biomass production per unit land area or resource input such as fertilizer (Newton *et al.*, 2009). This is due to the processes of facilitation and competition, often both above- and below-ground (Brooker *et al.*, 2016), and effectively results in greater resource availability and utilization. These interactions can be beneficial in practical agricultural production from classical inter-cropping of different species through to cultivar mixtures or even near-isogenic line mixtures (Wolfe and Riggs, 1983). The benefit in agriculture is measured normally in terms of the main crop yield component such as the grain in cereals, or the foliage in the grazed pasture or when cut for silage. Commonly, the assumption is made that the whole plant community benefits from greater resource utilization (Brooker, 2017), but this is rarely measured in agriculture. However, the step-change increase achieved in resource utilization in the 'Green Revolution' was achieved largely through a change in the harvest index (HI), i.e. a higher proportion of assimilates was apportioned to the grain (Khush, 2001), as demonstrated in many breeding programmes including in barley (Bertholdsson and Kolodinska-Brantestam, 2009). Therefore, as the growth model of cereals grown in self-competition monocultures has been changed, it cannot necessarily be assumed that total biomass, rather than grain alone, will increase in non-self-competition in the mixtures of cereal cultivars. The biomass components, i.e. the HI, may also change in such mixtures.

There is an increasing demand for the straw component of cereals in some places, for instance where it is used for power production through burning, for whole-crop silage for anaerobic digestion or animal feed, and potentially directly digested for fermentation and alcohol production (Swanston and Newton, 2005). However, increased straw production is rarely demanded at the expense of grain yield. Nevertheless, it is pertinent to determine whether the mixtures are an appropriate approach for achieving efficient biomass production in this context and whether this is achieved through enhancing the straw, grain or both components.

In general, the grain yield of cereal mixtures increases with the number of component cultivars (Newton *et al.*, 1998). This is expected as diversity tends to be positively correlated with the biomass in species mixtures (Brooker *et al.*, 2008) and, furthermore, this leads to increased potential resource-use efficiency (RUE) (Pakeman *et al.*, 2015). Thus, cultivar mixtures that tend to show little diversity, such as spring barley in the UK, show less benefit from being grown in mixtures than winter barley (Newton *et al.*, 2009). The same relationship with diversity is found for mixture disease resistance, limiting the spread of pathogens and so resulting in less disease, and hence reduced yield loss (Newton *et al.*, 2009; Brooker *et al.*, 2016).

Straw length is commonly assumed to be correlated with straw yield, but the current authors could find no validation of this relationship for commercial cultivars of cereals. A combination of the number of stems and their length is more likely to be correlated with overall straw yield, but both parameters will be influenced by many agronomic factors. Straw heights of current cultivars are published in the UK Recommended Lists (RL) each year (AHDB, 2011, 2018), but the values are valid for specific trials analysed and comparisons therefore give only broad indications. Other trials, such as those at the Agri-Food Biosciences Institute (AFBI) in Northern Ireland, only publish relative straw lengths, relative straw yield and standing power characteristics (AFBI, 2016). For spring barley, AFBI straw yields are from fungicide-treated plots and are described as very low, low, intermediate, high or very high, and for winter barley, the trials also receive a plant growth regulator and are described as low, intermediate or high. However, most variety RL do not assess the straw yield as it is considered to be laborious to measure, much more variable than grain yield (Ethel White, personal communication, AFBI) and of low priority to most growers.

The current paper sought to test whether the straw yield as well as the grain yield was increased in cultivar mixtures, and if so, whether and how this was influenced by crop type (spring or winter sown), environment (trial site), fungicide and nitrogen inputs. It aimed to determine whether the increased component number affects the straw yield in the same way as the grain yield and to determine whether the HI changes indicate a mechanistic basis of these cultivar interactions.

Materials and methods

Barley cultivars and mixtures

A spring barley trial and three winter barley trials were carried out. The spring trial had 48 entries comprising 13 monoculture cultivars and 35 equal proportion mixtures by grain number. The cultivars were selected to represent the contrasting straw production characteristics using information gained from trials carried out by AFBI and agronomists where these data could be found (Table 1). Amongst the mixtures were two series of two-component mixtures, which comprised each cultivar with Waggon (11 entries) representing a medium (intermediate) straw biomass type, and each cultivar with Doyen (11 entries) as a low straw biomass type, plus Waggon with Doyen; six four-component, two five-component, two six-component and two seven-component mixtures. The four- to seven-component mixtures were selected based on different contrasting cultivar combinations with either Doyen or Waggon (Table 1). Thus, half the mixtures were with Waggon or Doyen, representing balanced comparisons with a medium or a low straw producer, respectively.

The winter trials had 60 entries comprising six monoculture cultivars and a balanced series of 54 equal proportion mixtures by grain number. The cultivars were again selected to represent the contrasting straw production characteristics using information gained from trials carried out by AFBI (Table 1). The mixtures comprised all two-component (15), three-component (20), four-component (13 of 15) and five-component (6) combinations of the monocultures. The actual mean grain and straw values from the trials below are cited alongside the cultivar designations for both winter and spring cultivars in Table 1 for comparison, to fill in missing information and to qualify designation corrections.

