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Adaptive winter wheat populations: development, genetic characterisation and application

by

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1 ABSTRACT

An increasingly fluctuating global climate is creating mounting problems for production in agricultural systems. One possible way to buffer these changes is with the use of genetically diverse composite cross populations. Here we demonstrate in replicated field trials that composite cross populations of winter wheat when grown in organic conditions had a similar yield to currently used pure lines, but had higher yield stability than the pure lines. When grown under high input conditions the populations maintained their stability, but yielded significantly lower than the pure line varieties, indicating that the populations are potentially more suitable to low-input and organic than to conventional systems. However, in separate pseudoreplicated on-farm trials conducted across the UK the improved stability shown in the replicated field trials was not repeatable.

The replicated field trials demonstrated that both populations and (complex) variety mixtures are more or less equivalent with regard to their agronomic performance. Preference for either populations or mixtures is therefore likely to follow other criteria than those based purely on agronomic performance.

The populations were tested to investigate the level of adaptation that may occur when grown continuously at the same specific sites for a number of years. The populations did not adapt to the cropping conditions under which they were grown; this was evident both from molecular data and from comprehensive field trials. Yearly fluctuations in weather conditions are likely to have counteracted any adaptation to the site-specific factors associated with cropping management and soil conditions.

The suitability of the populations for four different end uses were tested, these included bread making, malting, distilling and animal feed. For each end use the one or two most appropriate of the following populations were tested: a high yield population (YCCP), a high baking quality population (QCCP) and an all-rounder population (YQCCP). None of the populations were any more beneficial than current varieties for distilling or malting, however both the YCCP and YQCCP were found to be suitable for use as animal feed. In micronutrient tests the values observed for the population grain tested were generally comparable to values previously reported for winter wheat. On the basis of various agronomic and quality performance indicators, as well as marketability to end-users, the QCCP is seen as the most promising population among the three tested populations, and was singled out as being particular favourable for bread making.

2 SUMMARY

2.1 Introduction

Modern agricultural systems are under ever increasing pressure to produce more food for a growing population, whilst at the same time facing the challenges brought about from a changing global climate and the pressure to be more environmentally friendly and sustainable (FAO, 2009). It has been proposed that to combat these issues the focus should be on greater intensification of production concurrently with an attempt to reduce pressure on the environment, i.e. the concept of sustainable intensification (Garnett et al. 2013; Godfray & Garnett 2014).

The focus of much research and development in conventional wheat production is still predominantly on the use of monocultures of individual varieties selected for production under high inputs. However, when grown under low input conditions such varieties have considerable yield and quality shortfalls. This reduced productivity is the result of the interaction between varieties that have not been selected for production under low-input conditions, and the field environments that are often highly variable in the absence of a range of inputs (Jones et al, 2010). Most importantly, the predictions for increased climatic variation, together with higher prices for inputs will place additional demands on the crops we grow in all systems.

A significant expansion of the genetic variability of the wheat crop would help to buffer such environmental variation. Therefore one alternative to the use of such monocultures is to employ the concept of evolutionary breeding (Suneson 1956). Evolutionary breeding using Composite Cross Populations (CCP) involves the inter-crossing of appropriate parents in many different bi-parental and higher order combinations. The resulting hybrids are then bulked together for propagation and exposed to natural selection in the environments in which they are then grown. The locality of where the seeds are grown are varied over a number of growing seasons meaning that the large amount of genetic variation generated by the crosses is maintained. The end result should be populations that adapt to localities while retaining genetic variation and hence adaptability for further change.

This is a potential approach for generating wheat crops which perform stably under a wider range of climatic conditions, not only in terms of their yield but also in terms of their quality, while allowing a major reduction in synthetic inputs (Döring et al*.,* 2009). Because of the buffering and compensation effects within a CCP, they are predicted to show increased stability in comparison to genetically uniform monocultures. Indeed, such stabilizing effects in cereals exhibiting increased genetic diversity have been documented in a number of cases (Wolfe, 2000; Wolfe, 2001; Döring et al., 2010). The agronomic capabilities of the three CCP which are the focus of this current study, have previously been established (Döring et al., 2009) and they have been shown in field trials to perform better than the means of their individual parent varieties for a range of characters including grain yield (Wolfe, 2006).

Evolutionary breeding may embody agronomic advantages which confer improved stability of yield in variable environmental conditions, but this will not be sufficient incentive for farmers to adopt the use of

CCP at a wide scale. Therefore, it is also important to determine the processing and end-use capabilities of wheat CCP, which will be critical in determining whether there are likely to be potential markets, and therefore commercial benefit, for farmers in growing populations. In particular, bread making is a major end-use of the UK wheat crop, with approximately 42% by area being sown to varieties appropriate for bread- or biscuit-making, and 60% of flour produced from these used in the production of bread products (nabim, 2013). With unpredictable climate conditions making it increasingly difficult for UK farmers to consistently produce wheat with the qualities required by the milling industry, there is a serious need for quality wheat crops with higher resilience, which can meet milling industry specifications under a wider range of climatic conditions and a lower-input regime.

In a five year study we evaluated the performance of three existing CCP that had been generated as part of the Defra-funded project 'Wheat Breeding' – AR914 (Döring et al*.,* 2009). One of the CCP was generated by crossing parents with high yield potential (YCCP), another by crossing parents with good milling potential/end use quality (QCCP) and another comprising both parent sets (YQCCP; see section 3.2.1). In the current study, core field trials were conducted over four project years at four main trial sites in the UK to quantify the yield, quality and stability of performance of the CCP in comparison to: one another; their respective parental mixtures, and five standard appropriate nabim group varieties. In a separate experiment, on farm field trials at more than 20 sites were established to determine the performance and stability of performance of the CCP across a range of climatic conditions, relative to control varieties. Using the seed from the CCP grown in the core field trials, genotypic analyses were conducted using molecular markers to help determine the types and extent of genotypic evolution occurring as an indicator of potential adaptation of the CCP to the four different field sites. Further work was conducted to estimate the relationship between the genetic and phenotypic contributions of the individual parent varieties to the YQCCP. Additional investigations were conducted to investigate the ability of the CCP, and mixtures of the parent varieties of the CCP, to control disease and stabilise yields in fungicide treated and untreated plots. Separate studies were conducted in order to test whether it was possible to enhance specific performance characteristics of the CCP by either including 'extra' parental material or by methods of mass selection. Finally, investigations were conducted into factors relating to the commercial potential of four possible end uses for the CCP; bread making, malting, distilling and animal feed.

2.2 Materials and methods

The project was divided into nine work packages (WP). In replicated field trials (WP1) the performance and stability of performance of the three CCP were compared at four different locations over four years. Their performance was compared against each other as well as against varietal mixtures and against five standard appropriate nabim group varieties. In complementary experiments, on farm single replicate field trials (WP5) compared the YQCCP, as well as the YCCP and/or the QCCP, with Claire and a farm-specific variety, across a range of climatic conditions (ranging from Northumberland to Cornwall).

Genotypic (WP2) and phenotypic (WP3) analyses were conducted using the grain from the main replicated field trial. Further field trials were conducted on the YQCCP, a varietal mixture (consisting of all the parent varieties of the YQCCP) and a broad selection of modern varieties grown at three different conventional trial sites in plots with or without fungicide treatments, in order to investigate the CCP ability to control disease and stabilise yields (WP5). In additional studies, it was tested whether specific characteristics of the CCP could be improved, either by incorporating new parental material (WP6), which consisted of either Pegassos (bred for low-input growing conditions) or Xi19 (high grain quality), or by non-destructive mass selection methods (WP7), using size, density and colour as practical on farm seed sorting tools. Finally, the possible end markets for the CCP were investigated. The performance of the QCCP and the YQCCP for bread making was assessed, as well as the micronutrient composition of the grain (WP8). The potential of the CCP for malting, distilling and animal feed was also assessed (WP9).

2.2.1 Quantifying the of performance of the CCP in different environments

Field trials were carried out in two organic sites located at (i) Wakelyns Agroforestry (WAF) in Suffolk, and (ii) Sheepdrove Organic Farm (SOF) in Berkshire, as well as two non-organic sites located at (iii) Metfield Hall Farm (MET), adjacent to WAF in Suffolk, and (iv) Morley Farm (MOR), an experimental farm in Norfolk managed by NIAB TAG (see section 3.2.3). In total, six different CCP were investigated – the YCCP, QCCP, and YQCCP – and three male sterile composite cross populations ($YCCP_{ms}$, $QCCP_{ms}$ and $YQCCP_{ms}$), which were generated by artificially hybridising all the parents used to create the above CCP to characterised genetic male sterile lines. Performance comparisons were made between: a) each CCP (Y, Q, YQ, with or without male sterility); b) their respective parents in varietal mixtures (YMix, QMix, YQMix); and c) five standard appropriate nabim group varieties. Field trials were performed from 2003/04 to 2011/12, in each year using CCP and mixture seed that had been harvested in the preceding cropping year, thereby allowing for evolutionary adaptation of the genetically heterogeneous material to site-specific cropping conditions. Here we report the results of the four trial years 2007/08 to 2010/11; results of the previous cropping seasons have been reported elsewhere (Döring et al., 2009; Jones et al., 2010).

In the main trials, the seed of the CCP was always replanted at the same site at which it was harvested. In addition, two series of field trials were carried out to test whether CCP adapt to the conditions under which they are grown. The first of these series was conducted in the last trial year (sowing 2010) and is called “cross-over trial”; here, CCP with a history of having been grown for seven generations at constant sites (from 2003 sowing onwards) were not only grown at their home sites (i.e. main trials at two organic and two conventional sites, see above), but also at all the other trial sites. In the second trial series, called “shuttle trial”, the CCP were either grown at constant sites or at alternating sites, i.e. part of the seed from CCP was sent to another site and planted there; the following generation was then sent back to the site where the CCP had originally come from. For all trials, a variety of different field and post-harvest assessments were made including: establishment, head density, proportion of awned ears, straw height,

lodging, grain yield, Hagberg Falling Number (HFN), grain protein content, hardness, specific weight and Thousand Grain Weight (TGW).

WP5 aimed to complement WP1 trials, by growing the CCP over a very large geographic range on 27 sites between Cornwall and Northumberland (Figure 1). Organic and conventional farms were included in these on-farm trials to further expand the range of growing conditions encountered by the populations. Single replicate field trials compared the YQCCP, as well as the YCCP and/or the QCCP, with Claire and a farm-specific variety. The participating farmers were asked to sow the CCP and Claire in adjacent strips in a field of winter wheat of their own choice; the variety present in this field was recorded and was labelled as the farmers' "own variety"; this served as the additional pure line control variety for the comparison with the CCP. Performance was measured through (pseudo-replicated) assessments of plant height, head density, yield, and grain quality.

Figure 1. Map showing the locations of the participating farms

Within this WP an additional adaptation trial was conducted aimed at testing adaptation of the CCP over time through natural selection. Here the YQCCP that had been grown at one of most northern conventionally managed sites for three generations was distributed to five other farms (two organic and three conventional) in Southern England in the fourth trial year. In addition, seed of a YQCCP that had

been grown at an organic farm in Southern England was sent to the northern conventional site in the same year.

2.2.2 Analysis of genotypic evolution in the CCP

Seed from the YQCCP, which had been grown for eight years consecutively at one of four different experimental sites (see WP1), were sampled over time by collecting seed from the F_5, F_8, and F_{12}. The seeds were germinated and DNA was extracted using standard protocols. This allowed analysis of the extent of genotypic evolution in adaptation of the CCP to the different sites and management regimes. The molecular markers used for genotyping included 20 SSR markers, 15 single nucleotide polymorphism (SNP) markers, and a set of nine "perfect" markers (denoted as 'Perfect set'), that are tightly linked with major genes involved in plant height, vernalisation requirement, photoperiod response, and one marker linked the *1B/1R* chromosome translocation from rye. All markers chosen were selected to provide multiple alleles at each locus, so that chromosome segments in a plant could be attributed to sub-groups of parents or individual parents where unique alleles were carried. The data of the allele composition of individual plants in each population at each site and each generation were analysed using a number of different statistical packages.

2.2.3 Estimating the relationship between genetic and phenotypic contributions of individual parent varieties to the YQCCP

Seed from the field trials of four sites in WP1 were also used in this work package. Because these trials were on sites that differed in their management system, it was possible to investigate whether phenotypic traits of the CCP behave differently under organic and conventional management. Phenotypic traits tested in this work package are highly integrative over multiple genes (e.g. plant height); they reflect the sum of a multitude of complex genetic (and environmental) effects. The phenotypic traits assayed included yield components as well as traits considered to be important in plant competition (such as plant height). Plants at F_7 and F_{11} generations were picked at harvesting stage and bagged before taking phenotypic measurements. Additionally, at generation F_{11} plants were tagged in the field and scored for growth habit and ear emergence. This tagging made it possible to follow individual plants through emergence to harvest. To obtain insights into traits that could be of importance in the adaptation process, an association analysis of phenotypic traits with genetic markers was conducted in the form of a single marker association mapping. However, the aim of this association mapping approach was not to generally identify any loci that are associated with any of the measured phenotypic traits. The aim was particularly to find out about phenotypic properties that could have led to the selection of certain alleles. Thus only loci that were identified as potential candidates of selection were evaluated for associations.

2.2.4 Testing the ability of CCP to control disease and stabilise yields

Disease levels in the YQCCP and a YQ-mix (consisting of each of the 20 YQCCP parental lines mixed together in a 1:1 ratio) were compared to a range of wheat varieties, with differing disease susceptibility profiles, across three disease management programmes. The trials were conducted using a randomised split plot design with three blocks and were conducted over five seasons in commercial fields at three different conventionally managed NIAB TAG sites with contrasting soil types: Caythorpe (Lincolnshire); Morley (Norfolk); and Sutton Scotney (Hampshire). The three disease management programmes consisted of: no treatment; 'disease exclusion' with a high level of foliar fungicide input and a 'reduced input programme' with a lower level of fungicide input (equivalent to normal farm practice). Plots were monitored for disease at three different wheat growth stages and the total yield of each plot recorded.

2.2.5 Enhancing the performance of CCP

In an attempt to enhance specific performance characteristics of the CCP, additional parental material from two lines were incorporated into the three original CCP. This included Pegassos, a variety bred in Germany to yield well in low-input growing conditions, and Xi19, a variety bred by Limagrain UK Ltd. for high grain quality. Pegassos was crossed with each Yield and Quality variety from the original 20 CCP parent lines in a series of two-way crosses. Similarly, Xi19 was crossed with each Quality variety from the CCP parent population. The seed from all Pegassos crosses was harvested and mixed together, as was that from the Xi19 crosses, and a third combination mix of Pegassos and Xi19 was prepared. Seed from these crosses was added to seed from the corresponding CCP (YCCP for Pegassos, QCCP for Xi19 or YQ CCP for the mix of both) in a ratio by weight of 2/3 CCP to 1/3 Xi19 or Pegassos cross (1/6 of each where both crosses were included). Trials of these mixtures were carried out at the four core sites from WP1, with each entry repeated three times in a randomised complete block design. Total yield for each plot was recorded and grain samples were taken for protein content analysis.

Further experiments explored the potential efficacy of three non-destructive mass selection methods; size, density and colour as practical on farm seed sorting tools to create more valuable fractions of grain for marketing and as breeding tools to select for a higher frequency of heritable traits in subsequent generations of the population. Trials were conducted over five growing seasons on two neighbouring farms from the core farm sites described in WP1, WAF (organic) and MET (conventional). In all trials, assessments were made each season for each plot of grain protein content, HFN, TGW and total plot yield.

In the first part of the experiment, grain from the QCCP and YQCCP grown at WAF and MET was subjected to mass selection based on kernel colour, using a Satake Alpha Scan II. The grain was split into dark grains or light grains and compared to a control (unselected CCP). Seed were sown in unreplicated plots at each of the two sites. In subsequent years seed were sown in a randomised complete block trial with three replicates. After each of the four years of the experiment Dark-CCP and

Light-CCP were bulked from their respective blocks, assessments made, and each colour CCP was then subjected to further mass selection, i.e. Dark-CCP were sorted into a darkest and lightest fraction, as were Light-CCP, which formed the seed for the following generation.

In the second part of the experiment, grain from QCCP grown in 2009 at MET, was split into three size fractions (small, medium and large) using a seed dresser. These separate fractions and a control (unselected QCCP) were then drilled in a fully randomised complete block trial, with three replicates, at the MET site. Following harvest, each fraction was again split into small, medium, and large. The largest fraction of the Large-CCP, smallest of Small-CCP, and medium fraction of the Medium-CCP were then analysed for quality parameters and sown again in 2010. The grain was then harvested and again analysed for quality parameters.

In the final part of the experiment, seed was split into three fractions (heavy, medium and light) using a gravity separator. The process of trialling these fractions were identical to those used for the second part of the mass selection experiment.

2.2.6 Investigating possible end markets for the CCP

A programme of baking tests was designed to explore whether flours from the YQCCP and QCCP could produce a range of bread types, which met with industry and consumer standards, and whether they could be viable for farmers to grow as bread wheat. Baking tests were undertaken using industrial and small-scale artisanal methods (this included the Chorleywood method, sourdough and a small-scale yeast-assisted process) in order to assess loaf volume and crumb structure of bread made from QCCP and YQCCP wheat grown at both organic and non-organic farms, in comparison with bread made from standard commercial flour. This was done by subjecting CCP grown on a range of farms to replicated baking tests using a range of baking methodologies, evaluating the quality of the dough and loaves produced according to professional bakers' criteria, and comparing these outcomes with those of commercially marketed flours baked according to the same methodologies. A further objective was to test whether the addition of Yield (Y) parents to the Quality (Q) population would dilute the baking quality of the QCCP. Each baker was therefore sent same-site paired samples of YQCCP and QCCP flours to put through the baking tests and dough and loaf quality evaluations.

Additionally, micronutrient analyses were conducted on the CCP with the aim of investigating the effect of high within-crop genetic diversity on the nutritional value of the wheat crop. Analyses were conducted to determine the concentration of nine minerals: eight of these are nutrients (calcium, magnesium, molybdenum, selenium, iron, zinc, chromium and cobalt); the ninth, cadmium, is a toxin. Grain from the QCCP and YQCCP grown at both organic and non-organic sites was tested, and compared against a pure-line from the same organic site as well as two different pure-lines grown at two further organic and non-organic sites.

Malting analyses were conducted on grain from the QCCP, YCCP and YQCCP, grown over the four project years at the four core farms from WP1, to test their commercial potential for use in brewing. Analyses were conducted by Crisp Malting Ltd., who analysed the following parameters: total nitrogen, soluble nitrogen, enzymes levels, extract, colour assessment, wort viscosity and pH. Distilling analyses were conducted on the YCCP and the YQCCP from three of the four core farms from WP1 (SOF, MET & MOR). The Scottish Whisky Research Institute carried out analyses of moisture content, alcohol yield, residue viscosity, protein content and total nitrogen on 18 wheat samples from project year one. Animal feed quality was measured by analysing the frequency of the 1B/1R translocation was monitored. This translocation has been found to be correlated with reduced amino acid digestibility in animal feeds (Short et al., 2000). Analyses were conducted on the YCCP and YQCCP using different methods over three cropping seasons: 2007-08 (YQCCP only), 2008-09 and 2009-10 (YCCP and YQCCP). In project year one (2007-08) a disease scoring method was used based on testing for susceptibility to a leaf rust isolate which is avirulent on 1B/1R carriers, but virulent on 1B varieties. In years two (2008-09) and three (2009-10), however, a new molecular marker method was developed by Limagrain UK which used TaqMan probes to detect a single nucleotide polymorphism (SNP). This approach gave unambiguous classification of the presence of either the 1BS or 1RS chromosome arms.

2.3 Results

2.3.1 Performance of the CCP in different environments

Yield, quality and stability of performance of CCP

One of the main questions of the project was how wheat CCP perform in agronomic terms in comparison with currently used pedigree varieties. Our results showed that in the conventional system, grain yield was lower in the CCP than in the pure lines, but no significant difference was found in the organic system. This result indicates higher suitability of CCP for organic and low-input systems than for conventional agriculture. Future CCP could be generated with a stronger reliance on high-yield parents; also, the existing CCP could be subjected to a process of (artificial) selection to enrich the population and increase its yield potential. Grain protein content was consistently higher in the CCP than in the pure lines. Thus, if future CCP are based on higher-yielding parents, potential trade-offs with grain protein content would have to be considered carefully.

In addition, the CCP showed a number of advantages over the pure lines during the growing season, namely an increased leaf area index, taller plant height and higher straw mass, indicating that the competitive ability of the wheat crop against weeds was higher in the CCP than in the pedigree varieties. CCP were also characterised by higher yield stability than the tested pure line varieties, and tended to have greater stability of grain protein content.

An alternative way of increasing genetic diversity of a wheat crop in the field is the use of variety mixtures. In this study we trialled complex variety mixes which were equivalent to the CCP in terms of the genetic starting point, but the CCP exhibited much greater genetic diversity than the mixes. Despite this difference in genetic diversity both performed equally for most parameters, e.g. in most cases, no differences between CCP and mixes were observed for grain yield, grain protein content, protein yield, as well as a range of pre-harvest parameters (leaf area index, plant height, fungal diseases).

In additional trials conducted as part of this work package , no effects of adaptation were observed for the CCP or mixtures. Material grown at its home site did not show any significant advantages in terms of grain yields or other parameters over material that had been grown under different conditions for the past few generations.

Participatory farm trials of CCP

A main difference between pure lines and CCP, consistently observed for the three different CCP across environments was greater plant height in the CCP, and an associated tendency of increased lodging, though the extent of lodging was small and did not impact on grain yield. On average, the YQCCP yielded around 10% less than the common control variety Claire. To a large degree this yield difference is likely to reflect the parentage of the YQCCP, which was created by crossing 12 high-quality parents and 9 high-yield parents, whereas Claire is classified as a high-yield type. For the QCCP however, yield differences in comparison to the pure line controls were small and not significant, despite the inclusion of relatively old parents in the QCCP; this may be seen as a result of the identity of the varieties chosen by the farmers. Thus, the highest potential for practical use of CCP is seen in the high-quality QCCP, though consistently low HFN values in the CCP mean that there are some quality limitations. In contrast to expectations, and to results obtained in WP1, stability of yield and of other parameters was not significantly higher in the in the CCP than in the pure lines in the field trials of this WP. Furthermore, the analysis shows that contrary to expectations, the YQCCP was not consistently better than Claire in low yield environments.

As in WP1 and WP2, the WP5 adaptation trial showed no consistent effect that would suggest adaptation to site or management conditions. In the case of the on-farm adaptation trial, the observed results may have been a consequence of differences in seed quality. Reduced seed quality of the North-CON YQCCP may have had a bigger effect on grain yield than adaption to site conditions, as the North-CON YQCCP was also lower in grain yield at the North-CON site than the organic YQCCP from the South. Also, the time of three generations may have been too short for adaptation to take place. Especially in relation to the environmental fluctuations over years within each site, there may not have been a consistent direction of environmental factors influencing the CCP.

2.3.2 Genotypic evolution in the adaptation of CCP

There were no clear signs of differences in selection pressure resulting in changes in allelic composition in the YQCCP between different sites or agronomy regimes. A few specific loci showed significant changes in allele frequency over time but the magnitude and direction of changes of allelic composition was of equal magnitude at each site, suggesting that an adaptation process did take place over time, but that it may not have affected the populations differently at the four sites and instead occurred in the same direction at all sites.

In comparisons of the founding population with the 11th generation of the YQCCP, four loci showed higher than genome-wide changes at all four field sites: *1B1R.1B*, *PpdD1.2D*, *RhtB1.4B* and *RhtD1.4D*. Two loci showed changes at the WAF and SOF sites but not at the MET and MOR sites: *PpdB1L5.2B* and *Xgwm626.6B*. The general phenotypic effects of the alleles of the loci that underwent some form of selection were an increase in plant height over time and delay in ear emergence, suggesting that plant height has been a major driving force in the evolutionary process of the YQCCP.

2.3.3 Genetic and phenotypic contributions of individual parent varieties to the YQCCP

The general pattern that was discovered when looking at the trends over all 7 loci that were identified as undergoing selection was an increase in number of tillers per plant, plant height, grain number, grain weight and a change towards later ear emergence and a more upright growth habit. However, when the effects of each locus were investigated separately, the pattern became much more complex. Therefore, two different potential patterns could be identified from the data: (1) If the phenotypic effects of the locus that has undergone strongest selection, are considered the phenotypic characteristics of adaptation are: increased plant height, reduced grain weight and number, and later ear emergence; however, (2) if the sum of phenotypic effects of all seven loci are considered, the pattern of phenotypic characteristics also shows increased plant height, coupled with increased biomass and later ear emergence, but towards increased grain weight and number.

2.3.4 Ability of CCP to control disease and stabilise yields

In comparison to commercially available wheat varieties, the YQCCP and YQ-mix were ranked consistently highly in terms of their ability to maintain yield in response to disease in all four years of trials. They also rated similarly to those varieties that had the greatest yield tolerance in response to a reduction of their green leaf area by disease. However, they were also typically amongst the lowest yielding, indicating a low yield potential. This may make them appealing to farmers who have timeliness issues regarding fungicide sprays, or are organic or at least less dependent upon high levels of fungicide inputs. Yields for the YQCCP and the YQ-mix appeared to be the most stable, which may appeal to farmers wanting insurance against dramatic yield losses associated with high disease pressure.

2.3.5 Performance of CCP with additional genetic material

The first part of this study investigated the addition of new genetic material into the CCP through crosses with varieties predicted to increase yield (Pegassos) or quality (Xi19). There were significant differences in terms of grain yield and protein content between experimental sites; however within sites there were few significant differences between those CCP which had additional material incorporated and those CCP without this additional material. In essence, the inclusion of this new genetic material did not significantly improve either the yield or grain quality characteristics of any of the CCP.

In further studies mass selection tools were evaluated as on-farm grain processing tools to separate higher quality grain, and investigate as to whether they could be used as a breeding tool to improve the quality of the CCP. It was possible to use phenotypic traits of grain, which are linked to desirable quality characteristics (e.g. grain protein and TGW), such as colour, density and size, to be selected for using on farm mass selection tools. However, after re-sowing and harvesting the progeny of these fractions, only the selection methods for grain colour and size showed any consistent difference between those fractions that were most different. There was also rarely any consistent improvement of significant magnitude of the selected fractions over the un-selected controls. Therefore, the current selection methods investigated show limited potential as breeding tools to improve the quality or yield of a population. The results suggest these differences in desirable traits have only limited heritability and at relatively small magnitudes, suggesting that mass selection methods need to be improved for them to be useful as breeding tools for populations.

2.3.6 Possible end markets for the CCP

The indication from the narrative comments received from the bakers involved in the project was that the CCP are capable of producing bread-making flours of a commercially acceptable standard, and that in unblended flours, site effects were particularly strong on various quality parameters. The bakers found that the CCP flours produced loaves with a range of colour and texture characteristics, with site-related environmental, crop management and storage factors, including grain moisture, HFN and protein, content being identified as more important for quality than the within-crop genetic diversity. In terms of the QCCP-YQCCP comparison, QCCP flours tended to be slightly more positively evaluated in bakers' narrative reports than YQCCP flours. Amongst the six organic tests where bakeries included a control flour, the QCCP was top-ranked three times, the YQCCP twice and the control once. Amongst the five non-organic tests where bakeries included a control, control samples were always ranked above CCP samples. In a comparison between CCP flours and commercial controls, there was no significant difference between mean loaf height (a proxy for loaf volume). There is a strong indication from these results that the populations can produce dough with gluten properties that meet industry standards and produce loaves which rise as well as those from commercial flours. Overall, flours from the CCP were not consistently worse than commercial alternatives as bread–making material.

In micronutrient tests the values observed for the CCP grain tested were generally comparable to values previously reported in the literature for winter wheat sample sets. The QCCP showed higher accumulations of molybdenum than the YQCCP but otherwise there were no differences in the accumulation of micronutrients measured between the two CCP. However, in comparisons to pure line varieties, the CCP accumulated significantly more magnesium and zinc, and tended to accumulate more of all other minerals except iron.

In malting tests there were very few significant differences detected in comparisons between years, sites and populations with respect to the parameters measured by micro-malting tests. In terms of overall values for key malting parameters, the results indicated that these CCP have limited potential for use in malting.

In distilling tests the alcohol yield of the grain tested was not significantly affected by crop management system or growing site. In fact, the only factor that had an impact on the alcohol yield was the population type, with the YCCP giving a higher yield than the YQCCP, which may be due to the higher nitrogen levels in the YQCCP. However, the CCP produced a relatively low alcohol yield in comparison to pure line wheat varieties already commercially available and therefore these CCP have limited potential for distilling.

In tests of the CCP's suitability as animal feed, the frequency of the 1R chromosome decreased in advanced generations of both the YCCP and YQCCP compared to the starting frequencies, and this could be observed as continuing in the majority of the populations from the F_7 to F_8. This suggested strong selection against this chromosome segment. In the YQCCP samples, the frequency of the 1R arm was usually less than 10% of the seed. At this frequency, the arm is unlikely to impart any significant detrimental effect on quality due to the dilution effect of the 1BS arm. Therefore, these results suggest that the use of the seed of the YQCCP for bread making and the YCCP for animal feed will not be impaired by the low frequency of the rye genes.

2.4 Conclusions and implications

A number of conclusions on CCP emerge from this research project:

1) Replicated field trials showed that the CCP had a reduced grain yield in comparison to currently used pure lines under conditions of high input, while yield differences were not significant under organic cropping conditions. This means that CCP are potentially more suitable to low-input and organic than to conventional systems.

2) The three different populations were tested to investigate their potential end uses: A high yield population (YCCP), a high baking quality population (QCCP) and an all-rounder population (YQCCP). On the basis of various agronomic and quality performance indicators, as well as

marketability to end-users, the QCCP is seen as the most promising population among the three tested populations.

3) The CCP showed increased yield stability in comparison to pure lines in replicated field trials, but on-farm participatory trials across the UK could not confirm this result.

4) The CCP did not adapt to the cropping conditions under which they were grown; this was evident both from molecular data and from comprehensive field trials. Yearly fluctuations in weather conditions are likely to have counteracted any adaptation to the site-specific factor associated with cropping management and soil conditions.

5) Field trials showed that both CCP and (complex) variety mixtures are more or less equivalent with regard to their agronomic performance. Preference for either CCP or mixtures is therefore likely to follow other criteria than those based purely on agronomic performance.

3 TECHNICAL DETAIL

3.1 Introduction

3.1.1 Background

Conventional UK wheat crops are composed of monocultures of individual varieties selected for production under high inputs. Consequently, when grown under low input conditions such varieties have considerable yield and quality shortfalls (4 to 5 t/ha modal range). This reduced productivity is the result of the interaction between varieties that have not been selected for production under low-input conditions, and the field environments that are often highly variable in the absence of a range of inputs (Jones et al 2010). Most importantly, the predictions for continued increases in climatic variation, together with higher prices for inputs will place additional demands on the crops we grow in all systems.

A significant expansion of the genetic variability of the wheat crop would help to buffer such environmental variation. This could be achieved by a massive increase in the number of varieties grown in monoculture. However, this would require a major investment by breeders with no equivalent return, the material would be unable to adapt to future change, and there would be no interaction among the varieties. The alternative is to grow plant genotypes together so that they can complement and compensate for each other under different environments (Tilman et al., 2006; Ceccarelli et al., 2001; Almekinders & Hardon, 2006). The simplest approach is to use mixtures of varieties (Finckh & Wolfe 1998), but the potential for genotypic interactions is limited because of the relatively small number of mixture components. A more complex, but potentially more robust and resilient solution is to use CCP as in the 'evolutionary breeding' concept proposed by Suneson (1956), for barley.

Evolutionary breeding using CCP involves inter-crossing appropriate parents in many different bi-parental and higher order combinations. The segregating progenies from the crosses are bulked and then exposed to natural selection in the localities in which they are to be grown. Large amounts of genetic variation are exposed to different localities over several years of natural selection and seed multiplication, thus expanding the genetic variation while limiting the effect of the environmental variation. Some degree of mass selection, positive or negative, can be applied to guide the natural selection, but the end result should be populations that adapt to localities while retaining genetic variation and hence adaptability for further change.

In the Defra project AR 0914 (Döring et al., 2009), six wheat CCP were developed from a total of 20 parent varieties: YCCP and $YCCP_{ms}$ (nine high yield parents without and with male sterility), QCCP and $QCCP_{ms}$ (12 high quality parents, without and with male sterility; one of these parents was also included in the high yielding group) and YQCCP and $YQCCP_{ms}$ (all 20 parents without and with male sterility). The CCP, and their equivalent physical mixtures, were grown over a number of generations in the field at two organic and two non-organic sites. The results of this project provided evidence that, across these different environments, the CCP performed better than the means of their parents for a range of

characters including grain yield (Wolfe et. al., 2006). More importantly, this improved performance was expressed across low and high yielding environments, unlike in most of the parents.

In a five year study across multiple locations in the UK we evaluated the performance of these existing CCP over a wider range of spatial and temporal variation, transferred the CCP to farmers to facilitate a commercial evaluation of their potential and determined their processing capabilities.

3.1.2 Aims and objectives

The overall aim of the project was to improve the yield, quality, and stability of performance of winter wheat for low input management under environmental conditions that are forecast to become more unpredictable and more costly to ameliorate through inputs. This was achieved by:

- Determining the yield, quality, and stability of wheat CCP under high and low input management over a wide range of environmental conditions through phenotypic monitoring and analyses;
- Genotypic analyses using molecular markers to help determine the relative importance of different genotypes, gene complexes and genes for phenotypic performance and adaptation; and
- Developing the application, processing and marketing capabilities of the CCP for a range of end users.

Therefore, the specific objectives of the present study were to:

1. Quantify the yield, quality and stability of performance of CCP over the four project years at four main trial sites.
2. Determine the types and extent of genotypic evolution in the adaptation of CCP to region, management and year.
3. Estimate the relationship between genetic and phenotypic contributions of individual parent varieties to YQ CCP selected under different regional and management conditions.
4. Estimate the extent to which disease restriction in mixtures and CCP differ from each other and contribute to yield stability.
5. Determine the performance and stability of performance of CCP among 24 farms across a range of climatic conditions relative to the variety Claire and the standard farm variety, in all project years.
6. Compare the performance of the CCP with or without either Pegassos or Xi19 as extra parents, at four main sites, in project year 4.
7. Determine the effect of mass selection on CCP.

8. Determine the bread-making quality of flour from the CCP.

9. Determine the acceptability of CCP for use in malting, distilling and animal feed.

3.1.3 Scope

There were amendments to the scope initially planned for the project due to a number of limitations, in particular disease resulting from high levels of bunt (*Tilletia tritici*) infestation in the UK during 2011 (Project Year 4).

More specifically, in work package 8, which looked to determine the bread making quality of flour from the CCP, it was not ultimately possible to fully test all hypotheses, primarily due to limitations of the sample set. No trial or participatory site within the study was growing the full range of parent varieties from the Q or YQCCP at any stage of the project. Moreover, high levels of bunt infestation impacting many areas of the UK in Project Year 4 (when testing was planned) rendered tests impossible in that year as the grain was unsuitable for human consumption. The tests were delayed until harvest 2012, after a project extension was granted, when the same CCP were being grown but in the context of different trials. The sample selection in this year could not follow the pattern of Trial Years 1, 2 and 3 because following completion of the four official trial years, participatory farms were no longer growing CCP. Twelve samples were selected to enable the comparison of YQ with QCCP and comparison of these CCP with one pure line from the same site, as well as the comparison of CCP from multiple sites with pure lines from multiple sites, as described in the methods (see section 3.2.11).

In work package 9, which looked to determine the acceptability of CCP for use in malting, distilling and animal feed, the distilling tests were only conducted in the first year of the project and not in every year. This was because no significant differences in alcohol yield were shown between the organic and conventionally grown wheat samples, and due to the very high cost of the tests it was agreed that these would not be carried on after year 1 and that the resources would instead be put towards other work within the proposal.

3.2 Materials and methods

3.2.1 Generation of CCP

The CCP used in the project were created in 2002 at the John Innes Centre, Norwich, UK, from a 20 x 20 half diallel crossing programme. Breeders' records and a collection of 800 wheat varieties held in the John Innes Centre gene bank were used to identify varieties that perform well under lower-input conditions. From these, twenty varieties were selected based on phenotypic characteristics and the distribution of DNA marker microsatellite variation. Coefficients of ancestry were used to choose varieties that cover a wide range of relevant geographical and character variation. Of the twenty parent lines, nine were selected to include varieties that have high yield potential, and twelve were selected to include varieties that have good milling potential (one of these parents was also included in the high yielding group). The chosen parents were inter-crossed in all combinations to produce 190 crosses (Table 1).

Table 1. The 190 crosses made between parental varieties to create the YCCP, QCCP and YQ CCP.

	Bezostaya	Cadenza	Hereward	Maris Widgeon	Mercia	Monopol	Pastiche	Renan	Renesansa	Soissons	Spark	Thatcher	Buchan	Claire	Deben	HTL	Norman	Option	Tanker	Wembely
Bezostaya		*yq*	*yq*	*yq*	*yq*	*yq*	*yq*	*yq*	*yq*	*yq*	*yq*	*yq*	Y	Y	Y	Y	Y	Y	Y	Y
Wembley	*yq*	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	Y	Y	Y	Y	Y	Y	Y	
Tanker	*yq*	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	Y	Y	Y	Y	Y	Y		
Option	*yq*	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	Y	Y	Y	Y	Y			
Norman	*yq*	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	Y	Y	Y	Y				
HTL	*yq*	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	Y	Y	Y					
Deben	*yq*	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	Y	Y						
Claire	*yq*	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	Y							
Buchan	*yq*	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ	YQ								
Thatcher	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q									
Spark	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q										
Soissons	Q	Q	Q	Q	Q	Q	Q	Q	Q											
Renesansa	Q	Q	Q	Q	Q	Q	Q	Q												
Renan	Q	Q	Q	Q	Q	Q	Q													
Pastiche	Q	Q	Q	Q	Q	Q														
Monopol	Q	Q	Q	Q	Q															
Mercia	Q	Q	Q	Q																
Maris Widgeon	Q	Q	Q																	
Hereward	Q	Q																		
Cadenza	Q																			

These crosses were then separated into three sets; one comprising crosses from parents with high yield potential (36 crosses), hereon referred to as YCCP; one comprising crosses from parents with good milling potential/end use quality (66 crosses), hereon referred to as QCCP; and one comprising both parent sets (190 crosses), hereon referred to as YQCCP (Wolfe et al., 2006, Döring et al., 2009).

3.2.2 Project overview

The project was organised into 9 work packages. Studies were conducted to determine the yield, quality, and stability of the CCP under high and low input management over a wide range of environmental conditions by conducting phenotypic monitoring and analyses, at four main trial sites (see 3.2.3; WP1) and also at more than 20 on farms sites across a broad geographic area (WP5). A specific objective of the work in the first work package was to gather comparative data on disease and weed levels to illustrate the ecological effects of populations and mixtures relative to each other and to standard pedigree line varieties. In the on-farm trials the objective of the work package was to determine the performance and stability of performance of CCP across a range of climatic conditions (ranging from Northumberland to Cornwall), relative to the variety Claire plus one other farm standard variety. Separate investigations were conducted on the ability of varietal mixtures and CCP to control disease and stabilise yields in fungicide treated and untreated plots (WP4).

Further, studies investigated the types and extent of genotypic evolution of the CCP as an indicator of their potential adaptation to different geographic locations (WP2), and from the same material, estimated the relationship between genetic and phenotypic contributions of the individual parent varieties to the YQCCP (WP3). The CCP tested in these studies were subjected to different environmental conditions by being grown at four different sites (two organic and two conventional; see 3.2.3) over 11 generations. In work package 2 the investigations attempted to track changes over time in allele frequency using molecular markers in order to ask the question as to whether these populations had undergone natural selection, having originated from the same ancestral population and having subsequently been grown at four different sites without any artificial selection. The aim of work package 3 was to elucidate phenotypic properties in the CCP that may have led to the selection of certain alleles, and then follow these back to the parent.

In order to test whether it was possible to enhance specific performance characteristics of CCP by including ‘extra’ parental material, two additional parents, wheat varieties Pegassos and Xi19, were crossed with the three CCP (WP6). Pegassos was bred to yield well in low-input growing conditions, whereas Xi19 is a variety bred for high grain quality. Therefore, it was tested whether the yield of the CCP could be increased with the addition of Pegassos crosses and quality of the CCP increased with the addition of Xi19 crosses. Further, mass selection studies were used to investigate whether it is possible to enhance the performance of a CCP by selecting from that CCP an elite sub-population to suit a specific end-use, while retaining sufficient diversity to confer adequate buffering capacity against unpredictable growing conditions (WP7). The work package explored the potential efficacy of three non-destructive mass selection methods; size, density, and colour, as practical on-farm seed sorting tools to create more valuable fractions of grain for marketing and as breeding tools to select for a higher frequency of heritable traits in subsequent generations of the population.

One of the key aims of this project was to develop the application, processing and marketing capabilities of the CCP for small-scale and industrial production of selected products in the human and animal food

chains. Therefore, studies were conducted to investigate the potential for using CCP in industrial and small-scale baking, as well as measuring the grains micronutrient value (WP8), and their suitability for use in malting, distilling and as animal feed (WP9).

3.2.3 Description of field sites

For trials in work packages 1, 2, 3, 6, 7, 8 & 9 the following field sites were used; two organic sites located at (i) Wakelyns Agroforestry (WAF) in Suffolk (52°39'N, 1°17'E), and (ii) Sheepdrove Organic Farm (SOF) in Berkshire (51°41N, -1°52'E), as well as two non-organic sites located at (iii) Metfield Hall Farm (MET), adjacent to WAF in Suffolk (52°41'N, 1°29'E), and (iv) Morley Farm (MOR), an experimental farm in Norfolk managed by NIAB TAG (52°56'N, 1°10'E).

Soils were characterised by clay content in the range of 13–40%. Soil type was medium to heavy at MET, MOR and WAF, and light to medium at SOF. Further details on soil texture, soil pH and nitrogen applications at the conventional sites are described in a previous study (Jones et al. 2010).

In work packages where additional sites were used those sites are described in the methods sections for those specific work packages.

3.2.4 Quantifying the yield, quality and stability of performance of CCP (WP1)

Trial entries

The trial sites used in WP1 were as described above (see section 3.2.1). The three CCP of winter wheat used in this work package were also as described above (see section 3.2.3). In addition, three **male sterile composite cross populations (CCP_{ms})**, were used. These were generated at the same time as the populations described in 3.2.1 by artificially hybridising all the parents used in the other CCP to characterised genetic males sterile lines (as females) obtained from two sources, RAGT (Shango derivatives: JB Plant 1, JB Plant 2, F2/F3 Sterile Bulk Population 2/77, JB NWH 65) and CIMMYT (F1TOPDMSO102 7 TURACO DMS, F1TOPDMSO102 10 GALVEZ S 87 DMS, F1TOPDMSO102 12 CUMPAS T88 DMS and F1TOPDMSO102 14 NING8201 DMS). The F_2 generations of these crosses were bulked as above to create $QCCP_{ms}$, $YCCP_{ms}$ and $YQCCP_{ms}$. To distinguish between these CCP_{ms} and the **non-male sterile populations** we denote the latter **as CCP_n**. When referring to **the three CCP_n and the three CCP_{ms} together**, the populations are called **CCP (without subscript**) (Table 2). The six CCP, three mixtures and all parental varieties were hand broadcast in single replicate plots of varying size at four locations in October 2003. There was enough seed available in autumn 2004 to begin standard replicated field trials.

In order to compare the performance of mixtures of homozygous lines with that of CCP, parental seed of equal proportions was also mixed in the same categories as those used to create the CCP to provide a Yield, Quality and Yield-Quality Mixture, subsequently called YMix, QMix and YQMix.

Pure line varieties included in the WP1 trials were two high baking quality varieties (Solstice and Spark), as well as three high yield varieties (Alchemy, Claire and Tanker). Further characteristics of the five pure lines are reported below in Table 3.

Table 2. Notation for the six tested CCP and the three corresponding mixtures; numbers in brackets denote the number of entries subsumed under the label.

Label	Explanation	Yield set (Y)	Both sets (YQ)	Quality set (Q)
CCP (6)	Both non-male sterile and male sterile CCP	YCCP (2)	YQCCP (2)	QCCP (2)
CCP_n (3)	Non-male sterile CCP	$YCCP_n$ (1)	$YQCCP_n$ (1)	$QCCP_n$ (1)
CCP_{ms} (3)	Male sterile CCP	$YCCP_{ms}$ (1)	$YQCCP_{ms}$ (1)	$QCCP_{ms}$ (1)
Mix (3)	Mixture of parents	YMix (1)	YQMix (1)	QMix (1)
Pure lines (5)	Pedigree lines	Alchemy	Alchemy	Solstice
		Claire	Claire	Spark
		Tanker	Tanker	
			Solstice	
			Spark	

Main field trials

Field trials were performed from 2003/04 (sowing autumn 2003, harvest summer 2004) to 2011/12, using in each year CCP and mixture seed that had been harvested in the preceding cropping year, thereby allowing evolutionary adaptation of the genetically heterogeneous material to site-specific cropping conditions to take place. Here we report the results of the four trial years 2007/08 to 2010/11, as this was the period covered by the project; results of the previous cropping seasons have been reported elsewhere (Boyd, 2007; Döring et al., 2009; Jones et al., 2010).

Table 3. Characteristics of the tested pedigree varieties; data compiled from various sources including AHDB Recommended List, Scottish Wheat Variety Database and information from breeders.

Variety name	nabim group	First year listed	Breeder	Parentage
Alchemy	4	2006	Nickerson	Claire x (Consort x Woodstock)
Claire	3	1999	Nickerson	Composite Cross
Tanker	4	2001	Elsoms	Beaver x Zodiac
Solstice	2 (1)*	2002	CPB Twyford	Vivant / Rialto
Spark	1	1991	Nickerson	Moulin x Tonic

*data not consistent from different sources

Adaptation trials

In the main trials, the seed of the CCP was always replanted at the same site at which it was harvested. In addition, two series of field trials were carried out to test whether CCP adapt to the conditions under which they are grown. The first of these series was conducted in the last trial year (sowing 2010) and is called "**cross-over trial**"; here, CCP with a history of having been grown for 7 generations at constant sites (from 2003 sowing onwards) were not only grown at their home sites (i.e. as part of the main trials at two organic and two conventional sites, see above), but also at all the other trial sites (

Table **4**).

Table 4. Trial design for the cross-over trial; shaded cells show the home-CCP; organic sites (Wakelyns, WAF and Sheepdrove, SOF) are marked in green, conventional ones (Metfield, MET and Morley, MOR) in blue.

	Trial site in 2010/11			
Site for last 7 generations	**MET**	**MOR**	**SOF**	**WAF**
MET	*MET*→MET	*MET*→MOR	*MET*→SOF	*MET*→WAF
MOR	*MOR*→MET	*MOR*→MOR	*MOR*→SOF	*MOR*→WAF
SOF	*SOF*→MET	*SOF*→MOR	*SOF*→SOF	*SOF*→WAF
WAF	*WAF*→MET	*WAF*→MOR	*WAF*→SOF	*WAF*→WAF

Thus, populations with four different histories were grown at each of the four trial sites. It was assumed that if CCP adapt to the conditions at which they are grown, the home-CCP would outperform the CCP whose seed came from a different site. Performance was measured as grain yield but also with other parameters (see below). Furthermore, this trial design allowed testing of whether there is an effect of management system on adaptation.

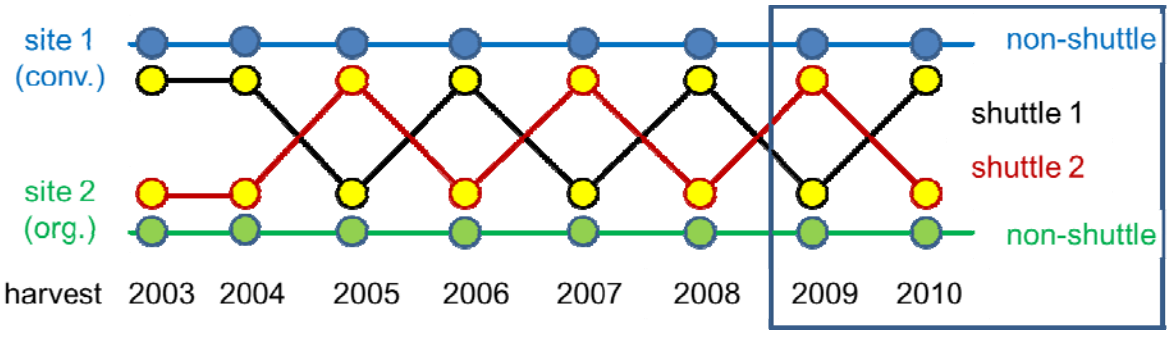

Figure 2. General set-up of the shuttle trial with the seed of the CCP being sent to alternating sites; the figure shows two sites only, but all combinations of all four sites were trialled.

In the second trial series, called "**shuttle trial**", the CCP were either grown at constant sites or at alternating sites, i.e. part of the seed from CCP was sent to another site and planted there; the following generation was then sent back to the site where the CCP had originally come from. The comparison of CCP from constant vs. alternating sites was run over several years for both the CCP_n and the CCP_{ms}; here we report the results of the last two trial years (see box in Figure 2), assuming that potential effects of adaptation become stronger over time.

Farm operations and trial design

Plots were drilled in October in all experiments (Table 5). The experimental design of each trial was a randomised complete block design with three replications. Plot size was 10 × 1.45 m at SOF, and 10 × 1.2 m at the other three sites. As an exception, double plot width was used in the last trial year at SOF. Seed rate was 200 kg ha^{-1} for the two organic sites and 170 kg ha^{-1} for the non-organic sites. Seed rate calculations were based on an average target plant density of 425 plants m^{-2}. Further details on farm operations are reported in Appendix A (section 4.1).

Table 5. Sowing dates for the WP1 trials

Year (sowing)	MET	MOR	SOF	WAF
2007	19 Oct	22 Oct	19 Oct	19 Oct
2008	13 Oct	14 Oct	Oct*	9 Oct
2009	12 Oct	16 Oct	15 Oct	14 Oct
2010	Oct*	Oct*	Oct*	Oct*

*exact dates not available

Field assessments and grain yield measurements

In early spring, crop establishment was measured as plants m^{-2} by counting individual wheat plants in two 0.25 m² sectioned quadrats per plot. Care was taken to perform these assessments before tillering so that individual plants could be well separated from each other. In addition, weed cover in the sectioned sampling squares was assessed as percentage of soil covered.

