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Blending of wheat for Resilience, Improved Distilling quality and Greater Environmental Stability (BRIDGES)

by

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Contents

Abstract

Mixtures of cereal varieties have shown benefits for controlling disease, increasing yield and improving stability across sites and seasons, particularly for winter barley. Preliminary investigations also suggested that both grain and alcohol yield may be improved in soft winter wheat varieties for distilling. This market is much less concerned with varietal purity than is spring barley for malting.

This project aimed to determine whether the same benefits of mixtures could be obtained using current Recommended List varieties of wheat from nabim Groups 3 and 4. Wheat varieties were grown in monocultures, or two- and four-way mixtures, over four sites with full and reduced fungicide treatments. At one of the sites, more varieties and mixtures were included and trials were grown at two nitrogen rates. Yield, thousand grain weight and predicted spirit yields were determined and stability across sites was analysed.

Overall there were some benefits from mixtures and no negative effects. Several interactions between individual mixtures and the environment, fungicide and nitrogen applications were also beneficial, although there were a few negative interactions. Stability in yield, thousand grain weight and predicted spirit yield was demonstrated and probably represents the main reason for recommending variety mixtures for distilling.

Comparison with commercial crops demonstrated that our trials were producing predicted spirit yields acceptable for distilling, while comparison of NIR data, adjusted for moisture content, showed slight, but consistent differences between calibrations. This suggested that our data might slightly underestimate alcohol yield from a highly efficient commercial distilling process. High input conditions (fungicide and nitrogen) at one site elicited most benefit from mixtures, indicating the potential for synergies to be to be enhanced given the right agronomy.

Summary

Mixtures of varieties have been used to build greater ‘buffering’ or resilience into cereal crops where biodiversity in compensatory and competitive interactions can be beneficially exploited. This has been proven to increase yield, reduce disease and give greater stability in many crops. Soft wheat has occasionally been grown and distilled in Scotland as mixtures, on an *ad hoc* basis, but usually with just two or three components. Achieving commercial significance would require concerted action across the entire supply chain from seed producers through growers to end-users. Overall more litres of spirit would be produced for the same or lower inputs, reducing the carbon footprint on the farm through fewer inputs and field operations and at the distillery by optimising the quality and consistency of supply.

At present, mixture components are selected by their performance as individual varieties and mixtures are generally reconstituted prior to each sowing. There is thus a need to establish and predict how varieties perform and interact within mixtures if the mixture is to be re-sown, or to give practical agronomic recommendations on optimal mixture composition. Mixtures increase the stability of enhanced yield response and disease reduction and do not require homogeneous blending as evidence from winter barley shows that ‘patchy’ mixtures work as well if not better than homogeneously mixed seed.

Varieties selected with specific desirable traits, particularly high grain and alcohol yields and disease suppression – can be most effectively exploited in mixtures/blends with other high quality components which will compensate for deficiencies. Such crops will be more resilient, substantially reducing risk in developing lower input crop production systems to reduce environmental impact and carbon footprint. Additionally, distilling performance would be improved through the deployment, as mixture components, of high quality varieties that would not otherwise be cultivated in monoculture for agronomic reasons. Less grain would be required for a given alcohol production, thus reducing energy requirement and waste for disposal and providing environmental as well as economic benefit to the distilling industry.

Specific project objectives were:

1) to develop a method of predicting mixture component performance using standard tester varieties in a multi-site 2-component mixture series and to compare the effectiveness of this with selection based on performance in monoculture.

2) to develop a commercially-relevant stability test for yield, grain quality and disease control in a mixture across multi-site farm crops, and demonstrate whether wheat mixtures could show greater consistency across sites in addition to enhanced performance.

For this initial study, distilling quality parameters were predicted using NIR. This provides an opportunity to compare the effectiveness of a calibration developed against a standard laboratory analysis with one developed for intake testing in a specific distillery test series.

Materials and Methods

Field trials were grown at four sites using a common set of 20 entries: Alchemy, Glasgow, Istabraq, Zebedee, Robigus and Monty grown as monocultures and the other 5 in an equal proportion mixture with Alchemy and similarly with Glasgow. The remaining entries were four-component and one six-component equal proportion mixtures. A full fungicide programme and a minimal or no fungicide programme was used. At one site there were 24 more trial entries and two nitrogen treatments. The additional trial entries were Riband, Consort, Nijinsky and Ambrosia, and these were tested in equal proportion pairs with Alchemy and with Glasgow as for the other varieties. Some entries were also re-sown from mixtures harvested the previous year. Trials were sown using standard procedures, harvested and dried to constant moisture and weighed. Any disease observed was recorded using standard trial protocols.

Following harvest, grain from each plot was assessed for a range of parameters, utilising NIR calibrations. Predicted Spirit Yield (PSY) was determined by a calibration that explains around 80% of the variation in alcohol yield and the grain dimensions, mean length and mean width were determined using a Marvin digital seed analyser, the thousand grain weight (TGW) being the main parameter used.

Statistical analysis used analysis of variance and REML to test for significant spatial effects across the area of the trial and series of contrasts were calculated from the variety means to test the significance of particular comparisons, such as monocultures versus mixtures, monocultures versus two-way mixtures, balanced so that each variety has equal weight in all comparisons. Yield sensitivity was determined from the linear regression of grain yield for each variety and mixture against a site or sub-site mean yield.

Data were recorded at intake, from 10 commercial crops in 2007 and 12 in 2008. Samples were assessed for alcohol yield, using the NIR calibration employed at the distillery for intake samples.

Results

There was significant variation between varieties/mixtures, sites and fungicides for yield, thousand grain weight (TGW) and predicted spirit yield (PSY). Monocultures varied in mean yield (i.e. over sites) by ~9%, 2-component mixtures by ~6%, and the complex mixtures by ~2.5%, but the overall means of each group did not differ significantly from each other. Monocultures varied in TGW by ~14%, 2-component mixtures by ~10%, and the complex mixtures by ~4%, but again the means of each group did not differ significantly from each other. The PSY data for sites other than SCRI gave no significant interactions at any level, but at the SCRI site with more replication, the two-component mixtures giving 6% more yield than the mean of the monocultures and the four-component mixtures 8% more.