Trial design, assessment and analysis

The spring barley trial had two fertilizer treatments and two replicates sown as a randomized split-plot design with fertilizer being the whole-plot treatment and cultivar as the sub-plot treatment. The fertilizer treatments were a full rate (N1.0) of 154 kg/ha nitrogen (N) and half rate (N0.5) applied as 22-4-14 (7.5 sulphate [SO_4]) in two splits and will be referred to as nitrogen treatments. The trial was sown at Balruddery farm (see below) with an eight-row Hege drill with a plot width of 1.55 m and a length of 6 m reduced to 4.8 m after plot definitions. Every plot was assessed for disease on a 1–9 scale, where 1 represents no disease and 9 is fully necrotic (Newton and Hackett, 1994), starting when the disease increased above trace levels and then at approximately 2-weekly intervals. Disease scores were converted to percentage leaf area of infection before analysis and the area under the disease progress curve (AUDPC) was calculated (Van der Plank, 1963). The plots were sampled for both straw and grain yields and the remaining plots were subsequently harvested with a plot combine. For plot sampling, three 0.5 m rows were cut at random from the inner rows of each plot, threshed and the straw and grain weights recorded after air drying for at least 1 week. The remaining plot was harvested with a plot combine and the straw was collected in a tarpaulin straight from the back of the combine and weighed immediately using a weigh cell in the field. The grain was dried at constant moisture before being weighed.

The winter barley trial had two fungicide treatments, two fertilizer treatments and three replicates sown as a randomized split-split-plot design with fungicide treatment as the whole-plot treatment and fertilizer as the sub-plot treatment. Fungicide treatment was either untreated (*f*0) or a full-rate programme (T1 Siltra Xpro 0.5 l/ha + Bravo 1.0 l/ha + Vegas (or Cyflamid) 0.25 l/ha; T2 Siltra Xpro 0.6 l/ha + Bravo 1.0 l/ha) (*f*1) and fertilizer levels were a full rate (N 1.0) of 188 kg/ha and a half rate (N 0.5) applied as 30-0-0 (19 SO_4) in two splits. The trial was sown at two locations in the 2014–15 season, on Hartwood Home Farm in North Lanarkshire, Scotland (55°48′48.3″N, 3°49′43.0″W) and on Balruddery Farm near Dundee, Scotland (56°29′08.6″N, 3° 08′05.8″W), and again at Balruddery Farm (56°28′53.8″N, 3° 06′35.7″W) in the 2015–16 season. Other trial details were the same as the spring barley trial except that only whole-plot assessments were made. Hartwood is predominantly grass pasture grazed by sheep where the mean June temperature (Springburn) is 13 °C and the annual rainfall is 1124 mm, with 1203 h of sunshine. The trial was sown into freshly ploughed-up pasture. Balruddery is an arable farm with sandy loam soil with low organic matter where the mean June temperature (Mylnefield) is 13 °C, the annual mean rainfall is 722 mm and 1426 h of sun. The previous crops were peas in 2014 and winter oilseed rape in 2015. For all trials, herbicide and manganese treatments were applied as appropriate to the trial field and no growth regulators were used.

Statistical analysis

Each trial was analysed using a mixed model and REML so that the spatial effects across the trial sites could be included if necessary. The fixed effects were the fertilizer, entry and their interaction for the spring barley trial, and fungicide, fertilizer, entry and all interactions among these for the winter barley trials. The random effects were the design structure of blocks, whole plots and sub-plots together with the effects of row and/or bed if these improved the model fit. The best model was selected using the minimum BIC (Schwarz, 1978). A set of contrasts compared the mean response of mixture plots with those of the corresponding monoculture plots weighted according to their

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Table 1. Barley straw and grain yields from prior information and trial means

A. Spring barley							
		Straw[a]					
Entry	Cultivar	AFBI	Trial[e]	Straw designation	Straw length (cm)[b,c]	Grain yield (t/ha)	Straw yield (t/ha)
1	Waggon	Intermediate		M	74[c]	6.59	4.98
2	Doyen	Low		L	71[c]	5.89	4.14
3	B83-12/21/5	–	(Med)	M	–	5.32	4.80
4	Aramir	–	(V Low)	vL	–	3.32	3.46
5	Triumph	–	(Low)	L	–	5.11	4.21
6	Optic	–	(Low)	L	76[c]	5.02	4.24
7	Derkado	–	(Low)	L	–	5.04	4.24
8	Carlsberg	–	(Low)	L	–	3.82	4.18
9	DkxB83/T		Tall(/Med)	M	–	4.55	4.72
10	FabelSejet		Tall(/Med)	M	–	5.44	4.91
11	Riviera	Intermediate		M	81[d]	5.60	4.70
12	Static	Low		L	76[d]	5.92	4.26
13	Chalice	Low	(Med)	L	72[d]	5.82	4.67
Entry	Cultivar combination/composition			Straw designation combination			
14	1 + 2	2-comp-Ser1Ser2		ML			
15	1 + 3	2-comp-Ser1		MM			
16	1 + 4	2-comp-Ser1		MvL			
17	1 + 5	2-comp-Ser1		ML			
18	1 + 6	2-comp-Ser1		ML			
19	1 + 7	2-comp-Ser1		ML			
20	1 + 8	2-comp-Ser1		ML			
21	1 + 9	2-comp-Ser1		MM			
22	1 + 10	2-comp-Ser1		MM			
23	1 + 11	2-comp-Ser1		MM			
24	1 + 12	2-comp-Ser1		ML			
25	1 + 13	2-comp-Ser1		ML		Ser1 total = 15 M 7L	
26	2 + 3	2-comp-Ser2		LM			
27	2 + 4	2-comp-Ser2		LvL			
28	2 + 5	2-comp-Ser2		LL			

(*Continued*)