The Leaf Area Index (LAI) was measured three times per growing season (median growth stage: 31, 52 and 64) in all plots with a SunScan Canopy Analysis System (from Delta T Devices, Cambridge, UK). This was done in all four years at the conventional sites, but in only 1 or 2 years at the organic sites because of confounding effects of weeds on LAI measurements.

During the growing season, assessments were performed on four foliar diseases as % infected area of flag leaf, namely *Septoria tritici* leaf blotch ssp., yellow rust (*Puccinia striiformis* f.sp. *tritici*), brown rust (*Puccinia triticina*) and powdery mildew (*Blumeria. graminis* f. sp *tritici*). Total disease of the flag leaf was then determined as the summed percentage of all four foliar diseases. In addition, damage caused by the

cereal leaf beetle (*Oulema melanopus*), senescence (yellowing not caused by any particular disease) and green leaf area (GLA) were also scored on the flag leaf as percentage area. Aphids were assessed on the ear as % of the ear infested. All these pest and disease assessments were performed on 10 randomly selected plants, at growth stage 75 to 84, in early July.

Prior to harvest, further measurements were taken in two adjacent rows, on a length of 0.5 m each. These included the number of stems, the straw mass in g; and the grain mass in g. These parameters were used to calculate straw mass per stem, grain mass per stem, the harvest ratio (grain/straw) and the harvest index (grain/(grain+straw)).

Prior to trial harvest, head density (heads m^{-2}) was determined by counting all ears in 1 m² sampling quadrats; in addition, the proportion of awned ears was determined (%), and for 10 randomly selected plants per sampling quadrat, straw height was measured (cm) to the base of the ear. Lodging was assessed as the percentage of plants in the following four lodging classes: 0, 1–30, 31–60 & 61–90 degrees from upright.

Plots were harvested with a trial combine harvester, and further processed at the experimental station at Wakelyns, where the grain was cleaned and weighed. Moisture content of the grain was determined and grain yield was adjusted to 15% moisture content.

Post-harvest measurements

Post-harvest measurements included the determination of Hagberg Falling Number (HFN), grain protein content (%), hardness, specific weight, and thousand grain weight (TGW, in g at 15% moisture content). Measurements of grain protein content followed standard procedures using a near-infrared device at Doves Farm Ltd.

Grain diseases were analysed microscopically for bunt (*Tilletia caries*, number of spores), *Septoria* sp. (% seeds infected) and *Microdochium nivale* (% seed infected) for selected trial entries.

Statistical analysis

All statistical tests were run with R, v. 3.0.1 (Crawley, 2007; R Core Development Team, 2013). For comparing means of the trial entries, mixed effects models were used with site as random factor and genotype (e.g. CCP vs. pure line, or the comparison among different populations) as fixed factor.

For assessing stability of yield, protein and protein yield, various parameters were calculated. These included measures of stability based on Genotype x Environment interactions (Annicchiarico, 2002) and regression-based stability measures (Finlay & Wilkinson, 1963; Eberhart & Russell, 1966) which is based on calculating deviations of individual data points from the regression line (Becker, 1981; Becker & Léon, 1988). Rank-based stability measures (Huehn, 1990) were included as well.

As a measure of variance across environments, corrected for the size of the mean, the coefficient of variation (CV) was used; the statistical test of whether two CVs are significantly different followed Sachs & Hedderich (2009). The slope *b* of the regression of genotype against mean yield in each environment was determined.

Further, a novel measure of stability was used, based on Taylor's Power Law (Cohen, 2013). Specifically, means (m) and variances (s^2) of yields over environments were calculated; following Taylor's Power Law (TPL), the regression of $y=\log(s^2)$ against $x=\log(m)$ was determined and the deviations from this regression line can be interpreted as an index of yield variability corrected for the scaling between mean and variance. Here, the smaller the residuals from the regression line, the higher the yield stability.

3.2.5 Genotypic evolution in adaptation of CCP (WP2)

For bread wheat, successful production currently relies on the selection of varieties that are well adapted to the chosen environment. Selection for these high performing genotypes has been imposed on segregating populations within breeding programmes, the outputs of which are homozygous varieties, genetically 'fixed' at all loci. By contrast, the idea of evolutionary plant breeding is to maintain genetic diversity through to the released variety; here, a segregating population displaying maximum allelic diversity at each locus is the basis of the farmed crop. As the crop is harvested and re-sown on the same farm, selection pressure will be imposed so that alleles and allelic combinations that are beneficial within that environment may increase in frequency. Conversely, deleterious alleles and combinations may be reduced in frequency.

This process of natural selection could be twofold: First, due to environmental differences at sites, different phenotypic characteristics could be advantageous. In this scenario, different genotypes and alleles would be selected for at different sites, as a consequence of environmental differentiation between sites. Second, insofar as environmental conditions are common among the sites, natural selection would lead to the same changes of genotypic and allelic composition at all sites where the population is grown over several generations. This would be particularly so if the ancestral population was not well adapted to environmental factors shared by the test sites; or if the genotypes of the ancestral populations had not been adapted to being grown within a population. The first process will lead to spatially differentiated populations; these populations would show site-specific genetic differences. The second process, i.e. the general change of genotypic and allelic composition over time can be termed temporal differentiation and would affect populations from all sites equally.

The main difficulty when investigating processes of natural selection is the occurrence of genetic drift, i.e. the random change of allele frequencies resulting from the sampling of gametes over generations in a finite population (Hedrick, 2005). Genetic drift can result in temporal as well as spatial differentiation – even when the populations may not encounter any natural selection. Genetic drift is – and temporal and spatial differentiation of populations can therefore be – solely a function of time and population size.

However, the fact that genetic drift affects all loci equally while natural selection only operates at certain loci should make it possible to separate the effects of drift and selection and thus also to determine whether observed spatial and temporal differentiation is solely due to genetic drift or the outcome of natural selection.

The methodological approach to separate these two processes is to reverse and exploit the problem that genetic drift is merely a function of time and population size: population size can be deduced as a function of time and the amount of genetic drift. The population size can be determined by using only seemingly neutral loci that show low and equal levels of temporal change of allele frequencies. Subsequently, the expected amount of genetic drift can be calculated from the derived population size. Loci that have undergone positive or diversifying selection should then show increased, or decreased, levels of changes of allele frequencies (Goldringer & Bataillon, 2004).

Population sampling

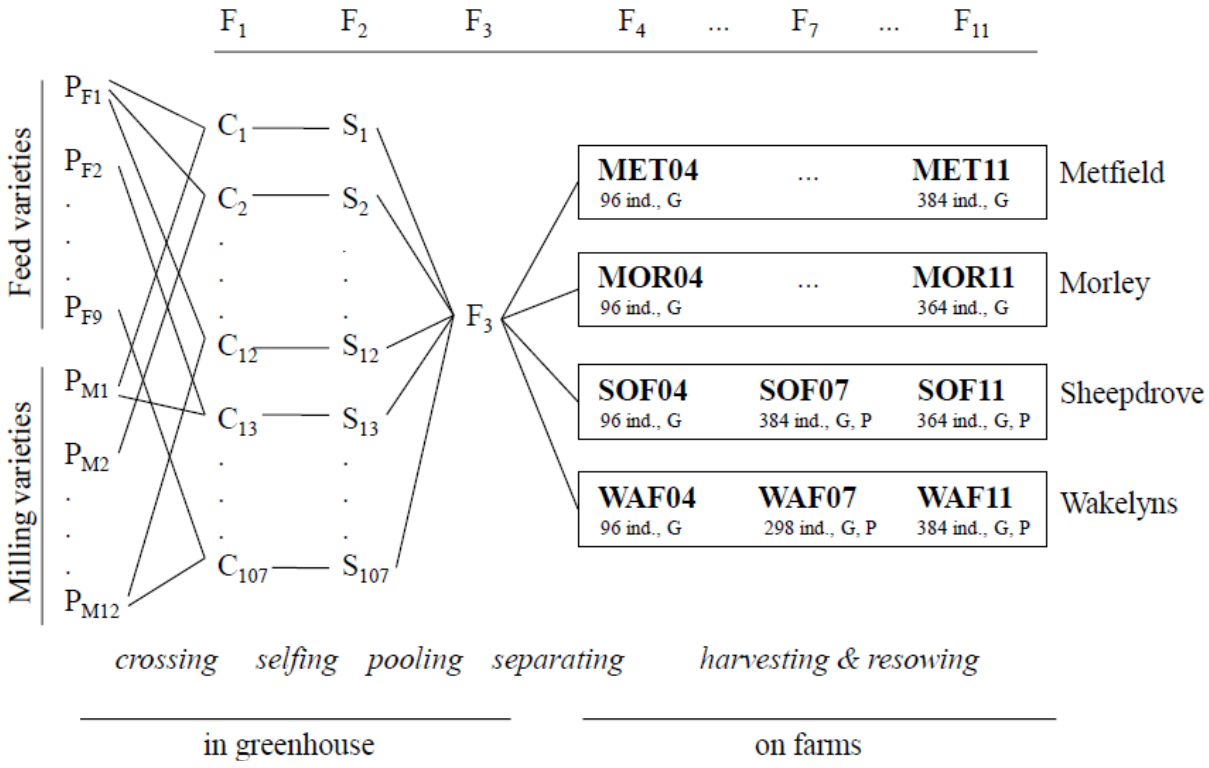

Figure 3. Scheme for generation of the founding population and overview of sampled populations (bold fonts). Below the sampled populations the number of sampled individuals is given and whether they were genotyped (G) or phenotyped (P). The population code consists of the three-letter site code and the number of the generation (e.g. MET11 = generation F11 continuously grown at Metfield).

As explained in section 3.2.1, the YQCCP were created by inter-crossing two sets of varieties: (1) 9 feed varieties and (2) 12 bread-making quality varieties. Each parent was crossed with all others in a half diallel cross design. F_1 plants were grown and allowed to self-fertilise, and the resulting F_2 seed counted and pooled. The CCP were then grown up to F_3 in the field at WAF. F_4 seed was harvested and then

equally distributed between the MET, MOR, SOF and WAF sites. At each site, seed was harvested every summer and a random sample re-sown at the same site in the autumn. This iterative process was continued up to F_{11}. The process is summarised in

Figure **3**. Plant individuals were sampled from the harvested seed of CCP at F_4 (96 individuals per site), F_7 (384 at SOF and 298 at WAF, but none from the conventional sites), and F_{11} (384 at MET and WAF, 364 at SOF and MOR). The sampled F_5, F_8, and F_{12} seed were then germinated and DNA was extracted using standard protocols (see below).

Genotyping

Genetic markers

The genetic locations of loci detected by molecular markers used for genotyping are shown in Figure 4. They include 20 SSR markers, 15 single nucleotide polymorphism (SNP) markers, and a set of 9 "perfect" markers (denoted as 'Perfect set'), that are tightly linked with major genes involved in plant height (*RhtB1*, *RhtD1*, (Ellis et al., 2002)), vernalisation requirement (*VrnA1prom* and *VrnA1y*, both provided by C. Ravel (INRA, France)), photoperiod response (*PpdD1* (Beales et al., 2007*)* and *PpdA1Cdex, PpdB1L5, PpdD1D2*, all provided by D. Laurie (John Innes Centre, UK)), and one marker linked to the *1B/1R* chromosome translocation from rye (sequence provided by S. Berry (Limagrain, UK)).

SSR markers were resolved by ABI 3730 capillary electrophoresis and the SNP markers using LGS SNPline. The 20 SSRs and 15 SNPs were carefully chosen to cover all chromosomes and most chromosome arms of the 21 chromosomes of wheat, so that as much as possible of the whole genome could be scanned.

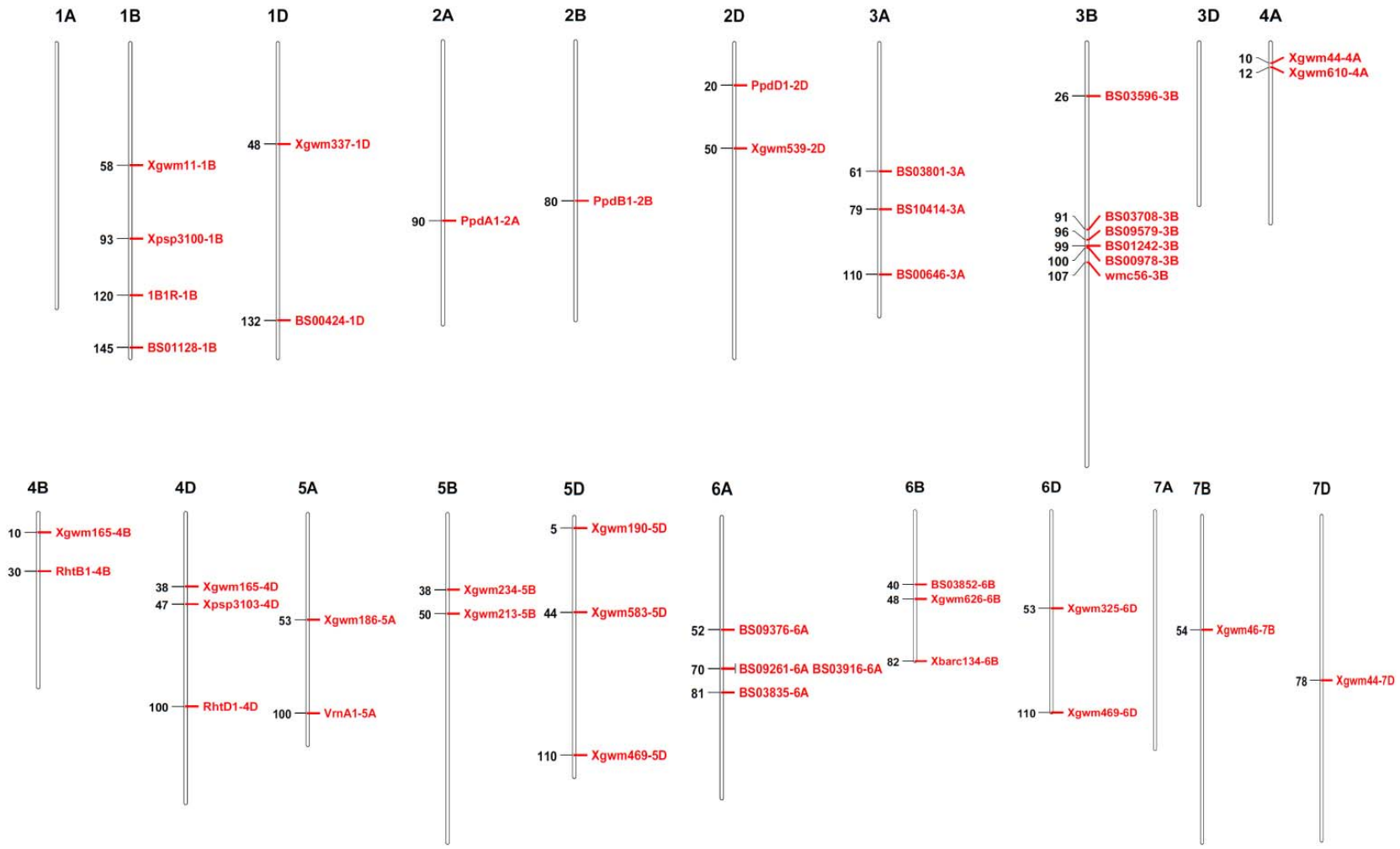

Figure 4. The loci detected in bread wheat by SSR (20) and SNP (15) molecular markers used in this study. SSR markers were resolved by ABI 3730 capillary electrophoresis and SNP markers using LGS SNPline. In addition, we used a set of 9 markers tightly linked with major genes involved in plant height, vernalisation requirement, photoperiod response and the *1B/1R* chromosome translocation from rye.

Sampling and DNA extraction

For the populations where phenotypic measurements were taken (SOF07, WAF07, SOF11 and WAF11) 5 seeds of each main tiller were germinated and 0.5–1 cm leaf tissue from each of the 2-week old seedlings cut and subsequently pooled, in order to decrease the chance of genotyping a single seed that could be due to outcrossing.

From the other populations 500 seeds (MET11 and MOR11) and 150 seeds (MET04, MOR04, SOF04 and WAF04) randomly drawn from seed bags containing the harvest of whole plots were germinated and 3 cm leaf tissue cut from 2-week old seedlings. DNA samples from the parental varieties were also taken from 2-week old seedlings, whereas for each replication leaf tissue from a different seedling was used for DNA extraction.

DNA was extracted from 96 individuals from the generation the 04 populations (MET04, MOR04, SOF04 and WAF04); from 298 individuals from WAF07; from 364 individuals from MOR11 and SOF11; and from 384 individuals from SOF07, MET11 and WAF11. For PCR reactions, parents were included on MOR11 and SOF11 and twice on WAF07 to fit onto 384-well plates. Additionally, parental lines were analysed when pre-assessing markers so that parental genotypes were available from 5 replications. The method for DNA extraction followed a modified protocol of Palotta (2003).

PCR protocols

Polymerase chain reaction (PCR) was performed in a HydroCycler 16 (KBioscience, Hoddesdon, UK) in a final volume of 5µl with 10–20 ng genomic DNA, containing 0.02 µM of the forward primer, 0.2 µM of the reverse primer and of the M13 tails and 1X HotStar Mix (Qiagen, Germany) for tailed primers, and 0.2 µM of both primers, 800 µM dNTP, 1X Buffer (Qiagen) and 0.02 µM Taq polymerase (Roche Molecular Biochemicals, Mannheim, Germany) for the directly labelled primers.

The cycling conditions were: after 3 min at 94°C (15 min with HotStar polymerase), 45 cycles were performed with 1 min at 94°C, 1 min at either 50, 55, or 60°C (depending on the individual microsatellite), 2 min at 72°C, and a final extension step of 10 min at 72°C. PCR products were pooled before electrophoresis on a 3730 DNA Analyser (Applied Biosystems). Data were collected and analysed with GeneMapper software (Applied Biosystems).

Using the allele sizes of the parental lines that were included in each run, allele sizes from the individuals from the CCP were standardised in order to allow for comparisons over generations. PCR SNP-Markers PCR reactions were performed in a HydroCycler 16 (KBioscience, Hoddesdon, UK) in a final volume of 5µl, containing 1X KASP Reaction Mix (KBioscience), 12µM of each allele-specific forward primer, 30µM reverse primer and 10-20 ng genomic DNA. The following cycling conditions were used: 15 min at 94°C; 10 touchdown cycles of 20 s at 94°C, 60s at 65–57°C (dropping 0.8°C per cycle); and 26–35 cycles of 20 s at 94°C, 60 s at 57°C.

Fluorescence detection of the reactions was performed using a Tecan Safire plate reader (Tecan Group Ltd, Männedorf, Switzerland) and the data were analysed with the KlusterCaller 1.1 software (KBioscience).

Analysis

All markers chosen were selected to provide multiple alleles at each locus, so that chromosome segments in a plant could be attributed to sub-groups of parents or individual parents where unique alleles were carried. The data of the allele composition of individual plants in each population at each site and each generation were analysed using different statistical packages, including the statistical programme R (R Core Team 2013).

Allele frequencies

Allele frequencies were calculated with the R-package *adegenet* (Jombart, 2008). Mutations at the SSR-markers were removed, as the focus was on differences of allele frequencies and mutations were considered as random and not of evolutionary importance in this study. It was assumed that mutations only become of evolutionary importance after a longer time than investigated in this study.

Table 6. Crossing scheme of parental lines and number of seeds of each cross that went into the foundling population

	Wembley	Tanker	Option	Norman	HTL	Deben	Claire	Buchan	Bezostaya
Bezostaya	417	363	516	674	636	785	558	198	–
Cadenza	1138	0	838	1316	1662	205	1003	1404	654
Hereward	37	937	453	369	0	0	973	342	618
M.Widgeon	1583	947	1077	111	1358	2067	1152	1012	402
Mercia	0	2569	1469	326	117	1524	485	1708	0
Monopol	0	824	1098	0	0	0	1392	412	555
Pastiche	0	452	199	290	345	967	1246	424	481
Renan	792	804	1009	993	1799	1220	1003	1259	500
Renesansa	0	456	1426	880	0	839	1011	649	191
Soissons	910	1519	1142	1688	1140	894	1800	1009	334
Spark	1888	1393	938	1386	2485	1939	2223	1828	578
Thatcher	0	688	721	803	560	2118	1240	0	326

The founding population was created by pooling seeds from all crosses of parental lines after one generation of selfing. The allele frequencies of the foundling population were generated based on the number of seeds of each cross that went into the pooled population (Table 6). The diploid genotypes of each cross were created using the genotypic data of each parent and the genotypes of each were added to the founding population containing 5000 individuals proportional to the number of seeds that went into the ’real’ pooled population.

Principal Component Analysis

Principal component analysis (PCA) was performed with the function dudi.pca from the *ade4* package for the R environment (Dray & Dufour, 2007). For all comparisons, only the loci that were available for all populations in the set of compared populations were used for the calculations.

F-statistics

For the purpose of investigating spatial and temporal differentiation of the populations several *F*-statistics were computed, including Nei's GST (Nei & Chesser, 1983) and Jost's D (Jost, 2008). Calculations were performed for each locus and over all loci, whereby the corrected gene diversity parameters were first averaged over all loci before computing the respective statistic. A further statistic, Weir and Cockerham's θ, was calculated, which uses a nested ANOVA approach to estimate within- and among-population variance components. Populations are weighted according to their sample size (Weir & Cockerham, 1984). The computation of θ was carried out with implemented functions in the R-package *hierfstat*, and calculation of Nei's GST and Jost's D was scripted by modifying the *basic.stats* function of the *hierfstat* package.

For all comparisons, only the loci that were available for all populations in the set of compared populations were used for the calculations. After comparing estimated θ values to the values produced by SpaGeDi, exact P-values were estimated using 20,000 permutations for individuals and genes for a 1-sided test with the null hypothesis that observed θ estimates were smaller than the expected values from the permutation test using SpaGeDi (Hardy & Vekemans, 2002).

Changes in allele frequencies

Standard statistical tests are not well suited to assess changes of allele frequencies over generations because the standard null hypothesis of sampling from the same population is violated (Waples 1989b). A statistical test for significance of changes of allele frequencies thus has to take account of genetic drift, which is dependent on the effective population size N_e. Effective population size is defined as the size of an ideal Wright-Fisher population undergoing the same rate of genetic change as the population under study (Wright 1969). The following method, often named 'temporal method', is based on estimating N_e indirectly from observed temporal changes of allele frequencies and the following equation follow the approach of Waples (1989a). The general logic relating effective population size to change of allele frequency is that alleles are drawn binomially and thus the variance of allele frequency due to drift after one generation is $V(P) = P(1 - P)/2N_e$, where P is the allele frequency at the previous generation. With the allele frequency P_0 at generation 0, after t generations of genetic drift, the variance is

$$V(P_t) = P_0(1 - P_0)[1 - (1 - 1/2N_e)^t] \qquad \text{(eqn 2.1)}$$

Because allele frequencies have to be estimated from sampling S_0 and S_t individuals at generation 0 and t, respectively, the binomial sampling variance has to be included. If the measured allele frequencies are

x and y at generation 0 and t, the sampling variances are $V(x) = P(1 - P)/(2S_0)$ and $V(y) = P_t(1 - P_t)/(2S_t)$. If we assume sampling before reproduction (sampling plan 2 in Nei and Tajima (1981)) combining the variance due to genetic drift (eqn. 2.1) and the sampling variances using conditional probability yields the variance of the difference of the measured frequencies

$$V(x - y) = P_0(1 - P_0)[(2S_0)^{-1} + 1 - \{1 - (2N_e)^{-1}\}^t(1 - (2S_t)^{-1})]. \quad \text{(eqn 2.2)}$$

One measure for the change of allele frequencies is the standardised variance of gene frequency change $(P_0 - P_t)2/(P_0(1 - P_0))$ (Nei and Tajima 1981). Waples (1989a) has elaborated a test, which is based on the confidence interval of a variance. The following test for Fc was used to estimate confidence intervals of genome-wide F_c for the subsequent calculation of effective population size:

$$(1 - \alpha)\ \text{CI for}\ F_c = [nF_c/(X^2_{\alpha/2[n]}), nF_c/(X^2_{1-\alpha/2[n]})] \quad \text{(eqn. 2.3)}$$

with $n = \sum K - 1$ being the number of independent alleles and denoting the degrees of freedom for the 2 distribution. However, it has been found that distributions from pure drift simulations depart from a X^2 distribution for differing values of N_e, t, sample sizes, number of loci and starting frequencies (Goldringer & Bataillon, 2004; Waples, 1989a). In order to detect loci that depart from expected changes changes of allele frequencies Goldringer & Bataillon (2004) have thus suggested to first estimate a genome-wide F_c that is based on all loci except the one to test and derive N_e from this estimator, and then produce an expected distribution of F_c, based on the estimated genome-wide N_e, the allele frequency in generation 0 of the loci to test and the sample sizes.

For this purpose a function for the R environment has been programmed that simulates genetic drift by sampling $2N_e$ gametes in each generation and replicates the whole process 3000 times. The function returns a P-value, which is calculated as the share of observations that yield a greater F_c value than the F_c value of the locus to be tested. This is equivalent to a 1-sided X^2 test.

3.2.6 Genetic and phenotypic contributions of individual parent varieties to CCP (WP3)

Natural selection yields changes of genotypic and allelic composition of a population but is based on the differential phenotypic appearance and properties of individuals. As the phenotype of an individual is the product of its genotype, the environmental conditions, and of the interaction of both, natural selection acts on genes that are associated with the individual's phenotype. If loci that have undergone natural selection can be identified and if associations of these loci with particular phenotypic traits are known, it is possible to discover phenotypic properties that confer local adaptation to a given environment (Kawecki & Ebert, 2004).

Phenotyping

Figure **3** shows how many individual phenotypes were analysed at each generation/environment. Table 7 shows the phenotypic traits that were measured; these included yield components as well as traits considered to be important in plant competition (such as plant height). From the SOF and WAF sites in generations F_7 and F_{11} 500 single plants were picked at harvesting stage and bagged before taking phenotypic measurements. Additionally, at generation F_{11} plants were tagged in the field and scored for growth habit and ear emergence. This tagging made it possible to follow individual plants through from emergence to harvest. At SOF the plants were tagged in rows and columns within plots and at WAF plants were tagged in a row/column design within one plot. After measurements were finished, ears were cut off and threshed by hand. Seeds from the leading and remaining tillers were subsequently bagged separately before carrying out measurements on seeds.

Statistical modelling for association analysis

To obtain insights into traits that could be of importance in the adaptation process, an association analysis of phenotypic traits with genetic markers was conducted in the form of a single marker association mapping. However, the aim of this association mapping approach was not to generally identify any loci that are associated with any of the measured phenotypic traits. The aim was particularly to find out about phenotypic properties that could have led to the selection of certain alleles. Thus only loci that were identified as potential candidates of selection were evaluated for associations.

Table 7. Description of phenoypic traits. Last three columns show for which set of populations data was taken. Gen. 07 = generation 7, Gen. 11 = generation 11

Trait	Unit	Gen 07	Gen 11	Parents
Number of tillers per plant	count	yes	yes	yes
Plant height	cm	yes	yes	yes
Grain weight per tiller	g	yes	yes	yes
Grain number per tiller	count	no	yes	yes
Straw weight per tiller	g	yes	yes	yes
Above ground biomass per tiller	g	yes	yes	yes
Grain weight of main tiller	g	yes	yes	no
Grain number of main tiller	count	yes	yes	no
Harvest ratio (grain/straw)	ratio	yes	yes	yes
Thousand grain weight, whole plant	g	no	yes	yes
Thousand grain weight, main tiller	g	yes	yes	no
Awned (1=awned, 0=not awned)	scoring	yes	yes	no
50% ear emergence, after May 1st	days	no	yes	no
Growth habit (0=prostrate, 5= upright)	scoring	no	yes	no
Mean grain area, main tiller	mm²	no	yes	no
Mean grain length, main tiller	mm	No	yes	no
Mean grain width, main tiller	mm	No	yes	no

Statistical model

In order to correct for spurious associations due to population structure and relatedness among genotypes, next to a simple ANOVA, a mixed model approach was employed. The statistical methods mainly followed the approach of Yu et al. (2006). The basic mixed model equation was: $y = \mathbf{X}\alpha + \mathbf{P}v + \mathbf{Z}u + e$, where y is a vector of phenotypic observations, **X** a matrix representing the genotypes, α a vector of allele effects to be estimated, **P** a matrix containing p principal components from PCA and v a vector of subpopulation effects. The terms $\mathbf{X}\alpha + \mathbf{P}v$ contain the fixed effects of the model, which are estimated by best linear unbiased estimates (BLUE). The random effects part $\mathbf{Z}u + e$, where u is a vector containing the random deviates due to genome-wide relatedness, **Z** is a matrix relating y to u, and e is a vector of residual effects, is solved by the best linear unbiased prediction (BLUP) method (Kennedy et al. 1992). The vectors u and e are assumed to be normally distributed with null mean and variances of $Var(u) = 2\mathbf{K}V_g$, where **K** is a matrix of relative kinship coefficients that represents the degree of genetic covariance among individuals and V_g is the genetic variance, and $Var(e) = \mathbf{R}V_R$, where **R** is a matrix in which the diagonal elements are the reciprocal of the number of observations for which each phenotypic data point was obtained, and V_R is the residual variance. Solving the mixed model equations and calculating *P*-values was done with the package *TASSEL* (Bradbury et al., 2007), using the option P3D, where the genetic and residual variance are estimated once for each trait. This approach has the same statistical power as estimating these parameters for each marker-trait combination and is much faster in computing (Zhang et al. 2010). Different mixed models to correct for population structure that included either the different **P** matrices or the **K** matrix or both were compared. The models used to account for genetic structure in the CCP are summarised in Table 8.

Table 8. Summary of models used to correct for population structure and relatedness among genotypes

Model	Description
ANOVA	Simple analysis of variance model without any correction
P25	With Principal components that explain 25% of variance
P75	With Principal components that explain 75% of variance
K	Mixed model with kinship as genetic covariance
P_{25}+K	Mixed model with kinship and P_{25} as fixed effects
P_{75}+K	Mixed model with kinship and P_{75} as fixed effects

Phenotypic observations

As the plants of the populations were tagged in a certain design (as described above), phenotypic observations from the populations were corrected for design/plot effects with a mixed model approach taking all design effects taken as random, because assignment of plants to their field position was

completely random. The model terms for observations at SOF were *response = plot + plot * row + plot * column* and at WAF *response = row + column*. The residuals of the mixed models were used as phenotypic observations for the association mapping equations.

This approach was chosen as all genotypes are different and not replicated. It is assumed to be complementary to a two-step approach and it can be shown by adding a random design term **Di** to the association mapping model equation, which yields y = **Di** + **X**α + **P**v + **Z**u + e. After subtracting this term from both sides of the model, y − **Di** is left on the left side, and the right side is again the same as in the original model. This left term equals the residuals from model that is used to correct for design/plot effects. This two-step approach will produce an only slightly higher type I error rate than when the phenotypic analysis and the association analysis is performed in one step (Stich et al., 2008).

Phenotypic observations from the parental lines were also corrected by a mixed model approach. After comparing several possible reduced models using Schwarz' Bayesian information criterion, or BIC (Schwarz, 1978), and the REML deviance, which is simply negative twice the REML criterion, (Smyth, 2002), produced by the *lmer* model function in R, the following model terms were chosen for calculating adjusted entry means, that were taken as response in the association mapping model equation:

*response = var + site + year + site * year + var * year +var * site+var * site * year +year * site * block*; *var* being the genotypic effects of varieties.

Although this model did not yield the best model selection parameters for all traits, it was employed for all traits for the sake of ease. The genotypic effect of *var* was taken as fixed and all other effects as random (Patterson, 1997). Solving the mixed model equations was done with the R-package *lme4* using the REML method (Bates & Maechler, 2010).

Population structure

A common method to correct for population structure is to identify groups within a set of genotypes and to calculate the probability of membership to these groups using the program STRUCTURE (Pritchard et al., 2000a). Subsequently these probabilities are used as a **Q** matrix as a fixed effect in the association model (Pritchard et al., 2000b). In typical association studies varieties or ecotypes are tested, that are expected to have some form of underlying population structure due to e.g. common ancestors or adaptation to certain environmental conditions. However, for two reasons this technique was not employed in this study: (1) in a CCP that has been generated through extensive inter-crossing of genotypes, one would not expect such form of population structure, and (2) running the STRUCTURE algorithm is computationally very intensive and depends on certain *a priori* or supposed information about the range of number of groups for which the structuring shall be tested.

Thus, a PCA was employed to summarise patterns of relatedness instead of a STRUCTURE approach, as suggested by Price et al. (2006). According to Zhao et al. (2007) this PCA approach performs similarly and they suggest that it could replace the STRUCTURE approach. The first *p* principal components were selected, that explained more than 25% and 75% of the molecular variance for the $\mathbf{P}_{25}$ and for the $\mathbf{P}_{75}$

matrix, respectively (Stich & Melchinger, 2009). The percentage of explained variance of each component was calculated as the ratio of the eigenvalue divided by the sum of all eigenvalues. PCA was carried out as explained above (section 2.2.3).

Kinship

The kinship matrix **K** includes the degree of genetic covariance between pairs of individuals. A kinship coefficient is often defined as the probability of identity by descent of the genes compared (Ritland, 1996). Estimators of kinship were produced with the software package *SpaGeDi* (Hardy & Vekemans, 2002), using only loci that had pairwise LD (linkage disequilibrium) values of $R^2 < 0.5$ (Mamidi et al. 2011). Kinship coefficients were calculated using the method proposed by Ritland (1996), because these estimators show lower sampling variance and are thus more powerful to detect genetic structure than, for example, the method proposed by Loiselle et al. (1995), (Vekemans & Hardy, 2004). Kinship coefficients between individuals *i* and *j* are defined as $K_{ij} = (Q_{ij} - Q_m)/(1 - Q_m)$, where Q_{ij} is the probability of identity in state for random loci from *i* and *j*, and Q_m is the average probability of identity by state for loci from random individuals from the sample. Negative values between individuals were set to 0, as this indicates that they are less related than random individuals (Hardy & Vekemans, 2002; Yu et al., 2006), and the diagonal in the kinship matrix, which reflects the self-relatedness, was set to 1.

Model Evaluation

To evaluate the different models to correct for population structure, *P-P* plots were produced in which observed *P*-values are plotted against expected *P*-values. The expected P-values were calculated as $r(p_i)/n$, where $r(p_i)$ is the rank of the *P*-value (p_i) observed for the *i*th marker after sorting *P*-value according to size, and *n* is the number of markers (Stich et al., 2008). The logic behind this calculation is that the *P*-values for associations with a trait of a set of random markers should follow a uniform distribution (Yu et al., 2006). As a measure for this deviation, mean squared differences (MSD) between expected vs. observed *P*-values were calculated as:

$$MSD = [\sum^{n}{}_{i=1}(p_i - i/n)^2]/n$$

where the same variables apply as for the calculation of expected *P*-values.

Marker-trait associations

For the assessment of significance of marker-trait associations plots were produced, where *P*-values of the simple ANOVA model and the model that showed the lowest MSD value for each combination of trait and population were plotted. Because the objective of this study was to get insights about phenotypic properties of a potential selection process, only loci that were identified as potentially subject to selection were finally evaluated for association.

3.2.7 Disease resistance and yield stability of CCP (WP4)

Field trials were conducted over five seasons in harvest years 2008, 2009, 2010, 2011 and 2012. Experiments were delivered by NIAB TAG in conjunction with the NIAB TAG National Agronomy Centre Initiative (supported by The Morley Agricultural Foundation) at the following conventionally managed locations on contrasting soil types:

- Caythorpe (Lincolnshire); brashy calcareous soil over limestone (typically Elmton 1 series).
- Morley (Norfolk); sandy loam over chalky boulder clay (typically Ashley series).
- Sutton Scotney (Hampshire); shallow calcareous soil over chalk (typically Andover 1 series).

Winter wheat varieties, with differing disease susceptibility profiles, were sown in commercial fields alongside the YQCCP and a YQ-mix (which consisted of each of the 20 YQCCP parental lines mixed together in a 1:1 ratio). Seed harvested from the YQCCP and YQ-mix at each site was used as sowing seed in the subsequent season. Over the course of the research there were some changes in the specific varieties sown (mainly due to changes in the availability of varieties), however, a core of eight varieties was retained throughout the project (Table 9).

Table 9. Inclusion of winter wheat varieties in the research programme at Caythorpe, Morley and Sutton Scotney. Year indicates year of harvest.

Variety	2008	2009	2010	2011	2012	All years
Ambrosia	✔	✔	✔			
Battalion			✔			
Claire	✔	✔	✔	✔	✔	✔
Einstein	✔	✔	✔	✔	✔	✔
Gatsby	✔	✔	✔	✔	✔	✔
Humber	✔	✔	✔	✔	✔	✔
Oakley	✔	✔	✔	✔	✔	✔
Solstice	✔	✔	✔	✔	✔	✔
Stigg				✔	✔	
Stirling				✔	✔	
Timber	✔	✔				
Varietal mix (YQCCP)	✔	✔	✔	✔	✔	✔
Varietal mix (YQ-mix)	✔	✔	✔	✔	✔	✔

Several approaches were taken to investigate the effect of three different fungicide regimes on varietal performance at each site in each growing season. The approaches were as follows:

- Untreated; where no foliar fungicides were applied.
- A ‘disease exclusion programme’; this regime targeted a high level of foliar fungicide input in an effort to minimise the impact of disease on crop performance.

- A ‘reduced input programme’; with a lower level of fungicide input, this approach could be considered to be more closely aligned to farm practice (*cf*. the other approaches used).

In any individual season all three sites received the same fungicide inputs at comparable crop growth stages. Efforts were made to maintain a consistent fungicide treatment regime between seasons, however, some differences between seasons did occur due to changes in the availability and approval of specific fungicides. The products used and their active ingredients are outlined in Table 10 and Table 11. All fungicide application dates are as outlined in Table 12.

Table 10. Fungicide treatment programmes used in specific seasons. T= spray timing. GS = Zadok’s growth stage for cereals.

Fungal Programme	T0 (GS 30)	T1 (GS 32)	T2 (GS 39)	T3 (GS 65)
2008 & 2009				
Disease Exclusion	Cherokee + Flexity (1.0 + 0.5 l/ha)	Proline + Bravo500 (0.8 + 1.0 l/ha)	Opus + Comet + Bravo500 (0.75 + 0.5 + 1.0 l/ha)	Swing Gold (1.0 l/ha)
Reduced input	Cherokee (0.6 l/ha)	Proline + Bravo500 (0.4 l/ha + 1.0 l/ha)	Opus + Comet + Bravo500 (0.5 + 0.25 + 1.0 l/ha)	Swing Gold (0.6 l/ha)
Untreated	-	-	-	-
2010				
Disease Exclusion	Cherokee + Flexity (1.0 + 0.5 l/ha)	Proline + Bravo500 (0.8 + 1.0 l/ha)	Opus + Comet + Bravo500 (0.75 + 0.5 + 1.0 l/ha)	Firefly (1.5 l/ha)
Reduced input	Cherokee (0.6 l/ha)	Proline + Bravo500 (0.4 l/ha + 1.0 l/ha)	Opus + Comet + Bravo500 (0.5 + 0.25 + 1.0 l/ha)	Firefly (0.75 l/ha)
Untreated	-	-	-	-
2011 & 2012				
Disease Exclusion	Cherokee + Flexity (1.0 + 0.5 l/ha)	Proline275 + Bravo500 (0.72 + 1.0 l/ha)	Opus + Comet200 + Bravo500 (0.75 + 0.5 + 1.0 l/ha)	Firefly155 (1.5 l/ha)
Reduced input	Cherokee (0.6 l/ha)	Proline275 + Bravo500 (0.36 l/ha + 1.0 l/ha)	Opus + Comet200 + Bravo500 (0.5 + 0.25 + 1.0 l/ha)	Firefly155 (0.75 l/ha)
Untreated	-	-	-	-

Table 11. List of active ingredients and commercial products used within fungicide treatments.

Product name	Active ingredient(s)	Active content	Formulation	Manufacturer
Bravo500	chlorothalonil	500 g/L	SC	Syngenta
Cherokee	chlorothalonil, cyproconazole and propiconazole	375 g/L, 50 g/L and 62.5 g/L	SE	Syngenta
Comet	pyraclostrobin	250 g/L	EC	BASF
Comet200	pyraclostrobin	200 g/L	EC	BASF
Firefly	fluoxastrobin and prothioconazole	50 g/L and 100 g/L	EC	Bayer CropScience
Firefly155	fluoxastrobin and prothioconazole	45 g/L and 110 g/L	EC	Bayer CropScience
Flexity	metrafenone	300 g/L	SC	BASF
Opus	epoxyconazole	125 g/L	SC	BASF
Proline	prothioconazole	250 g/L	EC	Bayer CropScience
Proline275	prothioconazole	275 g/L	EC	Bayer CropScience
Swing Gold	dimoxystrobin and epoxiconazole	133 g/L and 50 g/L	SC	BASF

Table 12. Fungicide application dates for all sites and seasons.

Location / Timing	T0 (GS 30)	T1 (GS 32)	T2 (GS 39)	T3 (GS 65)
2008				
Caythorpe	04/04/08	29/04/08	15/05/08	09/06/08
Morley	08/04/08	08/05/08	22/05/08	10/06/08
Sutton Scotney	27/03/08	02/05/08	28/05/08	17/06/08
2009				
Caythorpe	31/03/09	30/04/09	14/05/09	03/06/09
Morley	06/04/09	28/04/09	28/05/09	12/06/09
Sutton Scotney	03/04/09	01/05/09	23/05/09	12/06/09
2010				
Caythorpe	07/04/10	26/04/10	19/05/10	11/06/10
Morley	14/04/10	11/05/10	03/06/10	21/06/10
Sutton Scotney	08/04/10	05/05/10	26/05/10	25/06/10
2011				
Caythorpe	07/04/11	27/04/11	18/05/11	09/06/11
Morley	28/03/11	06/05/11	20/05/11	14/06/11
Sutton Scotney	06/04/11	21/04/11	13/05/11	07/06/11
2012				
Caythorpe	27/03/12	23/04/12	24/05/12	19/06/12
Morley	30/03/12	17/05/12	28/05/12	02/07/12
Sutton Scotney	30/03/12	01/05/12	22/05/12	13/06/12

All experiments used a randomised split plot design with three blocks (with variety as the main plot). Plots were all established using a plot drill and were 2 m wide and 12 m long; the central 10 m length of each plot was harvested by a plot combine. All fungicide treatments were applied using a knapsack boom sprayer, at 200 L ha^{-1} spray volume, with flat fan nozzles. With the exception of fungicides, all inputs were the same as those applied to the commercial crop being grown at that location, keeping with the best local practice.

3.2.8 Participatory farm trials of CCP (WP5)

Participating farms

A total of 27 farms took part in the on-farm field trials of the project, with 21 and 6 being under organic and conventional management, respectively (Table 13). However, not all farms were able to participate in all four trial years. Fifteen of the 27 farms remained in the trial programme over at least three of the four

years, whereas the remaining 12 farms were either unable to continue participation in the project after one or two years, or were recruited later to compensate for those sites that did not continue participating.

In some cases, logistic constraints meant that the wheat crop had to be harvested by the farm before samples could be taken, so that data from these cases is not available. In total, data from 73 farm-year combinations (environments) could be included in the analysis. As mentioned above, the study sites cover a wide geographic range in England, from the South-West to the North (Figure 1**,** above).

Trial entries and trial design

At the beginning of the trial programme, the participating farmers selected which of the three CCP (YQCCP, QCCP or YCCP) was to be trialled on their farms. They received seed of these CCP from the Organic Research Centre. In the first trial year, i.e. sowing in autumn 2007, the CCP seed that was given to the farmers was a mixture of four CCP with different histories; i.e. these CCP came from two organic and two conventional sites (see site description in WP1). This was necessary because of the amount needed to supply all farmers with CCP seed. In addition to the CCP, seed was provided of the nabim Group 3 variety Claire which was used as a control variety. This variety was chosen as it was a well-established and widely grown variety in the UK, both by conventional and organic farmers. Seed was sent to the farmers in first autumn of their participation; they then kept the seed from the following harvest for sowing in the subsequent trial years.

Table 13. Farm details of participating farms for the first three years of the study. Ticks indicate the variety/population was grown on that farm in that year

						2007/08					2008/09					2009/10				
Nr.	**System**	**Pair**	**Certification**	**Region**	**County**	**Claire**	**Own**	**YQ**	**Y**	**Q**	**Claire**	**Own**	**YQ**	**Y**	**Q**	**Claire**	**Own**	**YQ**	**Y**	**Q**
1	CON	1	LEAF	East	Norfolk	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
2	ORG	1	SA	East	Suffolk	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
3	ORG	-	SA	East	Cambridgeshire	-	-	-	-	-	✓	-	✓	✓	✓	✓	-	✓	✓	✓
4	ORG	-	SA	East	Cambridgeshire	✓	-	✓	-	-	✓	-	✓	-	-	✓	-	✓	-	-
5	ORG	-	OF&G	East	Norfolk	✓	✓	✓	-	-	-	-	-	-	-	✓	-	✓	-	-
6	ORG	-	OF&G	East	Suffolk	✓	✓	✓	-	-	✓	✓	✓	-	✓	✓	✓	✓	-	✓
7	CON	-	TAG	East	Norfolk	✓	✓	✓	-	-	✓	✓	✓	-	-	✓	✓	✓	-	-
8	ORG		SA	East	Norfolk	-	-	-	-	-	-	-	-	-	-	✓	✓	✓	✓	✓
9	ORG	2	SA	North	Loughborough	✓	✓	✓	✓	✓	-	-	-	-	-	-	-	-	-	-
10	CON	2	none	North	Nottinghamshire	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
11	CON	3	none	North	N. Umberland	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
12	ORG	3	OF&G	North	N. Umberland	✓	✓	✓	✓	✓	-	-	-	-	-	✓	✓	✓	✓	✓
13	CON	-	none	North	N. Umberland	✓	✓	-	✓	-	✓	✓	-	✓	-	✓	✓	-	✓	-
14	ORG	-	SA	North	N. Yorkshire	✓	✓	✓	-	✓	-	-	-	-	-	-	-	-	-	-
15	ORG	-	Demeter	North	Yorkshire	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	CON	4	none	West	Berkshire	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
17	ORG	4	SA	West	Berkshire	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	-	-	-	-
18	ORG	-	SA	West	Oxfordshire	-	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-
19	ORG	-	SA	West	Berkshire	✓	✓	✓	-	✓	✓	-	✓	-	✓	✓	✓	-	✓	✓
20	ORG	-	SA	West	Gloustershire	✓	✓	✓	-	✓	✓	✓	-	✓	✓	✓	✓	-	-	✓
21	ORG	-	SA	West	Devon	✓	✓	✓	-	-	✓	✓	✓	-	-	-	-	-	-	-
22	ORG	-	SA	West	Gloustershire	-	-	-	-	-	-	-	-	-	-	✓	✓	✓	✓	✓
23	ORG	-	SA	West	Wiltshire	✓	✓	✓	✓	✓	-	-	-	-	-	-	-	-	-	-
24	ORG	-	SA	West	Berkshire	✓	✓	-	✓	✓	-	-	-	✓	-	✓	✓	-	✓	-
25	ORG	-	SA	West	Wiltshire	-	✓	✓	-	-	✓	✓	✓	-	-	✓	-	-	-	✓
26	ORG	-	SA	West	Somerset	✓	✓	✓	-	-	-	-	-	-	-	-	-	-	-	-
27	ORG	-	SA	West	Devon	✓	✓	-	✓	-	✓	✓	-	✓	-	-	-	-	-	-

The participating farmers were asked to sow the CCP and Claire in adjacent strips in a field of winter wheat of their own choice; the variety present in this field was recorded and was labelled as the farmers' "own variety"; this served as the additional pure line control variety for the comparison with the CCP. The farmers own variety was classified according to the nabim baking quality grouping and comparisons were made between YCCP and group 3 or group 4 varieties on the one hand, or between the QCCP and group 1 varieties on the other. As the CCP seed available on each farm multiplied over the years, the width of the strips tended to increase over time, depending on the farmers' propensity to get (and stay) involved in the project.

In addition, three organic and three conventional farms grew all three CCP (YQ, Y, and Q) alongside Claire and their own variety, resulting in five adjacent strips at each of these six farms.

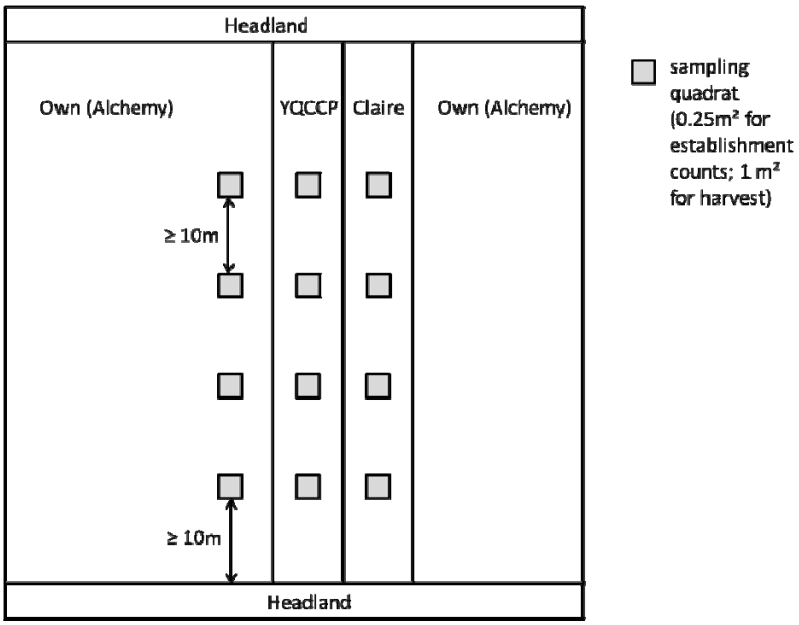

Figure 5. Schematic representation of the on-farm trial set up

Because of practical and technical reasons, the on-farm trials could not be designed in a fully replicated and randomised manner, but followed a pseudo-replicated trial design. Specifically, for all assessments and measurements, four sampling areas were selected in each strip. Sampling areas were chosen with a minimum distance of 10 m from field edges and the headland and also with the same minimum distance between sampling areas within a strip (**Figure 5**). In addition, depending on the width of the strip, the sampling area was placed at least 1 m from edge of strip; when the strip was wider, quadrats for sampling were mostly taken in the middle of the strip. Corresponding quadrats in adjacent strips were placed at the same distance from field edge, i.e. lines linking the quadrats were perpendicular to strip edge.