Ranking the monocultures showed considerable variation in their yield performance between sites and the sites also showed very different mean yield and yield range, the latter varying from 0.6t/ha at Cauldshiel to 3.61t/ha at SCRI. Glasgow and Istabraq always high and Alchemy always ranked low, but Monty and Robigus, and to a lesser extent Zebedee, showed great variation in their ranking between sites. The mixtures by contrast showed less instability in rank order. The Glasgow-Istabraq-Zebedee-Alchemy (GIZA) and Glasgow-Istabraq-Robigus-Alchemy (AGIR) mixtures performed best overall.

Yield sensitivity across all sites and treatments varied considerably between varieties, and also mixtures. The varieties Robigus, Zebedee and Alchemy had the highest sensitivity scores (above 1.2), indicating a relatively strong response to increasing mean site yield. By contrast, Glasgow and several of the two-component mixtures had a low sensitivity score (less than 0.9), indicating a relatively shallow response to increasing site fertility. Across all variety and mixtures there was a significant negative association between yield sensitivity and yield.

In the 48 entry trial the yield, TGW and PSY data range of the mixtures compared with monocultures showed a similar reduction to that in the multi-site trial. Comparing all the mixtures with the mean values of their constituent monocultures, significant increases in yield were observed both with (3.7%) and without (3.1%) fungicide for the high nitrogen but not the low nitrogen treatment. Analysing only the two tester pair mixture series together (all varieties with Glasgow and all varieties with Alchemy) and the balanced 3-component mixtures, these mixtures yielded significantly more only for the high fungicide high nitrogen interaction, 2.9% and 2.8% respectively. Under the high fungicide treatment the mixtures gave significantly higher TGW at both low (2.8%) and high (2.4%) nitrogen treatments but no differences under low fungicide treatment. The mixture pairs with Glasgow showed similarly significant effects, 3.8% and 3.0% respectively.

Comparing all the mixtures with the monocultures, significant increases in PSY were observed with fungicide and high nitrogen (0.4%) and without fungicide with low nitrogen (0.4%). Under low fungicide, low nitrogen Alchemy-Robigus, Glasgow-Robigus and Glasgow-Nijinsky all showed significant increase in PSY, 1.1%, 0.95 and 1.1% respectively. Re-sown mixtures were compared with their newly-constituted mixtures and were shown to give similar benefits in yield, TGW and PSY.

The mean PSY of the commercial crops in 2007 was 444 l/t with a 3.5% variation and 453 l/t in 2008 with a 5.6% variation. The NIR values obtained at the distillery generally averaged 4% higher when the laboratory data were adjusted for moisture content.

Conclusions

Previous work on cereal variety mixtures has demonstrated considerable yield increases in barley, up to 16% in winter crops, though lesser increases in spring crops. Winter wheat trials with mixtures had given positive interactions for grain and alcohol yield and all mixtures tend to show greater yield stability. These trials in a single year demonstrated relatively small overall benefits and no negative effects. Stability in yield was demonstrated in the mixtures and probably represents the main reason for recommending variety mixtures for distilling. Thus the unpredictable performance characteristics of varieties such as Alchemy and Glasgow would not be a problem in mixtures and consistency of yield and quality could be assured.

The implications of the sensitivity analysis are that some types of high yielding variety mixtures (or variety) could be selected for a better consistency in yield across site conditions. The addition of other factors such as soil residual nitrogen or fertiliser nitrogen would be a useful way to develop the understanding of yield sensitivity.

Comparison with commercial crops demonstrated predicted spirit yields that would certainly be acceptable for distillery intake. Comparison of data adjusted for moisture content indicated that the type of calibration that might be mounted on an NIR measurement machine in a plant breeder's laboratory would be suitable in screening lines for acceptability at distillery intake.

High input conditions (fungicide and nitrogen) at the SCRI site showed most benefit from mixtures, indicating the potential for synergies to be to be obtained given the right agronomy. Even without optimised agronomy, mixtures conferred stability thus reducing risk for growers.

Experience has demonstrated that in some seasons, mixtures of cereals can produce minimal benefits whilst in others they can be much greater. Where disease is present at moderate or high levels mixtures can be particularly beneficial. In this work we reported the results from a single season. It is likely that further trials, particularly optimising the agronomy, would extend the range of beneficial interactions, particularly for disease control. Given the yield increases, reduced inputs of some treatments and increased predicted spirit yield, a full economic appraisal would at worst show no net cost of using mixtures, and at best some increased profit margins.

Technical report

Introduction

Crop production costs, particularly those associated with products derived from fossil fuels, are continually increasing. Cropping risk is likely to increase too, especially as one of the major early impacts of climate change is the increased likelihood of more extreme conditions. Commodity prices cannot be guaranteed and concerns about chemical residues entering the food chain or the environment are growing. The arable industry still relies, largely, on high-input cultivation, with heavy protection of individual varieties, to provide consistent raw materials for processing. This is now resulting in squeezing of profit margins, potentially adverse environmental impacts and, in the case of distilling wheat, reduced alcohol yield if grain protein levels are too high. The decline of end-user favoured old varieties such as Riband has further eroded the overall distilling quality of the crop, as potential replacement varieties often have agronomic and other limitations.

Many inputs are responses to threats such as pathogen or pest attack or poor crop growth, all of which would lead to yield loss if no action was taken. Constraints on yield loss potential may be achieved by adopting more precise, lower input systems where the threats have a disproportionately smaller impact. Another approach is to build greater 'buffering' or resilience into the system, particularly utilising heterogeneity deployment strategies i.e. mixtures, where biodiversity in compensatory and competitive interactions can be beneficially exploited. This has been proven to increase yield, reduce disease and give greater stability in many crops. Soft wheat has occasionally been grown and distilled in Scotland as mixtures, on an *ad hoc* basis, but usually with just two or three components. Achieving commercial significance would require concerted action across the entire supply chain from seed producers through growers to end-users.