Table 1. (*Continued.*)

A. Spring barley							
		Straw[a]					
Entry	Cultivar	AFBI	Trial[e]	Straw designation	Straw length (cm)[b],[c]	Grain yield (t/ha)	Straw yield (t/ha)
29	2 + 6	2-comp-Ser2		LL			
30	2 + 7	2-comp-Ser2		LL			
31	2 + 8	2-comp-Ser2		LL			
32	2 + 9	2-comp-Ser2		LM			
33	2 + 10	2-comp-Ser2		LM			
34	2 + 11	2-comp-Ser2		LM			
35	2 + 12	2-comp-Ser2		LL			
36	2 + 13	2-comp-Ser2		LL		Ser2 total = 4 M 18L	
37	1 + 3 + 7 + 9	4-comp		MMLM			
38	2 + 3 + 7 + 9	4-comp		LMLM			
39	1 + 6 + 10 + 13	4-comp		MLML			
40	2 + 6 + 10 + 13	4-comp		LLML			
41	1 + 5 + 6 + 7	4-comp		MLLL			
42	2 + 5 + 6 + 7	4-comp		LLLL			
43	1 + 4 + 9 + 11 + 12	5-comp		MvLMML			
44	2 + 4 + 9 + 11 + 12	5-comp		LvLMML			
45	1 + 4 + 6 + 8 + 10 + 12	6-comp		MvLLLML			
46	2 + 4 + 6 + 8 + 10 + 12	6-comp		LvLLLML		Complex-comp = 17 M 29L	
47	1 + 3 + 5 + 7 + 9 + 11 + 13	7-comp		MMLLMML			
48	2 + 3 + 5 + 7 + 9 + 11 + 13	7-comp		LMLLMML		7-comp = 7 M 7L 24 M 36L	

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B. Winter barley

Entry	Cultivar/mixture	Straw length[f]					Straw designation	Grain yield[h] (t/ha)	Straw yield[i] (t/ha)
		+PGR 2014	+PGR 2015	−PGR 2016	−PGR 2017	Mean[g]			
1	Cassata	0.88	0.89	1.01	–	0.95	High	7.16	8.59
2	Flagon	0.96	0.98	1.04	1.01	1.00	High	7.52	8.63
3	KWS-Meridian	1.01	1.03	–	–	1.06	Int.	8.12	7.84
4	KWS-Tower	0.88	0.89	0.99	0.94	0.93	Int.	7.92	9.13
5	Cavalier	0.82	0.83	0.90	–	0.86	Low	7.62	7.53
6	Retriever	0.84	0.85	0.95	–	0.90	Low	7.87	7.34
7–21	all 15 × 2-comp								
22–41	all 20 × 3-comp								
42–54	all 13 × 4-comp								
55–60	all 6 × 5-comp								

[a]AFBI designations – see Introduction for range values.
[b]Entries containing Doyen including 'series 2' are shaded.
[c]AHDB RL data were available: mean of 2005/6, 2006/7, 2009/10 and 2010/11.
[d]AHDB RL data were available: mean of 2005/6 and 2006/7.
[e]Bracketed values not in AFBI data.
[f]AHDB RL data 2014/15 and 2015/16 where all cultivars were listed uses PGPRs in trial agronomy. 2016/17 and 2017/18 RL data available without PGPRs also but not all cultivars were still listed.
[g]Weighted mean height.
[h]Mean of spring trial or all three winter trials.
[i]Mean of Balruddery trials.

proportion in the mixture. The significance of the sum of squares associated with each contrast was tested by comparing it with the residual sum of squares using an *F*-test (Snedecor and Cochran, 1980). The benefit of the mixtures was calculated as the difference between the mixture mean and that of its corresponding monoculture components weighted according to their proportion in the mixture plots, designated as Type. All analyses were carried out using GenStat Seventeenth Edition (VSN International Ltd, Hemel Hempstead, UK).

Results

Spring barley

The spring barley trial established well, straw and grain were harvested and weighed from all plots, and sub-sampling was carried out successfully. The only disease that established above trace levels was rhynchosporium (causal agent *Rhynchosporium commune*). This was assessed on three dates, 12 June, 23 June and 2 July (approximately growth stage (GS) 34, GS47 and GS51, respectively; Zadoks *et al*., 1974) and these scores, converted to percentage infection equivalents, were used to calculate the AUDPC. For all traits, there was a significant spatial effect across the trial plots. Grain yield (t/ha) also showed a significant spatial trend along the plots and these effects were included in the final model.

There was a strong cultivar effect on the yield of both grain and straw measured on the whole plot ($P < 0.001$) but the nitrogen effect was not significant and there was no significant cultivar × nitrogen interaction. Cultivar also had a significant effect on rhynchosporium infection ($P < 0.001$). For the sub-samples, the cultivar effect was significant for grain ($P < 0.001$) and straw ($P < 0.001$). The sum of the grain and straw from both whole plot and sub-samples, i.e. the total above-ground harvested biomass, also showed a strong cultivar effect ($P < 0.001$). In addition to the overall contrasts tested for the mixtures compared with the monocultures, the effect of the component number was tested, but excluding the seven-component mixtures as these were observed to often behave very differently from the less complex mixtures (Fig. 1).