Adaptation trial

In the WP5 adaptation trial, the YQCCP that had been grown at one of most northern sites for three generations was distributed to five other farms in the South of England in the fourth trial year. While the source site (called CON-North) was managed conventionally, two of the five receiving sites were managed organically (**Figure 6).** In addition, seed of a YQCCP that had been grown at an organic farm in South England was sent to the northern conventional site CON-North in the same year.

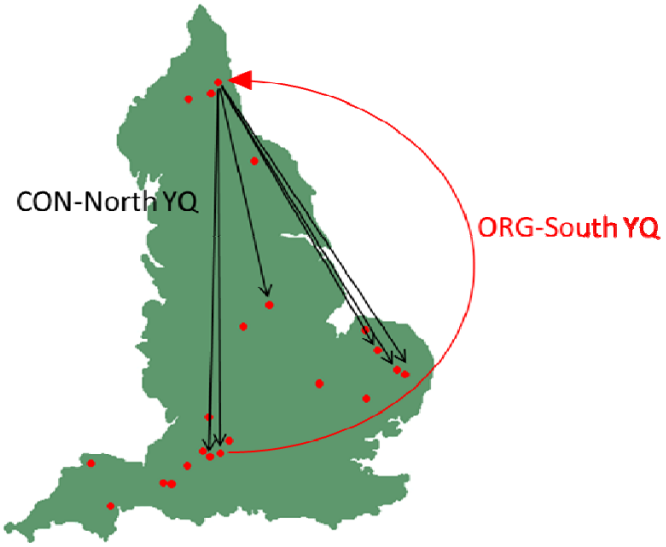

Figure 6. Schematic representation of the adaptation trial; the arrows show how the YQCCP seed with different histories was distributed in the last trial year

Farm operations

Sowing rates for CCP and control varieties were recorded by the farmers in most cases but when drilling was done by contractors, the information was not always available. Pre-crops and mechanical weeding operations were the same for the tested genotypes within each farm-year combination; the same was the case for plant protection on the conventional farms. No plant protection measures took place on the organic fields.

Field assessments and grain yield measurements

In early spring crop establishment was measured as plants m^{-2} by counting individual wheat plants per quadrat in four 0.25 m² quadrats per strip. Care was taken to perform these assessments as far as possible before tillering so that individual plants could be well separated from each other. Weed cover in the sectioned sampling squares was assessed as percentage of soil covered.

At trial harvest, head density (heads m^{-2}) was determined by counting all ears in 1 m² sampling quadrats; in addition, the proportion of awned ears was determined (%), and for 10 randomly selected plants per sampling quadrat, straw height was measured (cm) to the base of the ear. Lodging was assessed as the percentage of plants in four lodging classes. Ears from the sampling quadrats were cut and collected into strong paper sacks, taken to the experimental stations, threshed with a stationary thresher, and weighed. Moisture content of the grain was determined and grain yield was adjusted to 15% moisture content.

Post-harvest measurements

Post-harvest measurements included the determination of Hagberg Falling Number (HFN), grain protein content (%), hardness and thousand grain weight (TGW, in g at 15% moisture content). Measurements of grain protein content followed standard procedures using a near infra-red device at Doves Farm Ltd.

Statistical analysis

All statistical tests were run with R, v. 3.0.1 (Crawley, 2007; R Core Development Team, 2013). For comparing means of the trial entries, mixed effects models were used with farm as random factor and genotype (e.g. CCP vs. pure line) as fixed factor.

For assessing stability of yield, protein and protein yield, various approaches were followed. Generally, many of the established statistical measures of stability across environments are nearly meaningless in cases where only few genotypes are compared. In particular, measures of stability based on Genotype x Environment interactions (Annicchiarico, 2002) are not considered to be meaningful when only two genotypes are compared. For regression-based stability measures (Finlay & Wilkinson, 1963; Eberhart & Russell, 1966), one of the most commonly used stability parameters, which is based on calculating deviations of individual data points from the regression line (Becker, 1981; Becker & Léon, 1988). However, when only two genotypes are in the dataset, this parameter yields identical values for the two genotypes because of the symmetry of the data points around the regression line. Similarly, rank-based stability measures (Huehn, 1990) cannot be used in these cases either, as they are designed for comparing larger numbers of genotypes.

Despite these constraints limiting the choice of stability parameters for the analysis of the WP5 dataset, various measures of stability could be calculated. (1) As measure of variance across environments, corrected for the size of the mean, the coefficient of variation (CV) was used; the statistical test whether two CVs are significantly different followed Sachs & Hedderich (2009). (2) The CV within field was determined as a measured of small-scale fluctuations. (3) The slope *b* of the regression of genotype against mean yield in each environment was determined. (4) A novel measure of stability was used, based on Taylor's Power Law (Cohen, 2013). Specifically, means (m) and variances (s^2) of yields over

years within farms were calculated for farm. Second, following Taylor's Power Law (TPL), the regression of $y=\log(s^2)$ against $x=\log(m)$ was calculated; the deviations from this regression line can be interpreted as an index of yield variability, i.e. the smaller the residuals, the higher the yield stability. (5) The number of years in which performance (e.g. yield) is below k% of the average was used as a further measure of yield stability.

3.2.9 Performance of CCP with additional parent material (WP6)

Generation of CCP and trial entries

Initial seed crosses of Pegassos/Xi19 with the Y, Q and YQ CCP parents were carried out by the John Innes Centre, Norwich (JIC), and were completed at end of project year 1. Pegassos was crossed with each Yield and Quality variety from the original 20 CCP parent lines (see section 3.2.1) in a series of two-way crosses. Similarly, Xi19 was crossed with each Quality variety from the CCP parent population. This work was carried out at the JIC, and all seed from the progeny was grown in plots and bulked at WAF during 2008-09.

The seed from all Pegassos crosses was harvested and mixed together, as was that from the Xi19 crosses, and a third combination mix of Pegassos and Xi19 was prepared. In making up the mixtures of Xi19 and Pegassos crosses, thousand grain weight (TGW) was taken into account such that the numbers of seed of each type of cross included in the mixture was equal. Seed from these crosses was added to seed from the corresponding CCP (YCCP for Pegassos, QCCP for Xi19 or YQ CCP for the mix of both) in a ratio by weight of 2/3 CCP to 1/3 Xi19 or Pegassos cross (1/6 of each where both crosses were included) as follows:

YCCP (2/3) + Pegassos (1/3)
QCCP (2/3)+ Xi19 (1/3)
YQCCP (2/3) + Pegassos (1/6) + Xi19 (1/6)
QCCP + Pegassos + Xi19
QCCP + Pegassos

Trial design and location

Trials were carried out at four sites, two organic (WAF & SOF) and two conventional (MET & MOR; see section 3.2.1) over growing season 2009-10 with each of the five entries repeated three times in a randomised complete block design in plots 10.44m^2 harvested area. Trials at the conventional sites received nutrient, herbicide, fungicide, pesticide and plant growth regulator applications. Seed quantities were adjusted to give a target population after germination of 425 plants m^{-2}. The trials were drilled in October 2009 with 20 cm row spacing.

Plant assessments and measurements

Trials were harvested using a small plot combine to determine marketable yield in t/ha at 15% moisture content. Grain samples were taken for protein content analysis determined on a % dry basis using near infrared spectroscopy (Perten Instruments, model Inframatic 8600) on 100g subsamples of grain.

Statistical analysis

Data from SOF was excluded from the analysis due to particularly low yields and unreliable data (Figure 37). Data analyses were performed using the statistical software R, version 2.10.0 (R Core Development Team 2013). Grain yields and protein % dry matter were analysed by analyses of variance (ANOVA) to test statistical differences between trial entries. Fixed factors included system (organic/conventional), trial site and block. Step-wise model reduction was carried out using AIC values to determine best model. Residuals were then tested for normality using Shapiro test. Where there were significant effects, post-hoc Tukey Honest Significant Difference (HSD) testing was used to determine which factors differed significantly.

3.2.10 The effects of mass selection on CCP (WP7)

Field trial sites: location and set up

A randomised complete block trial with three replicates was conducted over five growing seasons (harvest year 2008 to harvest year 2012) at one organic (WAF) and one conventionally managed site (MET). Trails at WAF did not receive any chemical application and were managed according to organic regulations. Trials at MET received nutrient, herbicide, fungicide and pesticide applications. All treatments were sown to achieve a plant density of 425 plants/m^2 with 20 cm between rows. Plot dimensions were 10m by 1.2m. In all five years of the experiment seed was sown in October and grain was harvested using a small plot combine harvester in August.

In 2008, at F6, both YQCCP and QCCP were used for the mass selection trial reported here, after being grown at their respective sites (MET and WAF) for three consecutive years previously.

Grain Colour selection

Colour selection aims to exploit the link between grain colour and per cent protein content. Red wheats having higher protein than white wheats. However, it is unclear whether this link is genetic, therefore selectable, or whether environmental factors mainly influence grain colour and protein. After the 2008 harvest year (F6), grain from QCCP and YQCCP at WAF and MET was subjected to mass selection based on kernel colour. A Satake Alpha Scan II employed multiple high-resolution monochromatic charged coupled device (CCD) cameras to separate grain into two fractions (light and dark) based on kernel colour. Seeds in which optical signals exceeded the threshold were rejected by air ejectors, and

grain samples were passed through the sorter until they were stratified to a ratio of 30:60 dark:light for selection of the dark fraction, and 30:60 light:dark for the selection of light fraction. The resulting 6 treatments (Dark-, Light-, and unselected Control-CCP x 2 sites) were then drilled in October 2008. In the first year of the trial the treatments were unreplicated; in subsequent years they were sown in a fully randomised complete block trial with three replicates. Seed originating from WAF was sown in trials at WAF, and seed originating from MET was sown in trials at MET. In each year, after grain was harvested and relevant assessments had been made as below, Dark- and Light-CCP were bulked from their respective blocks and each colour CCP was then subjected to further mass selection, i.e. Dark-CCP were sorted into a darkest and lightest fraction, as were Light-CCP. After each selection event the darkest fraction from the Dark-CCP and the lightest fraction from the Light-CCP was assessed for quality parameters and retained to form the seed for the next generation, which was then sown alongside the unselected CCP. In 2011, selection was performed only on QCCP at WAF, which was then sown at WAF in a fully randomised complete block trial with six replicates.

Grain Size selection

A 7.5kg seed sample of QCCP, harvested from MET in August 2009, was split into 3 size fractions (small, medium and large) using a seed dresser (Model Röber GmbH, D-4950 Minden i/W). Yield and quality parameters were assessed as below before the three fractions were drilled in a fully randomised complete block trial with three replicates at MET. Grain from the progeny of each size-selected CCP was harvested in 2010, and analysed for yield and quality parameters. Each size fraction was then itself subject to a second round of mass selection for grain size using the seed dresser, where each of the three size fractions was separated into a large, medium and small fraction. The largest fraction of the Large-CCP, smallest of Small-CCP, and medium fraction of the Medium-CCP were then analysed for quality parameters and sown in 2010 in plots using the same experimental design as the previous year.

Density selection

A 15kg seed sample of QCCP, harvested from MET in August 2009, was split into 3 fractions (heavy, medium and light specific gravity) using a gravity separator (Specific Gravity Separator, Model SY100). The three density fractions were analysed for yield and quality parameters before being drilled in a fully randomised complete block trial with three replicates at MET. The progeny were harvested in 2010, analysed for yield and quality parameters and then each subjected to further mass selection by separating the grain of each of the three density fractions, once again, into the lightest, medium and heaviest fraction based on specific gravity. The heaviest of the Heavy-CCP, lightest of the Light-CCP, and medium fraction of the Medium-CCP were then sown in 2010 in plots using the same experimental design as the previous year.

Plant assessments and measurements

Protein content of grain was determined on a % dry basis (proteinDB) using near infrared spectroscopy (Perten Instruments, model Inframatic 8600) on 100g subsamples of grain. Hagberg Falling Number (HFN) was measured on the same 100g sub-samples using Perten Instruments model FN 1700. Thousand grain weight in grams (TGW) was determined by counting and weighing 500 grains per plot. Plots were harvested using a small plot combine and grain weighed to determine marketable yield in t/ha at 15% moisture content.

Statistical analysis

All data analyses were performed using the statistical software R, version 2.14.2 (R Core Development Team 2013). Analyses Of Variance (ANOVA) were carried out to test statistical differences between fractions. For colour selection, dark and light CCP were compared in two-way ANOVA with dark vs. light as levels, and also including the unselected control CCP as a third "colour" level. Due to the nature of CCP, year potentially has a cumulative effect on crop performance, as may the repeated mass selection at each generation; therefore year was included as a fixed effect in the linear models. Step-wise model reduction was carried out using AIC values to determine best model. Residuals were then tested for normality using Shapiro test. Where there were significant effects, post-hoc Tukey HSD testing was used to determine which factors fractions differed significantly.

3.2.11 Bread-making quality and micronutrient content of CCP (WP8)

Bread making requires wheat grain which exceeds minimum thresholds in protein content, HFN, specific weight and other measures to produce bread of a quality acceptable to consumers.

Baking tests

Sample selection

In all trial years, the sample set included within-farm pairs of YQCCP and QCCP samples (Table 14). Farms of sample origin included both trial hubs and participatory farms, organic and non-organic. In trial years one and two, samples were unreplicated within bakeries. In trial year three, bakers were sent two replicates of some samples.

Bakers used flours of their own choosing as controls in trial years one to three. Therefore, the control flour differed from bakery to bakery, sometimes including imported wheat with much higher protein content, and sometimes being limited to UK wheat. In this respect, the comparison between baker controls and the CCP should be treated with caution. Ultimately, it was decided by the consortium that the most relevant comparison would be between CCP and other all-UK flours. Because the blending of UK flours

with foreign flours is mainstream practice in the industry, and flours from CCP are likely to be treated in the same way if marketed, CCP flour only needs to perform as well as other UK wheat flours.

Table 14. Wheat CCP populations (QCCP and YQCCP) and pure line samples baked over the 4 year experimental period.

Trial year	Harvest Year	Farm	County	System	Number of samples		
					QCCP	YQCCP	Pure line
1	2008	Benham	Berkshire	Non-organic	1	1	0
1	2008	Causey Park	Northumberland	Non-organic	1	1	0
1	2008	Shackerdale	Nottinghamshire	Non-organic	1	1	0
1	2008	Metfield	Suffolk	Non-organic	1	1	0
1	2008	Wakelyns	Suffolk	Organic	1	1	0
1	2008	Sheepdrove	Berkshire	Organic	1	1	0
1	2008	Manor	Leicestershire	Organic	1	1	0
1	2008	Gilchesters	Northumberland	Organic	1	1	0
2	2009	Metfield	Suffolk	Non-organic	1	1	0
2	2009	Shackerdale	Nottinghamshire	Non-organic	1	1	0
2	2009	Benham	Berkshire	Non-organic	1	1	0
2	2009	Causey Park	Northumberland	Non-organic	1	1	0
2	2009	Wakelyns	Suffolk	Organic	1	1	0
2	2009	Sheepdrove	Berkshire	Organic	1	1	0
2	2009	Doves	Berkshire	Organic	1	1	0
2	2009	Lavenham	Suffolk	Organic	1	1	0
3	2010	Metfield	Suffolk	Non-organic	2	2	0
3	2010	Causey Park	Northumberland	Non-organic	1	1	0
3	2010	Benham	Berkshire	Non-organic	1	1	0
3	2010	Shackerdale	Nottinghamshire	Non-organic	1	1	0
3	2010	Sheepdrove	Berkshire	Organic	1	2	0
3	2010	Wakelyns	Suffolk	Organic	6	2	0
3	2010	EH Farms	Gloucestershire	Organic	1	1	0
4	2012	Morley	Norfolk	Non-organic	0	3	0
4	2012	[unknown]	[unknown]	Non-organic	0	0	3
4	2012	Wakelyns	Suffolk	Organic	3	3	0
4	2012	[unknown]	[unknown]	Organic	0	0	3

In trial year four, bakeries were sent three replicates of each sample. Five entries were selected, including WAF QCCP, WAF YQCCP (both organic), MOR YQCCP (non-organic), pure Paragon (organic, single site) and pure Hereward (non-organic, single site). Three samples of each entry were provided to each baker to allow for full within-bakery replication (although in some instances these were pseudo-replicates drawn from a single batch of seed, allowing only control for variation in the baking process rather than

variation in the field). This approach was taken to permit within-site comparison of QCCP against YQCCP (from WAF), inter-site comparison of YQCCP (WAF and MOR) and comparison of three population samples against two pure line samples standardised across bakeries.

Baking methods

Grain from selected populations or pure line wheat was sent to WH Marriage & Sons for roller-milling, and was stone-milled at either Letheringsett Watermill (trial years 1–3) or WAF (trial year 4). Stone-milled flour was distributed to all bakeries except WH Marriage & Sons, which used its own roller-milled flour. Each bakery received enough flour to bake at least two 400g loaves per sample, although only one loaf was used for evaluations. Participating bakeries changed year to year but always included WH Marriage & Sons, using the Chorleywood method, and Bread Matters, using sourdough. Other bakeries involved included Panary, Shipton Mills, Letheringsett Watermill (all using small-scale yeast-assisted process) and Wee Boulangerie (sourdough). A full description of bakers' methodologies is provided in Appendix B: Baking methods. The identity of each sample was known to bakeries in trial year one, but thereafter samples were coded and labelled with a number and 'organic' or 'non-organic'.

In 2009, 2010 and 2012, the HFN of every individual sample was tested. In all trial years, protein analysis was also undertaken. In addition WH Marriage & Sons performed a grain analysis of samples, including hardness, protein (% as is and % dry basis), screenings (%), impurities (%), moisture (%), bushel weight (kg), gluten quality (pass/fail) and *Fusarium* sp. (spores per 100g). Bakers provided qualitative feedback on criteria of their own choosing but which included dough properties such as gluten quality, water absorption and extensibility as well as loaf properties such as crumb and crust texture and colour, bread flavour. WH Marriage & Sons provided measurements of dough temperature (°C), proof time (min) and proof height (cm). In trial year four, bakers were asked to provide scores on qualitative criteria including crumb and crust colour and texture, flavour and overall recommendation.

It was proposed that loaf volume measurements should be carried out using seed displacement, but not all bakers had the necessary equipment for such a test. WH Marriage & Sons were able to measure both loaf volume and loaf height. In order to research whether loaf height could be used as a proxy for loaf volume, we tested the correlation between these two parameters in 56 tests using Pearson's Product Moment. We found a strong positive correlation (R^2=0.95) which was highly significant (critical value=0.354 for $p<0.01$ at df=54) and from this determined that it was justifiable to regard loaf height as an approximation of loaf volume as used by the industry.

In trial years one through three, tin loaf height and loaf volume measurements were provided by WH Marriage & Sons. From trial year two onwards, loaf height data for tin loaves was also available from Bread Matters, and all four bakers in trial year four were asked to provide loaf height data.

Data analysis

All data analysis was performed using the statistical software R, version 2.14.2 (R Core Development Team 2013).

Qualitative data

In trial years one through three, bakers provided verbal feedback on a range of dough and loaf parameters including gluten development, ease of handling, colour, texture and flavour. Each baker freely selected which parameters he or she would report on. This qualitative feedback was converted into quantitative data using a three-part coding scale (0 for a negative comment, 0.5 for a neutral one and 1 for a positive one). Scores from every parameter tested per loaf were combined, unweighted, into a single 0–1 score for comparison across samples.

In trial year four, bakers were asked to use specified parameters for qualitative evaluations and to provide numerical as well as verbal feedback (score between 0 and 5 on crumb texture, crumb colour, crust colour, flavour and overall recommendation). These scores were, again, combined and converted to a 0–1 scale for within- and between-year comparisons.

To compare CCP samples against controls, only data from trial year four was used. Because the data is ordinal, the main test was a Wilcoxon signed rank test with entries grouped into ‘Pure Line’ and ‘CCP’. To analyse the difference between YQCCP and QCCP quality scores, the Wilcoxon paired signed rank test was again used.

Loaf height

To test for population vs. pure line differences in loaf height, the only complete dataset was from trial year 4, when three populations (two organic and one non-organic) and two pure lines (one organic and one non-organic) were each tested in triplicate by all four bakeries. One-way analysis of variance, linear mixed effects models and Kruskal-Wallis tests were used, having divided the dataset into ‘Pure Lines’ (three entries, both organic and non-organic) and ‘Populations’ (two entries, both organic and non-organic) and taking ‘Pure/Population’ as the input variable with System (organic or non-organic) as a factor and loaf height variance (calculated with respect to within-bakery means) as the response variable.

Data from all four trial years was suitable to test for differences between YQCCP and QCCP loaves. Data from the first three trial years, when YQCCP and QCCP samples were being grown at a large range of farms, were analysed using linear mixed effects models with ‘CCP’ as the dependent variable, ‘height’ as the response variable and separate models being used for each baker. In trial year four, data from QCCP and YQCCP populations grown at WAF only were tested using one-way ANOVA and linear mixed effects models, with loaf height variance (within-bakery) as the response variable and population (QCCP or YQCCP) as the input variable.

Protein and Hagburg Falling Number tests

In all trial years, grain protein data was provided by WH Marriage & Sons. In trial years one and two, it was provided for some samples by Shipton Mills. Due to unbalanced experimental design, statistical comparison of populations against pure line or control samples was not possible. A comparison of protein content in QCCP and YQCCP samples was carried out on the pairwise data from all trial years using a one-way ANOVA (using type II sum of squares due to the unbalanced nature of the experimental design), with protein content as the response variable and year, baker (Marriage or Shipton), population type (QCCP or YQCCP) and growing system (organic or conventional) as fixed factors.

In 2009, 2010 and 2012, a subset of flour samples was tested for HFN. The HFN results for CCP were compared to those of pure lines for trial year four using one-way ANOVA (using type II sum of squares due to the unbalanced nature of the experimental design), with HFN as the response variable and baker, variety (QCCP, YQCCP, Paragon and Hereward) and growing system (organic or conventional) as fixed factors. A further pairwise Q-YQ comparison was made of HFN across all years using a univariate ANOVA (using type II sum of squares due to the unbalanced nature of the experimental design), with HFN as the response variable and baker, population type (QCCP or YQCCP) and growing system (organic or conventional) as fixed factors.

Micronutrient tests

Sample selection

The consortium decided that nutritional assessments should be made on wheat grain rather than bread as originally planned in the proposal. This was because the main subject of interest was the influence of the wheat genotype as opposed to that of the milling and baking processes. Testing the grain directly also makes results more widely relevant for applications of the CCP to uses in the human food chain other than bread making, such as pastry and biscuits. The main subjects of this study were the QCCP and YQCCP. Grain from the CCP grown at both organic and non-organic sites was tested, and compared against a pure-line from the same organic site as well as two different pure-lines grown at two further organic and non-organic sites.

Samples were taken from winter wheat planted in 2011 and harvested in 2012 on four farms in the east and southeast of England. Trial entries submitted for nutritional analysis are shown in Table 15. Solstice and Paragon are not among the parents of the CCP, but they and Hereward are all high-quality breadmaking varieties, i.e. equivalent in terms of intended end-use to the YQCCP and QCCP. When grain came from individual trial plots (QCCP and YQCCP), three replicates per entry and site were submitted. Where grain was only available from bulked lots, one replicate only was submitted to avoid pseudo-replication.

Table 15. Samples entered for nutritional analysis (WAF = Wakelyns Agro-Forestry, MOR = Morley Research Centre)

Entry (variety)	Diversity level	Site of origin	System	No. of replicates
Control 1 (Paragon)	pure	Unknown*	Organic	1
Control 2 (Hereward)	pure	Unknown*	Non-organic	1
Control 3 (Solstice)	pure	WAF	Organic	1
QCCP	CCP	WAF	Organic	3
YQCCP	CCP	WAF	Organic	3
YQCCP	CCP	MOR	Non-organic	3

*Supplied by Marriage's Millers, Essex

Micronutrient analysis

Following discussions within the consortium and guidance from industry partners, alterations were made to the originally planned programme of nutritional analyses carried out on samples selected. Assessment of vitamin B was abandoned due to the strong influence of milling and baking on final concentrations in bread, which obfuscates the influence of genotype and is the reason why vitamin B enrichment is not a prominent goal in wheat breeding programmes. Similarly, acrylamide tests were abandoned because current research demonstrates that soils sufficient in sulphur are more important than cereal genotype as a contributory factor to acrylamide formation in cooked grain products (Food Drink Europe, 2011). Phosphorous, potassium and silicon were also excluded from analyses on the basis that wheat is not considered an important dietary source of any of these elements. However cadmium, a soil contaminant, was added to the programme of tests since its uptake by wheat is thought to be influenced by genetic components (Zhao & Shewry, 2010, Greger & Löfstedt, 2004). Furthermore, it is important as an anti-nutritive factor, of which whole grain cereals are determined to be a primary source (EFSA, 2011).

Therefore, analyses were conducted to determine concentration of nine minerals. Eight of these are nutrients: calcium, magnesium, molybdenum, selenium, iron, zinc, chromium and cobalt. The ninth, cadmium, is a toxin. Two-hundred gram samples of wholegrain wheat were sent to Sciantec Analytical Services Ltd. Samples were ground to pass a 1 mm screen. Analysis for calcium, iron, magnesium and zinc was performed using Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES) following dry ashing and acid digestion. The minerals cadmium, cobalt, molybdenum and chromium were determined by ICP-OES following microwave digestion and selenium was determined by Atomic Fluorescence Spectroscopy, again following microwave digestion of the sample. Results were reported on an 'as received' basis.

Data analysis

Data was divided into subsets as follows:

- QCCP versus Solstice (pure line), both from WAF
- YQCCP versus QCCP, both from WAF
- Pure lines (Controls 1, 2 & 3 and Solstice) versus CCP (QCCP and YQCCP from WAF and MOR)

- Organic versus non-organic YQCCP (from WAF and MOR)

The sample means within subsets were compared using one-way analysis of variance with the diversity (for Pure-lines vs CCP) or entry (for QCCP vs Solstice, QCCP vs YQCCP and organic vs non-organic) as the dependent variable and mineral concentration as the response variable, using the statistical software R, version 2.14.2 (R Core Development Team 2013). Tests were performed separately for each mineral.

Across all samples, correlations between different mineral concentrations were tested using Pearsons product moment coefficient and compared with critical values to determine significance.

3.2.12 Acceptability of CCP for malting, distilling and animal feed (WP9)

Malting

The process of malting aims to control natural changes in the grain which occur during germination. In essence, germination is initiated and then halted when these changes have reached a specific point. It involves a number of stages, key of which are steeping, germination and kilning. During steeping the moisture content of the grain is increased to 44–46% to begin germination. The grain is then left to germinate for 4–5 days. During germination, the endosperm undergoes 'modification' where starch stores are mobilised to make them accessible to starch degrading enzymes in the brewery. Before excessive modification occurs, heat is applied through kilning which arrests any further changes. Depending on the type of malt required more heat can be used to fine-tune the flavour qualities and finish off the process of malt production.

The protein (nitrogen) content of the grain is one measure of quality and this determines the ultimate use of the malt; in brewing the tolerable range is relatively broad (9–12%) depending on the brewing process and type of beer to be brewed. Factors influencing grain quality act at various stages in the growth and processing cycle. For example, besides the choice of variety and crop management approach, the weather conditions during growth influence protein levels in the harvested grain (Agu & Palmer, 2003) and post-harvest moisture levels determine storage success.

Malting analyses

Crisp Malting Ltd. carried out the micro-malting tests throughout the project at their Ryburgh site in Norfolk. They micro-malted up to twelve samples in total per year (Table 16). The original plan as detailed in the original project proposal was to test the Q, Y and YQCCP from each of four hub sites (MET & MOR - conventional, SOF & WAF – organic) in each project year. In year 1 (2007-08), however, the crop at WAF failed due to weather conditions resulting in late drilling, followed by prolonged cold, further wet and high levels of slug damage. As a result, only three hub sites were assessed. In year 2 (2008-09) all CCP were tested from every hub site, but as had been observed in year 1 the results indicated that the QCCP was especially unpromising as a malting wheat so it was omitted from further tests. Instead, a fraction of

seed from the mass selection work (section 3.2.9) was substituted in the remaining years (2009-10 & 2010-11). This was the YQCCP LIGHT and it was chosen because WP6 had shown that the light fractions had a lower protein level than the dark fractions and therefore might be better for malting. The YCCP, YQCCP and YQCCP LIGHT were only tested from WAF and MET because the LIGHT selections were not included in trials at SOF or MOR.

Table 16. Micro-malting samples analysed in each project year by Crisp Malt Ltd.

Project Year	Sites	Samples micro-malted
1 (2007-08)	MET, MOR, SOF	YCCP, QCCP, YQCCP
2 (2008-09)	MET, MOR, SOF, WAF	YCCP, QCCP, YQCCP
3 (2009-10)	MET, WAF	YCCP, YQCCP, YQCCP Light
4 (2010-11)	MET, WAF	YCCP, YQCCP, YQCCP Light

A typical malting analysis includes a large number of complex parameters which are collectively used to evaluate the overall acceptability of the malt produced from the grain sample. Amongst these are total nitrogen, soluble nitrogen, enzymes levels, extract, colour assessment, wort viscosity and pH. The analyses were carried out according to the methods detailed in see Appendix C: Methods Of Malt Analysis. (supplied by Bruce Johnson, Crisp Malting). The maltsters interpret the results so as to characterise the grain according to three key factors: Protein levels, kilning control and quality.

Analysis was carried out using SPSS software. As the data were not normally distributed, the non-parametric Kruskal-Wallis test was used to evaluate whether there were differences according to management system (conventional or organic), site (MET, MOR, SOF or WAF) or population (YCCP, YQCCP, QCCP or YQCCP LIGHT).

The variables measured in micro-malting tests all help maltsters to judge the suitability of a grain for malt production. The following descriptions are largely based on information from two brewing websites[1], in addition to discussions with maltsters at Crisp Malting. The moisture post-kilning gives a measure of grain drying characteristics. It is important because greater moisture content increases the risk of mould and spoilage. In addition, when brewers purchase malt, they require a product that contains lower water levels and consequently higher levels of convertible starches and extractable sugars. Depending on the malt, the moisture content would typically be in the range of 3–5%, with 5% being a maximum. It should be noted, however, that the moisture of the finished malt product is unrelated to the crop management system employed, but is instead due to the kilning program used during malt production. Therefore it is not included in this report, which focuses on cropping system effects.

[1] http://www.byo.com/bock/item/1544-understanding-malt-spec-sheets-advanced-brewing; http://morebeer.com/brewingtechniques/bmg/noonan.html Both accessed 04/07/13

Total nitrogen is an indication of protein levels (total nitrogen = protein x 6.25). These levels need to be adequately high for fermentation, but low enough not to cause haze on chilling. In the UK brewing industry, minimal values are usually sought because protein adds little value other than the provision of enzymes and foam to beer. The acceptable total nitrogen values are usually less than 1.6%.

The soluble nitrogen ratio SNR is a measure of the level of protein breakdown/modification, which is a key part of malting process. The higher the SNR, the more modified the malt is (i.e. more protein has been broken down). To a certain extent, under or over modified malt can be adjusted by varying temperature and resting periods during processing, but values under 30% would be considered under-modified and undesirable in any potential brewing wheat. Diastatic power is an indication of the ability of starch reducing enzymes in the malt to convert starch to sugars. Of all the measures described in this report, diastatic power is perhaps the variable with the greatest level of tolerance. The desirable value depends on the type of beer to be brewed – it can be as low as 35–40 in British pale ale or 160 plus in North American beers. It is an important consideration when base malts are mixed with other grains or malts.

Extract is a very important measure because it is related to the yield that can be targeted by brewers. In effect, it is equivalent to the amount of sugars that are contained in the malt which act as the substrate for yeast. The values given by laboratory tests often need to be reduced to give a realistic indication of actual likely yields from a commercial brew house, because production breweries are not as efficient as lab equipment. The magnitude of any such reductions would be judged by maltsters on a case-by-case basis this would be decided by experienced maltsters. There are various ways of expressing extract; the method chosen for this report is based on the hot water extract (HWE) process. However, extract is more intuitively understood when expressed as % dry weight of extract, which is calculated by dividing the HWE value by 386. Typical standard dry weight values are close to 82%.

Distilling

Distillation in the context of the drinks industry is the process whereby ethanol is produced and separated from fermented grain for use in making alcoholic beverages, typically spirits. Here the focus was specifically on Scotch whisky. During the distilling process the cereal is cooked at a high temperature to release starch and liquefy the grain in order for it to be broken down by enzymes derived from barley malt into fermentable sugars. Yeast then acts on these sugars during fermentation to release ‘wash’, a product that contains 8–9% alcohol by volume. This is refined by distilling in continuous fractionating (Coffey) stills (Agu et al., 2006).

Moisture, alcohol yield, residue viscosity, protein and total nitrogen are important variables used in assessing distilling wheat quality. Aside from moisture playing an important role in cereal trading due to its impact on storability, there is evidence that moisture is an important parameter in alcohol

measurement and can affect alcohol yield (Agu and Walker, 2012). There is also compelling evidence of the influence water has on nitrogen (Miller, 2004), as well as its involvement in carbohydrate-protein binding (Clarke et al., 2001).

Distilling analyses

The Scottish Whisky Research Institute carried out analyses of moisture content, alcohol yield, residue viscosity, protein content and total nitrogen on 18 wheat samples (Table 17) from project year 1 (2007–08).

Table 17. Wheat population samples analysed for their suitability in distilling (2007–08)

Growing location	Population	Number of samples
Metfield (MET)	Standard YCCP	3
	Standard YQCCP	3
Sheepdrove (SOF)	Standard YCCP	3
	Standard YQCCP	3
Morley (MOR)	Standard YCCP	3
	Standard YQCCP	3
		TOTAL: 18

Residue viscosity relates to the downstream processing of co-products after distillation. The interactions between and amongst substrates present in wheat – protein and starch in particular, as well as other non-starch polysaccharides (NSPs) – will affect the residue viscosity of processed wheat. Starch is usually embedded in a protein matrix. Therefore to gain access to the starch during processing of cereals for alcohol yield, adequate hydrolysis of NSPs and proteins is required. It follows that when hydrolysis of proteins is limited, so too is hydrolysis of starch and excessively high protein levels will play a role in restricting access to starch. Un-hydrolysed starch and other materials (substrates) will show up during measurement of residue viscosity.

The analyses were performed according to standard SWRI methodologies (OP 302, 344 & 316). Moisture content was determined using an oven method, a wheat cooking method was used for alcohol yield and a glass viscometer was used to measure residue viscosity. Protein content was determined by NIR on a Foss 1241 spectrophotomer, based on the Foss Network model. Total nitrogen (TN; protein content/5.7) was calculated internally by the instrument (Agu et al., 2006; 2008).

Analysis was carried out using SPSS software. As was the case with the malting data, the distilling data were not normally distributed and therefore the non-parametric Kruskal-Wallis test was used to evaluate whether there were differences according to management system (conventional or organic), site (MET, MOR, SOF or WAF) or population (YCCP, YQCCP, QCCP or YQCCP LIGHT).

Animal feed

Many European winter wheat varieties carry a modified 1B chromosome, which has the 'short' arm of chromosome 1R (1RS) from rye fused to the 'long' arm of wheat chromosome 1B (1BL) instead of having the normal 'long' and 'short' arms of 1B. This modified chromosome is designated 1BL-1RS, or 1B/1R for short (Zeller et al 1973). The translocation originated in Eastern European varieties and occurred spontaneously by the introgression into wheat of rye genes from an adjacent field by natural cross pollination. The introgression probably dates to the end of the 19th century and must have been selected by farmers or breeders at that time because it gave agronomic improvements, notably disease resistance (the rye segment carries resistance genes for leaf rust (*Lr26)*, Yellow rust (*Yr9)*, Stem Rust (*Sr31)* and Powdery Mildew (*Pm8*)). Although these resistance genes have broken down to new virulences, recent experiments have also shown that the translocation can impart agronomic advantages by increasing biomass and yield by up to 1 t/ha in certain years and environments (Foulkes et al., 2007), and thus was maintained in breeding programmes because of this advantage.

However, although the 1B/1R translocation is good for yield productivity, experiments have also shown that it can be detrimental for quality traits. Notably, it results in 'sticky doughs' for bread making and high viscosity for animal feed (Graybosch, 2001), both traits that are undesirable for end-use purposes. Thus, at the present time, wheat breeders aim to remove it from breeding programmes aimed at producing varieties suitable for bread and animal feed.

In the CCP included in this project, three parent varieties carry the translocation: Buchan, HTL (a breeders' line) and Tanker. It was decided, therefore, to monitor the presence of the translocation over successive years and in different environments, to see if it would be at a frequency that could be an impediment for end-use quality in the CCP.

Animal feed analyses

Over the period of the project, the frequency of the 1B/1R translocation was monitored in the YCCP and YQCCP using different methods over three cropping seasons: 2007–08 (YQCCP only), 2008–09 and 2009–10 (YCCP and YQCCP).

The presence of the 1RS arm can be tested for in a number of ways. Initially, in year 1 (2007–08) a disease scoring method was used based on testing for susceptibility to a leaf rust isolate which is avirulent on 1B/1R carriers, but virulent on 1B varieties. This was carried out through the cooperation of Limagrain UK by their plant pathologist Paul Fenwick. 1B/1R carriers show a '00' (completely resistant, hypersensitive) reaction, whilst 1B carriers show reactions from '00CC' (some resistance with chlorosis) to a completely susceptible '4' reaction (Figure 7).

Figure 7. Pictures of detached wheat leaves showing a completely susceptible, type 4 reaction for a non-1B/1R plant, and a completely resistant 00 reaction for a completely resistant 1B/1R plant.

It was observed, however, that not all plants could be easily classified under this system, probably because of 'modifier' genes in some varieties, and thus an ordinate scoring method from 00 to 4 was used to evaluate individual plants:

00 *– completely resistant, hypersensitive, 'pin prick' reaction type, typical of Lr26 carriers (no chlorosis);*

0C *– another type of resistant reaction where lots of chlorosis is present (0CC is where there is even more chlorosis, sometimes typical of Lr37 varieties etc.);*

1 *– lots of chlorosis but a few very tiny pustules are present (1C – even more chlorosis);*

2 *– still basically a resistant reaction but medium sized pustules are surrounded by chlorosis (2C, 2CC etc.)*

3 *– a susceptible reaction, but less than fully susceptible. Big pustles with yellow halos.*

4 *– fully susceptible, pale green halos around large pustules.*

Whilst molecular methods were available at the beginning of the project, they were at that time costly and slow which precluded their use. In years 2 (2008–09) and 3 (2009–10), however, a new molecular marker method was developed by Limagrain UK which used a quick, cheap, assay using TaqMan probes to detect a single nucleotide polymorphism (SNP). This approach gave unambiguous classification of the presence of either the 1BS or 1RS chromosome arms, based on an A or G base difference in part of the sequence identified as discriminating between the chromosomes. These base differences were identified using proprietary probes and primers (also from Limagrain). Furthermore, this molecular method also

allowed the identification of heterozygous plants which the disease scores could not do and thus represented an unambiguous co-dominant marker.

3.3 Results

3.3.1 Quantifying the yield, quality and stability of performance of CCP (WP1)

Comparisons within populations

Pre-harvest parameters

In the main trials of WP1, **establishment** of the wheat crop (i.e. plant density in spring) tended to be lowest in the YCCP, and highest in the QCCP, with YQCCP being intermediate. However, differences between the three sets of populations were not significant when considered as a main effect (P>0.8). However, significant (P=0.029) three-way interactions between parent set (Y vs. YQ vs. Q), management system (organic vs. conventional), and male sterility (CCP_n vs. CCP_{ms}) indicated that crop establishment was subject to more complex effects.

For the CCP_n within the organic system there was a significant effect of the parent set, with the $YCCP_n$ showing 18.9% smaller plant density than the $QCCP_n$ (P=0.04). However, when the organic sites WAF and SOF were analysed separately, the effect of the parent set was no longer significant. There was no consistent relationship between crop establishment and grain yield.

Stability of establishment over environments did not show any consistent patterns among the three different parent sets (Table 18); the ranking of the Y, Q and YQ populations depended on the stability parameter used.

Table 18. Stability of establishment in the six populations; lowest stability among the six CCP marked in red font, highest in bold.

	b	CV	POLAR	s^2di	Ecovalence	Range/mean (%)
$YCCP_n$	0.92	67.5	0.009	22281	22529	288
$YQCCP_n$	0.98	67.5	-0.037	11512	11521	289
$QCCP_n$	1.01	69.4	-0.018	19297	19304	298
$YCCP_{ms}$	1.07	73.4	0.040	**10875**	**11033**	316
$YQCCP_{ms}$	1.08	74.2	0.044	15967	16208	314
$QCCP_{ms}$	0.94	**66.2**	**-0.038**	20232	20376	**285**

Further, there were no significant effects of management system (organic vs. conventional), parent set (Y, Q, YQ) or the type of the population (CCP_n vs. CCP_{ms}) on total **foliar disease** levels. Similarly, damage by the **cereal leaf beetle** (in % of flag leaf area affected) was not significantly affected by the three parent sets (Y, YQ, Q), neither in the organic nor in conventional fields. In both management systems, damage by this insect was small; at the organic sites, the damage (0.76±0.36% of leaf area) was slightly but not significantly (P=0.59) higher than in the conventional system (0.52±0.14%). In terms of **green leaf area**,

the Y set showed a significantly higher area than the Q set, but neither Q nor Y different from YQ (**Figure 8**).

Leaf area index (LAI) was measured three times per season. At all three times the QCCPs showed higher LAI values than the YCCPs. This effect was significant for the early-season measurement across all sites, and the mid-season measurement for the organic sites, but not for the late LAI; YQCCPs were not significantly different from the QCCPs or the YCCPs in any of the LAI measurements, with the exception of the mid-season LAI at the organic sites, where LAI was significantly smaller in the YQCCPs than in QCCPs.

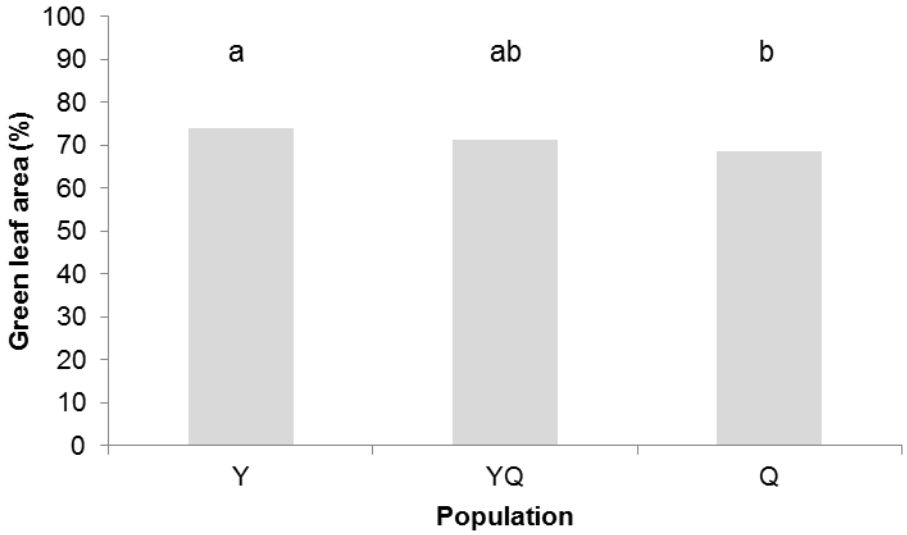

Figure 8. Green leaf area in the six populations, values pooled for CCP_{ms} and CCP_{n}. The letters Y, YQ & Q represent three different composite cross populations of wheat: a high yield population (Y), a high baking quality population (Q) and an all-rounder population (YQ). Columns with the same small letter are not significantly different.

Head density of the CCP was not significantly affected by the parent set. In the conventional system, the male-sterile CCP (CCP_{ms}) tended to show lower head density than the non-male sterile populations (CCP_{n}) (by -4.4±2.2%, P=0.0513); however, the difference between these two groups was not significant in the organic system (P=0.40).

Regarding **aphid** infestation of the wheat ears, overall numbers were low (mean: 0.6%, median 0.1%, proportion of plots without any aphid infestation: 47.5%); and no significant effects of parent set, male-sterility, or any interactions were observed. Despite the consistent differences between the YCCPs and the QCCPs for the leaf area index, there were no effects of the factor parent set on **weeds**. Furthermore, no significant effects were recorded for male-sterility or the interaction between parent set and male sterility. For the weed cover assessments, only the organic sites were investigated, as weed cover values

were zero in almost all plots of the conventional sites. There was a strong significant negative relationship between early weed infestation and grain yield; this was observed both across environments (**Figure 9**) and across genotypes (not shown).

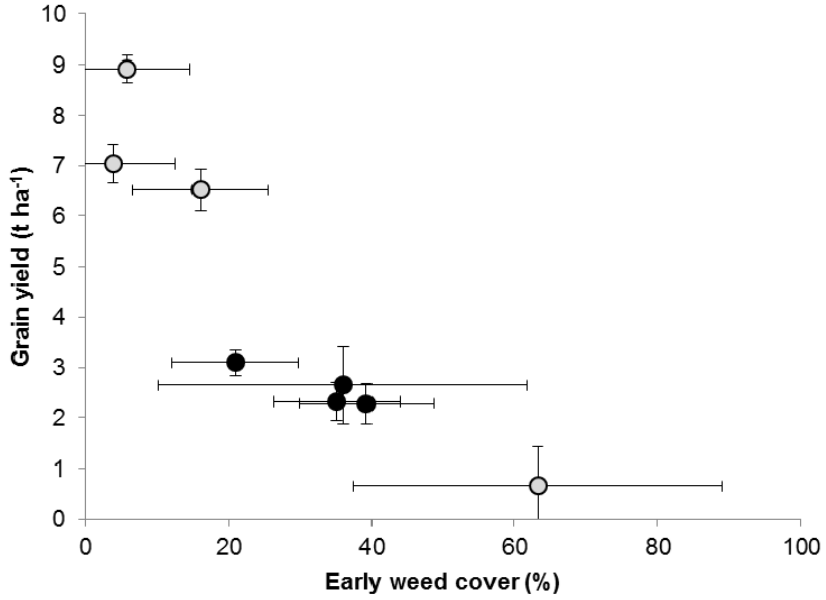

Figure 9. Early weed cover (%) and grain yield (t ha^{-1}) on organic sites. Each point represents one environment; black circles: SOF; grey circles: WAF; error bars show the Standard Deviation.

In terms of **plant height**, the order of the three parent sets was YCCP < YQCCP < QCCP (**Figure 10**). On average, the Y populations were 6.2±0.71 cm smaller than the Q populations. Within the parent sets, there was a highly significant (P<0.001) and consistent relationship between plant height and grain yield, with taller CCP showing higher grain yield (**Figure 11**); this was the case in both the organic and the conventional system, and also within all three parent sets CCP. Using the reciprocal regressions of plant height against grain yield, residuals were calculated to analyse whether the relationship between these two parameters changed over time. In particular, it was hypothesised that over time, CCP would allocate an increasing proportion to light competition, so that the residuals would increase over time, i.e. show an increase of plant height at a given grain yield. However, the data did not confirm this hypothesis. Although a trend of plant height residuals increasing over time was observed for the two organic sites, the two conventional sites showed either no consistent direction at all (MOR), or a decrease over time (MET).

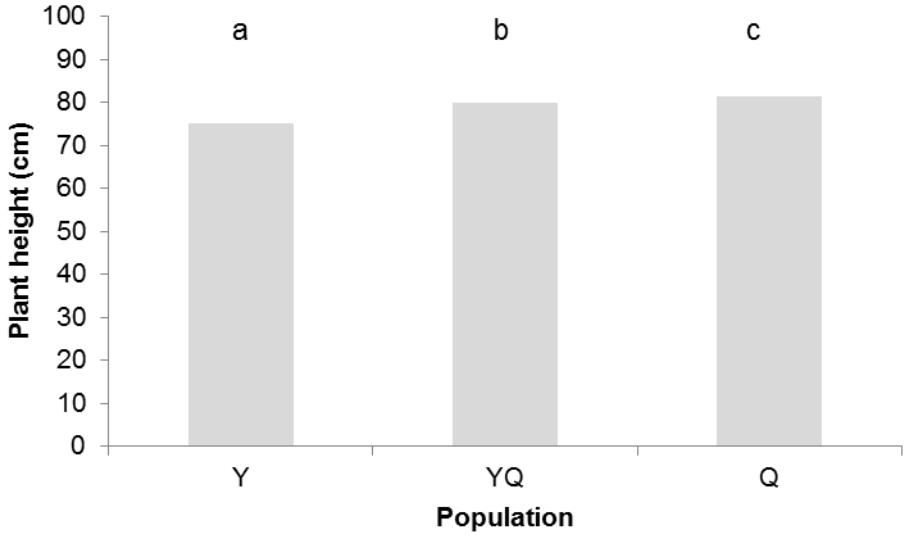

Figure 10. Plant height (cm) in the YCCP, YQCCP and QCCP, values pooled for CCP_{ms} and CCP_{n}. Entries with the same small letter above the column are not significantly different.