Combining both the above approaches may exploit further synergies, e.g. genotypes which have different positive environmental, weed or disease response characteristics mixed together. Under low fertility conditions many diseases are less problematic enabling fewer, later-timed or reduced-dose fungicides. Both weeds and crops will grow more slowly with less nitrogen, but the relative effectiveness of reduced herbicide doses is not known and will need to be integrated with sowing rate. The effectiveness would be enhanced if a mixture component with high early weed-competitive value was included. In distilling, wheat acts as a substrate for malt enzymes, so asynchrony in germination is not problematic. We need to assemble the key phenotypic traits of these interactions, determine their genotypic basis and devise tools to design optimum mixtures for achieving the desired environmental, grower and end-user objectives. Overall more litres of spirit would be produced for the same or lower inputs, reducing the carbon footprint on the farm through fewer inputs and field operations and at the distillery by optimising the quality and consistency of supply.

At present, mixture components are selected by their performance as individual varieties and mixtures are generally reconstituted prior to each sowing. There is thus a need to establish and predict how varieties perform and interact within mixtures, to determine compositional changes if the mixture is to be re-sown, or to give practical agronomic recommendations on optimal mixture composition. Mixtures increase the stability of enhanced yield response and disease reduction and do not require homogeneous blending as evidence from winter barley shows that ‘patchy’ mixtures work as well if not better than homogeneously mixed seed (Newton & Guy, 2009).

Varieties selected with specific desirable traits, particularly high grain and alcohol yields and disease suppression – can be most effectively exploited in mixtures/blends with other high quality components which will compensate for deficiencies. Such crops will be more resilient, substantially reducing risk in developing lower input crop production systems to reduce environmental impact and carbon footprint. The inherent synergies associated with mixtures/blends - enhanced disease control and better resource utilisation – would result in further improvement of yield and profitability, as well as stability, and can be detected as general and specific combining ability effects. Additionally, distilling performance would be improved through the deployment, as mixture components, of high quality varieties that would not be cultivated in monoculture for agronomic reasons. Less grain would be required for a given alcohol production, thus reducing energy requirement and waste for disposal and providing environmental as well as economic benefit to the distilling industry.

Specific objectives were:

1) to develop a method of predicting mixture component performance using standard tester varieties in a multi-site 2-component mixture series and to compare the effectiveness of this with selection based on performance in monoculture.

2) to develop a commercially-relevant stability test for yield, grain quality and disease control in a mixture across multi-site farm crops, and demonstrate whether wheat mixtures could show greater consistency across sites in addition to enhanced performance.

For this initial study, distilling quality parameters would largely be predicted using NIR. This provides an opportunity to compare the effectiveness of a calibration developed against a standard laboratory analysis with one developed for intake testing in a specific distillery test series.

Materials and Methods

Multi-site trials

Field trials were grown at four sites: Tibbermore (Perth), Cauldshiel (East Lothian), Swinton (Berwickshire) and SCRI (Dundee). A set of 20 entries were common to all trials (Table 1a). The varieties Alchemy, Glasgow, Istabraq, Zebedee, Robigus and Monty were all grown as monocultures and the other 5 in an equal proportion mixture with Alchemy and similarly with Glasgow. The remaining entries were four four-component and one six-component equal proportion mixtures (Table 1a). Two treatments were applied, a full fungicide programme and a minimal or no fungicide programme. The trial was laid out as a split-plot design, with fungicide application as the main plot treatment and monocultures or mixture as the split-plot treatment. At the Tibbermore, Cauldshield and Swinton sites plots measured 2.0 x 12.0 and there were two replicates. At the SCRI site plots measured 6.0 x 1.55 m and there were three replicates. At the SCRI site there were 24 more trial entries and two nitrogen treatments, a ‘full’ rate of 140 kg/ha and 80 kg/ha. The additional trial entries were Riband, Consort, Nijinsky and Ambrosia, and these were tested in equal proportion pairs with Alchemy and with Glasgow as for the other varieties. In addition there was a three-component series of some pairs of the first six varieties with Riband, Consort and Robigus, four more four-component mixtures, and a mixture of all the varieties. Two of the latter were re-sown from mixtures harvested the previous year and an additional three Glasgow pairs were also re-sown from harvested mixtures (Table 1b).

Table 1. Varieties and mixtures included in trials

Variety	**Type**
a) <u>Entries in Multi-site Trial</u>:	
Alchemy	Monoculture
Glasgow	Monoculture
Istabraq	Monoculture
Zebedee	Monoculture
Robigus	Monoculture
Monty	Monoculture
Alchemy+Glasgow	Tester pair 1/2
Alchemy+Istabraq	Tester pair 1
Alchemy+Zebedee	Tester pair 1
Alchemy+Robigus	Tester pair 1
Alchemy+Monty	Tester pair 1
Glasgow+Istabraq	Tester pair 2
Glasgow+Zebedee	Tester pair 2
Glasgow+Robigus	Tester pair 2
Glasgow+Monty	Tester pair 2
Glasgow+Istabraq+Alchemy +Robigus+Monty+Zebedee	Six-component
Glasgow+Istabraq+Alchemy+Robigus	Four-component
Glasgow+Istabraq+Alchemy+Monty	Four-component
Glasgow+Istabraq+Alchemy+Zebedee	Four-component
Glasgow+Zebedee+Robigus+Monty	Four-component
b) <u>Additional entries at SCRI site</u>:	
Riband	Monoculture
Consort	Monoculture
Nijinsky	Monoculture
Alchemy+Riband	Tester pair 1
Alchemy+Consort	Tester pair 1
Alchemy+Nijinsky	Tester pair 1
Glasgow+Riband	Tester pair 2
Glasgow+Consort	Tester pair 2
Glasgow+Nijinsky	Tester pair 2
Riband+Consort+Robigus+Nijinsky	SCRI extra Four-component
Riband+Consort+Robigus+Nijinsky +Glasgow+Istabraq+Alchemy +Robigus+Monty+Zebedee	Ten-component