The mixtures gave significantly ($P < 0.001$) more grain and straw yield than the monocultures as plots and as an overall biomass value (Table 2). The grain yields of the mixture sub-samples were significantly greater than for the monocultures also, but the straw yields of the sub-samples were not, reflecting the greater error associated with this sampling strategy. There was a significant ($P < 0.05$) positive correlation with the component number for sub-sample grain yield, straw per plot, straw sub-sample and total harvested biomass (excluding the seven-component mixtures). For grain yield per plot, the correlation was positive but of borderline significance level ($P = 0.07$). For AUDPC, there was a significant, negative correlation if the seven-component mixture was excluded indicating that a greater component number was associated with lower levels of disease (Table 2).

The spring barley HI was calculated as the grain yield divided by the total biomass × 100. There was a strong cultivar effect ($P < 0.001$) but the nitrogen effect was not significant and there was no significant cultivar × nitrogen interaction. The HI of the mixtures was significantly higher than for the monocultures ($P = 0.001$). The Type × nitrogen interaction was not significant. One five-component mixture (2 + 4 + 9 + 11 + 12) showed a significantly lower HI than expected ($P < 0.05$). The correlation with the component number was very close to zero, with or without the seven-component mixture.

The 13 cultivars chosen were selected to represent a diversity of phenotypes and straw yield in particular. The prior information available to make these selections was very limited so the actual mean yields are included in the table for comparison (Table 1). The two cultivars chosen for the two two-component mixture series differed consistently in straw yield in AFBI designation, straw length in the RL description and straw yield from the trial. Two of the three other AFBI data selections also performed as expected but Chalice gave higher straw yield than predicted. Overall, the more complex mixtures had 24 medium straw (M) cultivars and 36 low straw (L) cultivars, the Waggon two-component (Series 1) mixtures had 15 M and seven L cultivars, whereas the Doyen two-component (Series 2) mixtures had four M and 18 L cultivars. Whilst the two two-component series did not differ significantly from each other (data not shown), individual mixtures were identified amongst them and from the complex series that gave significantly more yield or less disease. For yield, these were more often from the Doyen series, suggesting more beneficial interactions when there were more L types (Table 2). If this was a factor contributing to the yield benefit, then the advantages of the complex mixtures might also be explained by their higher proportion of L types. The cultivar Aramir was more often present amongst these significantly higher yielding pairs than any other cultivar (data not shown). Individual mixtures with significantly less disease were more often from the Waggon series (Table 2).

Where there was a trend with the component number, the seven-component mixture often behaved differently (Fig. 1). For both plot and sub-sample straw weight as well as overall biomass, the seven-component mixtures had lower levels than would be expected from the monocultures, whereas all the less complex mixture means gave a positive interaction. For AUDPC, although disease levels were low, the seven-component mixtures had higher levels than expected from the monocultures, whereas all other component means were lower.

Winter barley

Disease was assessed and grain was harvested successfully from all plots. Straw was cut and weighed from the fungicide-treated plots only in the two 2015 trials, but for plots both with and without fungicide treatments in the 2016 trial. The AFBI designations were chosen so that there were two high, two intermediate and two low straw yield types. The two low and high types performed as expected, as did KWS Meridian, representing the intermediate category. However, KWS Tower gave the highest straw yield and so was wrongly designated under the trial conditions used in the current work. Overall, there were strong cultivar, nitrogen and fungicide effects as well as interactions. However, there were also strong side effects and spatial trends in some sites, so each trial was analysed separately.

For the 2016 trial at Balruddery, where straw weights were recorded in plots both with and without fungicide, there was a significant increase in grain yield in the mixtures overall compared to the monocultures (designated as Type) ($P < 0.001$, Table 3). There was no significant interaction between Type and fungicide, but there was a significant interaction ($P < 0.001$) between Type and nitrogen, with the mixture yield effects being significantly higher than for the monoculture yield under high nitrogen but not under low nitrogen. The monoculture effects are given for each treatment in Table 3. The straw yield increased significantly under the high-nitrogen treatment only. There was a