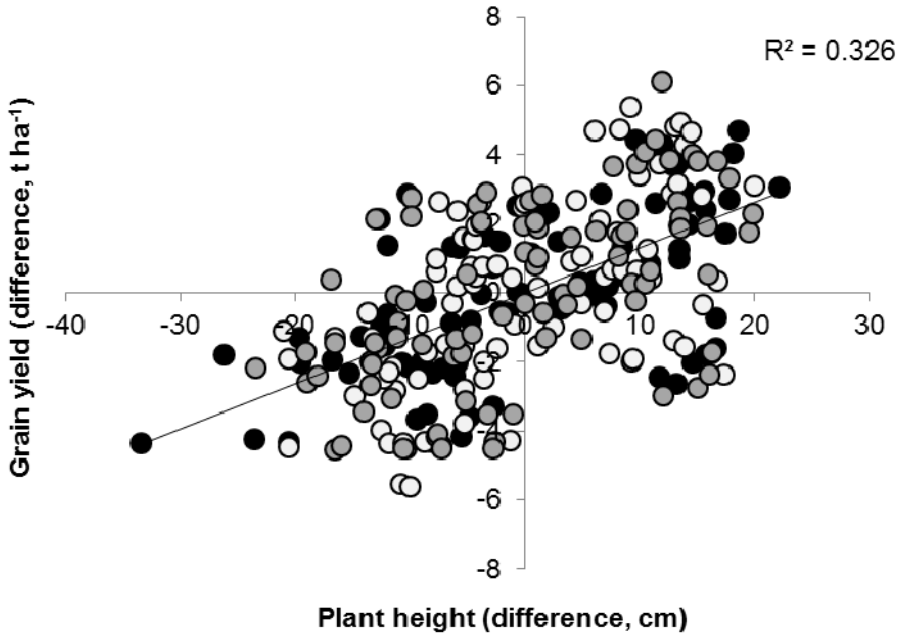

Figure 11. Relationship between plant height and grain yield, expressed as differences of individual plot values from system means (conventional and organic, respectively) for each parent set (Y, Q and YQ). Black circles: QCCP, light grey circles: YCCP, dark grey circles: YQCCP.

In most cases, **lodging** of the CCP was low, with means below 10°. The YCCPs were significantly lower in lodging than the YQCCPs and the QCCPs. Further, there was a significant effect of plant height on lodging, with taller plants lodging more ($P<0.001$); however, this effect was dependent on the year-site

combination, with no clear patterns detectable. There was no significant relationship between lodging and grain yield (P=0.4396).

Mean yield, thousand grain weight, harvest index

Grain yield was strongly affected by the cropping system, with yields substantially lower at the organic than at the conventional sites. Also, there was a significant interaction between the parent set and the cropping system (P<0.01). Interestingly, there were no significant differences between the three parent sets in the organic system, whereas the YCCP were different from YQCCP and from the QCCP at the conventional sites (**Figure 12**).

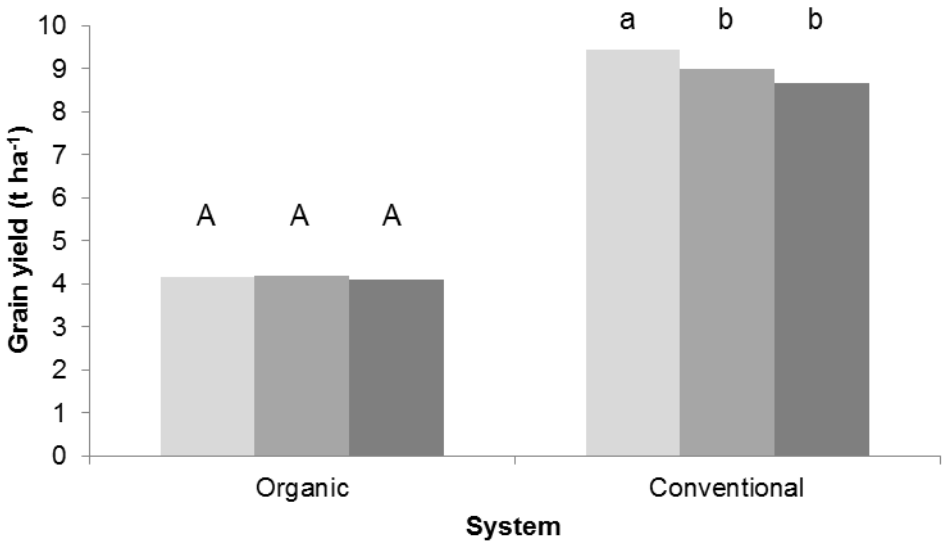

Figure 12. Grain yield (t ha^{-1}) of the CCP under organic and the conventional crop management, values pooled for CCP_{ms} and CCP_{n}. YCCP: light grey; YQCCP: medium grey; QCCP: dark grey columns. Statistical comparisons made within systems are indicated by upper case letters for organically grown CCP and lower case letters for conventionally grown CCP, therefore bars labelled with different letters of the same case indicate significant differences in grain yield between CCP for that system.

With regard to **thousand grain weight**, there were no significant differences between the parent sets; mean values were highest in the YQCCP (50.5 g), intermediate in the QCCP (50.4 g) and lowest in the YCCP (50.1g). The **Harvest index** was significantly lower in the YQCCP and QCCP than in YCCP, but not different between YQCCP and QCCP (**Figure 13**).

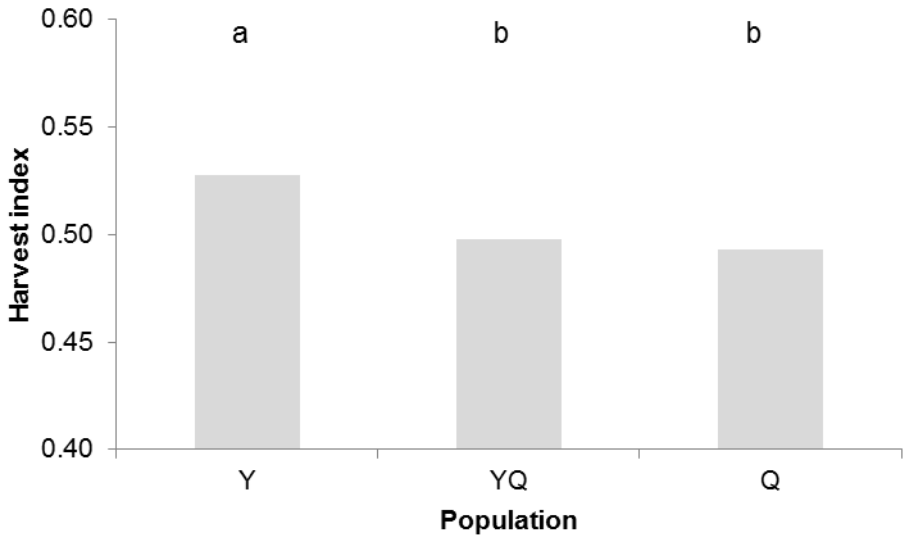

Figure 13. Harvest index in the YCCP, YQCCP and QCCP, values pooled for CCP_{ms} and CCP_{n}. Entries with the same small letter above the column are not significantly different.

Yield stability

For the CCP_{n}, there was a consistent order of the three parent sets in terms of grain yield stability; the QCCP was more stable than the YCCP for all stability parameters tested, and the YQCCP was always in between the other two populations (**Table 19**). For the CCP_{ms}, this pattern was repeated for three out of six stability parameters (*b*, s^2di and ecovalence), but not for CV, POLAR and the range/mean-ratio.

Table 19. Comparison of yield stability in the six populations; lowest stability among the six CCP marked in red font, highest in bold.

CCP	b	CV (%)	POLAR	s^2di	Ecovalence	Range/ Mean (%)
$YCCP_{n}$	1.07	56.92	0.05	11.58	11.64	186.49
$YQCCP_{n}$	0.99	54.01	0.01	3.87	3.87	171.79
$QCCP_{n}$	0.93	**50.78**	**-0.04**	1.53	1.60	**160.13**
$YCCP_{ms}$	1.04	52.58	-0.02	9.72	9.74	166.24
$YQCCP_{ms}$	1.00	52.89	-0.01	4.96	4.96	161.95
$QCCP_{ms}$	0.97	53.31	0.01	**1.10**	**1.11**	172.32

Grain quality

The analysis of the **protein content** of the six CCP revealed complex three-way interactions between male sterility, management system and parent set; these effects were mainly caused by the subset of the organic CCP_{n}, where effects of the parent set were not significant. In all other cases (both CCP_{n} and CCP_{ms} from the conventional system and organic CCP_{ms}) the order of the three parent sets was

consistently Y<YQ<Q (**Table 20**). In terms of protein content, the YQ populations were in no case significantly different from the average of the YCCP and the QCCP.

Table 20. Grain protein content (%) in the populations

	CCP_n				CCP_{ms}			
Protein	**Conventional**		**Organic**		**Conventional**		**Organic**	
YCCP	12.5	A	10.3	a	12.7	a	10.3	A
YQCCP	13.1	B	10.3	a	13.1	b	10.5	A
QCCP	13.6	C	10.4	a	13.5	c	10.9	B
Absolute difference between YQCCP and the average of YCCP and QCCP	0.07	ns	-0.02	ns	-0.03	ns	0.01	ns

Specific weight was significantly lower in the YCCP (76.5) than in the YQCCP (77.2) and in QCCP (77.4), but not different between YQCCP and QCCP. There were no significant differences in specific weight between the CCP_{ms} and the CCP_n or between organic and conventional management.

The QCCP showed the highest **Hagberg falling numbers** (HFN) among the three parent sets. This was the case in both cropping systems, and for both the CCP_n and the CCP_{ms} (**Table 21**), but differences were not significant among the three sets for the CCP_n under organic management. HFN for the YQCCP was always in between the other two populations, as expected.

Table 21. Hagberg falling numbers (s) in the 6 tested CCP

	CCP_n				CCP_{ms}			
	Conventional		**Organic**		**Conventional**		**Organic**	
YCCP	247	a	242	a	249	a	234	a
YQCCP	284	b	256	a	264	a	236	a
QCCP	301	b	257	a	291	b	265	b

Similar results were obtained for **Hardness** where the order of the populations was always Y<YQ<Q (data not shown). **Protein yield** was not significantly affected by the parent set; it was substantially lower in the organic than in the conventional system.

Stability of grain quality

When the QCCP, YCCP and YQCCP were compared for stability of protein content, there were two distinct patterns (**Table 22**). While ecovalence and the closely related s^2di showed highest stability in the QCCP (and lowest in the YCCP), this order was reversed for the regression slope *b* and the

range/mean ratio. POLAR values and the coefficient of variation (CV) did not show consistent orders of the three parent sets.

Table 22. Stability of grain protein content in the six tested CCP. Maximal stability within CCP_n and CCP_{ms} highlighted in bold, lowest stability in red font.

	b	CV (%)	POLAR	s²di	Ecovalence	Range/ Mean (%)
$YCCP_n$	0.89	**14.03**	**-0.015**	7.41	7.46	**45.83**
$YQCCP_n$	1.00	15.34	0.012	2.47	2.47	46.38
$QCCP_n$	1.16	17.35	0.069	**0.49**	**0.58**	51.94
$YCCP_{ms}$	0.95	14.87	0.021	5.11	5.11	**44.81**
$YQCCP_{ms}$	0.96	**14.61**	-0.043	2.07	2.08	45.80
$QCCP_{ms}$	1.05	15.53	**-0.044**	**0.96**	**0.97**	50.00

Comparisons of CCP with mixtures and pure lines

One of the main objectives of the project was to evaluate the agronomic performance of CCP against corresponding mixtures derived from the same parent varieties, and against commercial pure lines. The pure lines were chosen to be representative of the currently used spectrum of varieties used by organic as well as conventional farmers in the UK. Below we report the most important results of the main WP1 trials contributing to this objective.

Pre-harvest parameters

With regard to **plant establishment** in spring, no significant main effects of the diversity level was found (data not shown); significant two- and three-way interactions between diversity level, parent set and management system indicated complex responses of this parameter to growing conditions and the genetic background. The **stability of establishment** showed no consistent pattern across the different stability parameters; while some parameters showed highest stability in the pure lines (CV, POLAR, range/mean ratio), the opposite was the case for other parameters (s²di, ecovalence) (Table 23).

Table 23. Stability of establishment; maximal stability highlighted in bold, lowest stability in red font.

Diversity level	b	CV (%)	POLAR	s²di	Ecovalence	Range/mean (%)
Pure lines	0.94	**63.2**	**-0.052**	32052	32650	**264**
Mixes	1.05	68.4	0.025	**16836**	**17010**	296
CCP_n	1.00	68.1	0.016	18365	18424	292
CCP_{ms}	1.06	71.3	0.055	16963	17219	305

Plant height was significantly lower (by about 12 cm) in the pure lines than in the genetically diverse material, but among the CCP and mixes, there were no significant differences in plant height (**Figure 14**). **Lodging** was significantly higher in the diverse material than in the pure lines and this effect was stronger in the conventional than in the organic system (data not shown); however lodging had no significant effect on grain yield.

For **straw mass**, results strongly depended on model structure, so that consistent conclusions are difficult to obtain for this parameter. In linear mixed modelling significant two-way and three-way interactions were found among the three trial factors (management system, parent set and diversity level), and no significant main effects for the diversity level were observed (data not shown). However, alternative modelling revealed increased straw mass in the CCP (both CCP_n and CCP_{ms}) in comparison with the pure lines. This is in line with the results on plant height, since plants were taller in the CCP than in the pure lines and straw mass positively correlated with plant height.

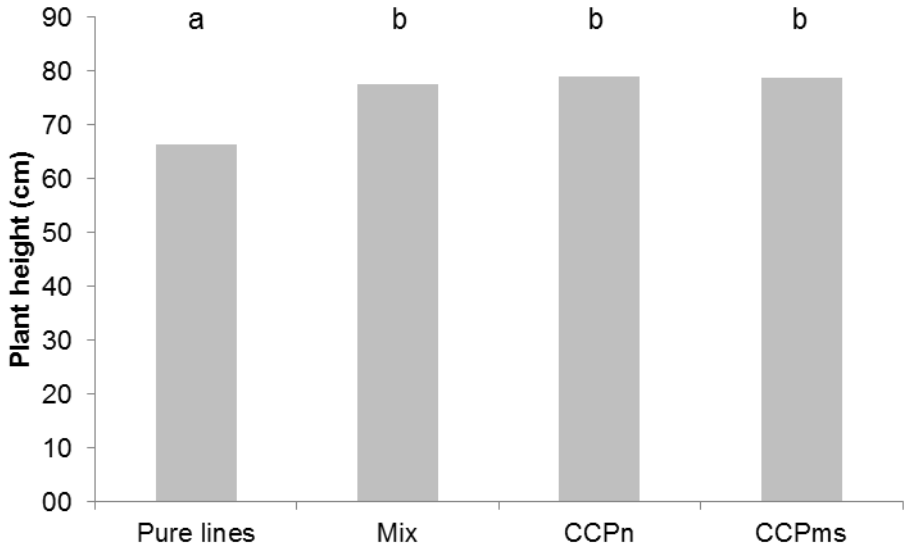

Figure 14. Plant height (cm) in the pure lines, mixes and CCP in the main trials. Entries with the same small letter above the column are not significantly different.

The **leaf area index** (LAI) was measured three times during the growing season. Results of the early-season LAI showed that the pure line varieties showed a highly significantly and substantially lower LAI than the CCP and the mixes. This was consistently the case for all three parent sets (Q, Y and YQ) (**Table 24**). This suggests that ground cover and competitive ability against weeds was higher in the CCP and Mixes than in the varieties. There were no significant differences between CCP and mixtures with regard to the early season LAI. Results from the mid-season and late-season measurements were consistent with the first LAI measurements; relative to the LAI in the CCP_n, the LAI in the pure lines

showed a reduction by 15% in the early season, 10% in mid-season, and 11% for the late season measurements.

Table 24. Leaf area index in spring, at the early-season measurement

	Parent set			
Diversity level	**Q**	**Y**	**YQ**	**Overall mean**
CCP_n	2.40	2.26	2.44	2.36
CCP_{ms}	2.44	2.24	2.36	2.35
Mix	2.57	2.08	2.26	2.31
Pure lines	2.08	1.96	1.99	2.01

Foliar diseases were found to be significantly higher in the diverse material (CCP and mixes) than in the pure lines; this was the case for both the organic and the conventional sites (Table 25). The most important disease across most trial environments was *Septoria tritici* leaf blotch (**Table 26**). The Sheepdrove trial site showed a slightly different disease spectrum, especially in year 2 when there was a severe infection with powdery mildew, and in year 4, when the diseases were dominated by yellow rust.

Table 25. Effect of diversity level on foliar diseases (backtransformed data); significance (***: P<0.001; ns: not significant) indicates difference from pure lines

Diversity level	**Conventional (%)**		**Organic (%)**	
Pure lines	3.2		8.5	
Mix	4.9	***	8.8	ns
CCPn	6.0	***	9.7	ns
CCPms	6.0	***	9.2	ns

Leaf damage caused by the **cereal leaf beetle** (*Oulema melanopus*) was generally small, with a median value of 0.1% affected leaf area, and nearly half of the plot values being 0% (**Figure 15**). According to linear mixed modelling on log-transformed values, the diversity level (CCP vs. mixes and pure lines) did not significantly affect the leaf damage caused by the cereal leaf beetle; this result is also confirmed by the graph showing the distribution of values for the different diversity levels. **Aphid** infestation of the wheat ears was very low, with 51% of plot records having no aphids at all.

Effects of the diversity level on **head density** of the crop were inconsistent and depended strongly on model structure, with no clear patterns emerging. Effects of site and year on head density were very pronounced.

Table 26. Different foliar diseases, as average percentage of flag leaf area infected, shown for the 16 trial environments

Year	Site	Septoria	Mildew	Yellow Rust	Brown Rust
Year 1	MET	2.8	0.2	0.0	0.0
	MOR	3.1	0.3	0.0	0.0
	SOF	8.3	0.0	0.0	0.0
	WAF	10.1	0.0	0.0	0.0
Year 2	MET	28.0	0.0	0.0	0.0
	MOR	3.1	0.0	0.0	0.0
	SOF		70.7		
	WAF	10.4	0.0	0.0	0.2
Year 3	MET	3.1	0.0	0.0	0.0
	MOR	2.9	0.0	0.0	0.0
	SOF	1.3	0.2	0.0	0.8
	WAF	2.6	0.0	0.9	0.4
Year 4	MET	0.9	0.0	0.0	0.0
	MOR	5.5	0.0	0.0	0.0
	SOF	1.0	0.0	6.2	7.3
	WAF	1.2	0.2	0.2	0.6
	Average	5.6	4.5	0.5	0.6

For **thousand grain weight** (TGW), effects of the diversity level depended on the management system and the parent set; in most cases, the diversity level had no effect on TGW, but for the Q parent set, pure lines showed a significantly lower TGW under both organic and conventional management.

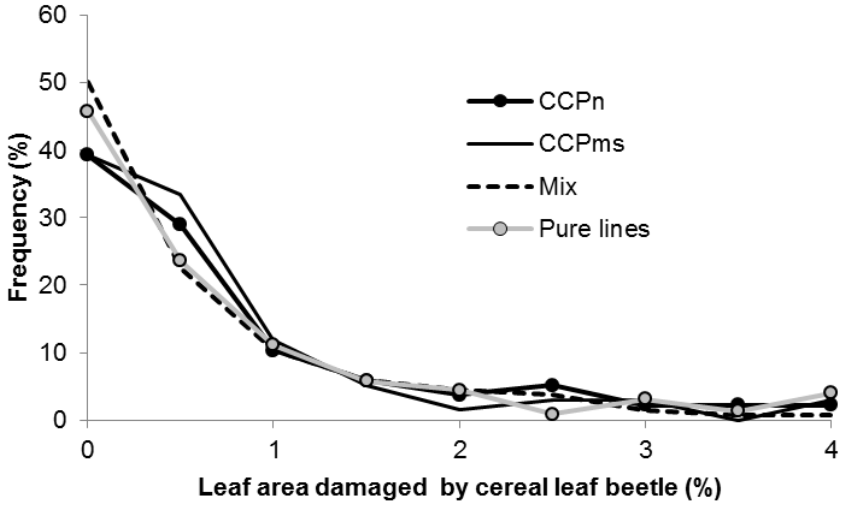

Figure 15. Percentage flag leaf area damaged by cereal leaf beetle (*Oulema melanopus*), shown as the frequency distribution of score values found for the CCP, the mixes and the pure lines.

Table 27. Thousand grain weight (g) in the different diversity levels; (*): P<0.1; *: P<0.05; **: P<0.01; ns: not significant

			Conventional			Organic		
	Parent set	**Diversity level**	**Estimate**	**S.E.**		**Estimate**	**S.E.**	
Means	Q	CCP_n	50.3	1.4		47.7	1.4	
	YQ	CCP_n	50.7	1.5		46.3	1.3	
	Y	CCP_n	50.3	1.5		46.2	1.3	
Differences between CCP_n and other entries	Q	Pure lines	-1.6	0.6	**	-2.8	0.9	**
		Mix	-0.6	0.6	ns	-1.8	0.9	*
		CCPms	-0.3	0.6	ns	-0.7	0.9	ns
	Y	Pure lines	0.1	0.5	ns	0.4	0.9	ns
		Mix	-1.0	0.5	*	0.8	0.9	ns
		CCPms	-0.5	0.5	ns	1.3	0.9	ns
	YQ	Pure lines	-1.1	0.5	*	-0.6	0.6	ns
		Mix	-0.6	0.5	ns	0.3	0.6	ns
		CCPms	0.1	0.5	ns	1.1	0.6	(*)

Grain yield

Linear mixed effects (LME) modelling of the grain yield data from the main trials showed significant two-way interactions between management system and parent set, between management system and diversity level, and between diversity level and parent set. In addition, there were some nearly significant three-way interactions among these factors. Separate LME modelling was therefore performed for the two systems and three parent sets. Significant effects of the diversity level were found for the conventional sites, but not for the organic sites (**Table 28**)**.** In particular, grain yields in the conventional system were significantly lower in the CCP and the mixes than in the pure lines. The highest relative difference was found for the Q parent set, where the yield of the CCP_n was 12.7% lower than the average yield of the respective pure lines (Solstice and Spark).

No significant interaction effects were observed when alternative modelling procedures were followed, using fixed effects only. The analysis of the main effects confirmed a yield reduction of the CCP in comparison to the pure lines (**Figure 16**). In addition, effect sizes of site and year were much stronger than of genotype.

Further data analysis showed that the effect of foliar diseases on grain yield was different for the two management systems (**Figure 17).** While an inverse relationship (higher yields at lower disease levels) was found in the organic system, there was a different behaviour in the conventional system, with yields being highest at intermediate disease levels. Interestingly, on the conventional sites the yield difference between pure lines and the diverse material (CCP and mixes) was smaller at higher disease levels than

at low and intermediate disease levels, indicating that CCP and mixes were more robust under high disease pressure than pure lines. However, these results are based on correlations only and therefore need to be treated with caution.

Table 28. Mean yields for pure lines (t ha^{-1}, average of the three high-yield and the two high-quality varieties) as well as mean differences between pure lines on the one hand and the CCP and the mixes on the other. Significances (***: P<0.001, **, P<0.01; *: P<0.05; ns: not significant) according to pairwise t-tests for individual comparisons with the pure lines. SE: Standard error.

System	Diversity level	Parent set								
		Q			YQ			Y		
		Estimate	SE		Estimate	SE		Estimate	SE	
CON	Pure lines (mean)	9.86	1.16		10.03	1.14		10.20	1.14	
	Mix (difference)	-1.25	0.18	***	-0.89	0.24	***	-0.22	0.27	ns
	CCP_n (difference)	-1.25	0.18	***	-1.19	0.24	***	-0.76	0.27	**
	CCP_{ms} (difference)	-1.14	0.18	***	-1.00	0.24	***	-0.69	0.27	*
ORG	Pure lines (mean)	4.32	1.59		4.32	1.73		4.33	1.67	
	Mix (difference)	-0.04	0.19	ns	0.30	0.20	ns	0.13	0.22	ns
	CCP_n (difference)	-0.11	0.19	ns	-0.17	0.20	ns	-0.38	0.22	ns
	CCP_{ms} (difference)	-0.31	0.19	ns	0.00	0.20	ns	0.13	0.22	ns

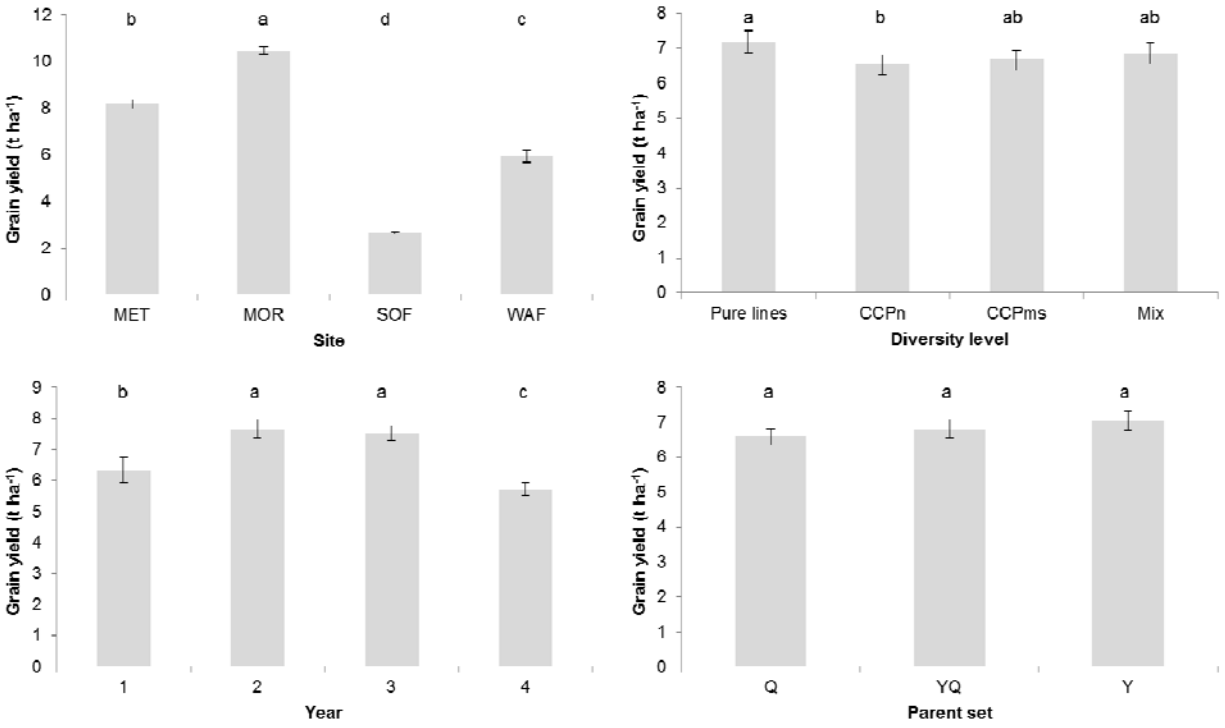

Figure 16. Grain yield (t ha^{-1}) in the main trials at four sites over four years; means and standard errors; bars with the same letter within a panel are not significantly different according to a Tukey HSD test.

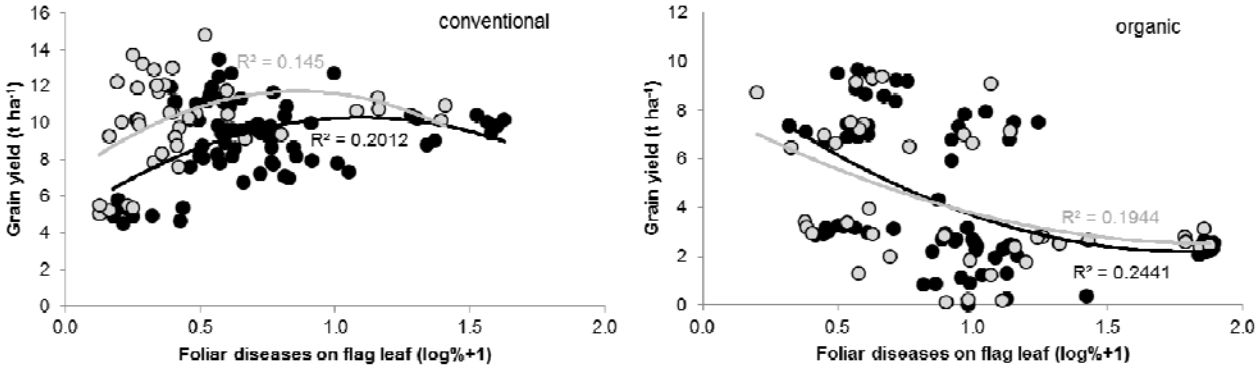

Figure 17. Relationship between foliar diseases on flag leaf (log-transformed) and grain yield (t ha^{-1}), displayed separately for the conventional and the organic sites. Grey circles and grey regression line: Pure lines; black circles and black regression line: CCP and Mixes. Each point represents three replicates from the same year and the same site.

Grain yield was positively correlated with head density for all diversity levels. However, the slopes of the regression lines were not significantly different among the four diversity levels (**Figure 18**). The common slope of the regression line of grain yield against was 0.53, i.e. for a reduction of head density by 0.1 units, grain yield would only be reduced by 0.053 units. The lack of difference among the diversity levels indicates that the ability of the wheat to compensate low head densities (by increased number of grains per head or by increased thousand grain weight) was similar for the pure lines, mixes and CCP.

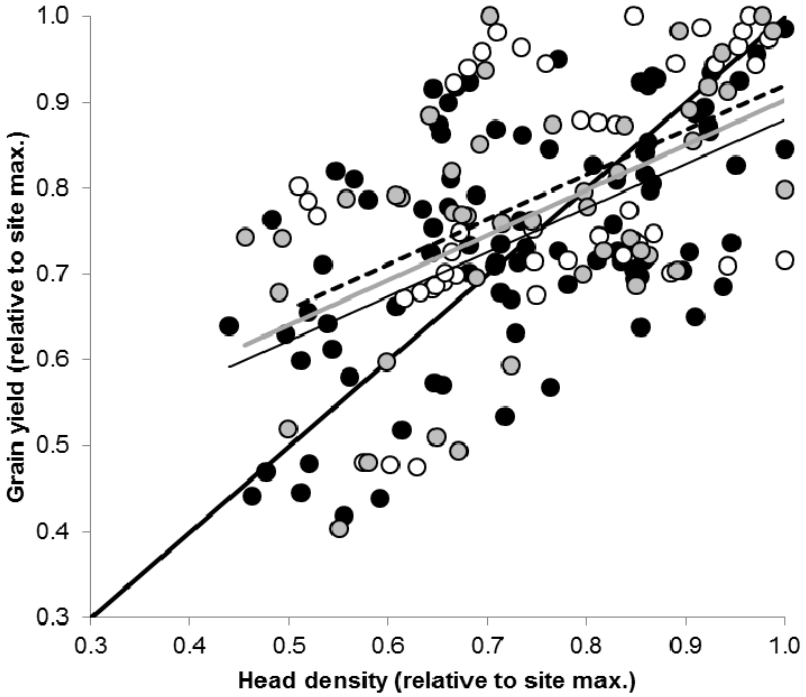

Figure 18. Relationship between head density (relative to each trial site's maximum value) and grain yield (also relative to site maximum) for pure lines (white circles and dashed line), mixes (grey circles and grey line), and CCP (black circles and thin black line); x=y is shown by the bold line.

There was also a positive correlation between thousand grain weight and grain yield (not shown), but this relationship was entirely attributable to site effects and disappeared when grain yields were expressed relative to site maxima. As for the relationship between head density and grain yield, the relationship between thousand grain weight and grain yield had similar slopes for all diversity levels.

Grain yield stability

Mean yields scaled with the variance of the yield over environments, so that POLAR stability could be calculated. Grain yield stability was consistently lowest in the pure lines, i.e. for all stability parameters measured, the variability of grain yield showed the highest values for the pedigree varieties (**Table 29)**. Among the pure lines, Tanker was the variety with the lowest dynamic stability (b=1.12), whereas the $QCCP_n$ was the most dynamically stable entry, i.e. with the lowest slope against environmental means (b=0.88) (Figure 19). Together with the results presented in Table 29, these results suggest that the CCP, and to a lesser degree the mixtures are more suited to low-yielding environments. Further, ecovalence and s^2di showed a consistent pattern of increased grain yield stability with increasing genetic diversity.

Table 29. Yield stability indices of CCP, Mixes and pure lines, averages over the parent groups; highest stability among the trial entries) is highlighted in bold, lowest stability in red font.

Diversity level	b	CV (%)	POLAR	s^2di	Ecovalence	Range/ Mean (%)
Pure lines	1.070	55.2	0.013	21.5	21.5	178.0
Mixes	0.976	**52.8**	**-0.018**	10.9	10.9	170.2
CCP_n	0.948	53.9	0.007	6.3	6.4	172.8
CCP_{ms}	0.955	52.9	-0.011	**5.6**	**5.6**	**166.8**

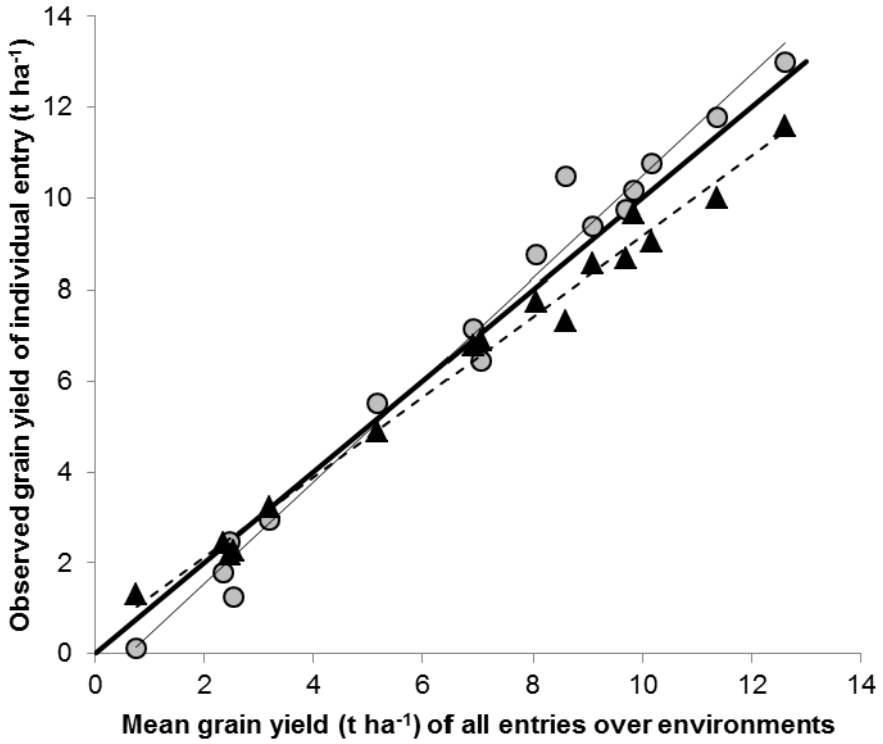

Figure 19. Grain yield of Tanker (grey circles, b=1.12) and $QCCP_n$ (black triangles, b=0.88) against environmental means of grain yield (in t ha^{-1}). Black bold line indicates x=y, i.e. b=1.

Grain quality

Protein content was consistently lower in the pure lines than in the diverse wheats. For all three parent sets and under both organic and conventional management, the pure line varieties showed a lower protein content than the CCP_n. Further, mixes and CCP_{ms} were only occasionally different from the CCP_n (Table 30). In terms of protein yield, the picture was less clear; under organic crop management, the CCP_n were not significantly different from the pure lines, while at the conventional sites the pure lines outperformed the $QCCP_n$ and the $YQCCP_n$ but not the $YCCP_n$ (Table 31). In most cases, the protein yield of the mixes was not significantly different from that of the CCP_n.

With regard to the **Hagberg Falling number** (HFN) and **hardness**, only the data of the Q parent set was analysed for differences between pure lines, mixes and CCP. HFN was significantly higher in the pure lines (Solstice and Spark) than in the CCP and the mixes. In contrast, hardness was not affected significantly by the diversity level.

Table 30. Mean protein content for CCP_n (%) as well as mean differences between CCP_n and the other entries. Significances (***: P<0.001, **, P<0.01; *: P<0.05; ns: not significant) according to pairwise t-tests for individual comparisons with the CCP_n. SE: Standard error.

		Parent set								
		Q			YQ			Y		
Syst.	**Diversity level**	**Estimate**	**SE**		**Estimate**	**SE**		**Estimate**	**SE**	
CON	CCP_n (mean)	13.61	0.27		13.10	0.42		12.50	0.37	
	CCP_{ms} (diff.)	-0.15	0.11	ns	-0.04	0.11	ns	0.19	0.13	ns
	Mix (diff.)	0.00	0.11	ns	-0.11	0.11	ns	-0.18	0.13	ns
	Pure lines (diff.)	-0.98	0.11	***	-0.69	0.11	***	-0.29	0.13	*
ORG	CCP_n (mean)	10.43	0.86		10.32	0.75		10.33	0.74	
	CCP_{ms} (diff.)	0.42	0.15	**	0.22	0.14	ns	-0.03	0.09	ns
	Mix (diff.)	0.20	0.14	ns	0.07	0.14	ns	-0.32	0.09	**
	Pure lines (diff.)	-0.40	0.14	**	-0.31	0.14	*	-0.30	0.09	**

Table 31. Mean protein yield for CCP_n (t ha^{-1}) as well as mean differences between CCP_n and the other entries; significances as in the previous table.

		Parent set								
		Q			YQ			Y		
Syst.	**Diversity level**	**Est.**	**SE**		**Est.**	**SE**		**Est.**	**SE**	
CON	CCP_n (mean)	1.278	0.066		1.246	0.085		1.284	0.082	
	CCP_{ms} (diff.)	0.011	0.027	ns	0.044	0.031	ns	0.009	0.038	ns
	Mix (diff.)	0.026	0.028	ns	0.073	0.031	*	0.064	0.037	ns
	Pure lines (diff.)	0.077	0.027	**	0.097	0.031	**	0.049	0.037	ns
ORG	CCP_n (mean)	0.564	0.319		0.570	0.324		0.523	0.306	
	CCP_{ms} (diff.)	-0.016	0.025	ns	0.043	0.031	ns	0.074	0.030	*
	Mix (diff.)	0.034	0.024	ns	0.059	0.031	ns	0.059	0.030	*
	Pure lines (diff.)	0.003	0.024	ns	0.000	0.031	ns	0.048	0.030	ns

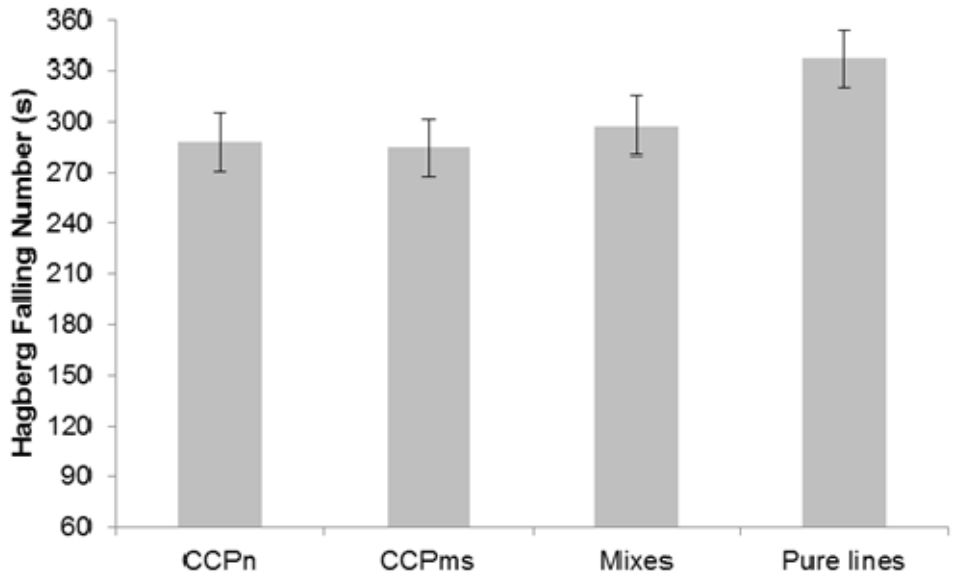

Figure 20. Hagberg falling number (s) for the high-quality entries (Q)

Stability of grain quality

Stability of grain protein content was determined with a range of stability indices. Large differences between the entries were detected for ecovalence (and the closely related s²di), as well as for the Power Law Residuals (POLAR); in contrast, the coefficient of variation (CV) and the range/mean-ratio showed only small variation among the four diversity levels (**Table 32**). Across most parameters, the CCP_{ms} showed the highest stability of the protein content among the entries. For four out of the six parameters, the pure lines were the least stable. However, for the regression slope *b* against environmental means, pure lines showed the lowest value, indicating that grain protein content of pure lines was comparatively high in low-performance environments, i.e where grain protein content was generally low. On the other hand, high (dynamic) stability is also commonly seen to be associated with values of |b-1| closest to 0, and according to this perspective, pure lines were less stable than the CCP_{ms} and the mixes.

Table 32. Stability of grain protein content; highest stability among the trial entries) is highlighted in bold, lowest stability in red font.

Diversity level	b	CV (%)	POLAR	s²di	Ecovalence	Range/mean (%)
Pure lines	0.97	15.35	0.03	10.97	11.00	48.49
Mixes	1.01	15.18	-0.01	5.02	5.05	46.87
CCP_n	1.04	15.57	0.00	3.48	3.53	48.05
CCP_{ms}	1.01	**15.00**	**-0.03**	**2.75**	**2.76**	**46.87**

Cross-over trial

With just one exception (green leaf area), there were no significant effects of the origin of the CCP on any of the pre-harvest parameters (

Table 33), i.e. there was no indication of the CCP having undergone significant adaptation over seven generations. Also, no adaptation effect was observed for the CCP in terms of grain yield (

Table **34**). However, although none of the effects was significant, the direction of change was consistent with the hypothesis of adaptation for foliar disease infection, thousand grain weight and grain yield (**Table 35**).

Further, no effects of origin were detected for the mixtures either (data not shown). In terms of relative change yield, coming from conventional going to organic was equivalent to -0.4%, whereas coming from organic going to conventional resulted in a 1.4 % change of grain yield.

Table 33. Significance values (P-value) for contrasts between home and away-CCP and for CCP coming from the same vs. a different system

	P-Value for contrast	
Response variable	**Home vs. away CCP**	**Same vs. different system**
Establishment	0.286	0.714
Total disease	0.241	0.788
Yellow rust	0.071	0.808
Septoria leaf blotch	0.803	0.597
Brown rust	0.502	0.897
Aphid infestation	0.698	0.873
Cereal leaf beetle	0.929	1.000
Green leaf area	0.454	0.034*

Table 34. Grain yield of the CCP in the cross over trial; absolute yields (t ha^{-1}), upper half of the table; and yields relative to the site mean (in %) lower half. Shaded parts of the table show the home-CCP.

Original site	**Site at which population was tested**			
Grain yield (t ha^{-1})	**MET**	**MOR**	**SOF**	**WAF**
MET	4.96	7.90	2.42	7.22
MOR	4.63	8.65	2.33	6.64
SOF	4.36	8.95	2.36	7.01
WAF	4.95	9.14	2.42	7.09
Average change in % of site mean				
MET	5.0	-8.8	2.7	3.2
MOR	-1.6	-0.1	-2.4	-5.1
SOF	-7.4	3.3	-2.0	0.5
WAF	3.9	5.6	1.7	1.4

Table 35. Home vs. away CCP: Absolute values for establishment, total disease, yellow rust, straw mass, thousand grain weight and grain yield.

	Establishment (plant m^{-2})	Total disease (%)	Yellow rust (%)	Straw (g m^{-1})	TGW (g)	Grain yield (t ha^{-1})
Home	320	5.2	1.4	111	50.9	5.77
Away	306	6.3	2.1	112	50.1	5.66
% Change	4.6	-18.2	-34.0	-1.3	1.6	1.8

Adaptation trial with shuttle populations

Data from this trial was used to test the hypothesis of whether populations adapt to site conditions under which they are grown. In particular, in case of adaptation of the CCP to site conditions, it was expected that shuttle CCP, having been grown at alternative sites over subsequent generations, would show weaker adaptation than those CCP that had been grown at one site continuously. A yield difference in favour of the home CCP over the shuttle CCP would then be interpreted as an indicator of adaptation. The analysis of the data showed that in fact the mean yield difference pointed in the opposite direction (0.46 ± 0.37 t ha^{-1}) but this difference was not significant (P=0.224). It was therefore concluded that the home CCP did not show any advantage over the CCP that had been grown at alternating sites. However, further data analysis revealed that the difference between home CCP and shuttle CCP was dependent on the general yield level. Yield levels were measured as differences of individual home CCP plots from the means of all 6 home CCP at each environments (CCP_n and CCP_{ms}, each from 3 blocks). High plot yields were associated with negative differences (P<0.001**; Figure 21**), i.e. the shuttle CCP showed an advantage over the home CCP in those cases were home CCP showed relatively low yield levels. This was the case both across and within environments.

This result suggests that the shuttle CCP were more stable within each environment than the home CCP; to test this further, the coefficient of variation (CV, %) within environments was calculated for both home and shuttle CCP. A mixed effects model of these data, with site as a random factor revealed no significant difference between the home CCP and the shuttle CCP (P=0.92). Further, no effects of male sterility and of year were detected; when site was used as a fixed factor in the model, there was also no effect of site on the within-environment CV.

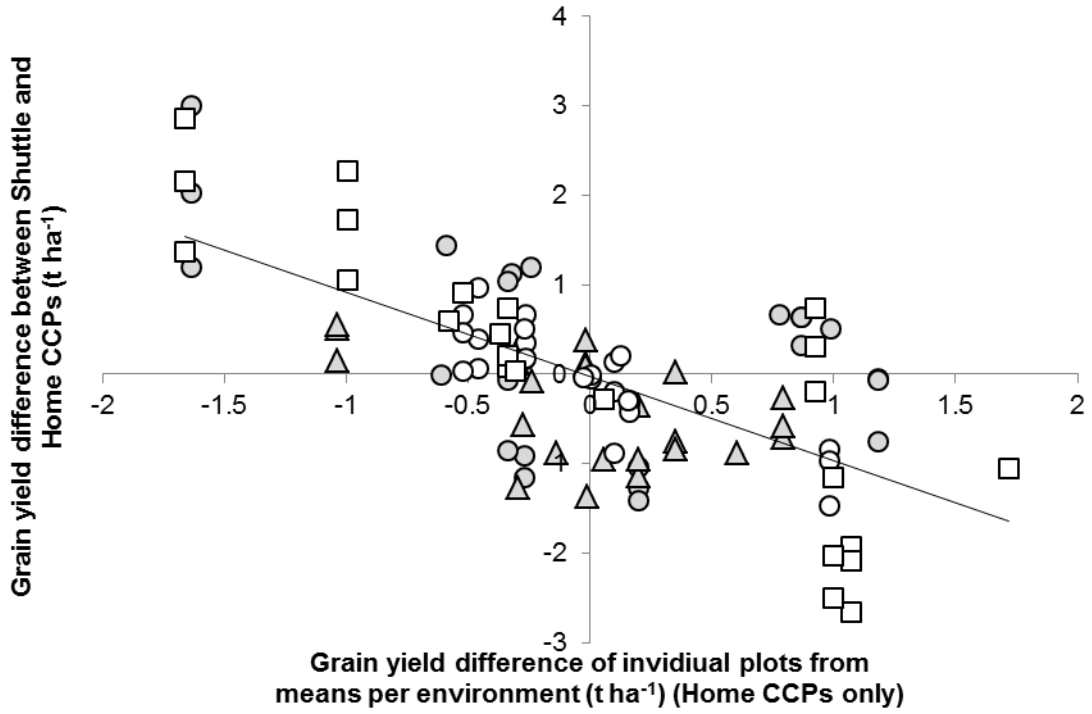

Figure 21. Relationship between (x) the absolute grain yield difference of the individual home CCP from the means of all home CCP at each environment (in t ha^{-1}) and (y) the grain yield difference between the shuttle CCP and the home CCP; white symbols: organic; grey symbols: conventional; grey circles: MET; grey triangles: MOR; white circles: SOF; white squares: WAF.

Grain diseases

Grain disease post-harvest was determined for selected trial entries for two of the field sites, namely MET as a conventional site and WAF as an organic site. Disease levels were low with respect to *Septoria* and *Microdochium* nivale, never exceeding threshold values (**Table 36**). However, infestation with common bunt (*Tilletia caries*) was extremely high in the last trial year, after already having exceeded threshold levels in the previous two trial years. Notably, bunt infection was also high in Claire in this year.

Table 36. Infestation of harvested CCP grains with *Septoria* (threshold level 5%), *Microdochium* (threshold level 10-15%) and Bunt (Tilletia caries, threshold level 1 spore per grain).

Harvest year	Site	Genotype	*Septoria* sp. (% seeds infected)	*Microdochium nivale* (% seed infected)	*Tilletia caries* (no. spores)
2008	Metfield	Q CCP	0	1.5	0.1
	Metfield	Q CCP	0	3	0
	Wakelyns	Q CCP	0	0	0
	Wakelyns	Q CCP	0	1.5	0
2009	Metfield	Q CCP	0	0	0.2
	Metfield	Q CCP	0	1	**1.2**
	Wakelyns	Q CCP	0.5	2.5	0
	Wakelyns	Q CCP	0	4	**1.7**
2010	Metfield	Q CCP	0	1	0
	Metfield	Q CCP	0	0	**1.3**
	Wakelyns	Q CCP	0	1.5	**3.5**
	Wakelyns	Q CCP	0	0.5	**1.8**
2011	Metfield	Claire	0	0	**56.0**
	Metfield	WAF YQ CCP	0	0.5	**381.7**
	Wakelyns	Claire	0	0	**806.3**
	Wakelyns	WAF YQ CCP	0	1.5	**1358.3**

3.3.2 Genotypic evolution in adaptation of CCP (WP2)

An example of the genotyping data obtained at a particular locus is shown in colour-coded allele form in

Table **37** for randomly selected individuals of the YQCCP from WAF for marker locus gwm11 on chromosome1B.

Such genotypic data were collected for each population, generation and site analysed and combined together to test whether genotypic frequencies had changed over years, sites in a way that could be attributed to the effects of selection.

Principal Component Analysis and F-statistics

According to the Principal Component Analysis (PCA) of the genotypic data, there were no clear signs of the CCP having undergone differential selection at the four different sites (Figure 22). Ellipses that capture the two-dimensional distribution for the first two axis overlap substantially at all 3 investigated generations.

Table 37. Random *Gwm11* YQCCP genotypes from F_7 generation harvested at WAF showing an example of the genotypic data obtained for each generation, population and site/year. The number in the box indicates the allele size in base pairs.