Glasgow+Istabraq+Consort	Consort three-component	
Glasgow+Zebedee+Consort	Consort three-component	
Glasgow+Alchemy+Consort	Consort three-component	
Glasgow+Istabraq+Riband	Riband three-component	
Glasgow+Zebedee+Riband	Riband three-component	
Glasgow+Alchemy+Riband	Riband three-component	
Glasgow+Istabraq+Robigus	Robigus three-component	
Glasgow+Zebedee+Robigus	Robigus three-component	
Glasgow+Alchemy+Robigus	Robigus three-component	
Glasgow+Istabraq+Zebedee	Three-component	
Alchemy+Glasgow	Tester pair 1/2	Resown
Glasgow+Istabraq	Tester pair 2	Resown
Glasgow+Zebedee	Tester pair 2	Resown
Glasgow+Istabraq+Alchemy+Zebedee	Four-component	Resown
Ambrosia	Monoculture	
Ambrosia+Consort+Deben+Robigus	Four-component	Resown
Ambrosia+Consort+Nijinsky+Robigus	Four-component	

Trials were sown using standard procedures, harvested and dried to constant moisture and weighed. Any disease observed was recorded using standard trial protocols. Following harvest, grain from each plot was assessed for a range of parameters, utilising NIR calibrations on an Infratec 1241 grain analyser (FOSS, Sweden). This type of machine uses a restricted range of wavelengths in the very near infra-red spectrum for transmission through whole grain samples (Wilson, 1993). Nitrogen and moisture contents were determined by standard calibrations (Lockyer & Wardlaw, 2008) and calibrations for hardess and starch content were also provided by the supplier of the equipment. Predicted Spirit Yield (PSY) was determined by a calibration that explains around 80% of the variation in alcohol yield (Sylvester-Bradley & Kindred, 2008), as measured by the method of Agu *et al*. (2006). The grain dimensions, mean length and mean width were determined using a Marvin digital seed analyser (GTA Sensorik GmbH) as described by Swanston *et al*. 2005. As grain number is counted during this process, weighing the sample beforehand permits thousand grain weight (TGW) to be determined. The ratio of mean grain length to width (L:W) was also calculated.

The large SCRI trial was analysed using analysis of variance, or a more complicated model can be fitted using REML to test for significant spatial effects across the area of the trial. These spatial effects include either local correlation effects (modelled by an autoregressive error process for each of beds and plots) or a larger scale effect modelled using random effects of bed and/or plot numbers. A likelihood ratio test based on the deviance of each model is used to identify whether the split-plot model is sufficient and, if not, to select the best spatial model. Once the best spatial model was identified, tests for the significance of the fixed effects variety, fungicide and their interaction were carries out. A series of contrasts were calculated from the variety means to test the significance of particular comparisons, such as monocultures versus mixtures, monocultures versus two-way mixtures, balanced so that each variety has equal weight in both comparisons, monocultures versus mixtures for specific comparisons of interest such as the average of Alchemy and Glasgow monoculture compared to their two-component mixture. The standard error of the contrasts was calculated from the residual mean square from the best spatial model.

The multi-site trial was a similar design with two replicates used at three other sites, using a subset of the variety mixtures and all three replicates from the SCRI trial for this subset. An over-sites analysis was conducted using REML, allowing the residual variation to differ among the sites where this proved a significantly better fit to the data. Spatial effects as described above were not investigated. This over-sites analysis allowed the assessment of site by variety interactions and contrasts were explored as for the previous trial. Only a single replicate was assayed for PSY from the Cauldshiel and Perth sites so these were analysed separately.

Yield sensitivity analysis

Yield sensitivity was determined from the linear regression of grain yield for each variety and mixture against a site mean yield. In this case, each site or sub-site was represented by the experimental blocks (two per trials) of fungicide treatments (high and low inputs) at each trial site (four sites). The regression procedure was based on the method of Finlay & Wilkinson (1963). Sub-site yields were simply the mean of each replicate block for each site and treatment combination. The average yield for each experimental block in each season provided a wide range of 'sub-site' means with which to examine variability in grain yield. The regression coefficient for each variety or mixture was used as the measure of sensitivity. A regression coefficient close to 1.0 indicates that grain yield changes parallel to changes in site yield; a coefficient above 1.1 indicates that grain yield responds strongly to an increase in site yield, whilst a value below 0.9 indicates a less sensitivity to changes in site conditions.

For each variety or mixture, the combined values of yield and yield sensitivity will indicate its overall performance in response to changes in sites or treatments.

Sampling of commercial sites

Data was recorded at intake, from 10 commercial crops in 2007 and 12 in 2008. Samples were assessed for alcohol yield, using the NIR calibration employed at the distillery for intake samples. As this was designed to assess the potential spirit yield from the sample rather than to compare the genetic potential of trial entries, spirit yields were given on an 'as is' basis. Representative sub-samples were also sent to SCRI for analysis, using the NIR calibration described above and results were adjusted to an 'as is' basis, from the moisture contents of the samples. This enabled comparison between the two calibrations, to ensure that the data being obtained from trials would be relevant to that obtained from the distillery intake laboratory.

Results

Multi-site trials

Trials at all four sites were carried out successfully. Disease levels were very low apart from an uneven net blotch infection on rep 2 full fungicide and rep 1 low fungicide at the Tibbermore site. The mean yield figures for all sites are shown in figure 1. There was significant variation between varieties/mixtures, sites and fungicides for yield, thousand grain weight (TGW) and predicted spirit yield (PSY). Monocultures varied in mean yield (i.e. over sites) from 8.71 to 9.62 t/ha (~9%), 2-component mixtures from 8.80 to 9.35 (~6%), and the complex mixtures from 8.89 to 9.12 (~2.5%), but the means of each group did not differ significantly from each other. Monocultures varied in TGW from 43.15 to 50.19 t/ha (~14%), 2-component mixtures from 44.78 to 49.89 (~10%), and the complex mixtures from 46.27 to 48.14 (~4%), but again the means of each group did not differ significantly from each other. The PSY data for sites other than SCRI gave no significant interactions at any level, but at the SCRI site with more replication, small but significant interactions were observed (see below). However, this does mean that PSY values of mixtures were, generally, the mean of the components, enabling varieties with high alcohol yield to be deployed to enhance the alcohol yield of mixtures.