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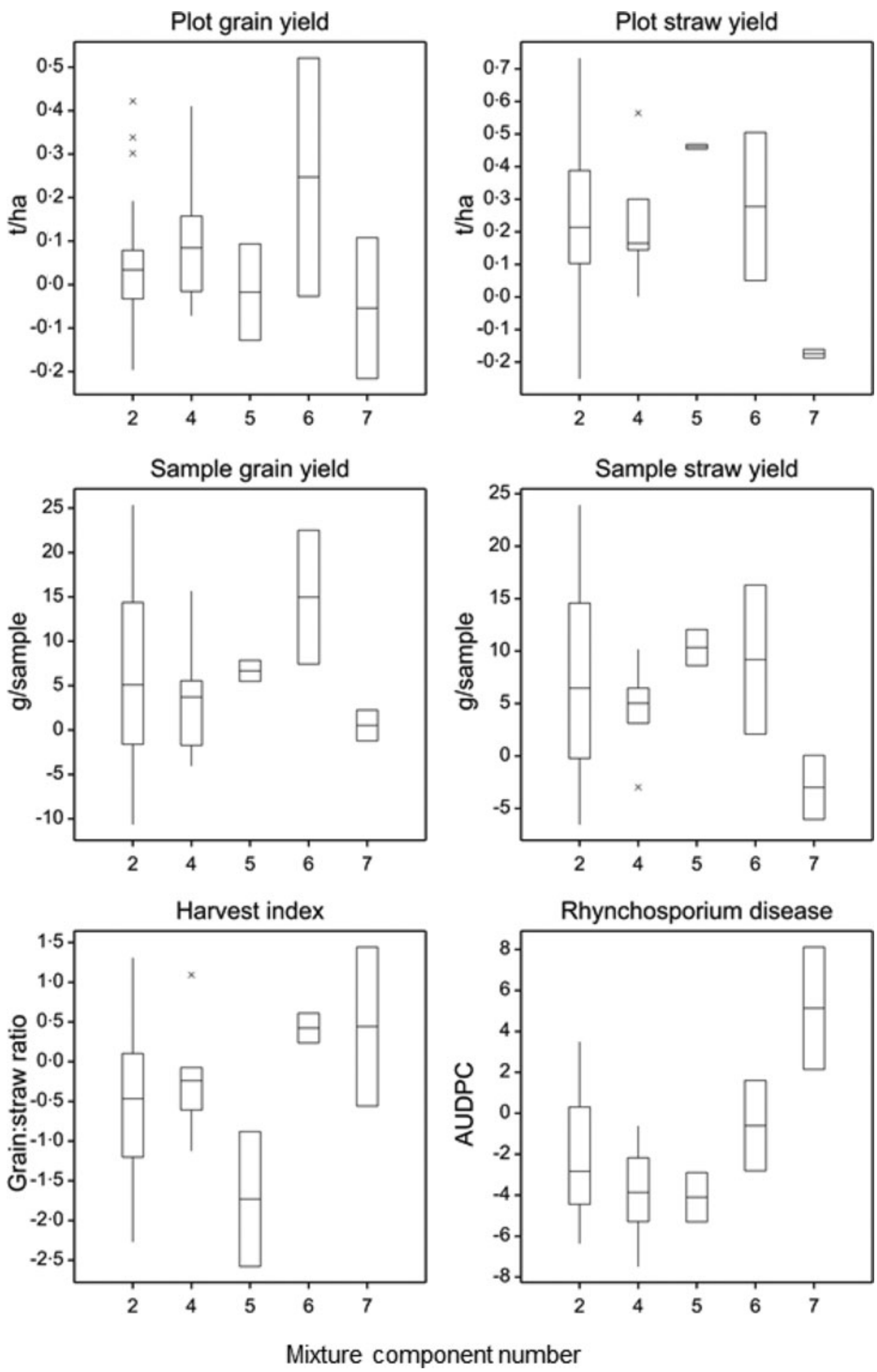

Fig. 1. Boxplots of mixture effects of spring barley yield components for each component number. The mixture effects are calculated as the difference between the estimated mixture mean from the REML analysis and the weighted means of the corresponding monocultures.

significant positive correlation between grain yield and component number in the mixtures under high nitrogen whether fungicide treated or not (Fig. 2; Table 4), but for straw, this trend was not significant under fungicide treatment and borderline significant ($P = 0.065$) under no-fungicide treatment (Table 4).

The 2015 trial at Balruddery also gave a significant increase in grain yield in the mixtures overall ($P < 0.001$), particularly at high nitrogen. There was also a significant interaction between Type and fungicide ($P < 0.05$), with a significant mixture effect without fungicide treatment but no significant effect with fungicide. However, in contrast to the 2016 trial, the straw yield (measured only with fungicide treatment) decreased significantly under the high-nitrogen treatment (Table 3). There was no significant effect at low nitrogen. There was a significant positive correlation of grain yield with the component number in the mixtures for the untreated plots and at high nitrogen levels (Fig. 2; Table 4), but for straw, the trend was not significant (Table 4).

The 2015 trial was also carried out at the Hartwood site with quite different, wetter and less sunny environmental conditions. Again, there was an overall increase in grain yield in the mixtures

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Table 2. Correlation of spring barley mixture effect with the component number (excluding the seven-component mixtures) for grain and straw yield and Rhynchosporium disease

	Mixture cf. Monoculture[a]	Correlation with Component number[b]	Significant two-component mixtures[c]	Significant complex mixtures[d]
Grain/plot	$P = 0.040$	0.269[‡]	Doyen x3	Doyen x3
Straw/plot	$P < 0.001$	0.395**	Doyen x3 Waggon x2	Doyen x3 Waggon x1
Grain sub-sample	$P < 0.001$	0.295*	Doyen x2 Waggon x2	Doyen x2
Straw sub-sample	$P = 0.171$	0.342*	Doyen x2 Waggon x2	Doyen x1
Harvested biomass	$P < 0.001$	0.403**	Doyen x4 Waggon x1	Doyen x2 Waggon × 1
Rhyncho. AUDPC	$P = 0.045$	−0.363*	Waggon x3	Doyen x1 Waggon x3
HI = Ratio	$P = 0.001$	−0.060	None	Doyen x1

[a]Significance of the contrast of mixture mean with monoculture mean.
[b]Significance of the correlation: ‡0.1 > P > 0.05; *0.05 > P > 0.01; **P < 0.01.
[c]Number of two-component mixtures containing Waggon or Doyen with a beneficial mixture effect.
[d]Number of four-, five-, six- or seven-component mixtures containing Waggon or Doyen with a beneficial mixture effect.