Plant	Allele			
YQW1			197	
YQW10	194			
YQW100		196		
YQW101		196		
YQW102		196		
YQW103		196		
YQW104			197	
YQW105		196		
YQW106		196		
YQW107		196		
YQW108				
YQW109			197	
YQW11		196		
YQW110			197	
YQW111		196		
YQW112				201
YQW113				201
YQW114				201
YQW115			197	
YQW116		196		
YQW117				
YQW118		196		
YQW119				201
YQW120				
YQW121			197	
YQW122		196		
YQW123		196		
YQW124		196		
YQW125				201
YQW126	194			
YQW127		196		

A slight trend towards spatial differentiation may be observed in a separation of the pair MOR and MET vs. the WAF and SOF population at generation 11 but this was not statistically significant. Investigation of the higher axis of the PCA showed no differentiation either (data not shown). Comparing changes over time within each site, *i.e.* temporal differentiation, showed no segregation either, meaning that through PCA no particular sign of change of allelic composition could be detected. However, *F*-statistics showed that there was significant change in genotypic difference in allelic composition over time (see below).

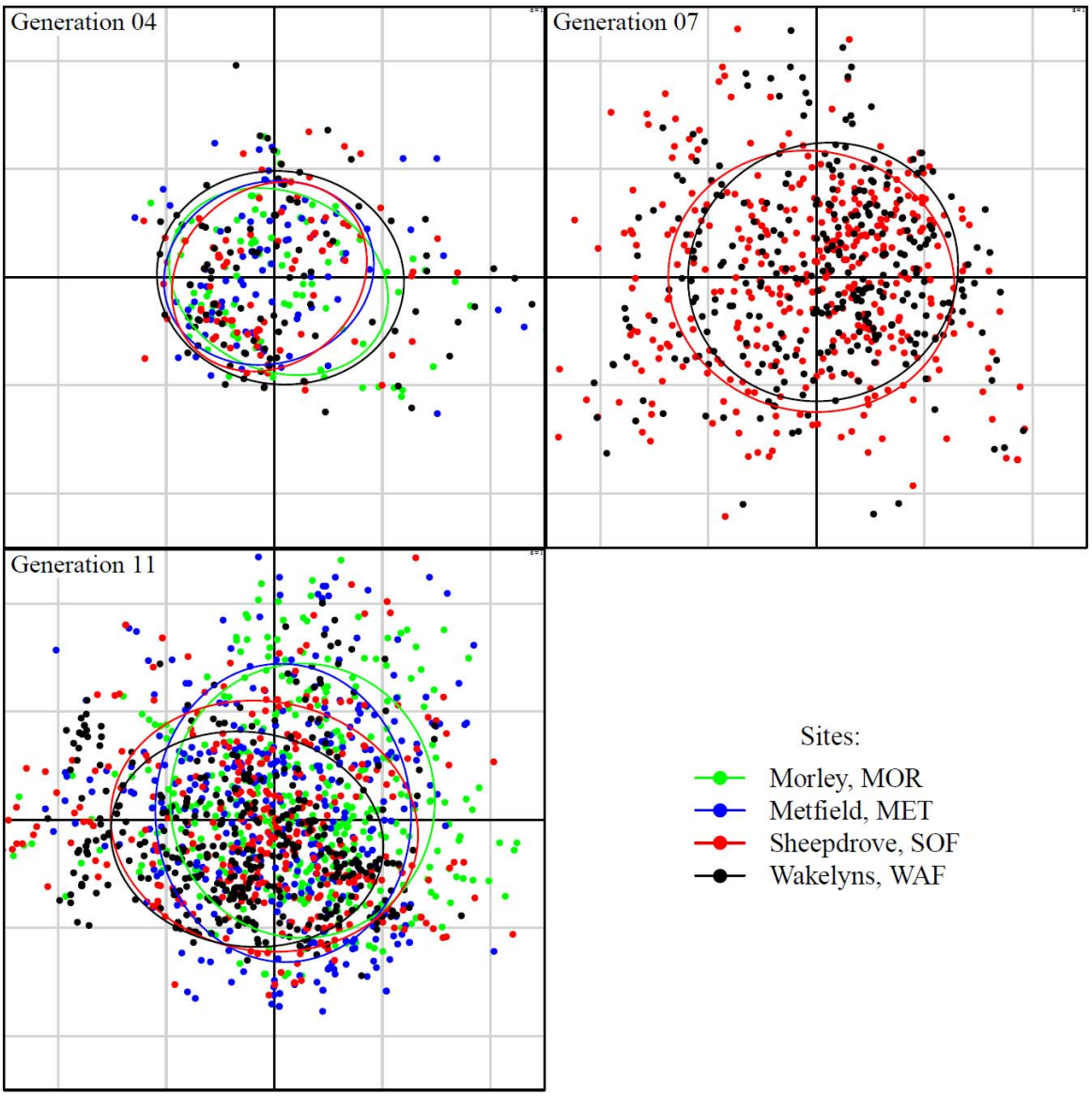

Figure 22. PCA of spatial differentiation of CCP at four sites. Plot of 1st (x) and 2nd (y) axes of principal component analysis of the populations available at each generation. Each dot represents one individual and the ellipses give an approximation of the distribution of the points in the two-dimensional space.

Changes in allele frequency

Effective population size derived from genome-wide *Fc* differed significantly when different pairs of generations were compared (Table 38). When comparing the populations at generation 11 to the founding population, for which allele frequencies were calculated from the parents' genotypes, effective population sizes between 107 and 130 were estimated.

However, when the founding population was paired with the populations from generation 07 the values were only 73 for the WAF population and 78 for SOF population. Values were even lower when

comparing generation 07 and 11 (50 and 54). However, within each pair of comparisons the values did not differ significantly between each site.

Table 38. Effective population size, estimated from genome-wide Fc weighted by number of alleles per locus. In parentheses the 95% confidence interval, based on X2 distribution.

Site	FND – Gen 07	FND – Gen. 11	Gen. 7 – Gen. 11
Metfield		130 (94, 174)	
Morley		113 (81, 151)	
Sheepdrove	78 (56, 105)	107 (79, 139)	54 (38, 73)
Wakelyns	72 (53, 97)	122 (88, 161)	50 (36, 68)

Examination of changes in allele frequency for each locus separately was carried out to identify outlier loci that could have been subject to selection. Four loci showed higher than genome-wide average *Fc* values at all of the different sites in the comparison of the founding population and generation 11: *1B1R.1B*, *PpdD1.2D*, *RhtB1.4B* and *RhtD1.4D*. Two loci produce an increased *Fc* value at WAF and SOF but not at MET and MOR: *PpdB1L5.2B* and *Xgwm626.6B*.

The locus *BS03916.6A* which was only run on the WAF and the SOF population showed an above average change at WAF. Of these loci, *X1B1R.1B*, *PpdD1.2D* and *RhtD1.4D* showed increased *Fc* values when comparing the WAF and SOF from generation 07 to the founding population. *PpdB1L5.2B* also showed higher than average values in both the WAF and SOF population when comparing allele frequencies of generation 07 and 11. Figure 23 shows the changes in allele frequency at these loci across sites and generations.

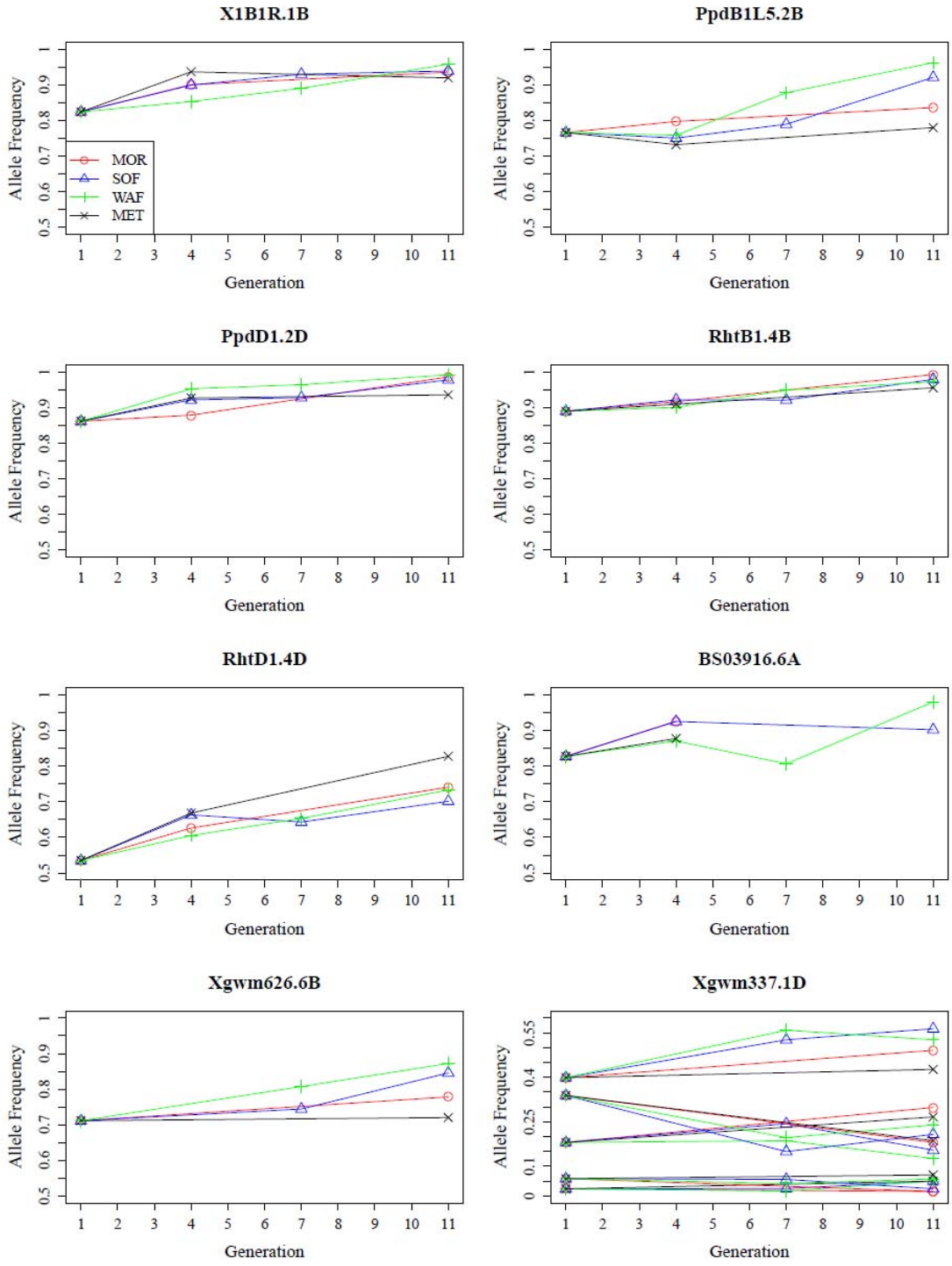

Figure 23. Changes of allele frequencies over generations for identified outlier-loci (first seven plots) and one SSR-marker as comparison. At the identified outlier-loci only the change of the major allele is shown. As these loci are all biallelic, the frequency of the other allele is 1 – the frequency of the plotted allele.

3.3.3 Genetic and phenotypic contributions of individual parent varieties to CCP (WP3)

Marker associations for tiller height are shown in Figure 24. A main signal can be seen for the association between plant height and *RhtD1*, the 4D semi dwarfing gene. With the *RhtD1-4D* marker and the trait plant height as a reference for investigating significance of marker-trait associations (MTA), because this association is highly expected, a significant association can be observed for all populations, except at population SOF07 (Figure 24). The frequency of significant *P*-values decreases with the degree of complexity of the correction model from around 20% at the ANOVA to 6.25% at the **P_{75}+K** model over all combinations of populations and traits. Particularly, extremely low highly-significant *P*-values are produced for the *RhtD1-4D* marker at SOF11 and WAF11 ($6.0*10^{-15}$ at SOF11 and $7.3*10^{-19}$ at WAF11, both for the ANOVA model).

However, when looking at Figure 24 (B) and (C) in detail, the *P*-values of the models that both include principal components to explain 75% of the molecular variance show a non-significant association whereas all 4 other models show a clear association. The difference between the correction models is not that pronounced for this locus at the set of parental lines (Figure 24;(E)). This observation of disappearing MTAs, when 75% of the molecular variance is included as the P75 matrix in the mixed-model, suggests that these models are over-parameterised and should be excluded when trying to identify significant MTAs. When excluding these two models, in 29 out of a total 58 PTCs the **P_{25} +K** yielded the lowest MSD, and the **K** model in 20 PTCs.

However, even after exclusion of the two **P_{75}** models, *P*-values show an unreliably high level and a high proportion of loci that show apparently significant associations. Different methods of correcting for multiple testing were tried, but none showed a reliable correction. It was thus decided to score significant associations visually on plots of *P*-values over loci for each trait. Only the loci that were identified as potentially subject to selection were evaluated for association. *RhtD1.4D* was associated with many of the traits that were measured. The only MTA that was scored in more than two of the mapping populations was the association of plant height with *RhtD1.4D*. Overall, most MTAs were only scored in one of the mapping populations.

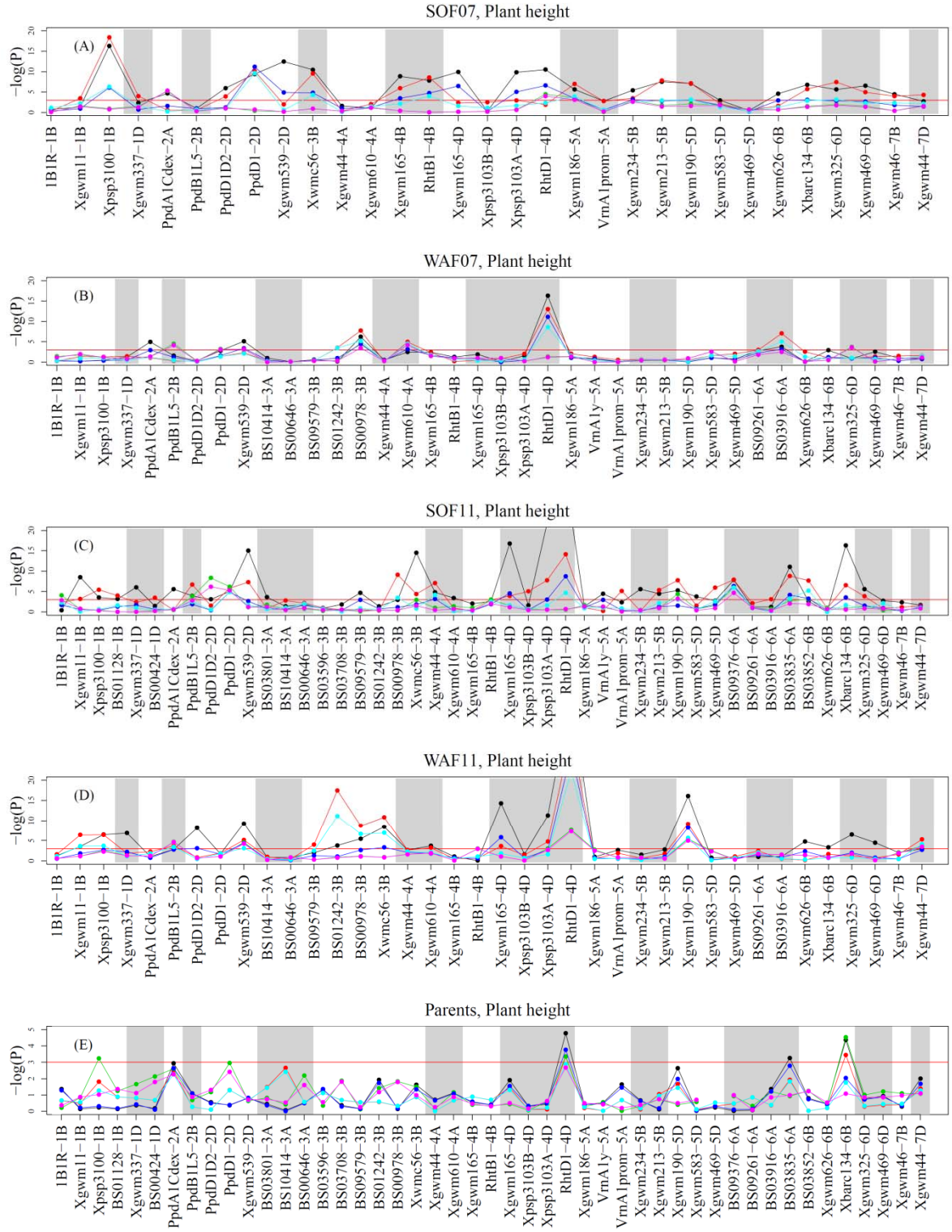

Figure 24. Plot of P-values of marker trait associations of plant height. All models of correction for population structure are shown. Colours are used to code the different models (black= ANOVA; red=P_{25}; green=P_{75}; blue= K; cyan= P_{25}+K; pink=P_{75}+K). The horizontal red line marks significance threshold of = 0.05. To maintain visibility of non-significant levels of *P*-values, highly significant values (-log(*P*)>20) are cut off.

In order to get insights into the effects of the alleles that have increased in frequency over time the effect estimates from the simple ANOVA association model were averaged over all 5 mapping populations. To

correct for effect, size values are shown as percentage of the mean of all phenotypic observations (Table 39). The effects on plant height were substantial at the 3 loci *PpdD1*, *RhtB1* and *RhtD1*, where selected allele has an increasing effect on plant height. The two loci, that were only selected for in half of the sites, *PpdB1L5* and *BS03916*, show a decreasing effect.

Grain number per tiller and grain number on the main tiller are increased by the selected allele at the loci *PpdD1*, *RhtB1* and *BS03916*, whereas the selected allele at *RhtD1* shows a decreasing effect. The only trait with which *X1B1R* showed an association was ear emergence with *X1B1R* having an effect for earlier emergence. In contrast, all the selected alleles of the remaining 6 loci show an effect towards later emergence. Grain weight and thousand kernel weight show a slight increase over all loci. The allele that has increased in frequency at locus *BS03916* has substantial reducing effect on harvest ratio, but also a reducing effect on plant height.

Table 39. Allele substitution effect on phenotype of the allele that was selected for at each locus. Values are the estimated effects (BLUE) from the ANOVA association model, averaged over all 5 mapping populations and expressed as % of the mean of all phenotypic observations; the mean given in the last column. The trend column shows the direction of the effect of the majority over the 7 loci and in parentheses the number of loci is given that have the same direction as the majority.

Trait / Allele (change in %	X1B1R	PpdB1L5	PpdD1	RhtB1	RhtD1	BS03916	Xgwm626	Trend	Phenotypic mean	
Tillers per plant	-0.7	7.6	28.8	7.4	-7.7	0	11.4	5	1.9	(count)
Plant height	1.3	-1.8	6.3	7.1	12.1	-5.2	1.5	5	77.8	cm
Grain weight / tiller	2.3	1.8	3.3	10.9	-4.4	1.7	0	6	1.67	g
Grain nr /tiller	0.2	0.9	8	14.4	-8	6.4	8.2	6	33.5	(count)
Straw weight /tiller	2	5.5	8.8	10.4	3.1	4.8	1.3	7	2.1	g
Harvest ratio	3.2	3.5	-4.1	-4.6	-16.2	-63.2	1.3	-4	0.92	(ratio)
Biomass / tiller	1.7	4.5	6	10	-0.2	3.1	1	6	3.7	g
TGW[a]	-0.2	3	5	4.6	0	-1	-3	4	49.6	g
TGW[a,b]	0.9	3.7	5.7	5.4	0.3	-1.3	-3.1	5	50.2	g
Grain weight[b]	5.1	4.2	9.8	9.4	-2	4.1	2.2	6	1.9	g
Grain nr[b]	6.2	1.9	4.6	5.3	-3.1	6.8	3.4	6	38.2	(count)
Ear emergence	-7	8.8	15.3	8.5	0.4	5.7	7.9	6	25.6	days
Growth habit	10.1	4.8	-4.4	-8.3	7	-1.9	4	4	2.8	(score)
Grain area[b]	-2.1	2.7	4.4	3.6	-3.1	-0.4	-1.2	-4	21.9	mm2
Grain length[b]	-1.3	1	-0.1	0.5	-2.8	-0.9	-2.3	-4	6.8	mm
Grain width[b]	-1.6	2	4.4	2.7	0.5	1	1	6	3.9	mm

[a] Thousand grain weight; [b]main tiller

3.3.4 Disease resistance and yield stability of CCP (WP4)

Harvest year 2008

At all three sites during 2007/08 the main disease present was septoria leaf blotch (*Mycosphaerella graminicola (synonym: Septoria tritici*; Table 40), some powdery mildew (*Blumeria graminis*) was also noted at Caythorpe and Morley but levels were low (data not shown). Disease pressure varied considerably between sites, with Sutton Scotney showing higher levels of infection than Morley and Caythorpe. Higher levels of fungicide input generally resulted in improved disease suppression across sites.

With regard to the specific sites:
Caythorpe – final disease assessments were carried out on the 26th June (GS 71). Disease pressure on this site remained very low. While septoria leaf blotch was the main disease present, limited levels of mildew were also recorded. Disease levels in plots where either the 'disease exclusion' or 'reduced input' programmes had been used were very low and only untreated plots displayed symptoms to any noteworthy level. Timber was the cleanest (i.e. the least diseased) variety in the trial.

Sutton Scotney – final disease assessments were carried out on the 10th July (GS 81). Disease pressure at the site was high and septoria leaf blotch was the main disease present. Untreated plots tended to have markedly higher levels of disease than either 'reduced input' or 'disease exclusion' programmes, with the disease differences between the latter two input levels generally being relatively small. Again, Timber was the cleanest variety in the trial.

Morley – final disease assessments were carried out on the 26th June (GS 77). Disease pressure, particularly in treated plots, at the Morley site was somewhat intermediate to that of Sutton Scotney and Caythorpe. Again septoria leaf blotch was the main disease present although limited levels of mildew were also recorded. Compared to the other sites there was relatively little difference in disease level between 'disease exclusion' and 'reduced input' programmes, with untreated plots displaying higher symptom levels. Timber and Gatsby were the cleanest varieties in the trial.

Despite differences in disease levels between sites, greater fungicide inputs consistently lead to an increase in yield. Individual varieties responded differently to the different treatments with Oakley benefiting the most from greater fungicide input, average yield increase across all three trials 1.67 t/ha (*cf.* 'disease exclusion' and 'untreated' programmes; (Table 40). Timber responded the least to the fungicide inputs with the highest level of fungicide inputs only resulting in an average yield benefit of 0.32 t/ha (Table 40). Disease levels in YQCCP and YQ-mix were similar to other varieties in trial; however final yields for both YQCCP and YQ-mix were lower than other entries across all fungicide regimes.

Table 40. Effect of location, variety and fungicide regime on Septoria (% leaf area on selected leaf layers) and yield (t/ha) in 2008.

Variety	Fungicide	Caythorpe				Morley					Sutton Scotney					Yield Mean (t/ha)
		Septoria			Yield (t/ha)	Septoria				Yield (t/ha)	Septoria				Yield (t/ha)	
		GS 39 Leaf 5 (%)	GS 71 Leaf 3 (%)	GS 71 Leaf 4 (%)		GS 41 Leaf 3 (%)	GS 41 Leaf 4 (%)	GS 77 Leaf 3 (%)	GS 77 Leaf 4 (%)		GS 39 Leaf 4 (%)	GS 39 Leaf 5 (%)	GS 81 Leaf 2 (%)	GS 81 Leaf 3 (%)		
Ambrosia	Exclusion	2.0	0.0	0.0	9.61	0.0	1.7	0.0	0.4	12.87	0.0	20.0	5.0	5.0	12.33	11.60
Ambrosia	Reduced	2.0	0.0	0.0	9.62	0.0	2.3	0.0	1.2	12.81	0.7	26.7	6.7	13.3	12.04	11.49
Ambrosia	Untreated	2.3	5.0	8.3	8.79	0.1	5.3	1.7	8.3	11.64	0.3	33.3	55.0	87.5	9.98	10.14
Claire	Exclusion	2.3	3.3	1.0	9.31	0.0	0.5	0.0	2.2	12.51	0.0	8.3	8.3	10.0	12.21	11.34
Claire	Reduced	1.7	0.3	1.7	8.97	0.3	1.5	0.0	1.2	11.83	0.0	18.3	6.7	11.7	12.01	10.94
Claire	Untreated	1.3	3.7	3.3	8.17	0.5	8.7	1.0	12.7	10.76	0.3	20.0	25.0	43.3	10.58	9.84
Einstein	Exclusion	1.7	3.3	0.0	9.98	0.0	0.5	0.0	0.5	12.79	0.3	23.3	6.0	23.3	12.36	11.71
Einstein	Reduced	2.0	0.0	0.7	9.56	0.0	3.3	0.0	0.9	12.76	2.0	28.3	6.7	25.0	11.84	11.39
Einstein	Untreated	2.0	5.0	20.0	9.11	0.5	7.7	1.2	8.0	11.89	2.3	41.7	55.3	80.0	10.14	10.38
Gatsby	Exclusion	2.0	0.0	0.0	10.05	0.0	0.3	0.0	1.0	12.24	0.0	7.7	3.7	11.7	11.76	11.35
Gatsby	Reduced	2.3	0.7	0.7	9.81	0.0	2.3	0.0	0.7	12.29	0.0	16.7	11.0	20.0	11.60	11.23
Gatsby	Untreated	2.7	0.7	1.0	9.23	0.0	8.7	0.4	6.7	11.84	0.1	13.1	28.0	40.0	10.68	10.58
Oakley	Exclusion	2.3	0.0	0.0	10.15	0.0	3.0	0.0	1.7	13.24	0.0	10.0	15.0	25.0	12.57	11.99
Oakley	Reduced	2.0	0.7	0.0	9.46	0.0	5.3	0.1	5.3	12.89	0.0	21.7	15.0	25.0	12.49	11.61
Oakley	Untreated	2.7	7.7	23.3	8.78	0.9	10.3	4.0	20.0	11.73	0.3	16.7	61.7	80.0	10.45	10.32
Solstice	Exclusion	2.7	0.0	1.0	9.20	0.0	2.3	0.0	1.0	11.99	0.0	20.0	10.0	11.7	11.75	10.98
Solstice	Reduced	2.3	0.0	1.7	8.89	0.1	1.7	0.0	2.3	11.69	0.0	21.7	10.0	23.3	11.64	10.74
Solstice	Untreated	4.3	10.7	13.3	8.12	0.0	3.7	1.5	10.7	11.21	0.3	31.7	53.3	75.0	10.29	9.87
Humber	Exclusion	1.7	0.0	0.0	9.81	0.0	2.5	0.0	2.7	12.77	0.0	14.3	5.7	11.7	12.03	11.54
Humber	Reduced	2.0	0.0	0.0	9.68	0.1	3.0	0.4	5.0	12.71	0.7	30.0	9.3	18.3	11.79	11.39
Humber	Untreated	2.7	1.7	34.7	9.45	0.1	7.0	2.8	13.3	12.53	2.0	46.7	53.3	dead	11.67	11.22
Timber	Exclusion	3.3	0.0	0.0	9.69	0.1	3.0	0.0	1.0	12.88	0.0	6.7	3.7	9.3	12.47	11.68
Timber	Reduced	2.3	0.0	0.0	9.48	0.1	3.5	0.0	2.0	12.58	0.0	7.3	3.7	13.3	12.14	11.40
Timber	Untreated	2.3	0.0	0.0	8.30	0.2	6.8	0.4	6.7	11.70	0.0	20.0	23.3	37.5	10.13	10.04
YQCCP	Exclusion	3.0	0.0	0.0	7.76	0.0	3.5	0.1	0.7	10.20	0.0	16.7	16.7	23.3	9.43	9.13
YQCCP	Reduced	3.3	0.0	1.0	7.45	0.1	5.1	0.0	4.3	9.95	0.0	21.7	30.0	36.7	9.43	8.94
YQCCP	Untreated	3.3	6.7	33.3	7.09	2.2	16.7	2.7	11.7	9.69	0.3	21.7	58.3	63.3	8.55	8.44
YQ-mix	Exclusion	3.0	1.0	0.0	7.42	0.0	3.1	0.0	0.9	10.59	0.0	15.0	26.7	20.0	9.46	9.16
YQ-mix	Reduced	3.0	0.0	0.0	7.21	0.1	5.7	0.4	3.3	10.30	0.3	30.0	41.7	50.0	9.45	8.99
YQ-mix	Untreated	3.7	1.0	0.0	7.07	1.9	10.0	3.7	11.7	9.77	0.3	36.7	53.3	45.0	8.87	8.57
LSD		1.53 P=0.09	5.26 P<0.01	30.02 P=0.66	0.570 CV=3.8%	1.14 P<0.05	5.40 P<0.001	1.26 P<0.001	4.11 P<0.001	0.376 CV=1.9%	1.26 P<0.05	13.87 P<0.001	14.66 P<0.001	18.53 P<0.001	0.400 CV=2.2%	- -

Harvest year 2009

The main disease present on all sites during 2008/09 was Septoria leaf blotch; although disease levels remained low in all varieties (Table 41). The lowest levels in untreated plots tended to be associated with Gatsby and Timber. As the season progressed other diseases were noted, specifically yellow rust (*Puccinia striiformis*) was observed on all sites in 'Untreated' Oakley plots (although this was controlled adequately by either 'Reduced' or 'Exclusion' fungicide programmes). Some powdery mildew was noted at Caythorpe; at the later assessment timing average levels of infection on final leaf 2 were quite low (below 1%) however higher levels of infection (5% on leaf 2) were found in the more susceptible varieties (data not shown). As expected, across all sites, 'Untreated' plots tended to show the greatest level of disease, the 'Exclusion' treatment the lowest level with the 'Reduced programme' demonstrating a somewhat intermediate levels of infection.

The yield potential varied across the three sites with Morley demonstrating the highest yields (averaging around 10.5 t/ha across all varieties and programmes), Caythorpe the lowest (averaging around 7.5 t/ha) with Sutton Scotney somewhat intermediate (averaging around 9.0 t/ha). The Caythorpe site suffered from drought stress later in the season, this resulted in the loss of several leaf layers and likely contributed to the lower yield on this site.

By averaging across all three sites the highest yields in 'Exclusion' and 'Reduced' programmes were found for Oakley and Timber (although those of Ambrosia were also comparable). In the 'Untreated' programme the highest yields were found for Timber. The greatest level of yield response across the sites (i.e. the yield difference between 'Untreated' and 'Exclusion' programmes) was found for Oakley; indicating the potential impact of yellow rust in this highly susceptible variety.

The yields achieved by the YQCCP and YQ-mix on the Sutton Scotney and Morley sites were lower than those achieved by other varieties, however on the lower yield potential site at Caythorpe this difference was much less pronounced; perhaps suggesting greater yield stability in these lines in scenarios where yield potential is more constrained. On each of the three sites the yield response level noted in the YQCCP and YQ-mix was always less than the average response across all the varieties and was similar to Gatsby and Timber (varieties with lower susceptibility to Septoria leaf blotch); although it should be noted that the yield potential of any given variety will also impact on the level of response that can be achieved.

Table 41. Effect of location, variety and fungicide regime on Septoria, Green Leaf Area (GLA) (% leaf area on selected leaf layers) and yield (t/ha) in 2009.

Variety	Fungicide	Caythorpe				Morley					Sutton Scotney					Yield Mean (t/ha)
		Septoria		GLA	Yield (t/ha)	Septoria				Yield (t/ha)	Septoria				Yield (t/ha)	
		GS 39 Leaf 3 (%)	GS 39 Leaf 4 (%)	GS 75 Leaf 3 (%)		GS 39 Leaf 3 (%)	GS 39 Leaf 4 (%)	GS 77 Leaf 2 (%)	GS 77 Leaf 3 (%)		GS 39 Leaf 5 (%)	GS 39 Leaf 6 (%)	GS 78 Leaf 2 (%)	GS 78 Leaf 3 (%)		
Ambrosia	Exclusion	0.7	1.7	83	7.90	0.1	2.0	0.0	0.1	11.16	0.7	5.0	2.0	3.0	10.38	9.81
Ambrosia	Reduced	1.0	3.0	79	7.87	0.1	4.0	0.0	0.0	10.98	2.0	8.0	1.7	3.7	10.26	9.70
Ambrosia	Untreated	1.7	4.3	65	7.48	0.5	10.0	0.7	3.7	9.84	1.0	6.0	53.3	70.0	9.25	8.86
Claire	Exclusion	1.7	5.0	63	7.59	0.5	2.0	0.0	0.0	10.90	0.0	5.0	1.3	2.3	9.60	9.36
Claire	Reduced	2.7	6.0	67	7.38	0.5	5.0	0.0	0.2	10.36	0.7	7.7	2.3	4.0	9.62	9.12
Claire	Untreated	4.3	8.0	50	6.83	0.5	10.0	0.5	5.7	9.43	0.0	7.0	13.3	60.0	8.76	8.34
Einstein	Exclusion	0.0	1.7	80	8.31	0.1	2.0	0.0	0.0	10.82	0.0	3.7	2.0	3.7	9.80	9.64
Einstein	Reduced	0.0	1.7	72	8.04	0.5	5.0	0.0	0.1	10.64	1.7	5.5	2.7	4.7	9.64	9.44
Einstein	Untreated	0.3	3.7	52	7.53	0.1	5.0	0.5	5.3	9.46	2.0	5.3	33.3	70.0	8.68	8.56
Gatsby	Exclusion	0.0	1.0	82	7.35	0.1	1.0	0.0	0.0	11.04	0.0	5.8	1.0	3.7	9.62	9.34
Gatsby	Reduced	0.0	1.7	72	7.23	0.1	0.1	0.0	0.0	10.52	0.0	5.3	1.0	3.7	9.31	9.02
Gatsby	Untreated	0.3	2.0	72	7.16	0.1	3.0	0.0	0.2	9.94	0.7	2.8	6.7	30.0	9.02	8.71
Oakley	Exclusion	0.0	1.0	75	8.15	0.0	1.0	0.0	0.0	12.17	0.0	5.0	1.0	1.7	10.58	10.30
Oakley	Reduced	0.0	2.3	63	7.50	0.1	3.0	0.0	0.4	11.83	0.0	4.3	2.7	5.0	10.57	9.97
Oakley	Untreated	0.3	1.7	17	6.56	0.1	12.0	4.0	8.3	8.90	0.3	7.3	30.0	dead	9.26	8.24
Solstice	Exclusion	1.7	3.0	47	7.16	0.1	2.0	0.0	0.0	10.71	0.3	5.7	2.7	3.5	9.65	9.17
Solstice	Reduced	1.7	3.0	43	6.77	0.1	2.0	0.0	0.1	10.31	0.0	7.0	3.0	11.7	9.37	8.82
Solstice	Untreated	2.0	5.3	23	6.82	0.5	8.0	0.2	5.3	9.08	0.7	8.3	43.3	dead	8.75	8.22
Humber	Exclusion	0.0	0.0	83	7.87	0.0	0.5	0.0	0.2	11.48	0.0	4.3	1.0	2.3	10.10	9.82
Humber	Reduced	0.0	2.0	83	7.71	0.1	1.0	0.0	0.1	11.14	0.0	8.3	2.3	3.7	9.93	9.59
Humber	Untreated	1.7	2.7	58	7.13	0.1	3.0	1.3	9.3	9.70	0.0	6.7	30.0	dead	9.02	8.62
Timber	Exclusion	1.0	2.7	82	8.39	0.1	1.0	0.0	0.1	11.33	0.0	1.7	3.0	5.3	10.03	9.92
Timber	Reduced	2.3	5.3	78	8.10	0.1	5.0	0.0	0.2	11.13	0.0	2.7	2.0	5.0	10.03	9.75
Timber	Untreated	3.0	6.3	62	7.95	0.1	3.0	0.2	3.7	10.67	0.2	2.7	6.7	36.7	9.62	9.41
YQCCP	Exclusion	1.0	2.0	82	7.08	0.1	1.0	0.0	0.7	9.65	0.0	3.7	4.3	5.0	7.99	8.24
YQCCP	Reduced	0.7	2.3	67	6.90	0.1	3.0	0.0	1.0	9.32	0.0	3.7	4.3	5.0	7.94	8.05
YQCCP	Untreated	1.0	2.7	30	6.70	0.5	8.0	2.1	11.7	8.70	0.0	3.7	21.7	60.0	7.67	7.69
YQ-mix	Exclusion	0.0	1.3	67	6.94	0.1	2.0	0.0	1.3	9.69	0.7	4.0	3.0	18.3	8.40	8.34
YQ-mix	Reduced	0.7	3.0	75	6.94	0.1	5.0	0.1	0.9	9.62	0.3	3.3	5.0	18.3	8.22	8.26
YQ-mix	Untreated	1.3	4.0	48	6.50	0.5	8.0	1.3	13.3	8.78	1.0	5.0	18.3	60.0	7.53	7.60
LSD		1.00	1.65	24.9	0.33	-	-	0.80	4.24	0.27	1.23	2.81	16.25	9.73	0.45	-
		P<0.001	P<0.001	P<0.001	CV=2.7%	-	-	P<0.001	P<0.001	CV=1.6%	P<0.05	P<0.001	P<0.001	P<0.001	CV=2.9%	-

Note: Yellow rust was also noted in untreated Oakley at all sites.

Harvest year 2010

The main disease present was septoria leaf blotch, although yellow rust was found at all three sites by GS 75, notably in untreated Oakley (15%–E98% on final leaf 2); although this was controlled adequately by either 'Reduced' or 'Exclusion' fungicide programmes (Table 42). In general, septoria leaf blotch levels were low. Dry conditions were evident in spring 2010, with Met Office data indicating that less than 50% of the monthly average rainfall was recorded in April over much of England (www.metoffice.gov.uk). All three sites suffered from drought conditions which had a greater impact on sites with thinner soils (e.g. Sutton Scotney). In general, across all sites, 'Untreated' regimes tended to show the greatest level of disease, 'Exclusion' regimes the lowest level, with the 'Reduced programme' demonstrating somewhat Intermediate levels of infection.

Yield potential varied across all three sites with Caythorpe demonstrating the highest yields (averaging ca. 10.5 t/ha across all varieties and programmes), Sutton Scotney the lowest (averaging ca. 8.0 t/ha) and Morley being somewhat intermediate (averaging ca. 9.5 t/ha). The greatest level of yield response across the locations was associated with Oakley; again indicating the potential yield loss associated with yellow rust infection.

Yields achieved by the YQCCP and YQ-mix on all three sites were generally lower than those achieved with other varieties. As with previous seasons the yield response level attributed to disease management in the YQCCP and YQ-mix was similar to that of the more resistant varieties (e.g. Gatsby) and was always less than the average response for all other varieties.

Harvest year 2011

While septoria leaf blotch was the main disease present in 2010/11 the exceptionally dry spring conditions limited both disease development and yield (

Table **43**). Met Office data for spring 2011 indicated that the spring was particularly dry over southern and eastern England, where less than a third of the normal amount fell widely, and across much of East Anglia only about 20% of the average rainfall was recorded.

With regards to disease data from each of the sites; at Caythorpe the disease levels were very low. By mid-May, septoria leaf blotch levels on leaf 4 were less than 5% infection on untreated plots and by early July there was no septoria leaf blotch noted on upper leaves in assessments (data not shown). Yellow rust was present, particularly in untreated Oakley plots. At Morley, disease levels were also low peaking at 4–7% septoria leaf blotch in untreated Oakley and Solstice on leaf 3 at GS 75 (5th July).

Table 42. Effect of location, variety and fungicide regime on septoria (% leaf area on selected leaf layers) and yield (t/ha) in 2010.

Variety	Fungicide	Caythorpe Septoria				Yield (t/ha)	Morley Septoria			Yield (t/ha)	Sutton Scotney Septoria				Yield (t/ha)	Yield Mean (t/ha)
		GS 39 Leaf 4 (%)	GS 39 Leaf 5 (%)	GS 75 Leaf 2 (%)	GS 75 Leaf 3 (%)		GS 39 Leaf 4 (%)	GS 71 Leaf 3 (%)	GS 71 Leaf 4 (%)		GS 45 Leaf 4 (%)	GS 39 Leaf 5 (%)	GS 65 Leaf 4 (%)	GS 65 Leaf 5 (%)		
Ambrosia	Exclusion	3.0	8.3	0.0	0.7	9.97	1.0	0.2	2.2	9.73	0.0	1.3	0.0	3.7	8.84	9.51
Ambrosia	Reduced	2.7	10.0	0.0	1.0	9.87	2.0	0.2	3.2	10.21	0.3	1.7	0.0	3.7	8.82	9.63
Ambrosia	Untreated	4.3	11.3	4.7	8.7	9.53	3.3	0.6	4.7	9.94	0.0	2.3	0.0	4.0	8.53	9.33
Claire	Exclusion	4.7	14.0	0.0	0.0	10.23	2.0	0.1	1.8	9.67	1.3	3.7	0.7	6.7	8.86	9.59
Claire	Reduced	2.7	13.3	0.0	0.0	10.04	2.7	0.2	2.8	9.76	1.3	4.3	2.0	10.0	8.41	9.40
Claire	Untreated	6.7	17.3	1.0	5.7	9.75	4.0	0.6	3.7	9.59	2.0	4.7	3.7	10.0	8.47	9.27
Einstein	Exclusion	5.3	22.3	0.0	0.0	10.00	1.7	0.1	2.3	9.56	1.0	4.3	0.0	5.0	8.48	9.35
Einstein	Reduced	5.0	28.3	0.0	0.0	10.16	2.3	0.2	3.0	9.59	0.7	3.7	1.0	10.0	8.37	9.37
Einstein	Untreated	13.3	20.0	4.0	8.3	9.82	4.3	0.4	4.7	9.24	0.7	4.3	2.3	16.7	8.23	9.10
Gatsby	Exclusion	0.0	3.7	0.0	0.0	9.31	1.7	0.1	1.7	9.59	1.0	3.3	0.0	3.7	7.88	8.93
Gatsby	Reduced	1.3	5.3	0.0	0.0	9.51	2.0	0.2	3.5	9.17	1.0	3.7	0.0	3.7	8.04	8.91
Gatsby	Untreated	2.3	4.3	0.3	2.7	9.43	3.0	0.9	3.3	9.00	1.0	3.7	0.0	5.3	7.93	8.79
Oakley	Exclusion	1.0	6.7	0.0	0.0	10.53	2.0	0.2	2.3	9.71	0.0	1.7	0.3	5.0	9.18	9.81
Oakley	Reduced	2.3	12.7	0.0	0.0	10.32	2.0	1.2	4.7	10.08	0.0	1.7	0.7	3.9	8.90	9.77
Oakley	Untreated	7.7	45.0	dead	dead	6.50	3.7	7.7	10.0	8.23	0.0	3.0	3.0	3.9	8.01	7.58
Solstice	Exclusion	2.3	9.3	0.0	0.0	9.59	2.3	0.1	1.4	8.96	1.0	3.3	1.7	5.0	8.13	8.89
Solstice	Reduced	4.7	12.3	0.0	0.0	9.71	2.3	0.1	3.2	8.71	1.0	3.0	3.7	10.0	8.10	8.84
Solstice	Untreated	2.7	14.3	1.0	1.0	9.24	4.0	0.5	6.3	8.80	1.0	4.3	3.7	18.9	7.79	8.61
Humber	Exclusion	5.3	25.0	0.0	0.0	10.37	1.7	0.1	1.9	9.82	0.3	3.0	1.7	5.0	8.70	9.63
Humber	Reduced	3.0	10.0	0.0	0.0	10.01	1.7	0.2	3.0	10.01	0.7	2.3	1.7	6.7	8.60	9.54
Humber	Untreated	7.7	35.0	2.0	5.3	9.78	3.0	0.5	4.7	9.95	0.7	4.3	5.0	11.6	8.65	9.46
Battalion	Exclusion	4.7	11.3	0.0	0.0	9.90	2.0	0.1	1.8	9.47	0.7	5.0	2.3	10.0	9.04	9.47
Battalion	Reduced	5.7	14.5	0.0	0.0	10.15	2.7	0.1	3.0	9.51	1.0	5.0	2.3	11.7	8.82	9.49
Battalion	Untreated	4.0	12.3	0.7	3.3	9.79	3.0	0.4	4.7	9.45	1.3	4.3	2.3	11.7	8.85	9.36
YQCCP	Exclusion	3.7	14.0	0.0	0.0	9.26	2.3	0.1	2.8	8.57	1.0	4.3	3.3	3.9	7.54	8.46
YQCCP	Reduced	6.3	18.3	0.0	0.0	8.80	1.7	0.2	3.3	8.00	1.0	5.0	3.3	3.9	7.36	8.05
YQCCP	Untreated	12.0	30.0	3.7	3.3	8.71	3.3	0.4	4.3	8.46	1.0	4.3	5.0	9.5	7.58	8.25
YQ-mix	Exclusion	7.7	18.7	0.0	0.0	9.06	2.3	0.1	5.7	8.29	1.0	5.0	1.7	8.9	7.34	8.23
YQ-mix	Reduced	10.3	40.0	0.0	0.0	8.92	3.3	0.2	6.7	8.62	1.0	5.0	0.0	8.3	7.42	8.32
YQ-mix	Untreated	10.0	50.0	4.7	4.7	8.36	3.0	0.5	5.3	8.53	1.0	5.0	3.3	13.9	7.36	8.08
LSD		5.79	11.44	1.55	2.17	0.598	1.26	0.88	2.44	0.535	0.82	1.67	3.36	7.03	0.510	-
		P<0.01	P<0.001	P<0.001	P<0.05	CV=3.8%	P<0.001	P<0.001	P<0.001	CV=3.5%	P<0.001	P<0.001	P<0.05	P<0.001	CV=3.8%	-

Note: Appreciable levels of yellow rust were noted in untreated Oakley, with lesser amounts in Solstice, Ambrosia, YQCCP and YQ-mix.

Table 43. Effect of location, variety and fungicide regime on septoria, Green Leaf Area (GLA) (% leaf area on selected leaf layers) and yield (t/ha) in 2011.

Variety	Fungicide	Caythorpe				Morley				Sutton Scotney					Yield Mean (t/ha)
		Septoria	GLA		Yield (t/ha)	Septoria		GLA	Yield (t/ha)	Septoria			GLA	Yield (t/ha)	
		GS 39 Leaf 4 (%)	GS 85 Leaf 2 (%)	GS 85 Leaf 3 (%)		GS 49 Leaf 5 (%)	GS 75 Leaf 3 (%)	GS 75 Leaf 3 (%)		GS 45 Leaf 5 (%)	GS 77 Leaf 2 (%)	GS 77 Leaf 3 (%)	GS 77 Leaf 3 (%)		
KWS Stirling	Exclusion	3.0	67	22	5.84	0.0	0.7	73	8.52	36.7	3.3	5.0	87	11.28	8.55
KWS Stirling	Reduced	3.0	60	18	5.76	0.0	1.5	75	8.42	36.7	11.7	16.7	70	11.09	8.42
KWS Stirling	Untreated	4.0	43	6	5.65	0.0	2.7	58	8.28	56.7	35.0	50.0	7	9.62	7.85
Claire	Exclusion	4.3	68	7	5.74	0.0	0.7	82	7.46	18.3	2.7	5.0	91	11.93	8.38
Claire	Reduced	5.0	67	17	5.64	0.5	0.8	77	7.67	23.3	3.3	9.3	84	11.72	8.34
Claire	Untreated	4.0	58	2	5.17	0.0	2.4	72	7.19	16.7	30.0	30.0	17	10.16	7.51
Einstein	Exclusion	1.0	70	15	5.53	0.0	0.3	67	6.70	23.3	3.0	7.3	89	9.49	7.24
Einstein	Reduced	2.0	77	23	5.52	0.0	1.7	62	7.79	33.3	4.0	8.0	87	9.02	7.44
Einstein	Untreated	2.3	62	8	5.13	0.0	2.0	67	6.36	36.7	36.7	53.3	22	8.25	6.58
Gatsby	Exclusion	1.3	73	30	5.78	0.0	0.7	83	9.05	15.0	2.0	6.7	87	12.04	8.96
Gatsby	Reduced	1.3	77	35	5.76	0.0	0.5	78	8.70	16.7	2.0	9.0	85	11.66	8.71
Gatsby	Untreated	2.3	70	15	5.63	0.0	1.7	73	8.02	10.0	4.3	10.0	85	10.95	8.20
Oakley	Exclusion	2.0	72	37	6.28	0.0	1.2	77	8.57	23.3	3.0	14.7	82	12.13	8.99
Oakley	Reduced	3.0	60	27	6.15	2.0	1.4	80	8.47	33.3	11.7	18.3	77	11.48	8.70
Oakley	Untreated	6.0	dead	dead	3.91	5.0	6.7	39	6.72	30.0	dead	dead	0	6.42	5.68
Solstice	Exclusion	5.0	70	23	5.14	0.5	0.4	75	7.50	25.0	2.7	6.7	88	10.51	7.72
Solstice	Reduced	6.0	62	20	5.13	0.5	1.4	72	7.62	43.3	6.7	10.0	68	10.13	7.63
Solstice	Untreated	7.7	63	8	4.86	2.0	4.3	52	7.31	30.0	25.0	40.0	13	8.81	6.99
Humber	Exclusion	2.3	80	50	5.77	0.0	0.2	87	8.10	23.3	0.7	6.3	90	11.73	8.53
Humber	Reduced	3.0	73	58	5.52	0.0	0.8	83	7.61	28.3	4.0	7.3	89	11.28	8.14
Humber	Untreated	7.0	70	40	5.07	0.0	2.0	75	7.79	26.7	48.3	50.0	10	10.29	7.72
Stigg	Exclusion	1.0	80	57	5.41	0.0	0.4	87	8.45	16.0	1.3	4.0	87	12.63	8.83
Stigg	Reduced	2.0	82	60	5.54	0.0	0.1	87	8.31	10.0	1.3	3.3	93	12.54	8.80
Stigg	Untreated	1.7	72	43	5.30	0.0	1.2	87	8.36	10.0	3.3	8.3	87	11.79	8.48
YQCCP	Exclusion	3.0	68	27	4.85	0.0	0.6	77	6.46	23.3	4.0	12.3	80	9.19	6.83
YQCCP	Reduced	3.7	67	32	4.91	0.0	3.0	78	6.51	30.0	5.0	9.0	72	8.95	6.79
YQCCP	Untreated	5.7	53	5	4.75	0.0	3.0	68	6.47	26.7	25.0	50.0	3	8.56	6.59
YQ-mix	Exclusion	4.0	55	12	4.85	0.0	0.0	67	6.38	33.3	4.3	8.3	85	9.16	6.80
YQ-mix	Reduced	5.3	67	23	4.99	0.0	0.8	60	6.66	26.7	6.7	16.7	77	9.06	6.90
YQ-mix	Untreated	6.0	50	3	4.86	0.0	1.0	57	6.37	33.3	22.5	dead	0	8.39	6.54
LSD		1.47	13.3	25.6	0.533	-	2.37	22.1	0.608	18.8	12.33	7.89	18.86	0.558	-
		P<0.001	P<0.001	P<0.001	CV=6.1%	-	P<0.001	P<0.01	CV=4.9%	P<0.001	P<0.001	P<0.001	P<0.001	CV=3.3%	-

Note: Appreciable yellow rust was noted in untreated Oakley and Solstice. Low levels of brown rust were noted Claire, Einstein, Humber, Solstice, on YQCCP and YQ-mix.