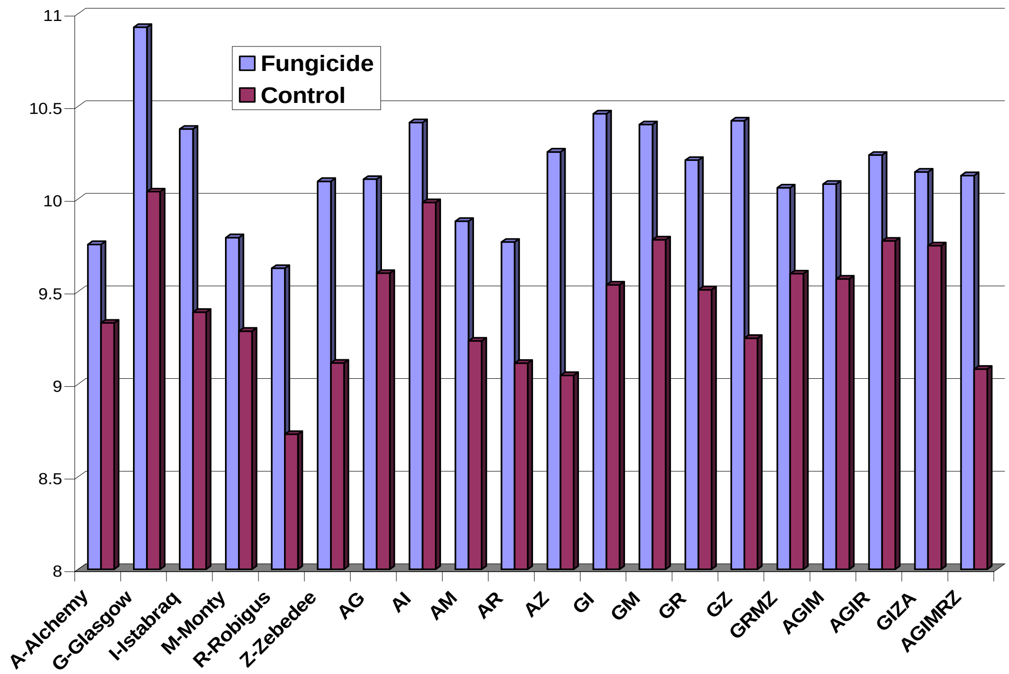

Figure 1. Mean yield of varieties and mixtures across sites for fungicide treated and untreated.

Only the SCRI site produced any significant yield changes for mixtures, the two-component mixtures giving 6% more yield than the mean of the monocultures and the four-component mixtures 8% more. For thousand grain weight the only significant interaction was for two-component mixtures at the Swinton site where there was a negative interaction of -1.7%, and the Glasgow-Monty mixture gave a significant increase of 5.2% at the Perth site.

Ranking the monocultures showed considerable variation in their yield performance between sites (Tables 2 and 3). The sites also showed very different mean yield and yield range, the latter varying from 0.6t/ha at Cauldshiel to 3.61t/ha at SCRI. Variance in the performance of Robigus across the sites was notable in contributing to this range. Glasgow and Istabraq always ranked either first, second or third and Alchemy either ranked fourth or fifth across all sites. However, Monty ranked sixth at Cauldshiel and Tibbermore whereas at SCRI it was second. By contrast Robigus ranked sixth at SCRI and Swinton whereas it ranked second at Caulshiel. Finally, Zebedee ranked first at Swinton but fourth or fifth at the other sites. Clearly some varieties exhibited considerable instability of yield. The mixtures by contrast showed less instability in rank order. The most complex mixture was the lowest ranking at three of the four sites. The GIZA and AGIR mixtures performed best and with the exception of GIZA at Swinton, were very stable.

Table 2. Mean monoculture and mixture yields at the four sites.

Cauldshiel		**Perth**		**SCRI**		**Swinton**	
Entry	**Yield**	**Entry**	**Yield**	**Entry**	**Yield**	**Entry**	**Yield**
M-Monty	11.10	AM	7.56	R-Robigus	6.56	AM	9.82
AM	11.25	M-Monty	7.71	AR	7.74	AG	9.94
AZ	11.26	A-Alchemy	7.80	GR	8.42	R-Robigus	10.11
GRMZ	11.26	Z-Zebedee	7.85	AGIMRZ	8.51	GM	10.18
GM	11.27	AGIMRZ	8.05	A-Alchemy	8.55	AZ	10.21
Z-Zebedee	11.33	GI	8.05	AZ	8.60	AR	10.29
AGIM	11.38	GRMZ	8.08	Z-Zebedee	8.73	A-Alchemy	10.31
GZ	11.48	AI	8.14	I-Istabraq	8.74	AGIMRZ	10.37
AGIMRZ	11.49	AR	8.15	M-Monty	8.94	AGIZ	10.39
AGIZ	11.49	GZ	8.18	AG	9.23	M-Monty	10.41
AG	11.51	AGIM	8.24	AGIM	9.23	AGIM	10.45
A-Alchemy	11.52	AGIR	8.33	GZ	9.23	GZ	10.45
G-Glasgow	11.55	AGIZ	8.33	GM	9.45	G-Glasgow	10.46
AR	11.58	R-Robigus	8.36	GRMZ	9.50	I-Istabraq	10.46
AI	11.62	AZ	8.53	AGIR	9.56	GRMZ	10.48
GR	11.63	I-Istabraq	8.57	AGIZ	9.57	AGIR	10.50
AGIR	11.63	AG	8.73	AM	9.60	Z-Zebedee	10.51
GI	11.67	GR	8.84	GI	9.70	GR	10.55
R-Robigus	11.68	GM	9.47	G-Glasgow	10.16	GI	10.57
I-Istabraq	11.76	G-Glasgow	9.76	AI	10.17	AI	10.85

Table 3. Yield ranking of varieties and mixtures across sites.