Table 3. Comparison of mixtures with their respective component monocultures (Type) for harvested traits in the winter barley trials

		Significance				Mixture – monoculture difference effects			
Trait	Trial	Type	Type.F	Type.N	Fung	Untr	N 1.0	N 0.5	S.E.D.
Grain yield	Balruddery-2016	<0.001	ns	<0.001	0.178	0.118	0.295	0.001	0.052
	Balruddery-2015	<0.001	0.026	ns	0.095	0.460	0.372	0.181	0.096
	Hartwood-2015	0.026	ns	ns	0.044	0.263	0.093	0.214	0.102
Straw yield	Balruddery-2016	ns	ns	0.010	0.067	0.114	0.257	0.074	0.097
	Balruddery-2015	0.006	n/a	0.032	0.428	n/a	−0.857	−0.100	0.145[a], 0.205[b]
	Hartwood-2015	ns	n/a	ns	0.159	n/a	0.253	0.058	0.258, 0.399
Rhynchosporium	Balruddery-2016	ns	n/a	0.005	n/a	−0.069	−0.315	0.178	0.083, 0.117
	Balruddery-2015	<0.001	n/a	0.078	n/a	−0.366	−0.486	−0.246	0.075, 0.106
	Hartwood-2015	<0.001	n/a	ns	n/a	−0.171	−0.214	−0.128	0.036, 0.051
Harvest index	Balruddery-2016	ns	ns	ns	0.42	−0.16	−0.08	0.34	0.249
	Balruddery-2015	0.002	n/a	0.058	1.38	n/a	2.30	0.47	0.399, 0.564
	Hartwood-2015	ns	n/a	ns	−0.10	n/a	−0.37	0.18	0.510, 0.897

[a]SED for effect under fungicide treatment.
[b]SED for effects under N1.0 or N0.5 and fungicide treatment.

($P < 0.05$), particularly in the without fungicide low-nitrogen treatments (Table 3), but no significant increase in the straw yield. There was a significant positive correlation of grain yield component number in the mixtures without fungicide treatment, and with low nitrogen (Fig. 2; Table 4).

Rhynchosporium levels were low in all three trials, about 1.2% in both Balruddery trials under no-fungicide treatments and 0.3% in the Hartwood no-fungicide treatment trial. In the Balruddery 2016 trial, there was a significant interaction between Type and nitrogen level, with a significantly lower disease in the mixture plots for high nitrogen only, but in the Balruddery 2015 trial and in the Hartwood 2015 trial, there were significant mixture effects ($P < 0.001$, Table 3). Furthermore, in both these trials, there was a significant negative correlation of rhynchosporium level with the component number (Table 4).

The total plot biomass (the sum of the straw and grain) and the HI were also analysed for these trials. For Balruddery in 2016, the biomass of the mixture was significantly greater than the monoculture component mean and had a positive correlation of 0.432 ($P < 0.001$) with the component number under high-nitrogen treatment but the HI was not significantly different between mixtures and monocultures (Table 4). However, in 2015 at Balruddery, the HI was increased in mixtures ($P < 0.01$), particularly under the fungicide-treated high-nitrogen treatment, and the correlation with the component number was 0.232 ($P = 0.075$). There was no significant difference between mixtures and monocultures for HI at the other two sites and no significant correlation with the component number.

Discussion

In both winter and spring barley, these trials again demonstrated that growing mixtures of cultivars frequently leads to significant increases in grain yield compared with the mean of the component monocultures with the same inputs. The amount of straw produced in mixtures was sometimes also significantly changed,