Septoria leaf blotch levels in all other varieties were <3% at this time and while some yellow rust was noted this was ostensibly in untreated Oakley. Disease levels at Sutton Scotney were higher than the other two sites, peaking at around 35–45% on leaf 2 in untreated plots at GS 77 (11th July); in all fungicide treated plots at this site disease levels were generally <10% septoria leaf blotch.

At Morley and Sutton Scotney the 'Untreated' regime tended to show the greatest level of disease, the 'Exclusion' approach showed the lowest, with the 'Reduced' programme demonstrating intermediate level of infection. At both sites disease levels in the YQ-mix and YQCCP were similar to those seen in other varieties.

Yield potential varied across the sites with Sutton Scotney demonstrating the highest yields (averaging about 10.5 t/ha across all varieties), Caythorpe the lowest averaging around 5.5 t/ha and Morley was intermediate (averaging around 7.5 t/ha). The spring drought conditions seem likely to have had the greatest yield limiting impact at the Caythorpe and Morley sites. The greatest level of yield response across the locations (i.e. the yield difference between 'Untreated' and 'Exclusion' programmes) was associated with the management of yellow rust in Oakley. The yields achieved by the YQCCP and YQ-mix were less than the site means at all locations. However, as with previous seasons the yield response levels in the YQCCP and YQ-mix were less than the average response across all the varieties, indicating a more robust disease resistance capacity offered by these lines (*cf*. other current elite lines included in these studies).

Harvest year 2012

Yield and disease data from all three sites in the 2011/12 season are presented in Table 44. Seasonal weather conditions during this season need particular consideration in the interpretation of this data set. Specifically, Met Office data from 2012 suggests that the UK had an exceptionally wet summer, especially during June and much of July (with only summer 1912 wetter in the series from 1910 and the equally wet June (with June 1860) in England and Wales since 1766). Further, the summer was duller than usual, with 82% of normal sunshine for the UK overall (amounts were below average in June (70%), July (81%) and August (95%)); suggesting this was the second dullest June recorded across the UK.

Septoria leaf blotch was the main disease present across the sites, but yellow rust was again prominent in Oakley and some brown rust (*Puccinia recondite*) was also recorded, notably at Morley. At Caythorpe appreciable levels of yellow rust were noted by GS 39 in Oakley (38% in untreated plots on leaf 3 with 5-7% in the other fungicide regimes) with lesser amounts on other varieties (less than 5%). However by GS 71 septoria leaf blotch was the main disease present with a high disease pressure noted in the study; with 15% (Gatsby) – 50% (Solstice) infection noted on untreated plots on leaf 2. Clear increments in both disease reduction and retention of green leaf area were apparent in all varieties in response to increasing intensity of fungicide programme. At Morley, high levels of rust were noted in both Oakley (yellow rust)

and Stigg (brown rust). As in other seasons, the intensity of fungicide regime improved both green leaf area retention and disease control later in the season, however, the disease pressure was lower at Morley than at Caythorpe and, consequently, differences were not as marked. Similar to Caythorpe, Sutton Scotney was also subject to high disease pressure with high levels of septoria leaf blotch apparent on all varieties on leaf 6 and GS 39 and leaf 2 by GS 75 (Table 44). As with Caythorpe and Morley sites, at GS 75, intensity of fungicide regime was associated with improved green leaf area retention and disease control in all varieties.

Seasonal conditions are likely to have impacted on the yield potential and response to fungicide regime at all sites. Caythorpe had the lowest peak yield, reaching around 7 t/ha with Stigg and Gatsby in disease exclusion programmes. In this low yield potential scenario the yields of both the YQCCP and YQ-mix, particularly in the reduced and untreated fungicide approaches, were very similar to those of the other elite varieties in the study. Yield potential at Morley peaked at around 9 t/ha, with several varieties achieving this level under the disease exclusion and reduced input scenarios; for many varieties in 2012 there was little difference in yield response between these two approaches at Morley. While the YQCCP and YQ-mix lines at Morley, again. yielded below the site average for all these approaches, in all three scenarios they out-yielded some of the elite lines (Table 44). Despite the high disease pressure, yields at Sutton Scotney peaked at around 8 t/ha, with the YQCCP and YQ-mix lines tending to yield less than the other elite varieties, but again demonstrating less of a response to increasing fungicide input compared to most other varieties in the study (and appearing similar to more disease tolerant lines such as Gatsby). Substantial ear blight (*Fusarium* spp.) was also apparent at Sutton Scotney in 2012; visual differences were assessed, and while some differences between varieties and responses to fungicide programmes were apparent the responses noted in the YQCCP and YQ-mix were similar to those of other varieties in the experiment.

Table 44. The effect of location, variety and fungicide on septoria, yellow rust, brown rust. Green Leaf Area (GLA) (% leaf area on selected leaf layers), fusarium (% ear infected) and yield (t/ha) in 2012.

Variety	Fungicide	Caythorpe					Morley					Sutton Scotney						Yield Mean (t/ha)
		Y. rust GS 39 Leaf 3 (%)	Septoria GS 39 Leaf 3 (%)	Septoria GS 71 Leaf 2 (%)	GLA GS 85 Leaf 3 (%)	Yield (t/ha)	B rust GS 77 Leaf 2 (%)	Septoria GS 77 Leaf 3 (%)	Y rust GS 77 Leaf 3 (%)	GLA GS 77 Leaf 3 (%)	Yield (t/ha)	Septoria GS 39 Leaf 5 (%)	Septoria GS 39 Leaf 6 (%)	Septoria GS 75 Leaf 2 (%)	GLA GS 75 Leaf 2 (%)	Fusarium GS 75 Ear (%)	Yield (t/ha)	
KWS Stirling	Exclusion	0.0	0.8	5.7	45	5.70	0.0	4.7	0.0	70	8.35	0.3	20.0	11.7	82	36.7	7.27	7.11
KWS Stirling	Reduced	0.0	1.2	14.3	23	4.89	0.0	6.3	0.0	60	8.04	8.3	20.0	23.3	67	40.0	7.01	6.65
KWS Stirling	Untreated	0.0	3.3	40.0	2	3.67	0.3	8.7	0.0	50	5.35	5.0	53.3	70.0	7	56.7	5.96	4.99
Claire	Exclusion	0.3	1.0	2.3	65	6.28	0.0	3.0	0.0	75	9.09	0.3	26.7	1.0	98	16.7	7.92	7.76
Claire	Reduced	0.7	1.0	7.0	48	5.80	0.0	5.7	0.0	65	8.50	0.0	28.3	6.7	88	21.7	7.54	7.28
Claire	Untreated	2.3	2.0	16.7	10	4.14	0.0	10.0	0.0	58	5.38	3.3	40.0	40.0	47	26.7	7.06	5.53
Einstein	Exclusion	0.3	1.0	5.7	55	6.62	0.0	7.0	0.0	68	9.26	2.3	53.3	6.7	88	16.7	8.40	8.09
Einstein	Reduced	0.7	2.0	13.7	25	6.25	0.0	7.7	0.0	67	8.92	2.3	60.0	20.0	70	16.7	8.14	7.77
Einstein	Untreated	0.7	2.7	40.0	2	4.58	0.1	10.0	0.0	63	6.31	6.7	56.7	80.0	3	33.3	6.62	5.84
Gatsby	Exclusion	0.0	0.2	1.7	74	7.09	0.0	1.3	0.0	82	9.43	0.7	33.3	1.0	98	6.7	7.27	7.93
Gatsby	Reduced	0.3	0.0	4.7	60	6.63	0.0	2.7	0.0	77	9.34	0.7	26.7	8.3	85	6.7	6.84	7.60
Gatsby	Untreated	0.3	1.0	15.0	37	5.53	0.0	6.7	0.0	67	7.83	10.3	40.0	26.7	63	10.0	6.64	6.67
Oakley	Exclusion	5.0	3.3	7.0	32	5.51	6.7	7.7	6.0	52	5.91	11.7	30.0	26.7	63	16.7	8.15	6.52
Oakley	Reduced	6.7	5.7	11.3	17	4.62	9.3	8.7	3.3	47	4.97	10.0	30.0	35.0	37	33.3	7.64	5.74
Oakley	Untreated	38.3	8.3	dead	0	1.80	13.3	12.3	4.3	23	2.17	20.0	dead	dead	0	66.7	2.42	2.13
Solstice	Exclusion	1.7	1.7	8.7	65	6.18	0.0	6.3	0.0	68	7.66	2.0	26.7	8.3	85	11.7	8.08	7.31
Solstice	Reduced	1.3	2.7	18.3	23	5.91	0.0	8.3	0.3	65	7.58	2.3	40.0	20.0	70	20.0	7.79	7.09
Solstice	Untreated	1.0	2.3	50.0	0	3.86	0.0	12.0	2.0	52	4.40	0.7	31.7	80.0	3	36.7	6.60	4.95
Humber	Exclusion	0.7	0.0	4.0	67	5.86	0.0	3.7	0.0	78	9.19	7.0	20.0	3.7	96	30.0	7.87	7.64
Humber	Reduced	0.0	0.7	8.7	42	5.20	0.0	6.7	0.0	72	9.11	7.7	30.0	18.3	73	40.0	7.45	7.25
Humber	Untreated	0.0	2.3	21.0	12	4.03	0.0	8.3	0.0	68	6.38	6.7	60.0	75.0	10	40.0	6.62	5.68
Stigg	Exclusion	0.0	0.0	0.0	82	7.02	9.3	8.0	0.0	37	5.83	2.0	30.0	2.3	97	20.0	8.45	7.10
Stigg	Reduced	0.0	0.0	0.7	72	6.53	15.0	8.3	0.0	35	4.35	0.7	45.0	2.3	97	30.0	7.71	6.20
Stigg	Untreated	0.0	0.0	dead	0	3.12	23.3	8.3	0.0	23	1.51	1.7	30.0	53.3	13	36.7	6.64	3.76
YQCCP	Exclusion	0.0	0.0	6.0	52	5.33	0.0	5.7	0.0	70	7.45	13.3	30.0	6.7	83	20.0	6.79	6.52
YQCCP	Reduced	2.0	0.7	8.7	47	5.24	0.2	8.0	0.0	65	7.20	13.3	30.0	23.3	67	23.3	6.51	6.32
YQCCP	Untreated	4.7	5.3	28.3	7	4.15	0.2	10.0	0.0	63	4.80	6.7	45.0	66.7	13	36.7	5.90	4.95
YQ-mix	Exclusion	0.3	1.3	4.3	60	5.38	0.0	5.7	0.0	68	7.23	2.3	40.0	10.0	85	16.7	6.83	6.48
YQ-mix	Reduced	0.7	1.7	9.3	35	5.10	0.0	7.0	0.0	65	7.02	3.3	40.0	26.7	63	23.3	6.40	6.17
YQ-mix	Untreated	2.7	2.3	25.0	8	3.94	0.2	8.0	0.0	62	4.39	11.7	46.7	70.0	13	33.3	5.76	4.70
LSD		3.59	2.27	6.82	20.5	0.376	3.32	3.41	1.84	10.4	0.50	11.86	24.35	12.07	16.4	9.50	0.369	-
		P<0.001	P<0.001	P<0.001	P<0.001	CV=4.4%	P<0.001	P<0.001	P<0.001	P<0.001	CV=4.5%	P=0.115	P<0.05	P<0.001	P<0.001	P<0.001	CV=3.2%	-

Note: Yellow rust and brown rust were noted at all sites.

Further analysis

Regression analysis was used to evaluate the behaviour of the YQCCP and YQ-mix in comparison to conventional wheat cultivars. Initially an analysis was run to investigate whether the YQCCP and YQ-mix differed from pure lines in terms of their tolerance to a reduction in Green Leaf Area which accompanies foliar diseases. A separate analysis investigated whether yield stability was greater for the YQ-lines than conventional lines.

There were no consistent trends regarding disease and crop yield between sites and years (data not shown). The lowest disease and highest yielding treatment ('disease exclusion') was used as a reference treatment for each trial to allow for comparison between other treatments ('untreated' and 'reduced input'). Season, site and variety altered the relationships between the reduction in green leaf area (GLA), observed as disease increases, and the reduction in crop yield were observed for individual trials and varieties (Figure 25, Figure 26, Figure 27).

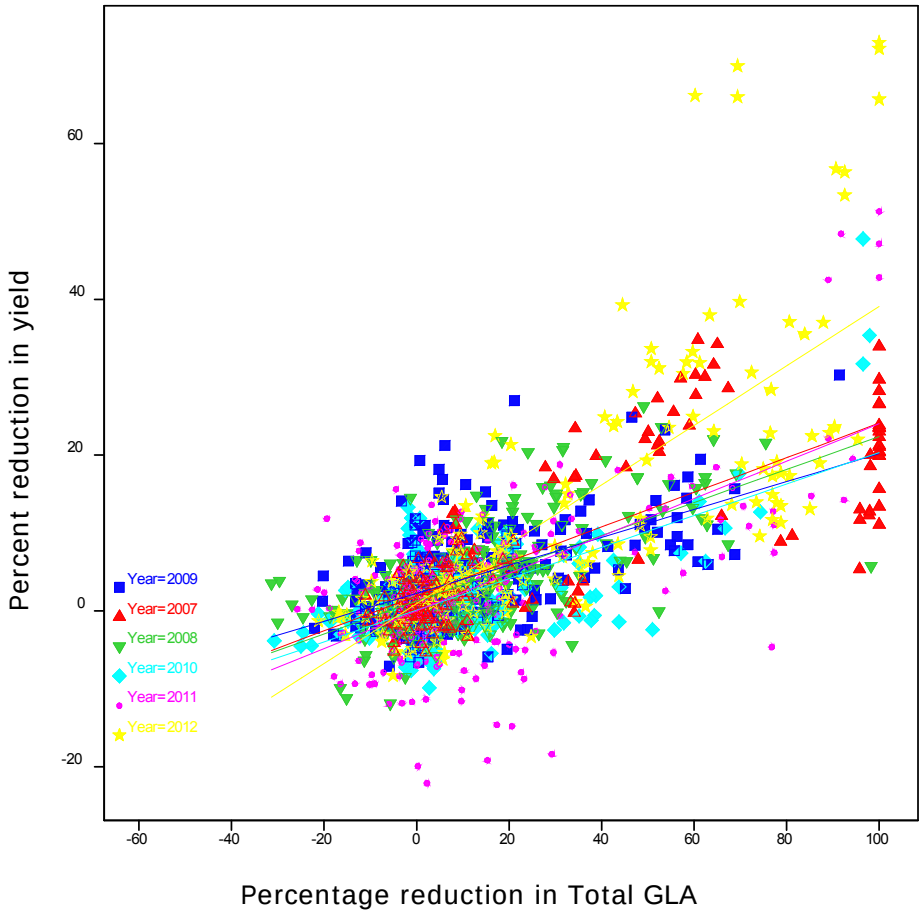

Figure 25. The relationship between the percentage reduction in green leaf area (total all leaf layers) and the percentage reduction in crop yield. Different seasons are indicated by contrasting colours. The best fit regression for each year (fitted separately) is shown as a line with the same colour.

A significant correlation was observed between GLA and crop yield for all six seasons despite an amount of within-year variability (Figure **25**; P<0.001). Further analysis revealed all the data except for the observations made in the 2011/12 season are best explained with a single relationship (Figure 25). The contrast between all the observations made from 2006/07 to 2010/11 with the 2011/12 season is important given the exceptional nature of the 2011/12 season in which a hard winter was followed by a spring drought and a wet summer.

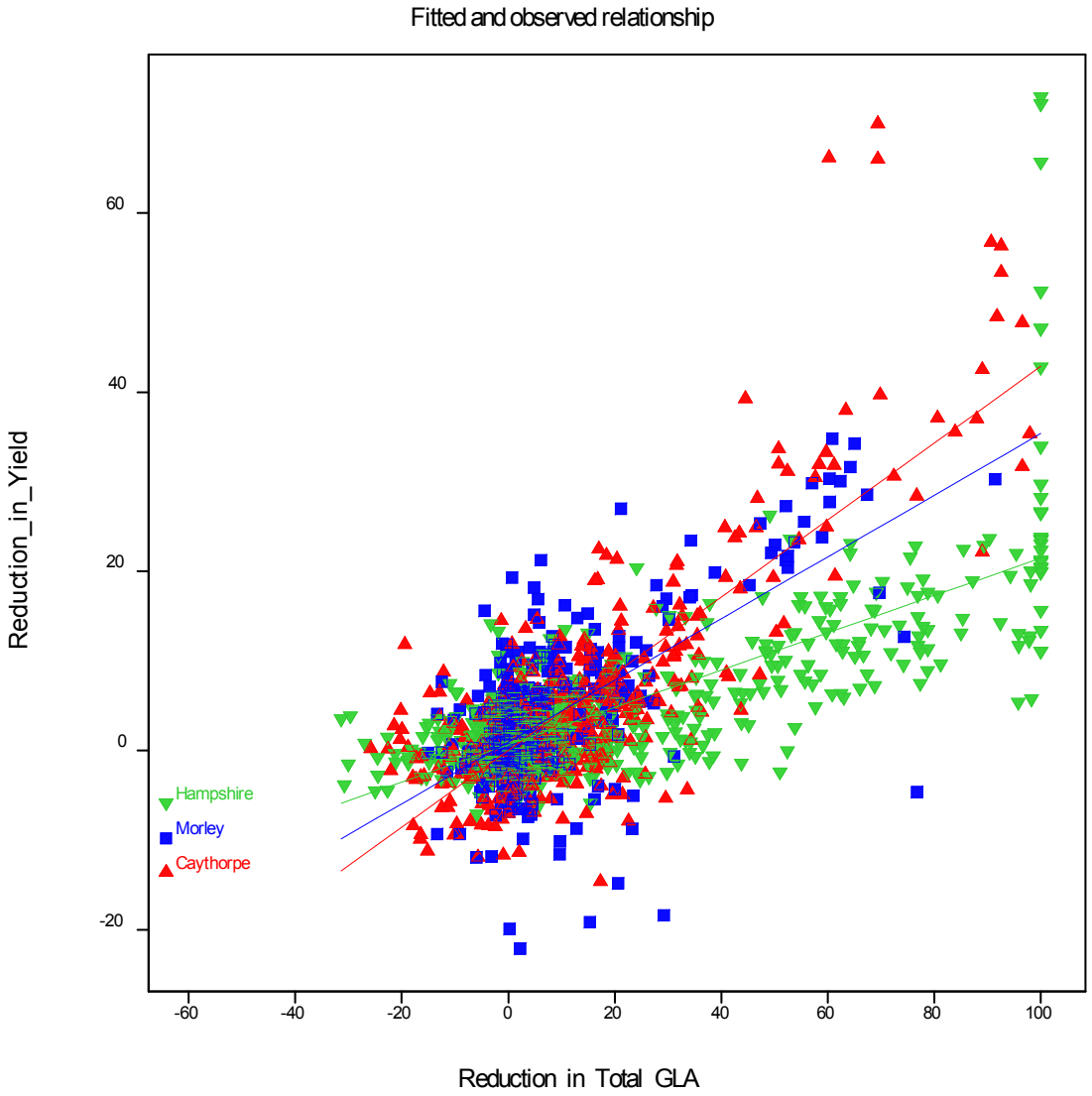

Figure 26. The relationship between the percentage reduction in green leaf area (total all leaf layers) and the percentage reduction in crop yield. Here data collected at different sites are indicated by contrasting colours. The best fit regression for each site (fitted separately) is shown as a line with the same colour.

A large effect of site on relationship between the reduction in GLA and crop yield was observed (Figure 26; P<0.05) for all three sites. The relationship observed at the Hampshire (Sutton Scotney) site was significantly different from combined data from the other two sites (P<0.05).

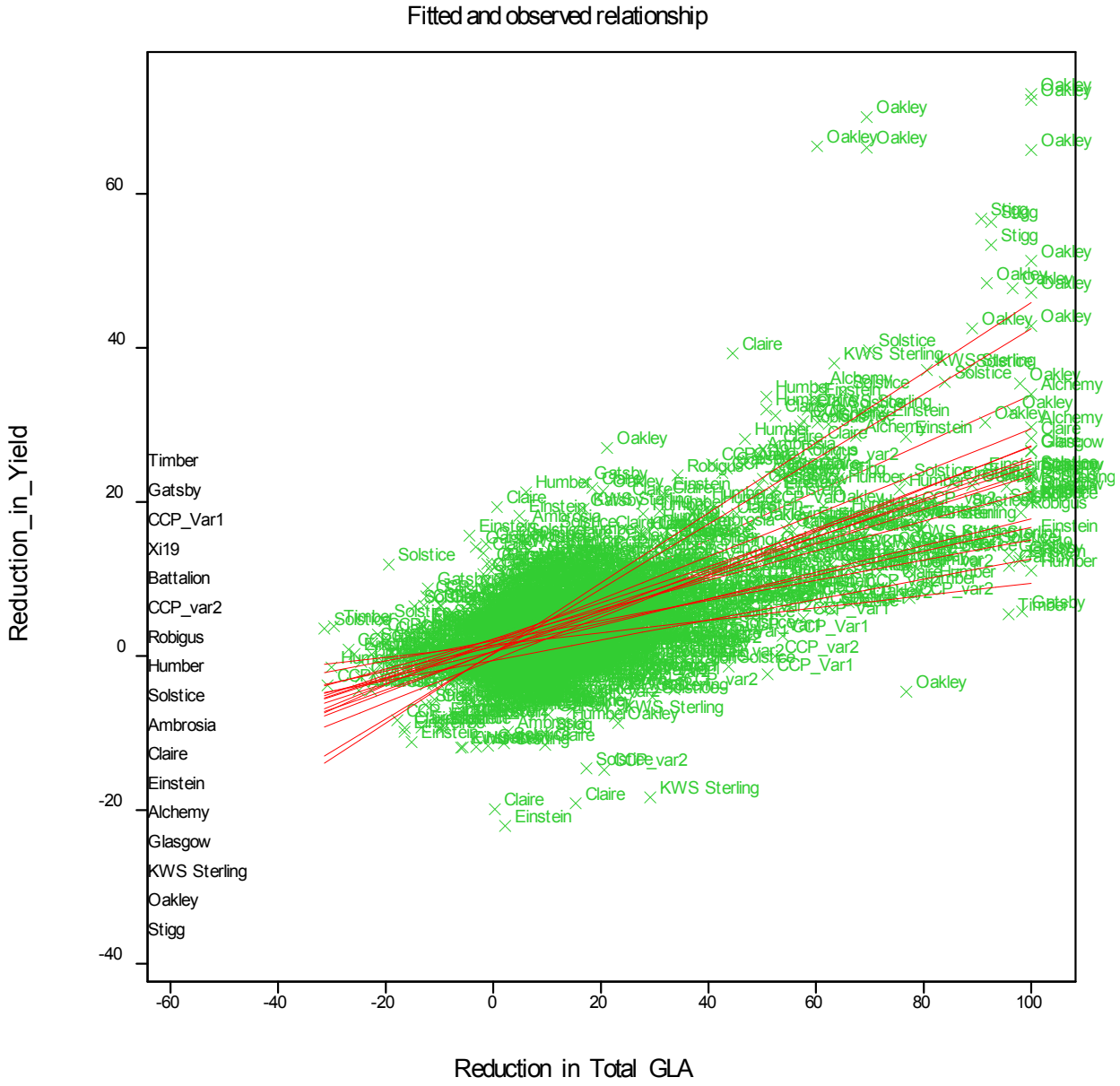

Figure 27. The relationship between the percentage reduction in green leaf area (total all leaf layers) and the percentage reduction in crop yield. Here data collected for individual varieties are compared and the best fit regression for each site (fitted separately) is shown.

The relationship between the reduction in GLA and crop yield differed between varieties (Figure 27; $P<0.05$). Further analysis indicates that the most parsimonious model used three regressions putting the varieties into three distinct groups. The first group, which displayed the greatest tolerance of the reduction in GLA associated with disease, consisted of Timber (the variety to show the greatest overall tolerance), Gatsby, Battalion, YQCCP, YQ-Mix, Xi19 and Solstice. The group which showed the lowest tolerance to reduction in GLA included Oakley and Stigg. The largest group of varieties was intermediate consisting of

Ambrose, Alchemy, Hunter, Robigus, Einstein, Glasgow, Claire and KWS Sterling. The CCP were placed in the group that proved most tolerant to reductions in green leaf area but did not show the greatest tolerance overall.

A comparison of the data from individual leaf layers supports the importance of the flag leaf and preceding leaf in determining yield. GLA reduction from the flag leaf was heavily responsible for the final crop yield supporting the findings from previous studies, such as Thorne (1965).

3.3.5 Participatory farm trials of CCP (WP5)

Pre-harvest parameters

Crop establishment, assessed as crop density in spring in each trial year was significantly lower in the YQCCP than in Claire ($P<0.05$); in relative terms, plant density was 7.6 ± 2.9 % lower than in Claire where mean density across all farms was 192 plants m^{-2}. Weaker establishment in the YQCCP did not lead to higher weed cover, though. Specifically, there was no significant difference between weed cover in Claire and the YQCCP ($P=0.71$). Also, crop establishment was not positively correlated with grain yield. In fact, there was a significant negative correlation between crop establishment and yield for Claire ($P<0.05$), whereas this relationship was not significant for the YQCCP ($P=0.19$). For the comparison between the QCCP and the OwnQ pure lines, there was no significant difference in crop establishment ($P=0.50$) or weed cover ($P=0.25$); the same was found when comparing the YCCP with the OwnY varieties ($P=0.11$ and $P= 0.81$ for crop establishment and weed cover, respectively).

Plant height, measured to the base of the ear in 10 plants per sampling area, was significantly lower in Claire than in the YQCCP ($P<0.001$, difference 16.8 ± 1.8 cm); the difference between Claire and the YQCCP tended to be greater on the organic farms (**Figure 28**), though the effect of management system on the difference between the two entries was not significant ($P=0.35$). Similar results were found for the comparison between the YCCP and the OwnY pure line varieties. For the QCCP vs. OwnQ, however, the height difference was not significant, because the OWnQ varieties in this case were dominated by a relatively old tall variety, Maris Widgeon. Over time, plant height did not increase significantly in the YQCCP, neither in absolute terms nor when determined as the absolute or relative difference between the YQCCP and Claire. This suggests that over this time period selection for tall genotypes was less important for mean plant height than other factors.

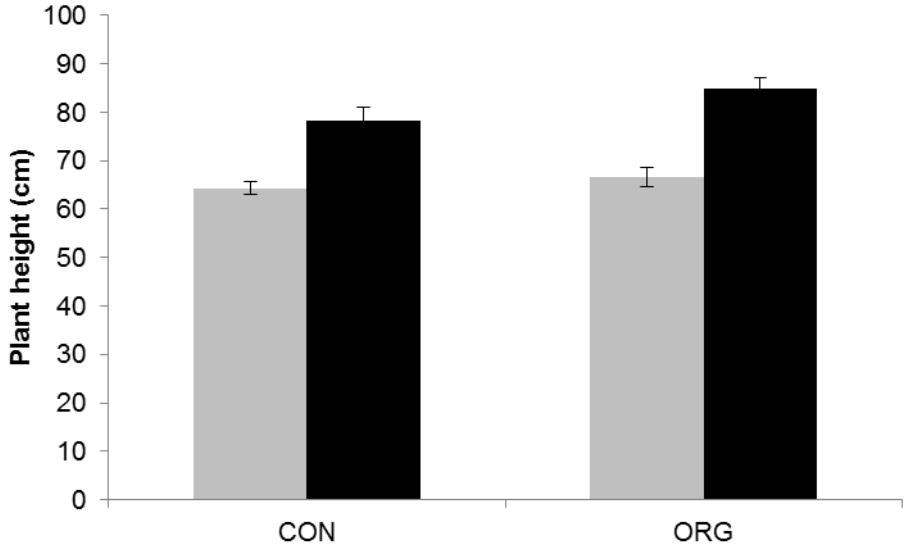

Figure 28. Plant height (cm) in Claire (grey bars) and in the YQCCP (black bars) for the conventional and the organic farms

In line with the greater plant height observed in the YQCCP than in Claire, lodging was significantly higher in the YQCCP than in Claire (**Figure 29**)**.** However, substantial lodging (>30°) only occurred in three out of 43 environments in the YQCCP; two of these cases were on conventional sites. Lodging was significantly smaller in the organic than in the conventional farms. There was a significant negative relationship between lodging and grain yield for Claire ($P<0.01$), but not for the YQCCP ($P=0.12$). Similarly to the comparison between Claire and the YQCCP, lodging was slightly but significantly higher in the QCCP than in the OwnQ varieties ($P=0.025$; mean difference 1.0°), and tended to be higher in the YCCP than in the OwnY varieties ($P=0.07$; mean difference 11.5°).

The proportion of awned ears in the YQCCP did not change significantly over the course of three years ($P= 0.84$).

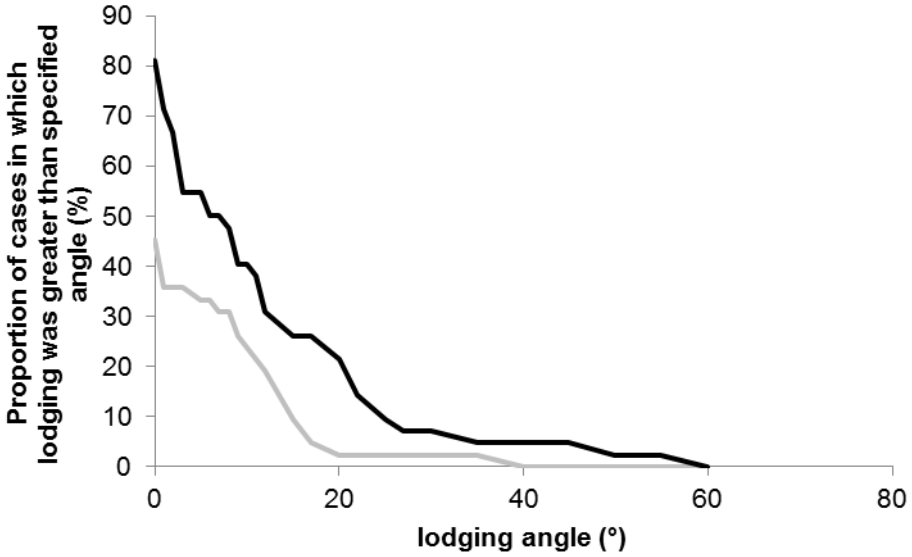

Figure 29. Lodging, measured as degrees deviation from upright position in Claire (grey line) and the YQCCP (black line)

Mean yield

Claire vs. YQCCP

In the comparison between the pure line variety Claire (nabim group 3) and the genetically diverse YQCCP in the on-farm trials, Claire showed a significantly ($p<0.001$) higher grain yield. On average across 53 environments, grain yield in Claire was 6.6 t ha^{-1} and 6.0 t ha^{-1} in the YQCCP. The yield difference of 0.65±0.16 t ha^{-1} (mean ± standard error) was equivalent to a yield reduction of 9.85%. However, in 11 out of 53 environments the YQCCP out-yielded the control variety Claire.

It was therefore investigated whether there were any factors that could further explain the yield difference between Claire and the YQCCP. In particular, it was tested whether the yield difference between the two trial entries was affected by the absolute yield level observed in each environment. When the yield difference between Claire and YQCCP was expressed in absolute terms, i.e. in t ha^{-1}, there was indeed a significant effect. In particular, the greater the absolute yield level measured in Claire the higher was the yield difference (**Figure 30**; $P<0.001$, df=48). In other words, in comparison with Claire, the yield disadvantage of the YQCCP only became evident at high yield levels. However, when the yield difference was expressed in relative terms (in %), the relationship between absolute yield level and this relative difference was not significant ($P=0.10$).

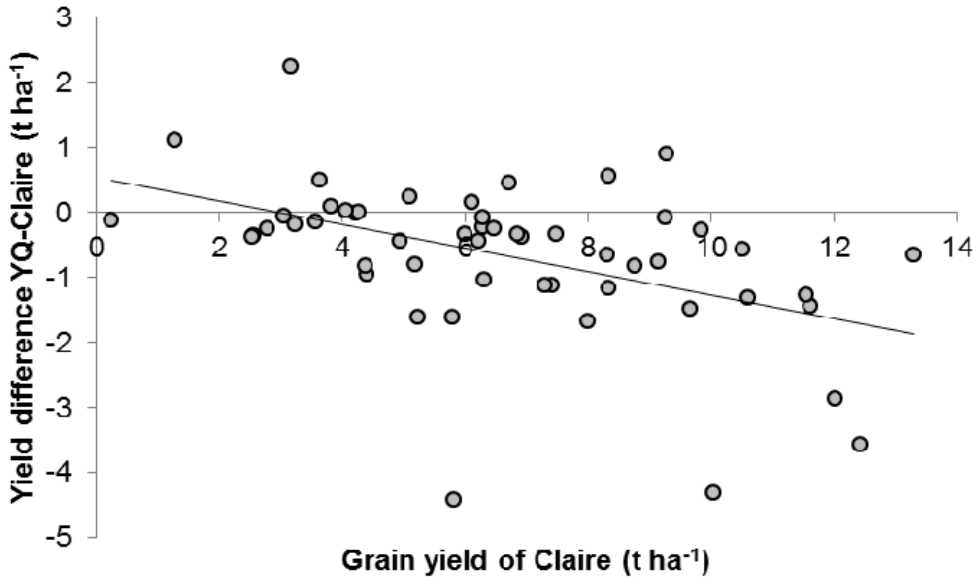

Figure 30. Grain yield difference between YQCCP and Claire, plotted against grain yield in Claire

YCCP and QCCP vs. Own variety

In addition to the comparison between the YQCCP and Claire, two further pairs were available for comparing CCP and pure lines in the on-farm trials. Specifically, the baking quality population (QCCP) was compared to those of the farmers' own varieties that possessed high baking qualities (nabim group 1, here labelled as "OwnQ"); and the yield population (YCCP) was compared to farmers' own varieties categorised as high-yield varieties (nabim group 3 and 4, here labelled as "OwnY"). According to the statistical analysis, grain yield was not significantly different between the QCCP and the OwnQ, whereas the OwnY pure lines significantly out-yielded the YCCP (**Figure 31**). The yield differences (means ± standard error) were -0.11 ± 0.27 t ha^{-1} for the high quality group (Q) and -1.33 ± 0.37 t ha^{-1} for the high yield group (Y). In relative terms, these differences amounted to -1.8% in the QCCP and -17.4% in the YCCP against the respective own varieties. In 4 out of 8 environments, the QCCP out-yielded the OwnQ whereas the YCCP out-yielded the OwnY only in 2 out of 14 environments.

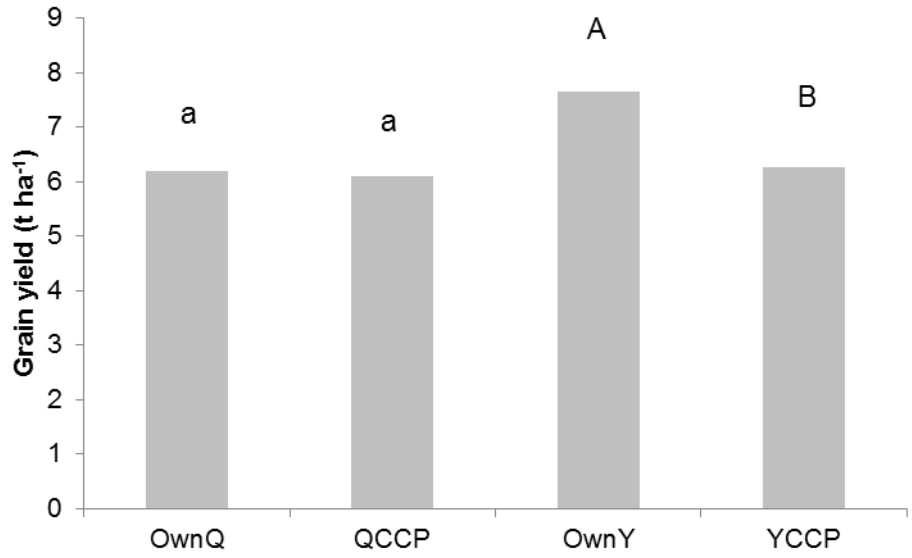

Figure 31. Grain yield in pure lines (farmers' own choice) and CCP. Y and Q designate the high yield and high baking quality entries, respectively. Statistical comparisons made between OwnQ and QCCP are indicated by lower case letters and between OwnY and YCCP with upper case letters; bars labelled with different letters of the same case indicate a significant difference in grain yield.

In all cases (i.e. both for the Q and for the Y comparison) this difference was statistically independent of the absolute yield level; this was the case both for absolute and relative differences. However, when both groups were considered together, the absolute yield difference between CCP and pure lines showed a trend (P=0.0794) of increasing yield difference with increasing absolute yield level of the farmers' own varieties. On the other hand, relative yield difference did not show any significant relationship with absolute yield levels (P=0.34).

Yield stability

Claire vs. YQCCP

Yield variability across all 53 environments, for which data from both Claire and YQCCP were available, was determined using the coefficient of variation. This measure was higher in Claire (45.7%) than in the YQCCP (45.0%), but the difference was not significant (z=0.09).

Temporal yield stability within farms was determined for 10 farms for which data from at least three years were available for both Claire and the YQCCP. According to the analysis of the Power Law Residuals (POLAR), there was a small and non-significant trend (P=0.306) of the YQCCP showing smaller residuals (i.e. greater stability over time) than Claire (**Figure 32)**. Similarly, yield CVs over years within farms

were only marginally smaller in the YQCCP (23.2%) than in Claire (25.4%), but again this difference was not statistically significant (difference -2.22 ± 1.78%, P=0.244).

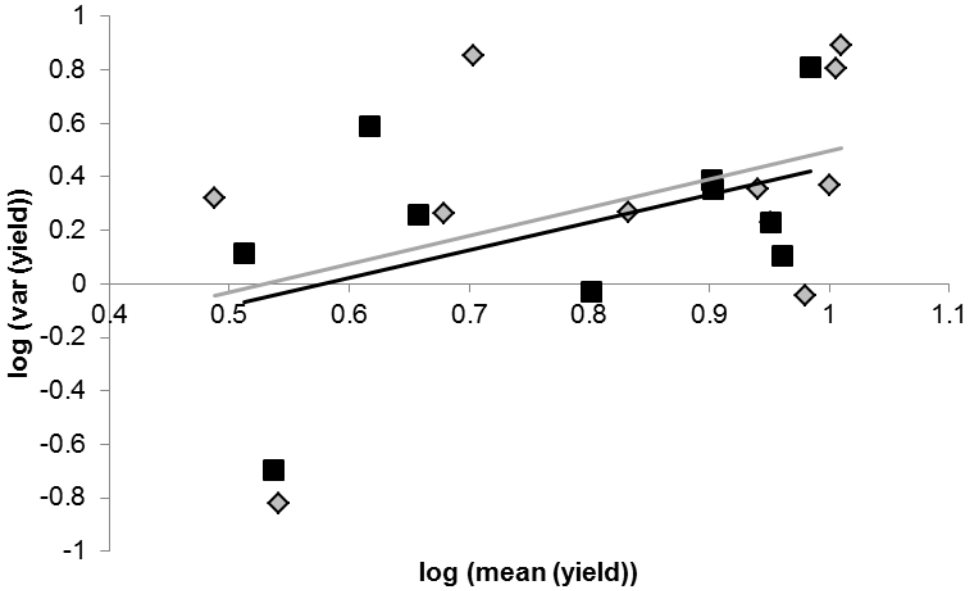

Figure 32. Relationship between log (mean) and log (variance) of YQCCP (black squares) and Claire (grey diamonds), according to Taylor's Power Law; variance and means of the grain yields are calculated across years within farms, so that each point in the graph represents one location x genotype combination.

As a further measure of stability, the slope *b* of the regression of genotype yields against average yields in each environment was determined. This slope was above 1 for Claire (*b*=1.062 ± 0.028) and (symmetrically, i.e. by the same amount) below 1 for the YQCCP (*b*=0.938 ± 0.028), and the difference between the two slopes was statistically significant (P<0.01). However, the deviation of the slope of the regression from *b*=1 is not usable as a measure of stability, because it is (necessarily) identical in the two trial entries.

The crossing of the two regression lines was determined at x=1.03 t ha^{-1}. For both YQCCP and Claire, the variance of the deviations from the regression line was identical (s^2d_i = 0.344). When one catastrophic outlier was excluded from the analysis (yield of both Claire and YQCCP below 0.25 t ha^{-1}), results changed only slightly, with b_{Claire}=1.064, b_{YQCCP}=0.936 and $x_{crossing}$=1.22 t ha^{-1}. One interpretation of the slope being greater in Claire than in the YQCCP is that yield of Claire responded better to high-yield environments than the YQCCP, a result already shown in **Figure 30**.

To determine the variability of grain yield within each environment, CVs were calculated over the four sampling quadrats. According to this analysis, Claire showed a smaller CV (15.1%) than the YQCCP

(18.3%). The difference between the two CVs (3.25 ± 1.92%) was statistically not significant (p=0.097, t-test). In 23 out of 53 environments, CV of the YQCCP was smaller than the CV of Claire; this proportion was not significantly different from random, i.e. 50% (P=0.41, binomial test). Similar results as for the CV were obtained for the Power Law Residuals, where the difference between YQCCP and Claire (0.076 ± 0.088) was not significant (P=0.39), and the number of environments for which POLAR were smaller for YQCCP than for Claire was 24 out of 53 (P=0.58).

YCCP and QCCP vs. Own variety

In comparison to the YQCCP, only few farms had chosen to grow the QCCP or the YCCP. Therefore, it was not possible to calculate temporal yield stability for the comparison between these CCP and the farmers' own varieties. CVs over all environments tended to be smaller in the QCCP (31.5%) than in the OwnQ variety (35.2%), whereas the opposite was the case for the high yield entries (50.1% for the YCCP and 42.2% for OwnY). However none of these differences were significant.

Yield stability within environments showed a similar pattern, with the CV of the QCCP (11.5%) being smaller than the OwnQ (16.0%) but the reverse being true for the comparison of the YCCP (16.5%) and the OwnY (13.8%).

Grain quality: Mean protein and protein yield, TGW and HFN

Claire vs. YQCCP

In 28 out of 30 environments, protein content was higher in the YQCCP than in Claire. On average, the grain protein content in the YQCCP was 1 %-point higher than in Claire; this difference was statistically significant (**Table 45**). In contrast, the number of environments in which protein yield was higher in YQCCP was 14, i.e. about half of the environments, and accordingly there was no statistically significant difference between the YQCCP and Claire for protein yield. The YQCCP showed a significantly lower HFN than Claire. However, the most parsimonious model for this data, with the management system as a further factor showed no significance of the difference between YQCCP and Claire (not shown). Thousand grain weight (TGW) was significantly higher in the YQCCP than in Claire.

With regards to grain yield, it was tested whether the difference between the two trial entries was dependent on absolute levels found in each environment. Neither for protein content nor for protein yield this was the case (P=0.160, df=27 for protein content; P=0.377 for protein yield, df=27), i.e. the difference in grain protein content between Claire and YQCCP was independent of the absolute level of protein.

Table 45. Grain protein content (in %), protein yield (t ha^{-1}), Hagberg Falling Number (s) and TGW (g) for Claire and YQCCP from on-farm trials.

	Grain protein (%)	Protein yield (t ha^{-1})	HFN (s)	TGW (g)
Claire	10.60	0.737	277	46.6
YQCCP	11.59	0.723	221	49.4
Difference	0.99	-0.014	-56	2.6
SE (diff)	0.12	0.030	15	0.7
P-value	<0.001	0.637 ns	<0.01	<0.001

YCCP and QCCP vs. Own variety

When comparing the protein content of the QCCP and the farmers' own choice high baking quality varieties ('OwnQ), the difference between the two (0.34 ± 0.27 %) was not significant (P=0.26) (**Figure 33**). However, in the high yield group, the YCCP was significantly different from the OwnY with regard to its protein content (difference 0.64 ± 0.26, P<0.05); in 8 out of 9 environments the protein content of the YCCP was higher than in the OwnY. In contrast, no significant differences between the QCCP and the OwnQ or between the YCCP and the OwnY were found for protein yield (**Figure 34**).

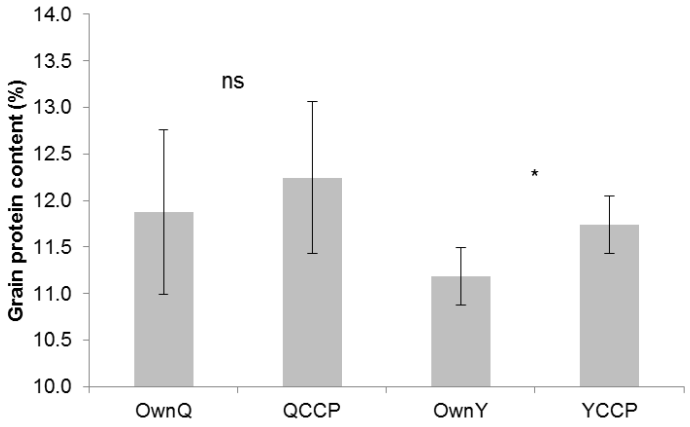

Figure 33. Grain protein content (%) in pure lines (farmers' own choice varieties) and CCP. Y and Q designate the high yield and high baking quality entries, respectively. The asterisk indicates a significant difference between OwnY and YCCP at P<0.05.

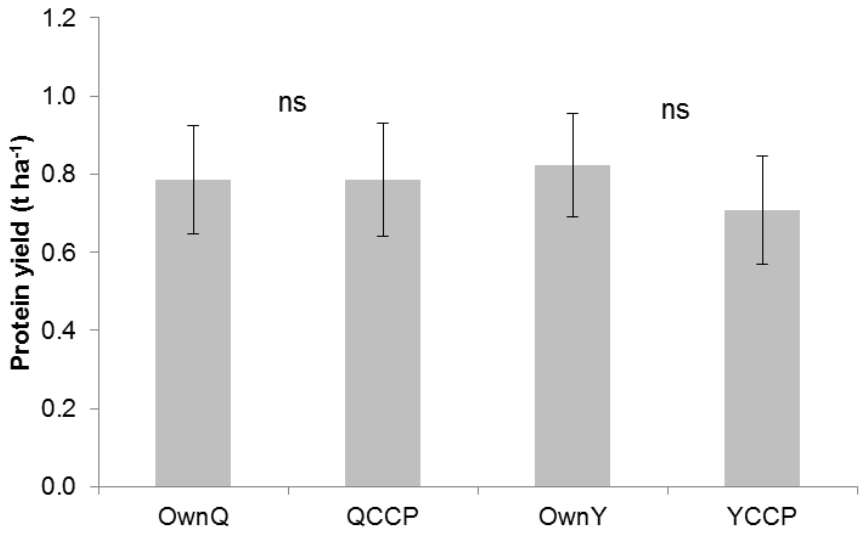

Figure 34. Protein yield (t ha^{-1}) in pure lines (farmers' own choice varieties) and CCP. Y and Q designate the high yield and high baking quality entries, respectively

Stability of grain protein and protein yield

Temporal variability of grain protein content, as measured by the coefficient of variation across years within farms tended to be slightly smaller in the YQCCP (7.3%) than in Claire (7.4%), but this difference (0.13 ± 1.05%) was not significant (P=0.898). For protein yield, temporal yield stability was also not significantly different between the two trial entries (P=0.221), with CVs being 20.5% for YQCCP and 17.6% for Claire. However, the number in which the CV of protein yield was smaller in Claire than in the YQCCP was 10 out of 12 environments, a proportion that was significantly different from random (P=0.04). Similar results were obtained when stability was measured with Power Law residuals (POLAR). The direction of the POLAR differences between YQCCP and Claire were the same as obtained from the coefficient of variation and were neither significant for protein content (P=0.299) nor for protein yield (P=0.189).

Regression-based stability showed significant differences between the YQCCP and Claire for protein content, with the regression slope *b* being significantly (P<0.001) smaller in Claire (*b*=0.861 ± 0.044) than in the YQCCP (*b*=1.14± 0.044). For protein yield, regression slopes were not significantly different from 1 for both the YQCCP and Claire (P=0.465).

Relationship between grain yield and protein content

There was a weak but significant positive correlation between grain yield and protein content (R^2 = 0.10, P<0.001), when a simple linear model was used that related the two parameters across all environments; this is thought to reflect the general response of both parameters to nitrogen availability and other growth-

promoting factors varying across the tested environments. However, more complex mixed models, using farm as a random factor revealed that the relationship between grain yield and protein content is in fact generally negative, i.e. higher yield levels tend to be associated with lower protein levels. Further analyses showed interactions between System (Organic vs. Conventional) and the relationship between grain yield and grain protein, such that the slope between the two parameters was less negative on the Organic sites.

The inverse relationship between grain yield and protein content was also highlighted when a subset of data was analysed from all environments where all three CCP, Claire and an own pure line variety had been grown (**Figure 35**). The analysis also reiterated the high yield potential of Claire, and the high protein content in the QCCP.