Varieties	Cauldshiel	Perth	SCRI	Swinton
A-Alchemy	4	5	5	5
G-Glasgow	3	1	1	3
I-Istabraq	1	2	3	2
M-Monty	**<u>6</u>**	**<u>6</u>**	***2***	4
R-Robigus	***2***	3	**<u>6</u>**	**<u>6</u>**
Z-Zebedee	**<u>5</u>**	4	4	***1***
Mixtures				
AGIMRZ	3	5	5	5
GRMZ	5	4	3	2
AGIM	4	3	4	3
GIZA	2	1	1	4
AGIR	1	2	2	1

Multi-site trials : stability analyses

Individual varieties were both the highest yielding (Glasgow, 10.48 t ha-1) and lowest yielding (Robigus, 9.18 t ha-1) crops when compared across all sites and treatments (Figure 1). The two-component mixtures: Alchemy-Istabraq, Glasgow-Monty and Glasgow-Istabraq along with the four-component mixture Alchemy-Glasgow-Istabraq-Zebedee all yield at least 10.0 t ha-1, whilst the two-component mixtures: Alchemy-Robigus and Alchemy-Monty along with the six-component mixture yield less than 9.6 t ha-1. Figure 2 illustrates the wide spread in yield of the individual varieties and the much narrower spreads of the mixtures, especially the four-component mixtures.

Yield sensitivity across all sites and treatments varied considerably between varieties, and also mixtures (Figure 2). Three varieties: Robigus, Zebedee and Alchemy had the highest sensitivity scores (above 1.2), indicating a relatively strong response to increasing mean site yield. By contrast, Glasgow and several of the two-component mixtures had a low sensitivity score (less than 0.9), indicating a relatively shallow response to increasing site fertility. Across all variety and mixtures there was a significant negative association between yield sensitivity and yield (R2 = 0.39, *F* significance = 0.003). In these data, at least, there is evidence for higher yielding crops to be associated with low sensitivity to changes in sites conditions i.e. a shallow response across sites and treatments. This contrasts with observations in spring barley where high yielding varieties and mixtures have been associated with strong responses to increasing site yield potential (Hoad and Wilson, unpublished).

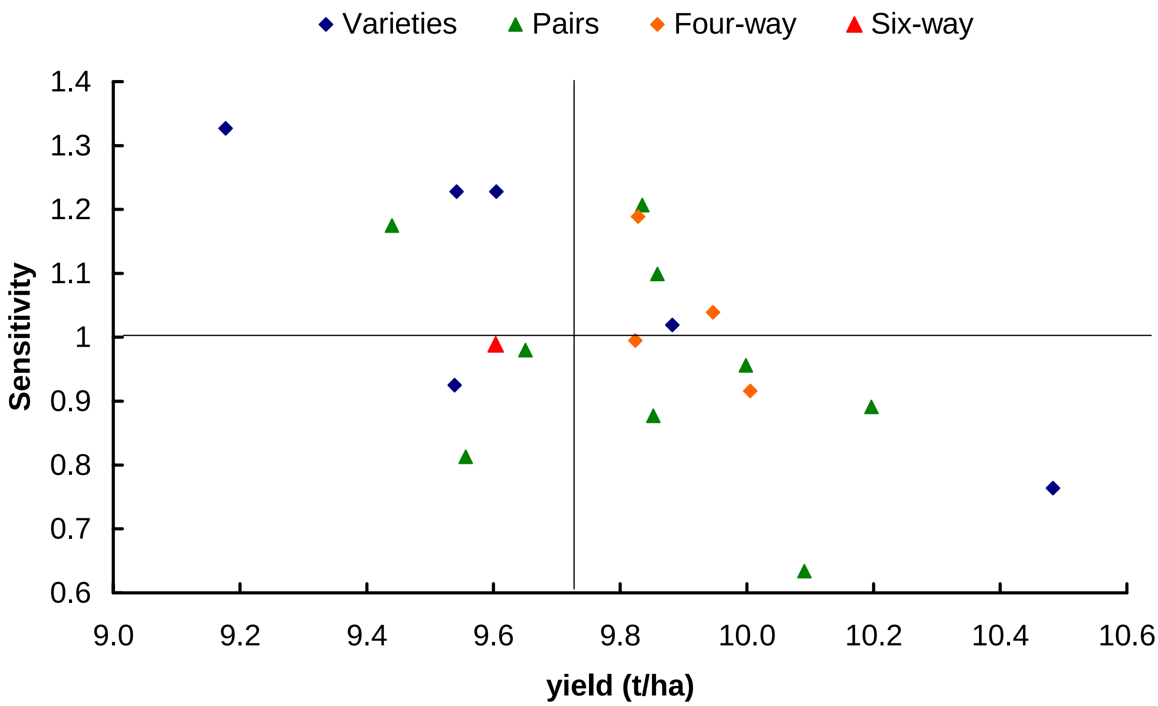

Figure 2. Yield sensitivity across all four sites and treatments for varieties mixtures with different complexity levels.

SCRI site nitrogen multi-entry trial

In the 48 entry trial the nitrogen and variety / mixture entry differences were highly significant but the fungicide effect was not (very little disease). There were significant spatial effects in this trial, and correction for these reduced the residual mean-square for the yield from 0.51 to 0.26 for example. The yield, TGW and PSY data range of the mixtures compared with monocultures showed a similar reduction to that in the multi-site trial. Comparing the same mixtures and monocultures, the monoculture range was ~28%, that of the pairs 17% and the complex mixtures ~7%.

Comparing all the mixtures with the mean values of their constituent monocultures, significant increases in yield were observed both with (3.7%) and without (3.1%) fungicide for the high nitrogen but not the low nitrogen treatment. Analysing only the two tester pair mixture series together (all varieties with Glasgow and all varieties with Alchemy) and the balanced 3-component mixtures, these mixtures yielded significantly more only for the high fungicide high nitrogen interaction, 2.9% and 2.8% respectively. Analysing the Alchemy tester pair series alone, this was still significant (2.6%) as was an individual mixture, Glasgow-Consort (7.4%).