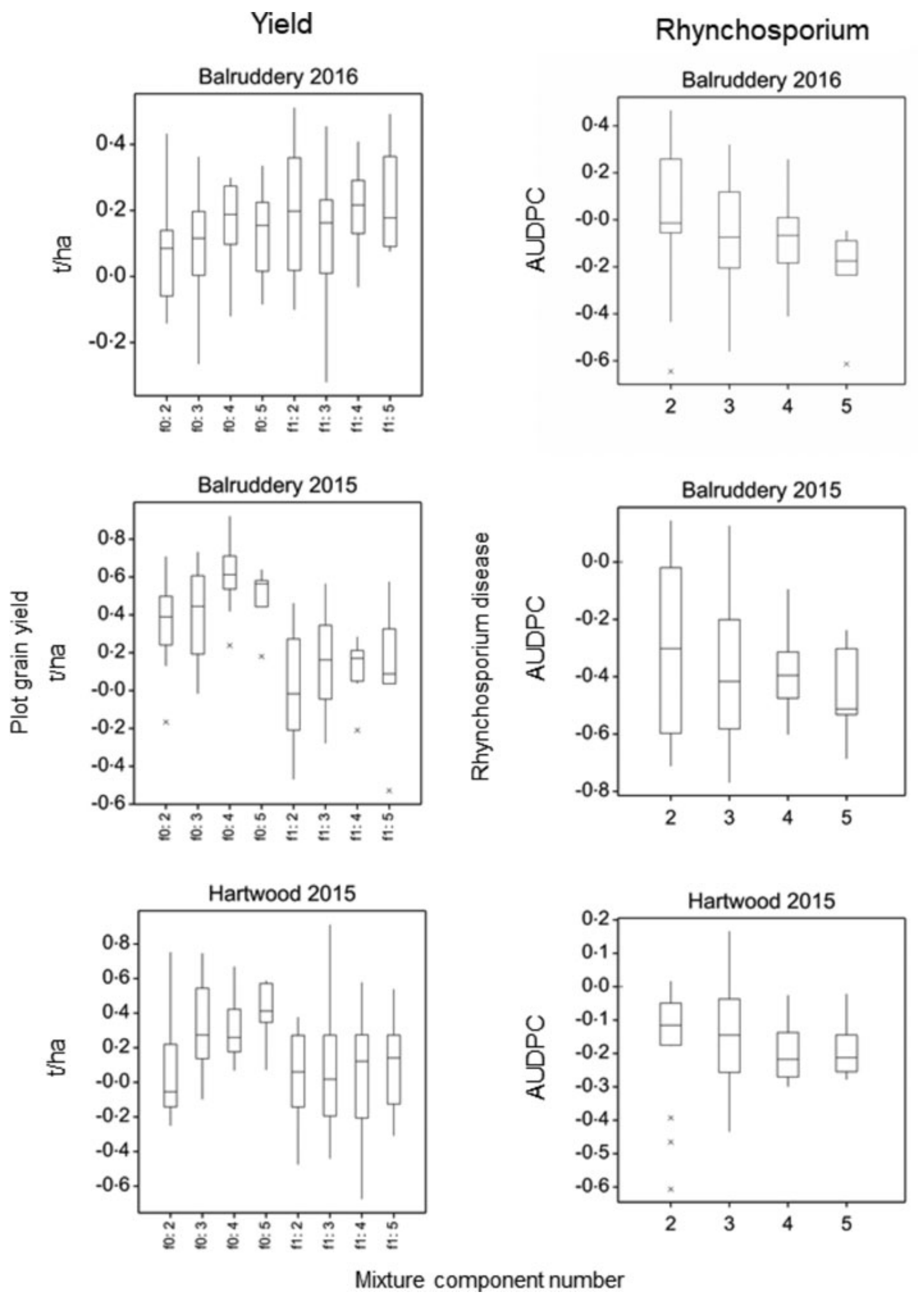

Fig. 2. Boxplots of mixture effects of winter barley grain yield and rhynchosporium disease for each component number means for three trials (site/year). The mixture effects are calculated as the difference between the estimated mixture mean from the REML analysis and the weighted means of the corresponding monocultures. For grain yield, the untreated (*f*0) and fungicide protected (*f*1) effects are shown separately.

but not always in a positive direction. The increases in grain yield were often correlated with increasing mixture component number but the changes in straw yield were less correlated. Where disease occurred at sufficient levels, again there was a mixture effect correlated with the component number but always negatively, i.e. disease was reduced in mixtures.

In these trials, some complex interactions were observed between cultivars in different combinations and environments. Previously, very clear correlations have been seen between increasing component numbers and increasing grain yield in winter barley (Newton *et al*., 1997, 2012). Although there are limited data for spring barley on the effect of component numbers, unpublished data clearly shows, for example, balanced three-component mixtures yielding significantly more than two-comp mixtures (Newton, unpublished data), and the current work confirmed this correlation with the component number for spring barley.

The Waggon two-component series had a 15:7 medium to low (M:L) straw designation ratio of component cultivars and the Doyen two-component series had a 4:18 M:L ratio. However, there were no significant differences between these series for any harvest measure. The more complex mixtures also had a higher proportion of L designation cultivars but as the series with contrasting ratios had no effect then it is more likely that the complexity has a stronger effect than any correlation with M:L ratio (Table 1), so no causal relationship between straw yield potential and mixture benefit could be derived. However, Doyen, which gives low straw yield, occurred more often amongst the particular individual mixtures that gave a significant increase in grain and straw yield than Waggon did. Conversely, Waggon mixtures were more frequent amongst the individual mixtures with significantly reduced rhynchosporium disease. Waggon had a resistance rating of 3 (very susceptible) whereas Doyen

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Table 4. Correlation of mixtures component number trends for harvested traits in the winter barley trials

Trial	Grain				Straw		Rhynchosporium			Harvest index
	Fung	Untr.	N1.0	N0.5	Fung	Untr.	Overall	N1.0	N0.5	Overall
Component number trend										
Balruddery GV (2016)	0.254‡	0.288*	0.499***	−0.012	0.083	0.240‡	−0.268‡	−0.299*	−0.057	0.050
Balruddery GC (2015)	0.199	0.537***	0.499***	0.198	−0.148	n/a	−0.394**	−0.400**	−0.208	0.232‡
Hartwood GC (2015)	0.050	0.447***	0.178	0.276*	0.100	n/a	−0.258*	−0.167	−0.244‡	−0.103

n/a, not measured.
Significance of the correlation: ‡0.1 > P > 0.05; *0.05 > P > 0.01; **P < 0.01.

was 7 (moderately resistant) (AHDB, 2011, 2018), so this may be a reflection of the monoculture infection levels and therefore the greater potential of the mixtures to reduce infection on this cultivar. Disease resistance apart, had the two cultivars expressed a larger difference in straw traits then a clearer trend may have been observed in their behaviour in the mixtures.

The spring barley trial reported here shows a trend towards increased effect with complexity, strongly for grain and sometimes for straw too, but only when the seven-component mixture is excluded. There were only two seven-component mixtures in this trial and they consistently departed from the trend of complexity shown by the two-, four-, five- and six-component mixtures for the traits measured. Hence, the effect of complexity was tested excluding these mixtures. Why these particular mixtures behaved differently is unclear. An understanding of the mechanisms of mixture complexity from experimental and modelling would suggest that a continuation of the same trend would be expected (Newton *et al*., 1997; Kiær *et al*., 2009). It seems unlikely that an optimum level of complexity exists, either side of which benefit is reduced, so this does not appear to be a feature of the level of complexity *per se*. Rather it seems to represent an interaction peculiar to these particular cultivars in combination. There were only two seven-component mixtures and they were not a complete, balanced series, both lacking cultivars 4, 6, 8, 10 and 12. Particular cultivars can have strong effects in mixtures (Newton *et al*., 2008). This may reflect differences in the ability of different cultivars to compete with, complement or facilitate the growth of other components of the mixtures. In fully balanced comparisons, the contribution of individual cultivars to particular traits can be quantified (Newton *et al*., 2008), but where trait expression is variable, these data may be too environment-dependent to be useful for the prediction of robust mixture performance.