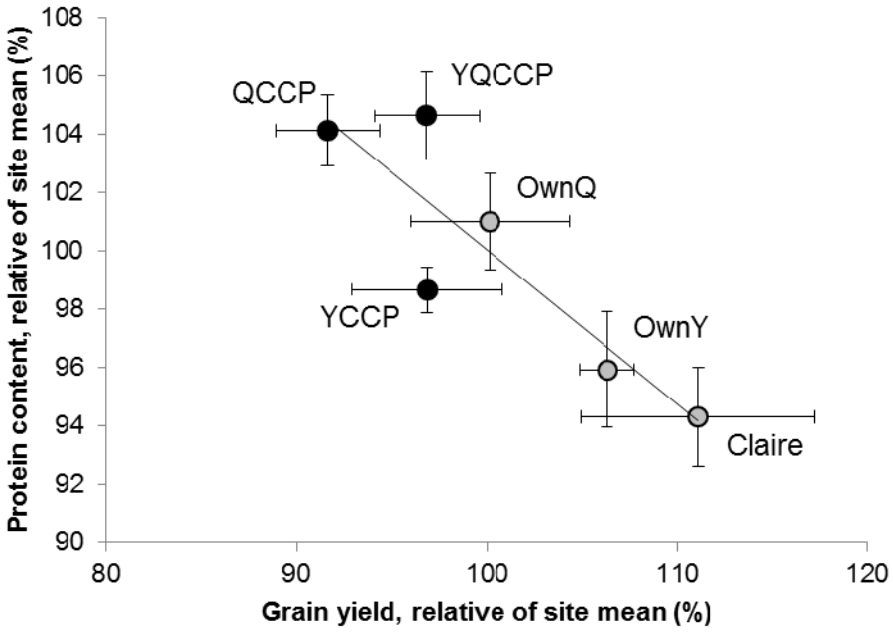

Figure 35. Relationship between grain yield (relative, %) and grain protein content (relative, %) for 9 environments in which all three CCP, Claire an own pure line variety had been grown. The data points and error bars show averages and standard errors. Black circles: CCP, grey circles: pure lines. The regression lines follows the equations y= ax+b, with a= 153.0±14.1, b= -0.53±0.14, Adj. R^2 = 0.728, P<0.05 and df=4.

Further, the YQCCP showed a remarkable position, as it had the same level of relative yield as the YCCP and the same level of protein as the QCCP; this finding suggests that there is a potential of the YQCCP to break the negative relationship between yield and protein. Indeed, the point representing the YQCCP is positioned above the regression line between yield and protein. However, this deviation was not

statistically significant; specifically, the relative protein content observed in the YQCCP was not significantly different from the expected value based on the regression (t-test, P=0.227).

Adaptation trial

The hypothesis for this trial was that in the case of local adaptation, at each site grain yield would be higher in the 'home' CCP than in the CCP that had been grown elsewhere in the previous years (the 'away' CCP). Indeed, this was the case in four out of six sites (**Figure 36**). Intriguingly however, at its own site the population that came from CON-North also performed worse than the away CCP (from the Southern organic site). This indicates that adaptation to local conditions did not consistently take place for the YQCCP over the course of the three previous generations.

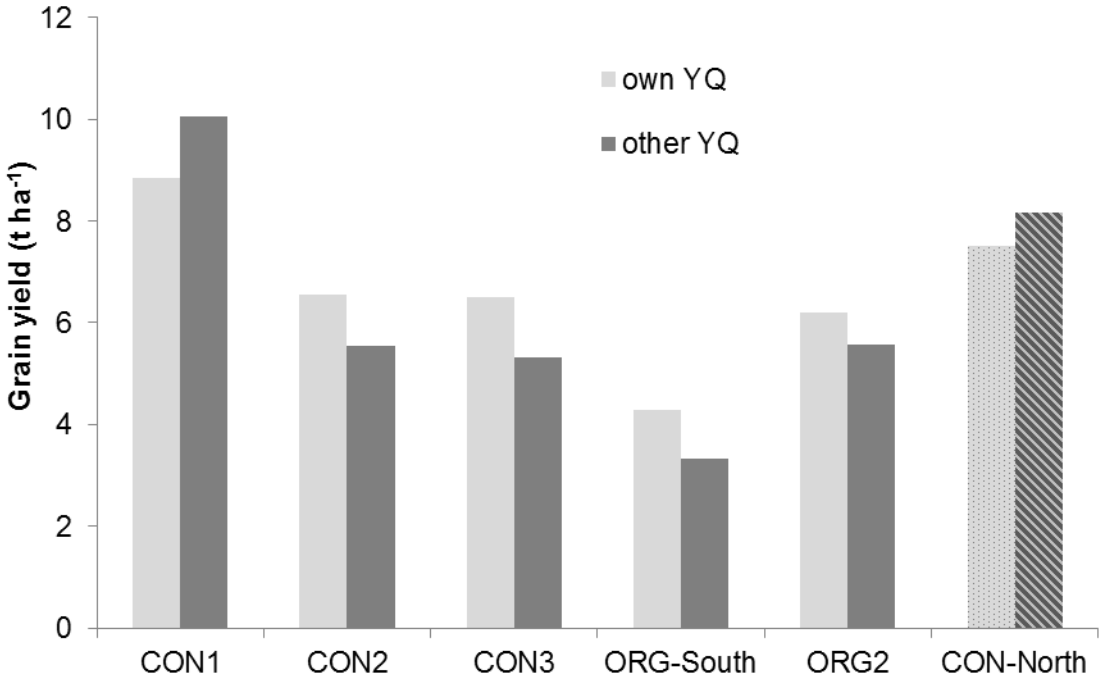

Figure 36. Grain yield of YQCCP with different growing histories; CON= conventional; ORG=organic management. Light grey bars show the population that had been grown at the respective sites for 4 consecutive years, whereas dark grey bars show populations coming from different sites. In particular, for all sites except CON-North, the seed from "other YQCCP" came from the CON-North site. For the CON-North site, CCP seed came from the ORG-South site. No error bars are shown because trial entries were not replicated in the field.

Summary

The main results are summarised below, showing trends and significant effects mean performance and stability (Table 46).

E

Table 46. Summary of results for the comparison between CCP and pure lines in the WP 5 on-farm trials. Brackets indicate non-significance of the difference; -: CCP < Pure line; +: CCP > Pure line. NA: Not available; ND: not determined.

			Stability		
Comparison/parameter		Mean	Temporal	Regression	Within field
Establishment	YQCCP – Claire	–	ND		
	YCCP – OwnY	(–)			
	QCCP – OwnQ	(+)			
Plant height	YQCCP – Claire	+			
	YCCP – OwnY	+			
	QCCP – OwnQ	(–)			
Yield	YQCCP – Claire	–	(+)	+	(–)
	YCCP – OwnY	–	NA		(–)
	QCCP – OwnQ	(–)	NA		(+)
Protein	YQCCP – Claire	+	(+)	–	(–)
	YCCP – OwnY	+	NA		
	QCCP – OwnQ	(+)	NA		
Protein yield	YQCCP – Claire	(–)	(–)	(–)	(+)
	YCCP – OwnY	(–)	NA		
	QCCP – OwnQ	(–)	NA		

3.3.6 Performance of CCP with additional parent material (WP6)

Grain yield and protein content differed significantly between the four sites (P<0.001) (Figure 37).

Although there were significant differences observed between trial entries, this was only between non-relevant comparisons. Crossing Xi19 and/or Pegassos into the populations (Y, Q, YQ) as additional parents provided no significant increase in grain yield or protein percentage dry matter (p>0.05) (Figure 38 and Figure 39).

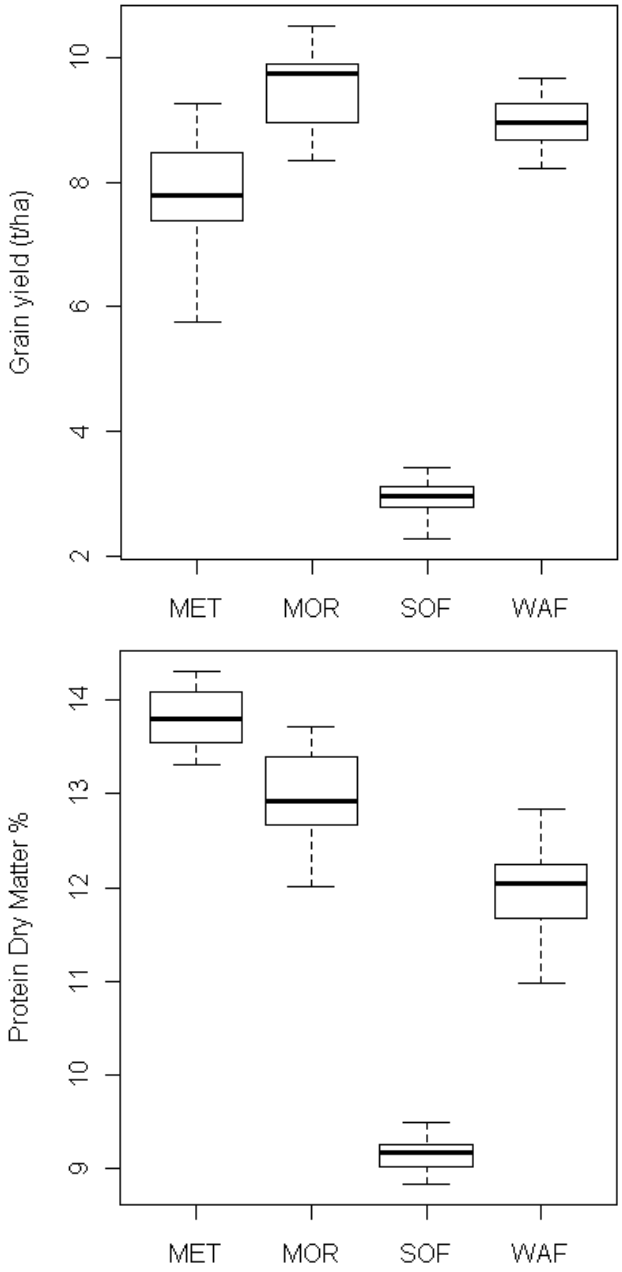

Figure 37. Average grain yields and protein content between the four sites. Box plots show mean, upper and lower interquartile range and range. MET= Metfield, MOR = Morley, SOF= Sheepdrove and WAF= Wakelyns. N=2 4/site.

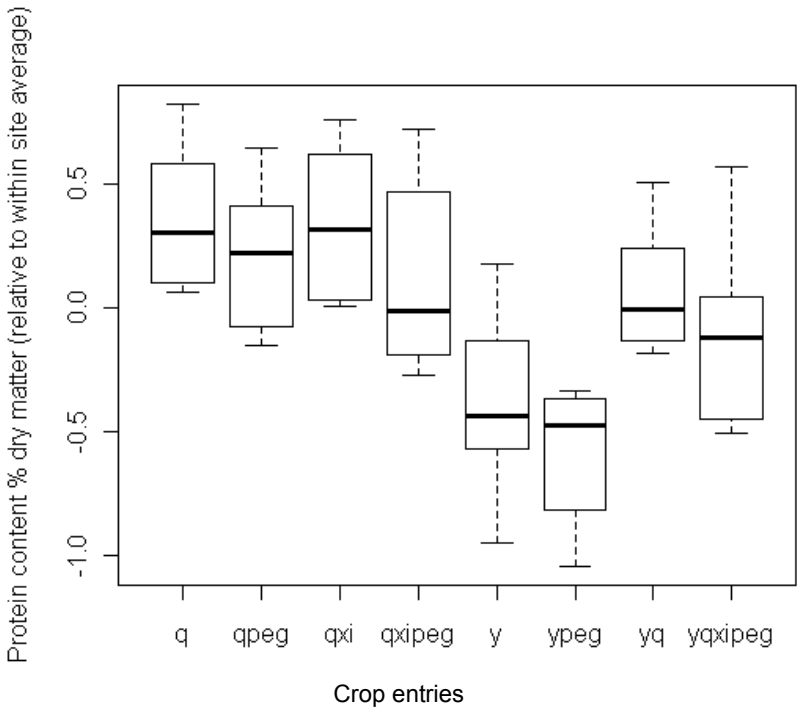

Figure 38. Relative differences in percentage dry matter grain protein content (relative protein content = plot protein content – average per site) between crop entries. Entries consisted of a quality composite cross population (q) and a yield composite cross population (y) into which the varieties Xi19 (xi) and/or Pegassos (peg) were crossed. Box plots show mean, upper and lower interquartile range and range. Values are adjusted relative to within site averages to account for a large site effect. N=3/entry/site.

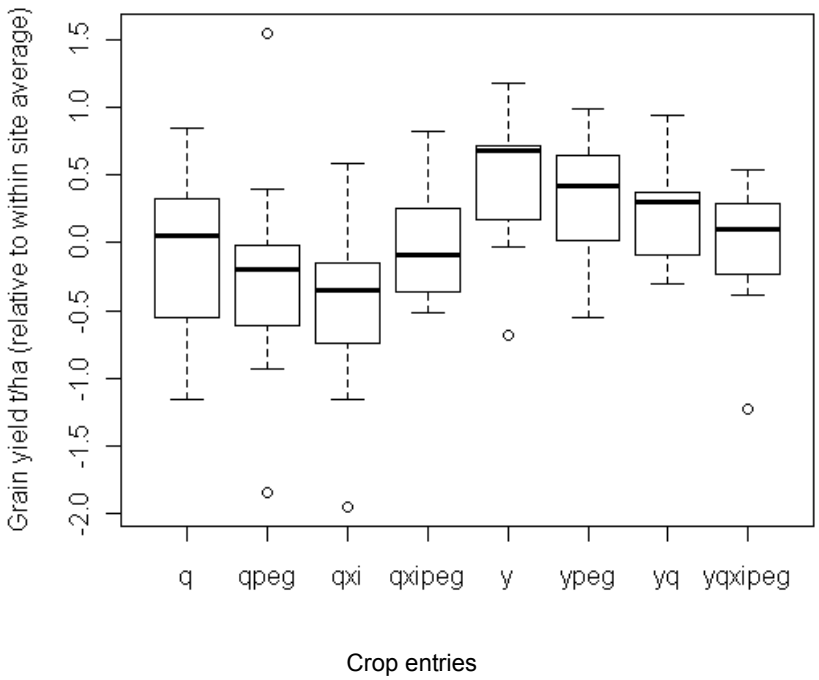

Figure 39. Relative differences in grain yield (relative yield = plot yield – site average plot yield) between crop entries. Entries consisted of a quality composite cross population (q) and a yield composite cross population (y) into which the varieties Xi19 (xi) and/or Pegassos (peg) were crossed. Values are adjusted relative to within site averages to account for a large site effect. Box plots show mean, upper and lower interquartile range and range. . N=3/entry/site.

3.3.7 The effects of mass selection on CCP (WP7)

Effect of grain colour selection

At harvest in 2008 proteinDB in the QCCP prior to mass selection was 10.37% and 13.4% at WAF and MET, respectively, and in YQCCP was 9.55% and 12.76% at WAF and MET, respectively. After the first round of mass selection (see "S1" in Figure 40) based on grain colour, proteinDB in the dark fraction of grain was consistently greater than in the light fraction. Mean proteinDB in the Dark-CCP was 0.16% greater than Light-CCP at WAF and 0.53% greater at MET. Compared to proteinDB in the CCP prior to any mass selection, Dark-CCP was on average 0.19% greater at WAF, and 0.3% greater at MET (Figure 40).

At harvest in 2009 (see "H1" in Figure 40) proteinDB in the progeny of the Dark-CCP was consistently greater than that in the progeny of the Light-CCP. Mean proteinDB in Dark-CCP was 0.11% greater than Light-CCP at MET, and 0.54% greater at WAF, the difference neared significance (P = 0.064; Figure 40) although trial plots were unreplicated in this year.

After harvest in 2009, the Dark- and Light-CCP were each subjected to a second round of mass selection (see "S2" in Figure 40) based on kernel colour. Selecting the darkest fraction of grain increased proteinDB by 0.14% in the Dark-CCP at MET, and by 0.85% at WAF; while selecting the lightest fraction of grain decreased proteinDB in the Light-CCP by an average 0.77% at MET and 0.82% at WAF. At sowing in October 2009, proteinDB in the Dark-CCP was thus 1.01% greater than Light-CCP at MET, and 2.21% greater than Light-CCP at WAF (see "S2" in Figure 40).

At harvest in 2010 ("H2" in Figure 40) proteinDB in the progeny from the second round of mass selection was significantly greater in Dark-CCP when compared to Light-CCP (P = 0.03); however, in a three-way ANOVA which included proteinDB of the unselected control CCP (control mean at MET 14.33% ± 0.16 s.e., control mean at WAF 12.15% ± 0.14 s.e.), neither the Dark- nor Light-CCP differed from the control (P = 0.17).

After harvest in 2010 Dark- and Light-CCP were subjected to a third round of mass selection based on kernel colour (see "S3" in Figure 40). Selecting the darkest fraction of grain increased proteinDB by 0.41% in the Dark-CCP at MET and by 0.27% in the Dark-CCP at WAF. Selecting the lightest fraction reduced protein in the Light-CCP by 0.64% at MET and by 0.48% at WAF. Thus after the third round of mass selection Dark-CCP were on average 1.28% greater than Light-CCP at MET, and 0.84% greater than Light-CCP at WAF.

At harvest in 2011 ("H3"), proteinDB in Dark-CCP was on average 0.14% greater than Light-CCP, but the difference was not significant (P = 0.098; Figure 40). However, again in a three-way ANOVA including proteinDB of the unselected control CCP (control mean at MET 15.04% ± 0.11 s.e., control mean at WAF 10.96 ± 0.05 s.e.), neither the Dark- nor Light-CCP were different to the unselected controls (P = 0.177, Figure 40).

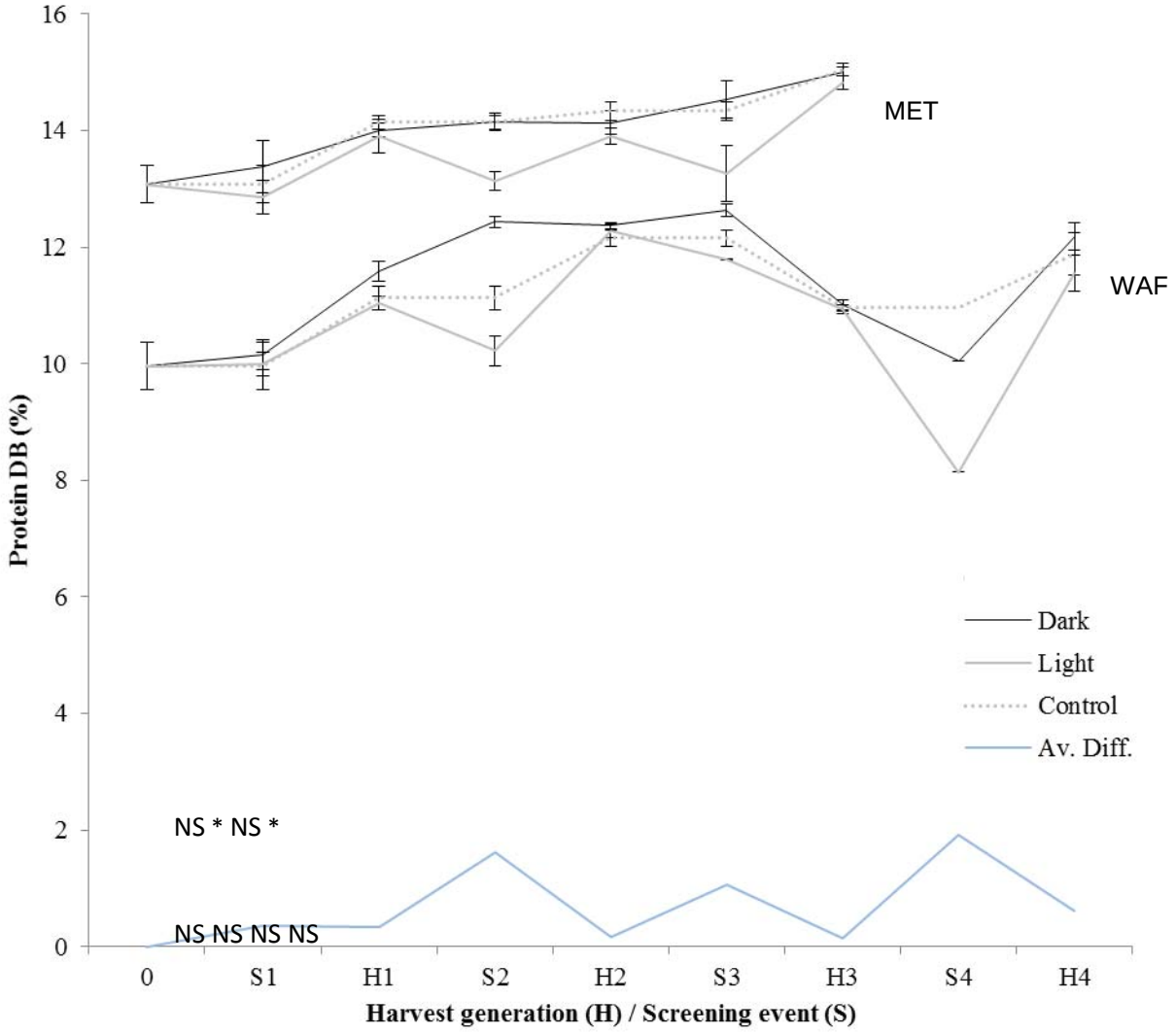

Figure 40. Mean protein (% dry basis) of two CCP of winter wheat grown at two sites. CCP had been grown at their respective sites for three years prior to generation "0". Generation "S1" shows proteinDB content in the Dark-, Light- and unselected fraction of grain immediately after the CCP were subject to mass selection based on kernel colour. Generation "H1" shows proteinDB content in grain at the subsequent harvest of the progeny from the previous mass selection, i.e. differences retained from "S" to "H" indicate grain colour and associated protein content were genetically controlled and heritable in the population. "Av. Diff." shows the difference between Light- and Dark-CCP at each generation averaged across both CCP and both sites. Error bars show standard error. ANOVA: *, P = <0.05; NS, P = >0.05. Significance values above the Av. Diff. line show results from two-way ANOVA comparing Dark-CCP with Light-CCP; significance values below the Av. Diff. line show results for three-way ANOVA comparing Dark-, Light-, and unselected Control-CCP.

After harvest in 2011, a fourth round of colour selection was performed ("S4"), but only on QCCP from WAF. Selecting the darkest fraction of grain reduced proteinDB in the Dark-QCCP by 1.06%, while selecting the lightest fraction reduced proteinDB by 2.81% in the light-QCCP (Figure 40). The Dark-QCCP for sowing was thus 0.58% greater than the Light-QCCP for sowing in October 2011.

At harvest in 2012 ("H4") proteinDB in the Dark-QCCP was 0.62% greater than proteinDB in the Light-QCCP at WAF (P = 0.045, Figure 40); but again neither the Dark- nor Light-CCP was different to the unselected control (P = 0.208).

Combining post-selection data from all years (i.e. those from "S" generations in Figure 40) in a linear model treating year as a fixed effect in order to investigate whether there was a difference in the effectiveness, in terms of selecting for the protein trait associated with kernel colour, of mass selection across years revealed a significant effect of site (P = 0.000***), colour (dark vs. light P = 0.000***), year (P = 0.000***), and also an interaction between Colour and Screening event (P = 0.053), where the difference in proteinDB between Light- and Dark-CCP immediately after use of the colour sorter varied over the four years, with colour selection being more effective in terms of a difference in proteinDB between Light- and Dark-CCP at the second and fourth screening events (see "Av. Diff." in Figure 40).

Effect of grain size selection

In 2009 TGW (g) after the first screening event for selection based on grain size was significantly different between size fractions and the unselected control CCP (P = 0.0003). Post hoc Tukey HSD testing revealed TGW of the largest size fraction was significantly greater than the small, medium and unselected control CCP (P = 0.004, P = 0.032, P = 0.0002, respectively; Figure 41), and the medium fraction was also larger than the unselected control (P = 0.009). There was no difference in TGW between the medium and small fractions (P = 0.49; Figure 41).

At harvest in 2010 there was a significant difference in TGW between the progeny of the three size selected fractions and the unselected control (P = 0.0077; Figure 41). Tukey HSD testing revealed TGW in the progeny of the large size fraction was significantly greater than in the progeny of the small and the medium size fractions (P = 0.007, P = 0.01, respectively), but there was no difference in TGW between the unselected control CCP and the progeny of either the large, medium or small size fractions (P = >0.1). There was also no difference in grain yield between any of the size fractions and the unselected control (P = >0.1).

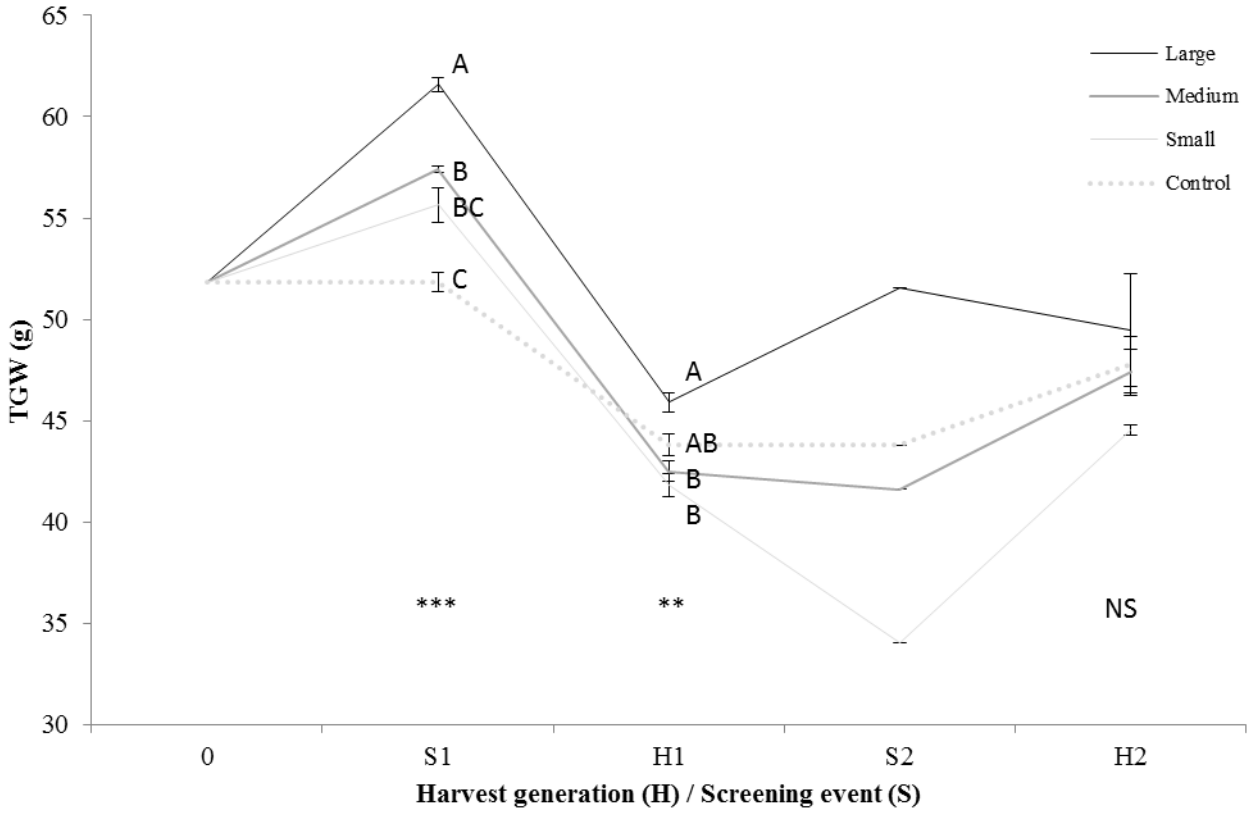

Figure 41. Thousand Grain Weight (TGW) in grams of QCCP before and after mass selection based on grain size using a seed dresser, and grown at MET. All size CCP originate from the same QCCP (Generation "0") that had been grown at MET for three years previously. Generation "S1" shows TGW in the three size fractions and control immediately after the first mass selection event. Generation "H1" indicates TGW in the progeny of those size selections when harvested the following year. "S2", shows TGW of the largest fraction from the Large-CCP, medium fraction of the Medium-CCP, and smallest fraction of the Small-CCP; these were then also sown and harvested the following year ("H2"). Error bars show standard error. ANOVA: *** P = 0, ** P = <0.01, * P = <0.05; P = <0.1; ns P = >0.05. Where effects were significant, treatments with different letters indicate significance at P<0.05.

After harvest in 2010, the three size fractions were subjected to a second round of mass selection based on grain size. The largest fraction of the Large-CCP had a TGW of 51.58, the medium fraction of the Medium-CCP had a TGW of 41.62, and the smallest fraction of the Small-CCP had a TGW of 34.04 (see "S2" in Figure 41).

At harvest in 2011 TGW in the progeny of the largest fraction of the Large-CCP was consistently greater than that in the Medium-, Small- and Control-CCP, but the difference was not significant (P = >0.1), or for the comparison between the Large-CCP and Small-CCP (P = 0.091), with TGW in the Large-CCP greater than the Small-CCP by an average of 4.93g (large mean 49.47 ± 2.80 s.e.; small mean 44.54 ± 0.24 s.e.). There was no difference in grain yield between any of the size selections in 2011 (P = >0.1).

Combining data from both harvest years revealed a significant main effect of size selection on TGW (P = 0.009) and no interaction with year (P>0.1); Tukey HSD revealed TGW of the Large-CCP was significantly greater than the Small-CCP (P = 0.005) and all other comparisons were non-significant (P = > 0.1). There was also a significant main effect of sowing year on both the TGW and grain yield, (TGW 2010 mean 43.44 ± 0.49 s.e., 2011 mean 47.30 ± 0.89 s.e. P = 0.0001; Yield 2010 mean 8.22 ± 0.17 s.e., 2011 mean 4.86 ± 0.17 s.e. P = 0.000***).

Effect of density selection

In 2009 the unselected Control CCP had a mean TGW of 51.83. After the first round of mass selection based on seed density there was a highly significant difference in TGW between the unselected control, heavy, medium, and light fractions (P = 0.0000***; see “S1” in Figure 42). Post hoc Tukey HSD testing revealed the TGW in the heavy fraction was significantly greater than that in the light and unselected control, but not different to the medium fraction, which was also significantly greater than the light fraction and the unselected control (P = 0.001, P = 0.01 respectively); there was no difference between the light fraction and unselected control (P = >0.1; Figure 42).

In 2010, there was no difference in TGW or grain yield in the progeny of the three density fractions or the control (P >0.1, see “H1” in Figure 42). After harvested grain was subjected to a second round of mass selection based on density (see “S2” in Figure 42), the heaviest fraction of the Heavy-CCP had a TGW of 48.95, compared to a TGW of 39.55 in the lightest fraction of the Light-CCP. The medium fraction from the Medium-CCP had a TGW of 43.69.

In 2011, there was no difference in TGW between any of the density selections or the control CCP, although TGW in the Control CCP and Small CCP were both greater than the TGW in the Heavy CCP, and the difference neared significance (P = 0.08, and P = 0.06, respectively). There was no effect of density selection on grain yield (P >0.1).

When combining data from both years there was a significant effect of sowing year on TGW and grain yield (P = 0.000***; P = 0.000***), but no effect of size selection (P = > 0.1).

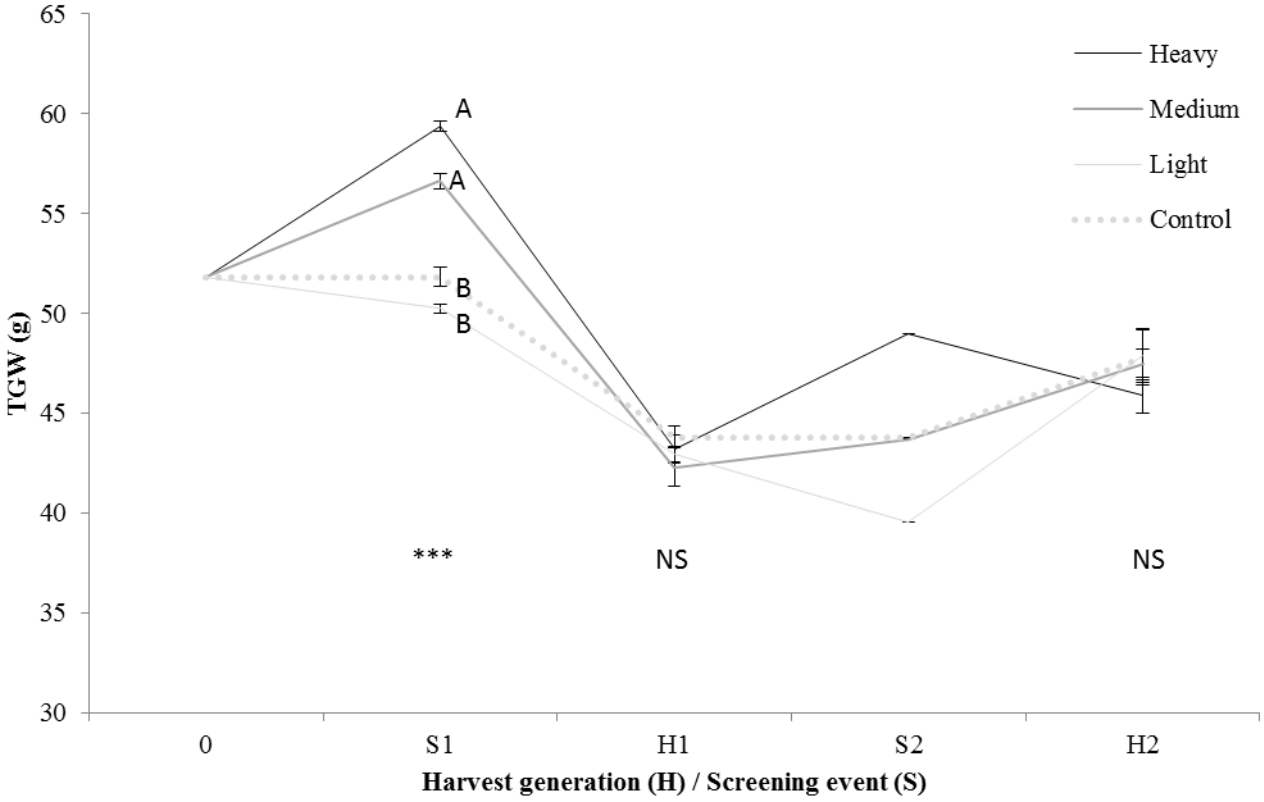

Figure 42. Thousand Grain Weight (TGW) in grams of QCCP before and after mass selection based on specific gravity of grain using a gravity separator, and grown at MET. All size CCP originate from the same QCCP (Generation "0") that had been grown at MET for three years previously. Generation "S1" shows TGW in the three weight fractions and control immediately after the first mass selection event. Generation "H1" indicates TGW in the progeny of those weight selections when harvested the following year. "S2", shows TGW of the heaviest fraction from the Heavy CCP, medium faction of the Medium CCP, and lightest fraction of the Light CCP; these were then also sown and harvested the following year ("H2"). Error bars show standard error. ANOVA: *** P = 0, ** P = <0.01, * P = <0.05; P = <0.1; ns P = >0.05. Where effects were significant, treatments with different letters indicate significance at P<0.05.

3.3.8 Bread-making qualities and micronutrient content of CCP (WP8)

Baking tests

Bakers' general narrative comments

Narrative comments from the bakers were submitted each year. These took into account parameters including dough properties, crumb texture, crumb colour, crust texture, crust colour, loaf height and flavour, although bakers commented only on those aspects which they found remarkable, rather than addressing each individual parameter. These narrative accounts are particularly important in benchmarking the performance of population samples against that of control flours, since in trial years one through three bakers recorded data on only two control loaves at maximum and often recorded no

control loaf data, meaning that rigorous statistical analyses of the population-control comparison are not possible for these years. Not all bakers provided narrative reports in all years.

The overall indication from narrative feedback is that CCP are capable of producing bread-making flours of a commercially acceptable standard, and that in single-variety (unblended) flours, site effects are strong on various quality parameters. In trial year one (harvest 2008), WH Marriage & Sons reported that all CCP samples produced “good doughs and handled very well”, although no single sample achieved both high protein and high hardness, and loaves tended to be somewhat “fragile” as a result. Bread Matters reported that no sample responded very well to the long fermentation, although “the flavour of all the samples, particularly the organic ones, was excellent.” In trial year two (harvest 2009), WH Marriage & Sons reported that “all [CCP] test bakes made good doughs and handled very well,” and that the crust colour of some CCP samples was superior to that of the control. Shipton Mills concluded that several CCP samples were “very good”. In trial year three (harvest 2010), the report from WH Marriage & Sons was even more positive, stating, “the breads made from the seven samples of [CCP] wheat were very impressive”. All four bakers submitted narrative reports in trial year 4, when CCP and pure line flours were tested in replicate. Bread Matters and Wee Boulangerie, which both used sourdough methods, found little variation between samples, including between CCP and control flours, and Wee Boulangerie concluded that “in terms of mechanical properties, all the flours were suitable for artisan bread making”. Panary also found that the predominant problem, lack of oven-spring, affected all samples regardless of whether they came from CCP or pure lines, although the conclusion was that all samples “were consistently producing bread that could be regarded as adequate” by professional standards, and that several CCP samples “could be quickly endorsed as being suitable to tradesman craft bakers willing to bake with English flour.” WH Marriage & Sons in trial year four included CCP samples among its top-ranked samples and concluded that with the exception of CCP samples from MOR, both control and CCP test bakes “performed well with only slight differences in crumb colour and texture”.

Bakers found that CCP flours produced loaves with a range of colour and texture characteristics, with site-related environmental, crop management and storage factors including grain moisture, HFN and protein content being identified as more important than the within-crop genetic diversity. For example, Panary in 2009 assigned ‘recommended’ status to all samples except for QCCP and YQCCP samples from Causey Park farm, Doves Farm and Lavenham. The poor performance of a Sheepdrove YQCCP sample in trial year 3 was linked by WH Marriage & Sons to “extremely low protein (6.6% as is) and Hagberg (close to 200 seconds)”. In trial year 4, negative evaluations of samples from MOR were quite consistent across bakeries.

In terms of the QCCP-YQCCP comparison, QCCP flours tended to be slightly more positively evaluated in bakers’ narrative reports than YQCCP flours. Samples which bakers singled out as top performers in narrative reports were QCCP as opposed to YQCCP in around 85% of cases.

Scored qualitative data

A qualitative score on a 0–1 scale was determined for each loaf baked by test bakers in each year, based on the parameters on which they had reported (see description of methods, see section 3.2.11).

A Wilcoxon signed-rank test conducted on the raw scores from trial year four revealed no significant difference between groups when entries were divided into 'Pure Line' (Paragon and Hereward) and 'CCP' groups (containing all QCCP and YQCCP samples) from either organic or non-organic systems (Figure 43).

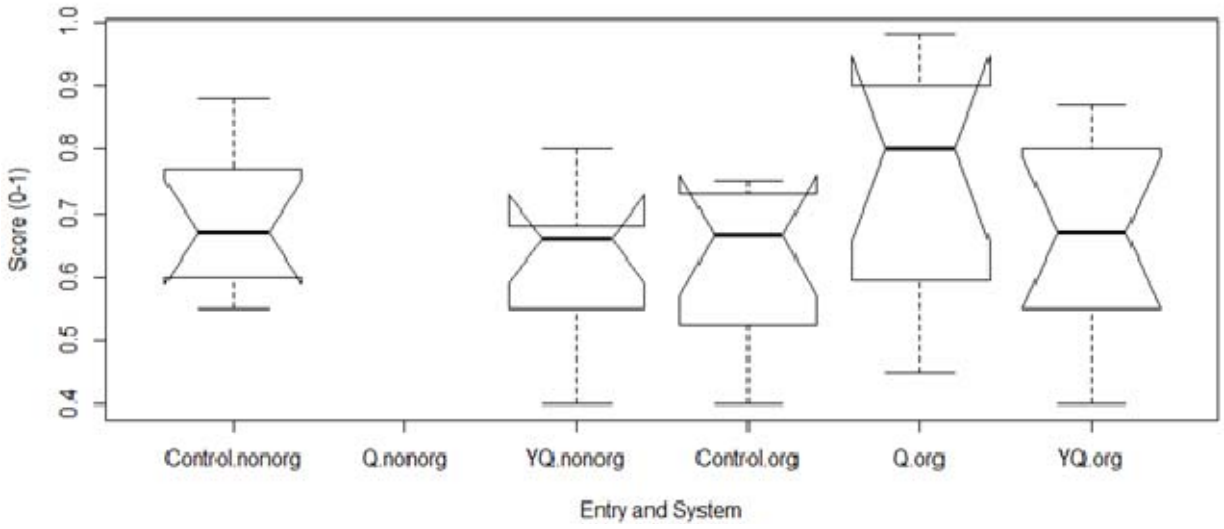

Figure 43. Test bake quality scores in trial year 4 of each entry within each system. Notches show the limits of the interquartile range. There were no non-organic QCCP samples.

As a further test, entries (YQCCP, QCCP or Control) were ranked within bakery and year according to the average score of all loaves baked from their flour. Amongst the six organic tests where bakeries included a control flour, QCCP was top-ranked three times, YQCCP twice and the control once. Amongst the five non-organic tests where bakeries included a control, control samples were always ranked above CCP samples (

Table **47**).

Table 47. The number of times over four trial years CCP and Control entries, respectively, were ranked top amongst all samples by bakeries performing tests which included both CCP and controls.

	Occurrences as top rank (out of cases where a control was included)	Total cases where a control was included
ORGANIC		
QCCP	3	6
YQCCP	2	6
Control	1	6
NON-ORGANIC		
Population	0	5
Control	5	5

A Wilcoxon paired signed rank test was conducted to compare QCCP and YQCCP sample scores across all four trial years (n = 64). The difference between the two groups is close to statistical significance (p= 0.053), with a trend for QCCP scores to be higher (Figure 44), reflecting the pattern seen in evaluations of other parameters.

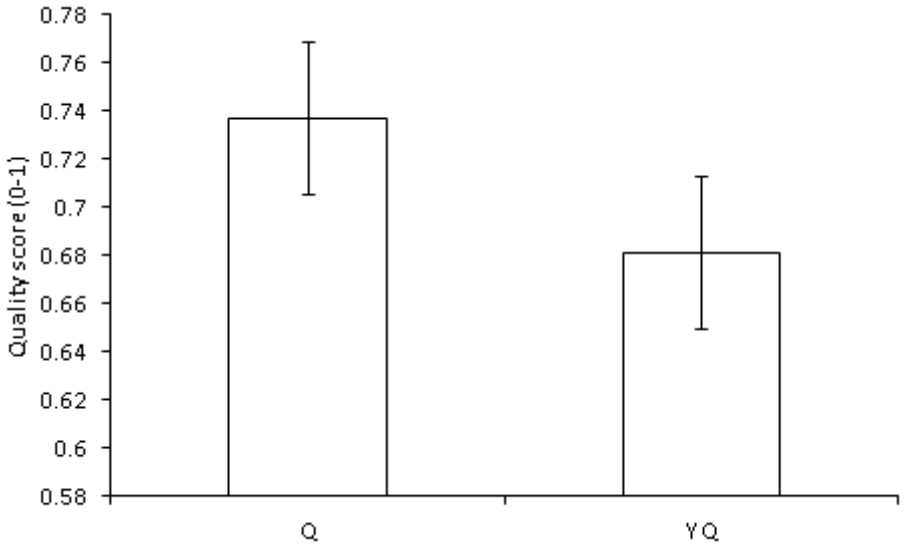

Figure 44. CCP sample quality scores averaged across samples, bakeries and all four trial years (± LSD.).

Loaf height

European consumers are thought to prefer voluminous bread, with a soft and chewy texture (e.g. Osman et al. 2012). For this reason and because it promotes more even cooking, the baking industry generally prefers strong flours with high gluten, able to produce well-risen loaves of large volume.

In a comparison between CCP flours and commercial controls using the replicated dataset from trial year four, there was no significant difference between mean loaf height (a proxy for loaf volume, see methods section 3.2.11) between loaves from pure line samples and those from CCP (YQCCP mean = 98mm, QCCP mean = 100mm, Pure Line mean = 98mm).

During the first three trial years, indications were that commercial control samples tended to produce larger loaves, although statistical significance of this effect is impossible to establish with confidence due to the insufficient number of control samples in those years.

Overall, there is a strong indication from these results that the populations can produce dough with gluten properties that meet industry standards and produce loaves which rise as well as those from commercial flours.

In comparisons between YQCCP and QCCP loaf heights using data from all trial years (within-bakery loaf height variance), no significant difference was established in any trial year, although there was a trend for YQCCP flours to produce slightly smaller loaves in three years out of four (Figure 45). Thus, it is possible to conclude that there is no significant quality impairment in terms of gluten properties and associated structural properties from the inclusion of Y parents in the YQCCP.

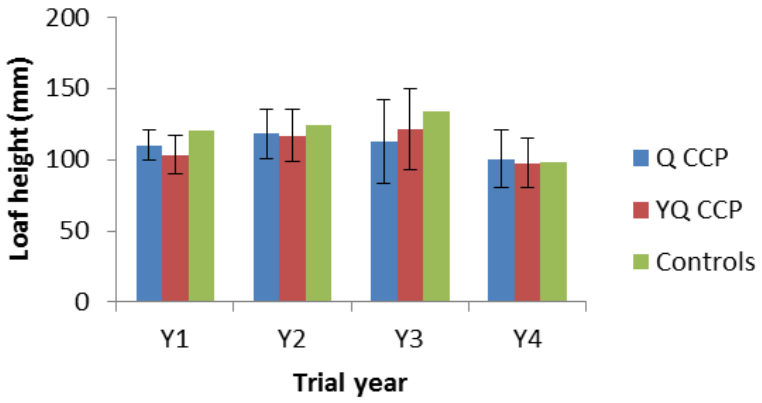

Figure 45. Loaf height (mm) measured in loaves from YQCCP and QCCP and from commercial (Control) samples in Trial Years 1–4. Coloured bar shows loaf height by entry averaged across all samples measured in that year, and error bars show one Standard Deviation to either side of the mean and are omitted where the number of data points was fewer than two.

An analysis of variance using trial year four data and testing Loaf Height (within-bakery variance) against Entry showed a significant interaction between System and Entry: the CCP make relatively larger loaves when compared to pure lines in an organic situation ($p<0.05$). This result should be treated tentatively because the test was unbalanced, with two organic CCP being compared against only one organic pure line originating from a different site. Nonetheless, it reflects findings from other evaluations within the baking tests that CCP outperform pure lines under the more challenging environmental conditions of an organic or untreated system.

Hagburg Falling Number (HFN) tests

Populations versus pure lines: tests on the dataset from trial year four, when two pure lines from two sites were tested along with two CCP from WAF and one from MOR, demonstrated that variety had a significant effect on HFN number ($F_{3,38}$=19.17, P < 0.001) and that Paragon produced a significantly higher HFN (mean HFN = 368s) than the YQCCP (mean HFN = 273s; Tukey HSD, P < 0.001), the QCCP (mean HFN = 302s; Tukey HSD P < 0.001) and Hereward (mean HFN = 294s; Tukey HSD, P < 0.001). The QCCP had a significantly higher HFN than that of the YQCCP (Tukey HSD, P = 0.045) and was not significantly different to the HFN for Hereward (Tukey HSD, P = 0.90). There was also a significant difference between the HFN of those varieties produced under conventional or organic conditions with organic conditions producing a significantly higher HFN ($F_{1,38}$=28.71, P < 0.001), however the balance of varieties was not equal between growing conditions, with Paragon only grown in organic conditions and Hereward only grown in conventional.

QCCP vs YQCCP: in paired datasets from trial years 1–4 there was no significant difference in HFN between the QCCP (mean HFN = 250s) and the YQCCP (mean HFN = 248s; $F_{1,57}$=0.005, P = 0.943).

Protein

QCCP vs YQCCP: A number of variables had significant effects on the protein levels of the wheat including: the year the wheat was grown in ($F_{3,50}$=5.92, P = 0.002); the baker that measured the protein level ($F_{1,50}$=15.63, P < 0.001); and the system (conventional vs. organic) that the wheat was grown in (Mean Conv. = 13.94 Mean Org. = 11.43; $F_{1,50}$=58.73, P < 0.001). However there was no difference in protein levels between the two CCP (Mean QCCP = 12.55, Mean YQCCP = 12.58; $F_{1,50}$=0.031, P = 0.86).

Micronutrients

Mineral concentrations in each of the entries tested are displayed in Table 48.

Table 49 displays the mean values for each entry along with reference values from datasets reported in the literature. Chromium was omitted due to levels in our sample set being below laboratory detection limits (0.1 mg/kg), and cobalt was omitted because the laboratory returned the same value for all entries (0.06 mg/kg). The values returned for the samples tested were generally comparable to values previously reported in the literature for winter wheat sample sets (Davis et al., 1984; Toepfer et al., 1972; Lorenz 1978; Pomeranz & Dikeman 1983; Monasterio & Graham; 2000, Zhao et al., 2009), although calcium concentrations exceeded reported values in the three sample sets available for comparison.

Only one reference value was available for cadmium, and this exceeded that of our samples by 2.5–5 times. The European Food Safety Authority has determined a maximum safe limit for exposure to cadmium in the diet of 2.5 µg/kg of body weight per week, i.e. 150 µg for a 60 kg adult (EFSA, 2011). Cadmium content of the samples tested for this study was between 0.02 and 0.04 mg/kg, or 2–4 µg/100g. Given a sample at the higher end of this range, a 60 kg adult eating 200 g wheat per day would be exposed to 56 µg of cadmium in a week from wheat alone, representing 37% of the safe limit. Since wholegrains have been identified as one of the main sources of cadmium in the human diet, this is probably a tolerable level, so none of the samples we submitted can be considered dangerously contaminated with cadmium.

No reference values were found for molybdenum, but there is other evidence to suggest that the values returned were not out of the ordinary: Momcilovic (1999) suggests that normal intake of molybdenum is 100 to 500 µg/day, coming predominantly from meat substitutes, wholegrain cereals and legumes; thus, an average adult consuming wholegrain wheat as a staple food might struggle to achieve their daily intake with one of the samples at the lower end of this range, but could easily achieve it with samples above 50 µg (assuming intake of 200 g of wheat per day). Site effects on molybdenum were very strong ($P < 0.01$), much stronger than 'diversity' effects (no significant relationship), with all samples from MOR being low in molybdenum.

Site effects were strongly evident when WAF and MOR were compared using sample means from the YQCCP samples. An ANOVA revealed that the MOR samples were significantly higher in iron and lower in molybdenum and selenium (all $P \leq 0.01$) than the WAF samples.