Under the high fungicide treatment the mixtures gave significantly higher TGW at both low (2.8%) and high (2.4%) nitrogen treatments but no differences under low fungicide treatment. The mixture pairs with Glasgow showed similarly significant effects, 3.8% and 3.0% respectively. Alchemy-Riband, Glasgow-Istabraq, Glasgow-Consort and Glasgow-Zebedee all showed similar levels of significantly greater TGWs than their expected means under high fungicide low nitrogen treatments, and Alchemy-Istabraq, Glasgow-Consort and Glasgow-Riband showed significantly greater TGW for various other treatment combinations.

Comparing all the mixtures with the monocultures, significant increases in PSY were observed with fungicide and high nitrogen (0.4%) and without fungicide with low nitrogen (0.4%). Under low fungicide, low nitrogen Alchemy-Robigus, Glasgow-Robigus and Glasgow-Nijinsky all showed significant increase in PSY, 1.1%, 0.95 and 1.1% respectively. Similar increases were recorded for Alchemy-Istabraq, Alchemy-Nijinski and Glasgow-Zebedee for particular treatment combinations. Even though PSY increases of around 1% seem small, that equates to about four litres of alcohol per tonne, and with half a million tonnes of wheat used for whisky production alone in Scotland, that is two million litres more of alcohol.

The tester pair series were able to detect where mixtures gave significant interactions overall with some fungicide-nitrogen combinations, particularly high nitrogen. However, effects were small and whilst individual pairs also gave some significant interactions, overall consistent patterns could not be discerned.

Re-sown mixtures were compared with their newly-constituted mixtures and were shown to give similar benefits (Table 4).

Table 4. Comparison of yield, TGW and PSY data for new and re-sown mixtures compared with their respective monoculture means.

	DWt New	DWt Resown	TGW New	TGW Resown	PSY New	PSY Resown
Glasgow+ Zebedee	5.9%	5.5%	4.0%	-0.1%	+0.4%	-0.3%
Alchemy+Glasgow	5.9%	1.3%	-0.2%	-0.4%	+0.1%	+0.1%
Glasgow+Istabraq	4.8%	4.2%	2.6%	0.3%	-0.0%	-0.0%
GIZA	2.5%	6.5%	1.5%	2.0%	-0.2%	+0.0%

Commercial crops

Ten commercial mixture crops were sampled in 2007 and 12 in 2008 (Figure 3). The mean PSY in 2007 was 444 l/t with a 3.5% variation and 453 l/t in 2008 with a 5.6% variation. These data were derived using the calibration described above, with the method of Agu et al. (2006) as the standard for alcohol yield and NIR spectra obtained from grain samples that had generally been dried after harvest. In addition, Predicted Alcohol Yields were recorded on a dry weight basis. The utility of the calibration was tested against predicted alcohol yields derived at distillery intake for some of the samples (Table 5). These predictions were derived for samples obtained from farmers (i.e. with a much wider range of moisture) and based on distillery performance. Additionally alcohol yields were recorded on an 'as is' basis, to evaluate the sample, as offered for sale, rather than at optimal moisture content. The NIR values obtained at the distillery generally averaged 4% higher when the laboratory data were adjusted for moisture content, though this was skewed by the high moisture in the Muir Freelands sample. This would have been detected as an 'outlier' value by the calibration on the laboratory-based machine and may therefore have affected the accuracy of prediction. The higher PSY from Caledonian Properties crops was higher than average in both instances (Table 5).

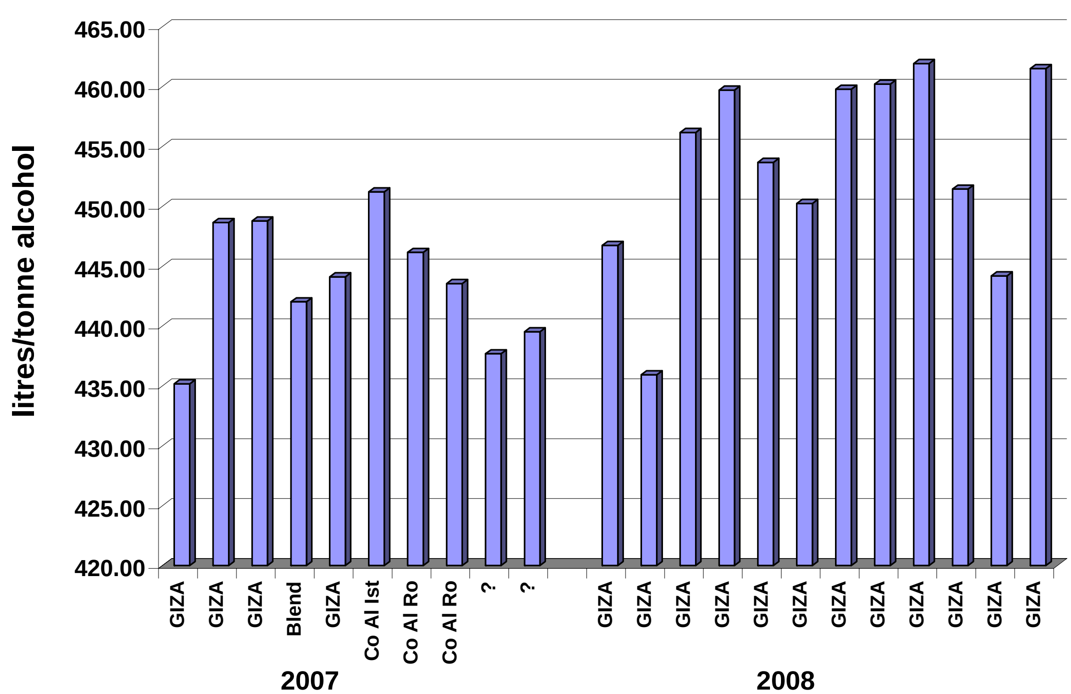

Figure 3. Predicted spirit yield using NIR method (l/t dry base) from commercial fields sampled in 2007 and 2008.