The spring barley trial data from the sub-samples and the whole plots showed the same trends, although with higher significance levels in the latter. Plot size is frequently raised as a caveat on efficacy measures of mixture experiments due to the perceived importance of edge effects in smaller plots (Newton *et al*., 2002). The sub-sample data, being derived from plot centres, will have no edge effect, comprising only plants in equal competition and facilitation on all sides, thus demonstrating that the small plot setup was a valid approach.

The HI analysis illustrates the consequences of the simultaneous change in grain and straw yield or otherwise, as it is a ratio of these. For the spring barley, this was influenced strongly by cultivar but not nitrogen and the HI of the mixtures was significantly higher than for the monocultures, although there was no correlation with the component number. That one five-component mixture showed a significantly lower HI than expected indicates that particular interactions can be unpredictable, as was the case for the seven-component mixtures for other traits.

The winter barley series of mixtures were balanced, all two-, three-, four-(13 of 15) and five-component equal proportion mixtures of the six monocultures being present and therefore effects of complexity were more transparent. The unexpectedly highest straw yield of intermediate straw-designated cultivar KWS Tower meant that there was no longer a balance of two high, two intermediate and two low straw types that might have helped identify a correlation between component cultivar straw yield and its performance in a mixture.

The 2016 Balruddery trial generally gave the data expected from previous experience (Newton *et al*., 1997), namely an

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increase in grain yield corresponding with an increasing component number. As the mixture effect is generally assumed to be due to better RUE through whatever interaction mechanisms operate between the components, it might be expected that the biomass of the crop increases as a whole and therefore the straw yield will increase too. This result did occur under the high-nitrogen treatment and the trend towards more straw with an increasing component number was also significant in the no-fungicide treatment. The comparable trial at Balruddery in 2015 gave a similar result for grain yield but the straw yields in the mixtures were significantly lower, particularly under high nitrogen. That the effect should be opposite in these two comparable trials under different environmental conditions indicates the strength of the cultivar × environment and the cultivar mixture × environment response. The environmental differences between these two trials in different years are likely to be predominantly abiotic stress, i.e. the weather, as the fields used were <2 km apart on very similar sandy loam soil with the same agronomic treatments and had very similar levels of the same disease pressure in these different years.

The third winter barley trial was about 100 km south-west on very different soil with a contrasting crop history and different weather again, even within the same season as the latter Balruddery trial discussed above. Here again, in the no-fungicide treatment, grain yield increased in the mixtures and there was a correlation between increasing component number and greater yield in the no-fungicide and low-nitrogen treatments. However, there were no significant effects on the straw or biomass overall, adding credence to the argument that cultivar × environment and cultivar mixture × environment interactions are strong, leading to a range of outcomes. The inconsistency of both grain and straw yield across the environments illustrates the plastic nature of barley as a crop, compounded by the complex ecological interactions when cultivars with different trait expressions are mixed. Source-sink prioritization reflected in changes in HI shows that sink prioritization occurs more in mixtures in the spring barley trial, whereas in the winter barley trials, only Balruddery 2015 showed the same trend.

The effects of mixtures on disease generally fitted the expected pattern from previous work. Not only are mixtures effective at reducing disease in the range of up to around 50% infection, but they are also more effective with an increasing component number. There was no correlation between greater disease reduction in mixtures and yield response *per se*, indeed the strongest yield (straw and grain) responses of the Balruddery 2016 trial had the least reduction in disease due to mixtures, but this is more likely to be attributable to the low overall disease levels.

In natural ecosystems, the biomass of diverse communities is often greater than the sum of its component species grown as monocultures (Brooker, 2017), but the seed or grain component is often not measured separately, and when it is, is a minor component of the total (Trinder *et al.*, 2013). In cereal crops, the opposite is the norm and the grain is the component measure that is often greater than the sum of its component species or cultivars in diverse mixtures. Overall the diversity–productivity relationship holds for both situations. However, the HI that describes this ratio of vegetative to seed components is the factor that has been manipulated in breeding and its response to diversity appears to be environmentally responsive in its expression. This in turn is the sum of competition, complementarity and facilitation effects that respond to environmental factors differentially. These factors can be considered as stress factors and these have been shown to alter the impacts of plant interactions on survival, growth and reproduction (He *et al.*, 2013). Furthermore, He *et al.* (2013) demonstrated that these effects differ depending on the type of stress, such as biotic, physical or resource, lifestyle such as annual or perennial, and developmental stage such as juvenile or adult, climate, and cropping system or ecosystem in an ecological context. Furthermore, the effects will depend on the assessment method or approach, duration and the length or nature of the stress gradient used. This was shown to a degree in the modifying effects of fungicide and nitrogen treatments as well as experimental environment, namely site and year in the current work. However, there is normally a benefit derived from the resilience conferred by using multiple components in mixtures for increased grain yield, reduced disease and sometimes increased straw yield, particularly under untreated and higher nitrogen treatments, but environmental interactions are high so benefits will not always be seen. Current analysis of multiple trials of cereal mixtures with comparable agronomic treatments across many seasons is under way to more clearly define the factors that result in beneficial interactions. This is needed to identify the factors that we can use to design mixtures that will be robust across environments.

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