Table 5. Spirit yield prediction comparison of NIR and distillery data.

Farm	**Distillery PSY**	**NIR PSY**	**Moisture adjusted**	**Percentage difference**
Middlemass Northrig	392	*447*	383	*-12.3%/*2.3%
Muir Freelands	389	*454*	352	*-14.3%/*10.5%
Caledonian Properties	397	*460*	390	*-13.7%/*1.8%
Caledonian Properties	397	*460*	392	*-13.7%/*1.3%
Keir & Cawder	390	*444*	374	*-12.2%/*4.3%
Mean	**393**	***453***	**378**	***-13.2%/*4%**

Discussion

Previous work on cereal variety mixtures has demonstrated considerable yield increases in barley, up to 16% (Newton & Guy, 2009a) and disease reductions of up to 75% (Newton *et al*., 2008a) in winter crops, though lesser increases in spring crops (Newton *et al*., 2009b). Winter wheat trials with mixtures had given positive interactions for grain and alcohol yield (Swanston *et al*., 2005) and all mixtures tend to show greater yield stability (Newton *et al*., 2009b). On the basis of this evidence the above trials were initiated in conjunction with WN Windsay and their contract growers.

Overall there were some benefits from growing varieties of winter wheat for distilling in mixtures, and there were no negative effects. This was reflected at a detailed level too where very few individual mixture-site-fungicide-nitrogen interactions were negative but more were positive. Stability in yield was demonstrated in the mixtures and probably represents the main reason for recommending variety mixtures for distilling. Thus the unpredictable performance characteristics of varieties such as Alchemy which performed well in 2005 but not 2006 (Swanston and Newton, 2009), and Glasgow which we understand performed well in 2006 but less well in some areas in 2007, would not be a problem in mixtures and consistency of yield and quality could be assured.

The implications of the sensitivity analysis are that some types of high yielding variety mixtures (or variety) could be selected for a better consistency in yield across site conditions. The addition of other factors such as soil residual nitrogen or fertiliser nitrogen would be a useful way to develop the understanding of yield sensitivity. Greater consistency in yield across sites and seasons would also have implications for distilling quality. Although a good distilling variety will give higher alcohol yield at any given protein level than a poorer quality variety (Kindred *et al*., 2008), there is a strong negative association between alcohol yield and grain protein (Kindred *et al*., 2008; Swanston *et al*., 2007). Unpredictable variations in yield at given nitrogen applications will have similarly unpredictable effects on protein content and, consequently, on alcohol yield. The limited data and the fact spirit yields were predicted rather than actual measurements, precluded the application of sensitivity analysis to the alcohol yield data, but this would be a very valuable exercise in a future project.

Comparison with commercial crops demonstrated predicted spirit yields that would certainly be acceptable for distillery intake. Comparison of data adjusted for moisture content indicated that the type of calibration that might be mounted on an Infratec in a plant breeder's laboratory would be suitable in screening lines for acceptability at distillery intake. Another trial which was not part of this project demonstrated that at a large plot scale (160m x 6m plots) wheat mixtures performed similarly whether homogeneously mixed or sown in various degrees of unevenness or patchiness (A.C. Newton, unpublished data). Anecdotal evidence indicated that unevenness of ripening could occasionally be a problem in some crops, though no samples or trial data, in the experiments described here, encountered this problem.

High input conditions (fungicide and nitrogen) at the SCRI site showed most benefit from mixtures, indicating the potential for synergies to be to be obtained given the right agronomy. Even without optimised agronomy, mixtures conferred stability thus reducing risk for growers.

Experience has demonstrated that in some seasons, mixtures of cereals can produce minimal benefits whilst in others they can be much greater. Where disease is present at moderate or high levels mixtures can be particularly beneficial. In this work we reported the results from a single season. It is likely that further trials, particularly optimising the agronomy, would extend the range of beneficial interactions, particularly for disease control. Given the yield increases, reduced inputs of some treatments and increased predicted spirit yield, a full economic appraisal would at worst show no net cost of using mixtures, and at best some increased profit margins. Costing reduced risk would further enhance the benefits.

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Declaration on data storage

(to be submitted along with final report)

Along with HGCA final reports, you are required to submit this declaration covering the location of all project records and archiving procedures employed. You are also asked to detail the format in which project records are stored to satisfy us that data are retained in a form that ensures integrity and security, and prevents unauthorised modification. This declaration commits you to store data for a minimum of seven years.

Data sets (add further rows as required)**:**

Name	Format	Location
BRIDGES2008_4sites.xls	Microsoft Excel	BBSRC BITS IT AGRINET Archiving Service Group=NewtonSCRI, Archive=BRIDGES, ID=20506017
BRIDGES_4sitesStats.doc	Microsoft Word	BBSRC BITS IT AGRINET Archiving Service Group=NewtonSCRI, Archive=BRIDGES, ID=20506017
BRIDGES_DZ.xls	Microsoft Excel	BBSRC BITS IT AGRINET Archiving Service Group=NewtonSCRI, Archive=BRIDGES, ID=20506017
BRIDGES_DZStats.doc	Microsoft Word	BBSRC BITS IT AGRINET Archiving Service Group=NewtonSCRI, Archive=BRIDGES, ID=20506017

I hereby confirm that all the information provided above is correct and the procedures followed will ensure that data is retained in an accessible format for a minimum of seven years as required for HGCA contracts issued from 2007 onwards. I understand that, as outlined in Section 7 of the Guidance notes for HGCA final reports, HGCA may require me to provide data in response to reasonable third party requests and that I will receive a fee for responding to such requests.

Lead scientist name: Dr Adrian C Newton

Lead scientist signature: **Date:** 27th April 2009

Signee name: Dr Neil Hattersley

Signee signature: **Date:** 27th April 2009