Table 48. Micronutrient concentrations for 9 minerals in 12 samples of winter wheat, February 2013

				mg/kg	%	mg/kg	%	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Entry	**diversity**	**Site**	**System**	**Cd**	**Ca**	**Fe**	**Mg**	**Mo**	**Zn**	**Cr**	**Co**	**Se**
Hereward	pure-line	n/a	organic	0.02	0.05	49	0.1	0.6	34	<0.1	0.06	0.13
Paragon	pure-line	n/a	non-organic	0.03	0.07	42	0.1	0.6	27	<0.1	0.06	<0.1
Solstice	pure-line	WAF	organic	0.04	0.05	27	0.1	1.1	32	<0.1	0.06	0.29
Q	CCP	WAF	organic	0.04	0.13	40	0.13	1.4	52	<0.1	0.06	0.37
Q	CCP	WAF	organic	0.04	0.05	31	0.12	1.6	40	<0.1	0.06	0.33
Q	CCP	WAF	organic	0.04	0.05	28	0.11	1.6	37	0.15	0.06	0.5
YQ	CCP	WAF	organic	0.03	0.05	29	0.11	0.8	35	<0.1	0.06	0.36
YQ	CCP	WAF	organic	0.04	0.08	31	0.13	0.9	39	<0.1	0.06	0.38
YQ	CCP	WAF	organic	0.03	0.07	29	0.12	1.2	39	<0.1	0.06	0.41
YQ	CCP	MOR	non-organic	0.04	0.11	47	0.12	0.4	38	<0.1	0.06	0.21
YQ	CCP	MOR	non-organic	0.03	0.14	40	0.11	0.4	36	<0.1	0.06	0.21
YQ	CCP	MOR	non-organic	0.03	0.08	43	0.11	0.4	34	0.15	0.06	0.13

Table 49. Grain mineral concentrations from two CCP and three pure lines, with reference values from the literature.

Source	Germplasm	Geographic origin	Cadmium mg/kg	Calcium per cent	Iron mg/kg	Magnesium per cent	Molybdenum mg/kg	Zinc mg/kg	Selenium mg/kg
[present study]	Pure lines (range), n=3	UK	0.02–0.04	0.05–0.07	27–49	0.10	0.6–1.1	27–34	0.10–0.29
[present study]	QCCP (range), n=3	UK	0.04	0.05–0.13	28–40	0.11–0.13	1.4–1.6	37–52	0.33–0.50
[present study]	YQCCP (range), n=6	UK	0.03–0.04	0.05–0.14	29–43	0.11–0.13	0.4–1.2	34–39	0.13–0.41
Davis et al. 1984.	404 samples of 231 bread wheat cultivars from three crop years and 49 growing locations (except chromium and selenium, one crop year only) (mean)	USA	-	0.04	79	0.133	-	47	0.074
Toepfer et al. 1972	Hard Red bread wheat	USA	0.1	0.037	44	0.18	-	24	0.5
Lorenz 1978	Hard and soft bread wheat samples originating from 16 states in the USA (range)	USA	-	-	-	-	-	-	0.02–1.09
Pomeranz and Dikeman 1983	Bread wheat varietal composites from 23 locations (range)	USA	-	0.041–0.054	44.1–53.9	-	-	26.1–31.8	-
Monasterio & Graham 2000	505 wheat lines including wild species, landraces, high-yielding bread wheat, durum wheat triticale and specialised lines (range within top 12 entries)	CIMMYT genebanks worldwide	-	-	43.5–56.5	-	-	44.5–64.9	-
Zhao et al. 2009	130 winter and 20 spring bread wheat lines (range)	Europe, Asia, Australia & the Americas	-	-	28.8–50.8	-	-	13.5–34.5	0.033–0.238

To evaluate the effects of crop genetic diversity on mineral accumulation ability, the samples were divided into two groups, Pure (pure lines Hereward, Paragon and Solstice, low genetic diversity) and CCP (QCCP and YQCCP, high genetic diversity). Comparison of these two groups by ANOVA revealed that the CCP accumulated significantly more magnesium ($P \leq 0.01$) and zinc ($P \leq 0.05$), and tended to accumulate more of all other minerals except iron (Figure 46). This tendency was similar in a within-site comparison using the two populations and Solstice from WAF.

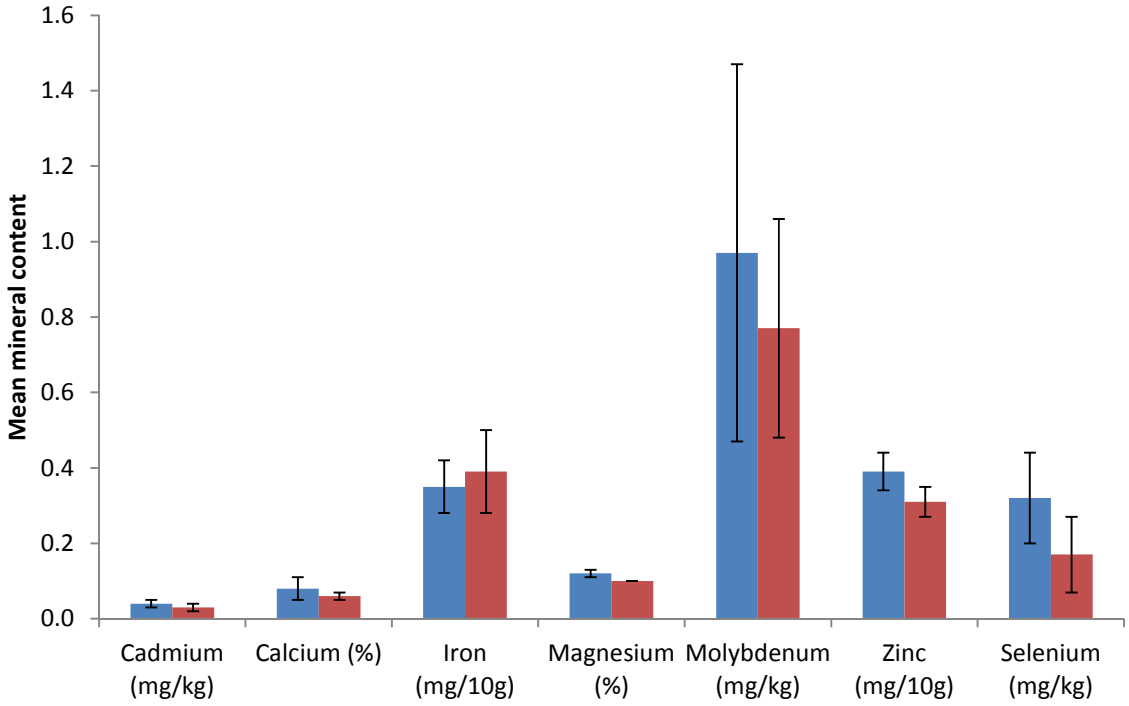

Figure 46. Mean values (± S.D.) for content of seven different minerals in CCP (blue bars) and pure line entries (red bars) tested. Chromium omitted due to levels below laboratory detection limits; cobalt omitted because all entries showed the same value.

At the same time, however, the only minerals among those tested here which are consistently reported in the literature as being subject to strong genetic influence are iron and zinc (selenium accumulation ability is reported to have a genetic component in some studies but not in others, see e.g. Šramková et al., 2009). The conflicting results regarding the accumulation of iron and zinc, therefore, might be thought to weaken the argument for a consistent effect on mineral accumulation ability from genetic diversity *per se*. Instead, the particular characteristics of the genotypes involved could be the primary factor of influence. Site effects also cannot be ruled out due to lack of balance within the dataset; there are only three pure line samples versus nine population samples, and two out of three pure line samples came from sites where the populations were not grown, making it impossible to definitively separate site effects from genetic effects.

Nonetheless, the results indicate that the genetic diversity of the CCP does not result in a depletion of their nutritional value as a source of micronutrients, rejecting one concern about their application for food uses.

For a comparison of the QCCP and YQCCP, ANOVA tests were carried out using only the grain harvested at WAF to eliminate the influence of site effects. For the only mineral in which there was a significant difference between QCCP and YQCCP treatments, molybdenum ($P \leq 0.01$), the QCCP showed higher accumulations than the YQCCP. While Q-YQ comparisons were non-significant for other minerals, the trend was for QCCP mean concentrations to be higher. The QCCP population was less genetically diverse than the YQCCP, and the YQCCP contained all the parents in the QCCP. This suggests that the strongest explanation for the results seen is that the Q parents were better mineral accumulators than the Y parents, and that their characteristics in this respect outweighed any influence of additional genotypic diversity in determining overall population grain mineral concentrations.

Direct correlations between internal genetic diversity and micronutrient concentration did not seem to be apparent in a larger study on mineral composition of wheat genotypes by Hussain et al. (2010), wherein larger numbers of wheat genotypes were tested across a larger number of sites in a balanced design. Nonetheless, the study was not directly concerned with the impact of diversity, and the diversity of the wheat materials tested was not measured and reported but instead must be estimated.

Tests on the CCP sampled in this study (YQCCP and QCCP from WAF) showed a strong and statistically significant ($R=0.97$, $P \leq 0.01$) correlation between iron and zinc. If, as suggested in previous studies (e.g. Zhao et al. 2009, White & Broadley 2009), different genetic mechanisms control iron and zinc uptake, this result suggests that both mechanisms are operating effectively within the populations.

Cadmium is a toxic element harmful to human health. In areas where soil is contaminated by industrial pollution or the use of contaminated groundwater for irrigation, it gets into the human diet via uptake by plants through cellular transport channels designed for other elements, particularly iron and zinc. Partly because of this association with iron and zinc, uptake of cadmium is thought to be subject to genetic control (Zhao & Shewry 2010). We might therefore expect to see a positive association within our sample set between grain content of cadmium and iron and/or zinc. Calculation of the correlation coefficients within population samples taken from WAF indicated only a weak and non-significant positive correlation.

3.3.9 Acceptability of CCP for malting, distilling and animal feed (WP9)

Malting tests

Comparisons between Management Systems

Data were pooled for the trial years and compared across sites. Significant differences were observed for all variables except total soluble nitrogen. Total nitrogen (dry basis) and diastatic power were both higher

under conventional management. In contrast, the soluble nitrogen ratio (dry basis) and extract (% dry basis) were higher under organic management (Table 50).

These patterns follow the normal responses obtained by maltsters; the higher diastatic power and lower extract in conventionally grown samples are due to their higher nitrogen content. The higher soluble nitrogen ratio in organically grown grain aligns with previous observations made at Crisp Malting.

Table 50. Median values (± S.D.) of standard micro-malting variables measured under organic and conventional management (pooled for years and sites).

	Organic		Conventional		
Variable	**Median**	**S.D.**	**Median**	**S.D.**	**P-Value**
Total nitrogen (% in dry malt)	1.82	0.21	2.38	0.15	<0.001
Total soluble nitrogen (% in dry malt)	0.48	0.04	0.48	0.04	N.S.
Soluble nitrogen ratio	25.70	3.02	20.15	2.19	<0.001
Diastatic power (°IOB)	113.00	16.11	140.00	20.47	<0.01
Hot water extract HWE (°L/kg 7M)	313.00	5.53	305.00	4.59	<0.001
Extract (% dry basis)*	(81%)	-	(79%)	-	-

*Extract (% dry basis) is calculated from the hot water extract figures using the formula % dry extract = HWE/386.

Comparisons between Years

When data were pooled for population and system and compared between years, only total nitrogen and extract did not differ significantly. Total soluble nitrogen, soluble nitrogen ratio and diastatic power all showed significant annual variation (Figure 47). Pairwise comparisons identified which pairs of years were sufficiently different from each other to account for this significant result (Table 51). To reduce the chance of type 1 error, adjusted p-values were used in these multiple pairwise comparison tests according to the method of Dunn (1964).

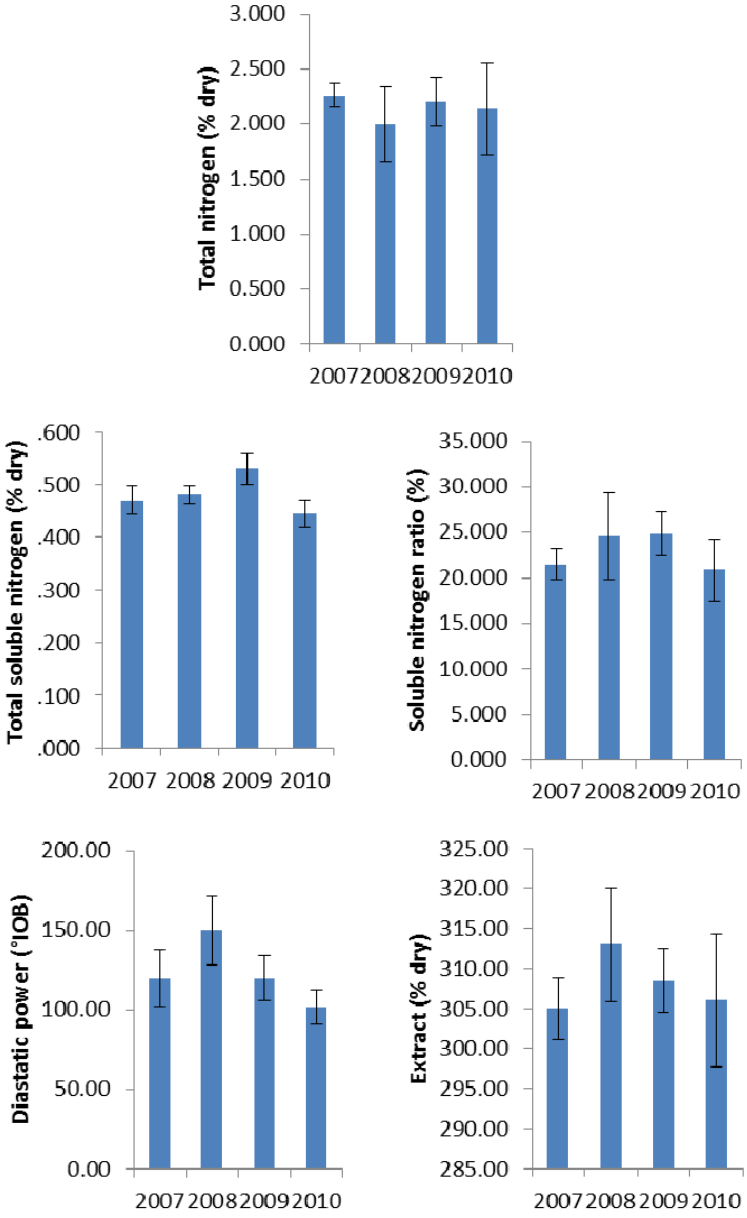

Figure 47. Annual variation in median total nitrogen (± S.D.), total soluble nitrogen, soluble nitrogen ratio, diastatic power and extract.

Table 51. Matrix showing pairs of trial years where significant differences were observed between micro-malting variables. Letter indicates which variable differed (A: total soluble nitrogen; B: soluble nitrogen ratio; C: Diastatic power)

Year	2007-8	2008-9	2009-10	2010-11
2007-8			A	A, C
2008-9	B			A, C
2009-10	B	A		A, C
2010-11				

Table 52. Median values of variables (± S.D.) at hub sites measured in malting tests (pooled by population and system). Medians followed by the same letter are not significantly from each other for the named variable.

Variable	Site	Median	Std. Deviation	P-Value
Total nitrogen (% dry)	MET	2.44a	0.13	P<0.001
	MOR	2.21b	0.12	
	SOF	1.82bc	0.36	
	WAF	1.82c	0.12	
Total soluble nitrogen (% dry)	MET	0.49a	0.04	N.S.
	MOR	0.48a	0.01	
	SOF	0.48a	0.03	
	WAF	0.49a	0.04	
Soluble nitrogen ratio	MET	19.55a	2.37	P<0.001
	MOR	21.35a	1.62	
	SOF	27.5b	4.97	
	WAF	25.7b	1.84	
Diastatic power (°IOB)	MET	129.5a	20.96	P<0.01
	MOR	148.5a	14.17	
	SOF	114b	4.55	
	WAF	106b	19.04	
Extract (L°/kg)	MET	304.5a	4.42	P<0.001
	MOR	306.5a	4.12	
	SOF	312ab	10.21	
	WAF	313b	2.39	

Comparisons between Sites

Significant differences were observed for all variables except total soluble nitrogen when data from the populations were pooled by population and system (Table 52). In all cases, the differences were between combinations of organic/conventional sites; pairs of sites under the same management system (MET & MOR; SOF & WAF) did not show any significant differences one to another.

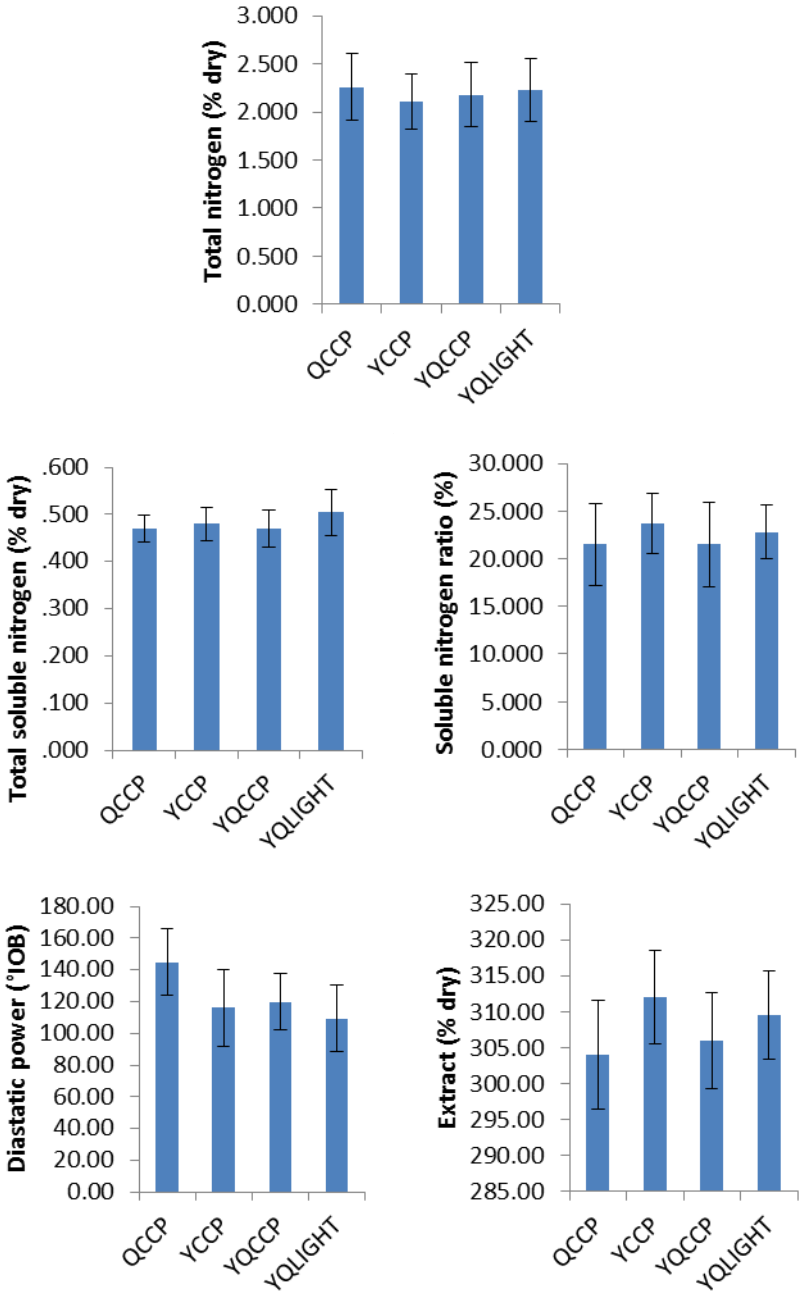

Figure 48. Median total nitrogen, total soluble nitrogen, soluble nitrogen ratio, diastatic power (± S.D.) and extract by population (pooled across sites and years).

Comparisons between Populations

When data were pooled across sites and years and analysed by population, no significant differences were observed in any of the variables measured in malting analyses (Figure 48). This was unexpected because the populations containing Q (quality) parents usually have higher levels of nitrogen than those without. However, there is relatively wide variation around the medians for each variable which accounts

the lack of significance; furthermore, the medians themselves are generally quite similar between populations.

Distilling

Comparisons between Management Systems

Management system had no effect on any of the variables except residue viscosity (P<0.01). The residue viscosity from samples grown conventionally was higher than those grown organically (mean value: 1.58mPAS, n=12 [conventional]; 1.55mPAS, n=6 [organic]).

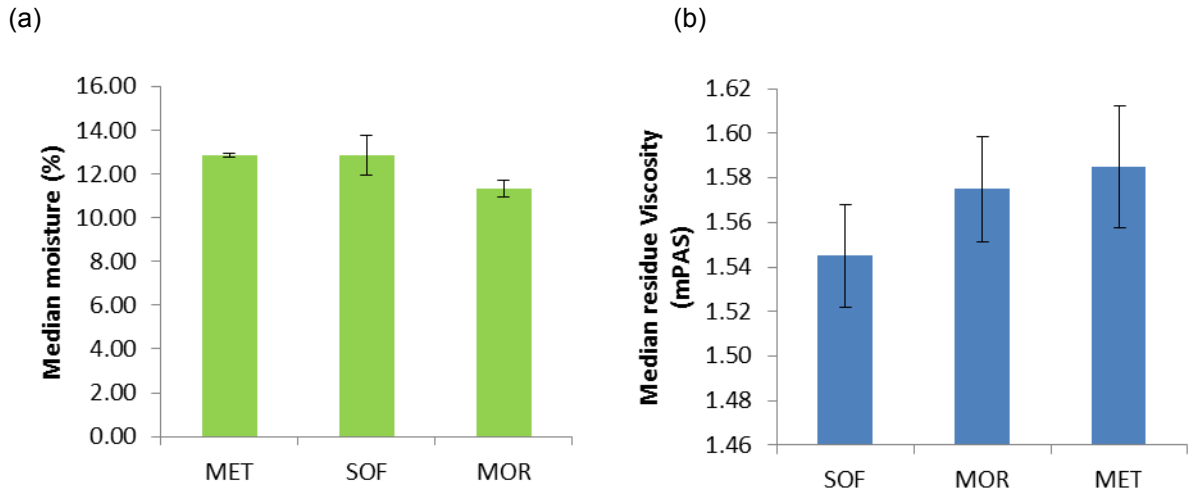

Figure 49. (a) median grain moisture (% ± S.D.) and (b) median residue viscosity (mPAS) measured from grain samples grown at Sheepdrove (SOF), Morley (MOR) and Metfield (MET).

Comparisons between Sites

The growing site had a significant effect on moisture (P<0.05) and residue viscosity (P<0.05), but did not impact on protein, total nitrogen or alcohol yield. Examination of pairwise comparisons showed that for moisture the difference was due to the variation between MOR and MET (median value: 11.30%, n=6 [MOR]; 12.84%, n=6 [MET] Figure 49a). For residue viscosity, it was due to differences between SOF and MET (median value: 1.55mPAS, n= [SOF]; 1.59mPAS, n=6 [MET], Figure 49b).

Comparisons between Populations

Moisture, protein, total nitrogen and alcohol yield all differed significantly between the two populations; only residue viscosity was unaffected by the population type. The results are summarised in Table 53.

Table 53. Mean values (± S.D.) for Moisture, Protein, Total nitrogen, Alcohol yield and Residue viscosity as measured in distilling tests carried out on the YCCP and YQCCP populations by SWRI

	YCCP		YQCCP		
Variable	Mean	Std. Dev.	Mean	Std. Dev.	P-Value
Moisture (%)	12.49	0.67	11.84	0.91	P<0.05
Protein (%dry)	12.03	0.42	12.9	0.58	P<0.01
Total nitrogen (%dry)	2.11	0.07	2.26	0.1	P<0.01
Alcohol yield (LA/tonne, dry)	438.11	3.59	430.33	6.07	P<0.01
Residue viscosity (mPAS)	1.57	0.04	1.57	0.03	N.S.

The trends for protein and total nitrogen followed expectations based on the parentage of the populations; the YQCCP has both yield and quality parents, the latter of which are bred for their higher protein (and nitrogen) content which is known to have an inverse effect on alcohol yield. The residue viscosity levels observed here were fairly typical for the harvest year.

Animal feed

Year 1 (2007-08)

In Year 1 the frequency of the 1B/1R wheat-rye translocation in the MET and SOF YQCCP populations grown under organic conditions in 2007-08 was compared with the starting frequencies in the F_2 YQCCP. Table **54** shows the contributions of each parent of the CCP originator varieties to the 1B/1R and non-1B/1R genotype frequencies based on previous knowledge and their reaction types in disease tests. Most non-1B/1R varieties showed a susceptible (2–4) score, but four (Renesansa, Bezostaya, Cadenza and Wembley) showed an intermediate (00CC) reaction, despite not being expected to have the rye arm. The relative frequencies are given in Figure 50. Table 55 shows examples of the scoring of different reaction types for 15 out of the 297 of the individual plants in the F_7 YQCCP population grown at MET in the 2007-08 season under organic conditions. This was based on assessing 4 progeny plants of each plant harvested as seed from single spikes.

Table 54. Frequency of the contributions of the parental varieties to the starting YQCCP F2 population

Variety	1BS/1RS	Frequency	Summed
Claire	1B	7.8%	
Deben	1B	7.1%	
Hereward	1B	2.1%	
M Widgeon	1B	5.4%	
Mercia	1B	4.6%	
Monopol	1B	2.2%	

Option	1B	6.2%	
Thatcher	1B	3.7%	
Spark	1B	8.3%	
Pastiche	1B	2.2%	
Soissons	1B	6.0%	
Norman	1B	5.2%	
Renan	1B	5.2%	66.0%
Renesansa	1B	3.1%	
Bezostaya	1B	4.7%	
Cadenza	1B	4.8%	
Wembley	1B	3.7%	16.3%
Buchan	1R	6.0%	
HTL	1R	5.6%	
Tanker	1R	3.7%	15.3%

If the susceptible and intermediate (1B) reactions are combined, the relative frequency of 1B to 1B/1R in the starting F_2 was 82.3% to 15.3% (with 2.4% missing data)

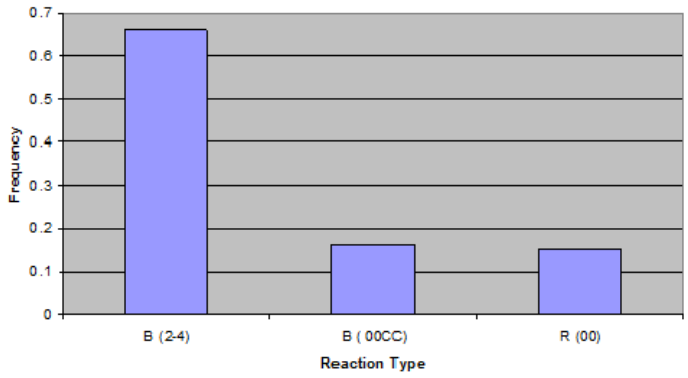

Figure 50. Frequencies of 1B and 1B/1R in the starting YQCCP population based on the relative numbers of F2 seed from parents with and without the 1R segment

Table 55. Year 1 1BS/1RS brown rust seedling test results, MET YQCCP

Plant	Score	Comments	1RS or 1BS
M1	4		B
M2	2/3C		B
M3	OCC	One plant	R
M4	OC		R
M5	4		B
M6	OCCNN		R
M7	OO		R
M8	4		B
M9	3		B
M10	OCN	One plant	R
M11	4		B
M12	3:OCCNN		Seg
M12	1:4		

M13	OCC	R
M14	O/1CCN	R
M15	2/3CC	B

The frequencies of the starting F_2 CCP and the F_7 MET and SOF YQCCP for the 2008 harvested lines are shown in Table 56 and presented graphically in Figure 51. These were based on assessing 4 progeny plants of each of 297 individuals of the MET YQCCP and 304 plants of the SOF YQCCP.

Table 56. Frequency of disease reaction type in the starting F2 CCP and F7 Metfield (M) and Sheepdrove (S) YQCCP grown under organic conditions in 2007–08.

Type	F_2	F_7 M Pop	F_7 S Pop
B (1–4)	0.66	0.64	0.70
B (00CC)	0.16	0.09	0.08
R (00)	0.15	0.11	0.11
Seg	-	0.15	0.04
*	-	0.01	0.08

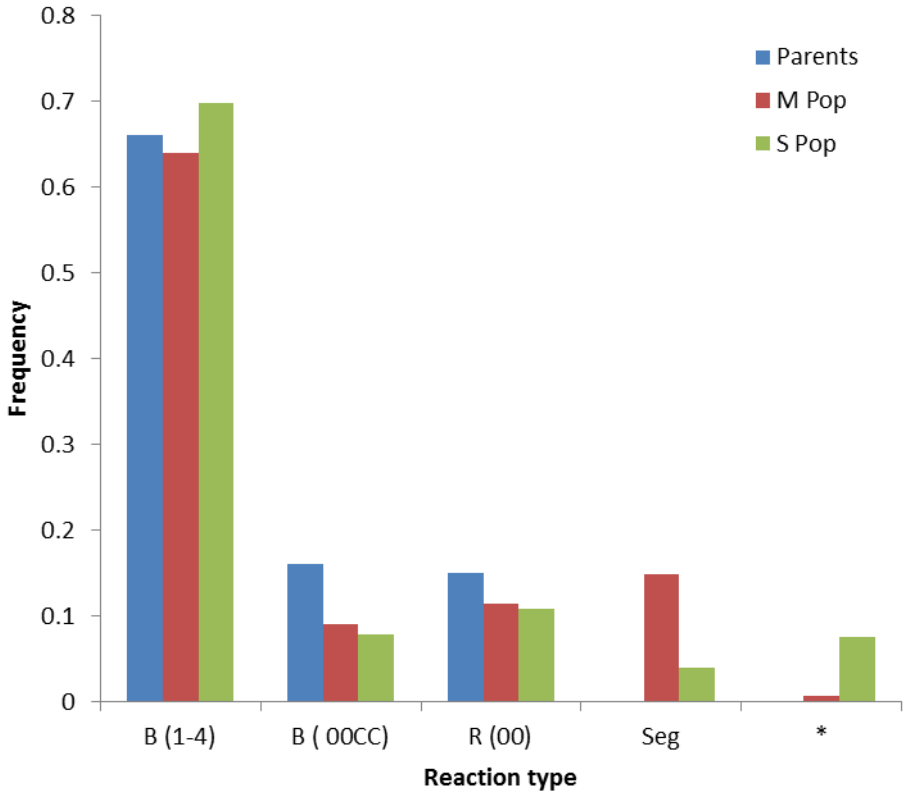

Figure 51. A comparison of 1B and 1B/1R chromosome frequencies in the YQCCP grown at MET and SOF in 2007-08

Analysis of these data shows that there is a slight, but not statistically significant, drop in the frequency of the 1B/1R chromosome in both the MET and SOF YQCCP after four generations of cultivation, hovering just over the 10% mark. There is no statistically significant difference in frequency between the two CCP (i.e. YCCP and YQCCP).

The low frequency of the 1B/1R chromosome, around 10%, in these populations is unlikely to have any major effect on the end-use quality, either for bread or for animal feed because of the dilution effect of the 1B chromosome.

Year 2 (2008-09)

For the 2008-09 harvest, because of the low cost of the SNP assay described in section 2.3, the number of tests was greatly expanded so that around 400 seeds from combine harvester samples were tested for the frequency of the translocation in each of the YCCP and YQCCP populations grown at 6 sites. Half of these sites were organic and half were conventional, grown in geographically close locations.

The populations tested were Benham (BEN) YCCP only (YQ lost); Causey Park (CPY) YCCP & YQCCP; Metfield (MET) YCCP & YQCCP; Wakelyns (WAF) YCCP & YQCCP; Shackerdale (SHA) YCCP & YQCCP; Sheepdrove (SOF) YCCP & YQCCP. An example of these data for 16 seeds of the Benham YCCP are shown in Table 57.

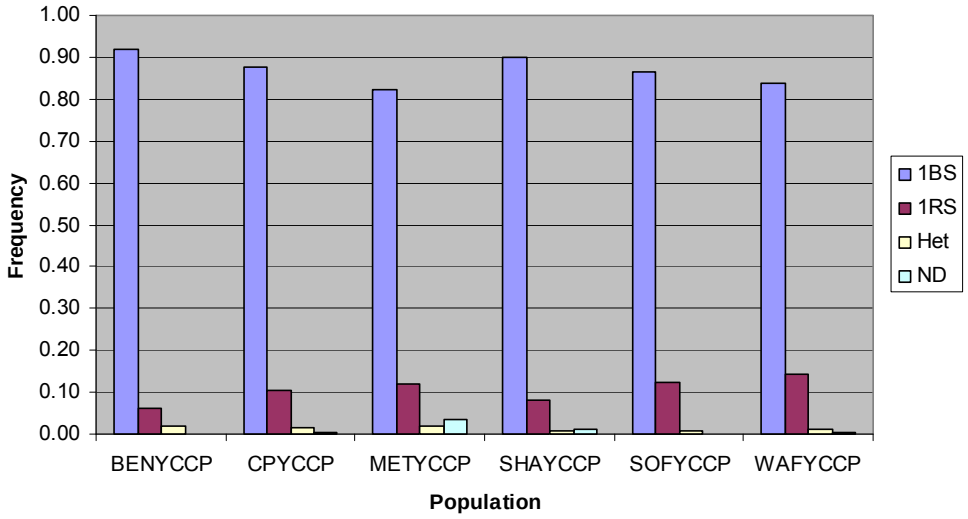

Figure 52. Frequencies of the 1B and 1B/1R chromosomes for the YCCP (2009 harvest) at the different sites.

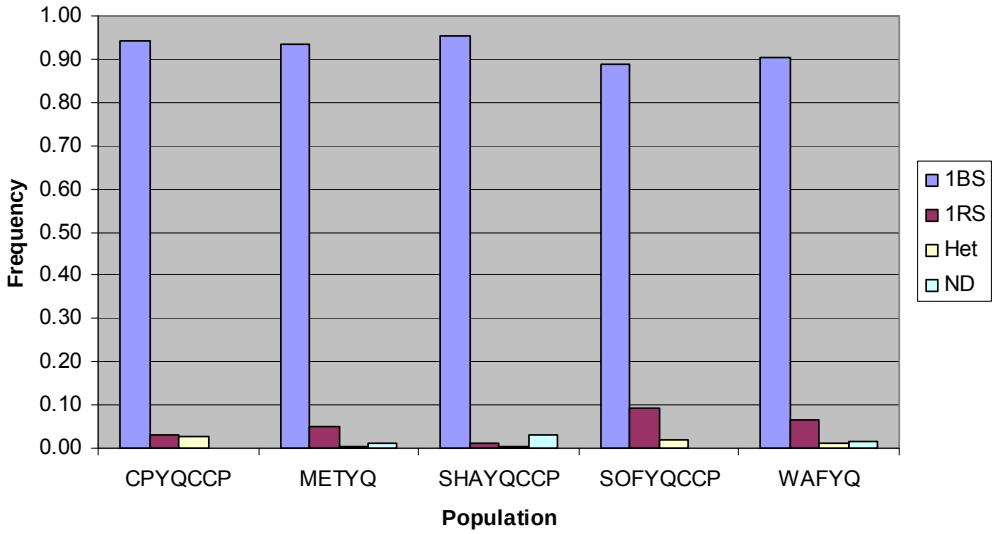

Figure 53. Frequencies of the 1B and 1B/1R chromosomes for the YQCCP (2009 harvest) at the different sites.

The overall results for each of the sites and populations grown in 2008-09 are shown in Table 58. These are presented graphically in Figure 52 and Figure 53. 1662 plants of the YCCP population and 1554 plants of the YQCCP were tested in this season. As expected at F_8, there is a very low frequency of heterozygotes observed (expected to be 1/128 = 0.78%), but it is generally higher than this at some sites, e.g. MET YCCP and CPY YQCCP, perhaps suggesting some selection of heterozygotes or some out-crossing in the populations.

Table 57. SNP results and 1B/1R classification for 16 random plants of the Benham YCCP.

Plant No.	Population	SNP	Class
68	BENYCCP	G:G	1BS
69	BENYCCP	G:G	1BS
70	BENYCCP	G:G	1BS
71	BENYCCP	G:G	1BS
72	BENYCCP	G:G	1BS
73	BENYCCP	G:G	1BS
74	BENYCCP	G:G	1BS
75	BENYCCP	G:G	1BS
76	BENYCCP	G:G	1BS
77	BENYCCP	G:G	1BS
78	BENYCCP	G:G	1BS
78	BENYCCP	G:G	1BS
80	BENYCCP	A:A	1RS
81	BENYCCP	G:G	1BS
82	BENYCCP	G:G	1BS
83	BENYCCP	G:G	1BS

Table 58. Frequencies of 1B and 1B/1R chromosomes from molecular testing of (a) YCCP and (b) YQCCP populations from the 2008-09 season.

(a)

Class	BENYCCP	CPYCCP	METYCCP	SHAYCCP	SOFYCCP	WAFYCCP	
1BS	228	175	274	263	187	313	
1RS	15	21	40	24	27	54	
Het	5	3	7	2	2	4	
ND	0	1	12	3	0	2	
Total	248	200	333	292	216	373	1662

(b)

Class	CPYQCCP	METYQ	SHAYQCCP	SOFYQCCP	WAFYQ	
1BS	249	357	296	185	344	
1RS	8	19	4	19	25	
Het	7	1	1	4	5	
ND	0	5	9	0	6	
Total	264	382	310	208	380	1544

The frequency of the 1B/1R chromosome in the starting YCCP F_2 population was expected to be 32.7% based on the numbers of seeds of the crosses contributing to this population and also since this population contained only nine parents, three of which were the 1B/1R carriers Buchan, HTL and Tanker. However, as can be seen in Figure 52, this frequency has dropped greatly to between 6% (BEN) and 15% (SOF), suggesting strong selection against this chromosome in these populations. The reason for this is unclear, but could be related to disease susceptibilities, since the rye resistance genes are no longer functional against UK rust isolates. There is a small significant difference between sites in the 1R frequency, but this is likely due to genetic drift rather than differential selection.

The frequencies in the YQCCP populations had also dropped from the initial starting frequency of 15%, with the highest being 8.6% (SOF) and the lowest being 1.3% (SHA) again suggesting strong selection against the chromosome. There is a small significant difference between the sites in the 1R frequency, but again this is likely due to genetic drift rather than differential selection. It is probable that disease susceptibility is a major reason for the selection against 1R at all sites. This is, in fact, beneficial with respect to the use of these populations for bread making.

A comparison of the 2008 and 2009 harvest data for the Metfield and Sheepdrove YQCCP populations showed a further drop in the 1B/1R frequencies from 11% to 5%, and 11% to 9%, respectively. Comparing the paired sites MET and WAF (which were adjacent to each other) for both the YCCP and YQCCP revealed no significant differences between the 1R frequencies. Thus the drop in 1R frequency is a characteristic of both organic and conventional agronomic systems and populations.

Year 3 (2009-10)

For the 2009-10 harvest, again around 400 seeds from combine harvester samples were tested for the frequency of the translocation using the SNP molecular marker test in each of the YCCP and YQCCP populations grown at 6 sites. These sites were the same as used in 2008-09.

The populations tested were Benham (BEN) YCCP & YQCCP; Causey Park (CAU) YCCP & YQCCP; Metfield (MET) YCCP & YQCCP; Wakelyns (WAF) YCCP & YQCCP; Shackerdale (SHA) YCCP only and Sheepdrove (SHE) YCCP & YQCCP.

Figure 54 and Figure 55 show the frequencies of the 1B and 1B/1R chromosome for the 2009-10 growing season for the YCCP and YQCCP, respectively. Comparisons between the 2009 and 2010 harvests are shown in Figure 56 and Figure 57 for the YCCP and YQCCP, respectively. Not all sites are included because of the one missing site in each of the years.

Generally, both populations showed a further fall in the 1R frequency, although there was a slight increase at two sites (BEN and MET) in the YCCP, and at MET in the YQCCP.

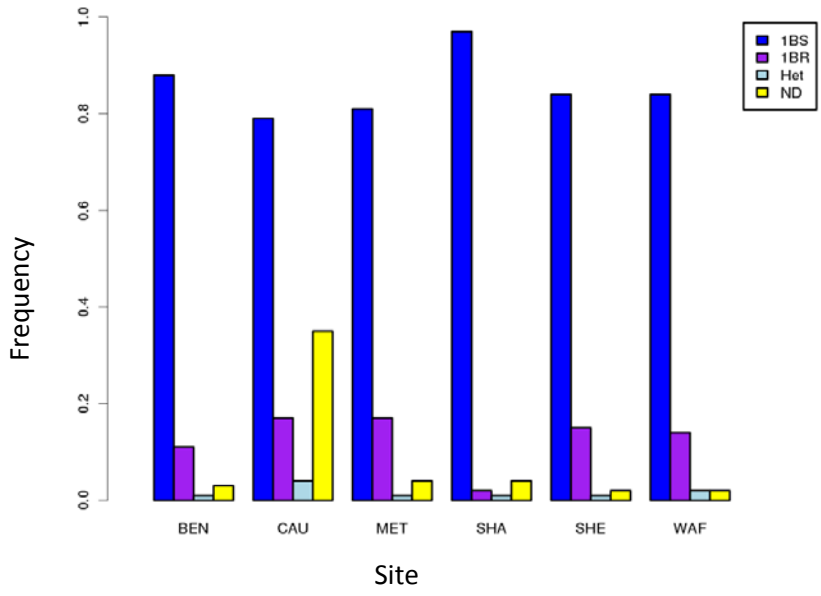

Figure 54. Frequencies of the 1B and 1B/1R chromosomes for the YCCP (2010 harvest) at the different sites.

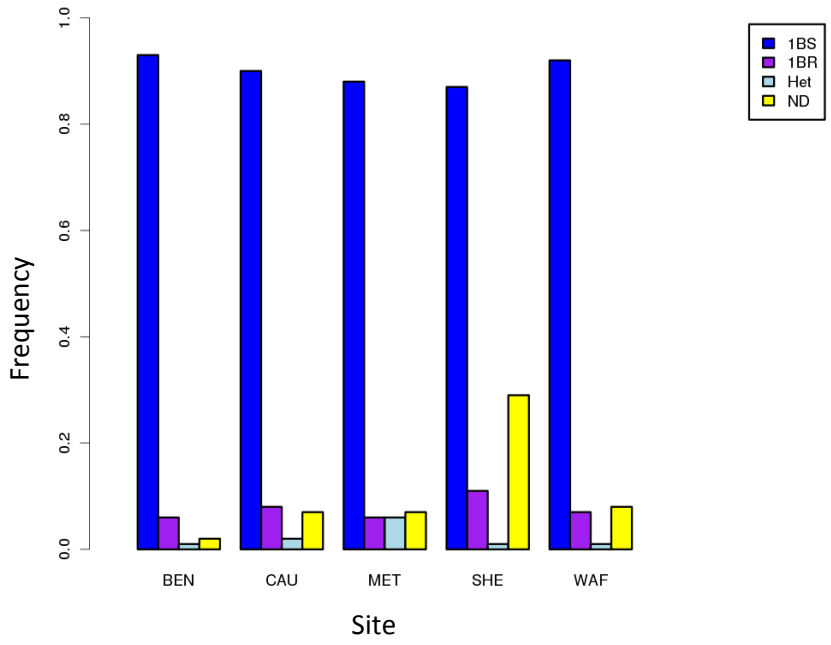

Figure 55. Frequencies of the 1B and 1B/1R chromosomes for the YQCCP (2010 harvest) at the different sites.

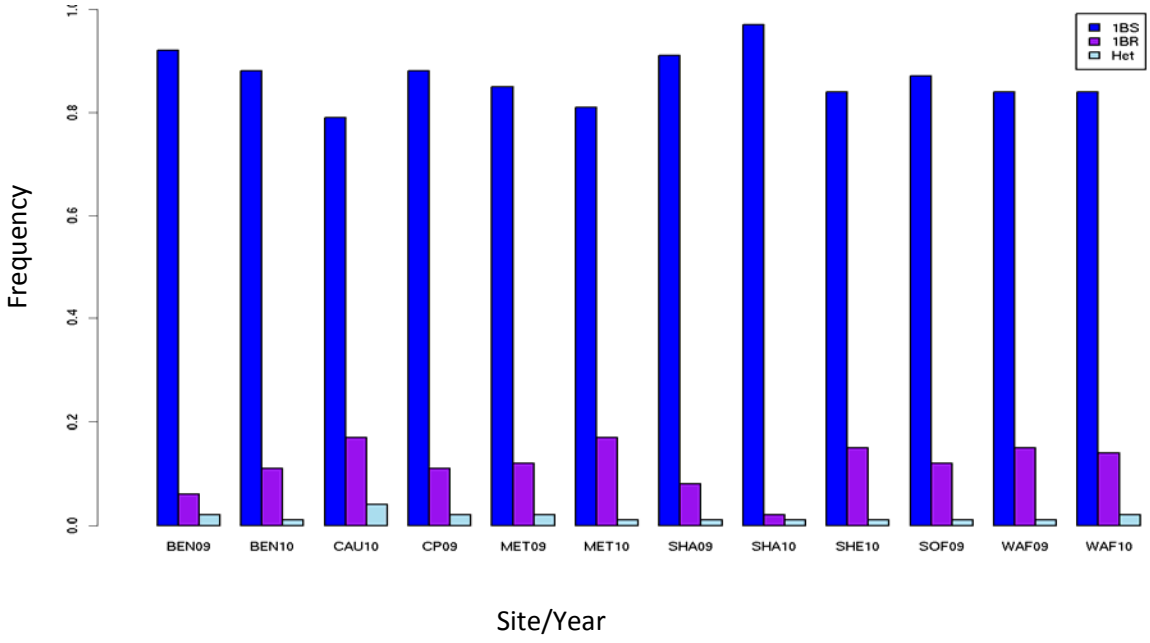

Figure 56. A comparison of the 1B and 1B/1R chromosome frequencies in the YCCP grown in different years and at different sites

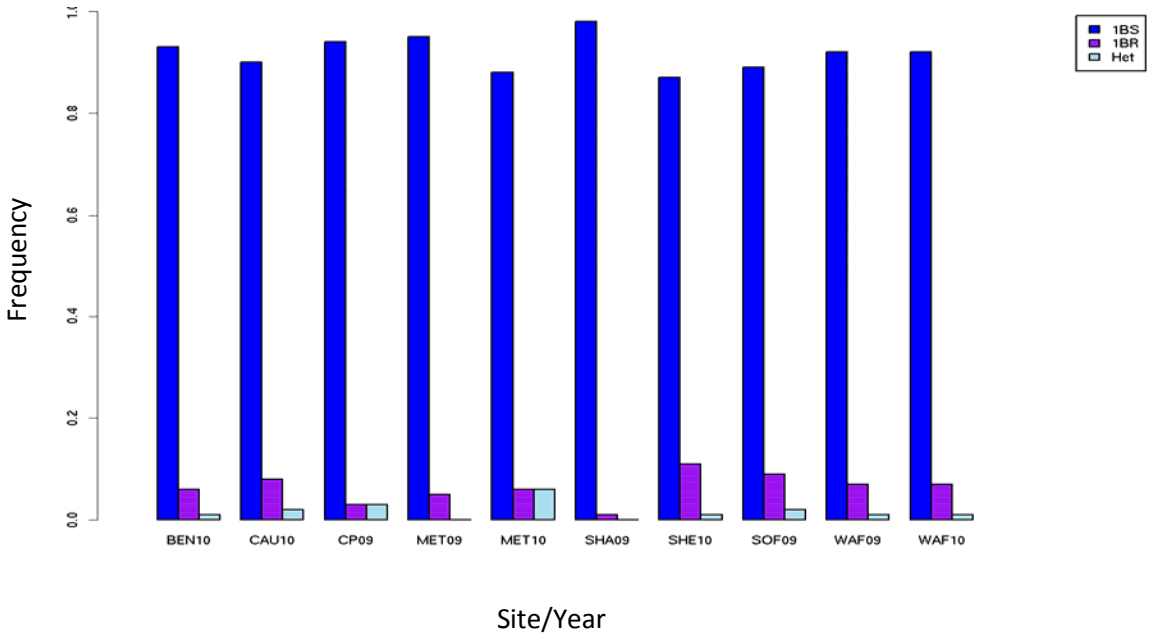

Figure 57. A comparison of the 1B and 1B/1R chromosome frequencies in the YQCCP grown in different years and at different sites

3.4 Discussion

3.4.1 Quantifying the yield, quality and stability of performance of CCP (WP1)

Comparisons within populations

Establishment tended to be lowest in the YCCP, and highest in the QCCP, with YQCCP being intermediate; however, these effects were not significant (Table 18, page 64). Similarly, stability of establishment over environments did not show any consistent patterns among the three parent sets. With regard to plant height, the observed order (Q>YQ>Y) reflects the properties of the parents used to create the CCP, with Q parents showing taller plant height than Y parents (Figure 10, page 67). Accordingly, the YCCP were also found to be significantly lower in lodging than the YQCCP and the QCCP, and as expected, taller plants showed greater lodging than smaller plants. However, lodging of the CCP was generally low and did not affect grain yield.

In terms of grain yield, there were no significant differences between the three parent sets in the organic system, whereas the YCCP were different from YQCCP and from the QCCP at the conventional sites, in the order expected (Figure 12, page 68). Further, the harvest index was significantly lower in the YQCCP and QCCP than in YCCP, but not different between YQCCP and QCCP (Figure 13, page 69). With regard to grain protein the order of the three parent sets was consistently Y<YQ<Q, as expected (except for the organic CCP_n) reflecting parent properties (Table 20, page 70). Similar results were obtained for Hagberg falling number and Hardness where the order of the populations was consistently Y<YQ<Q (Table 21, page 70). For the grain yield stability parameter *b*, a potential explanation for the high stability of the QCCP is that yield is not responding strongly to high inputs (or good nutrient availability at the organic sites; Table 22, page 71); this result may also be a consequence of the age of the parents (older in Q) (Jones et al., 2010).

Comparisons of CCP with mixtures and pure lines

One of the main questions of the project was how wheat CCP perform in agronomic terms in comparison with currently used pedigree line (homozygous) wheat varieties. Our results showed that in the conventional system, grain yield was lower in the CCP than in the pure lines, but no significant difference was found in the organic system (Figure 16, page 76). This result indicates higher suitability of CCP for organic (and possibly for low-input) systems than for high-input conventional agriculture. Thus, our study confirms the conjecture made earlier that CCP may be particular (or more) suitable for organic agriculture (Phillips and Wolfe, 2005; Dawson and Goldringer, 2012).

The highest relative yield difference between pure lines and CCP was found for the high quality (Q) parent set in the conventional system, where the yield of the CCP_n was 12.7% lower than the average yield of the high-quality pure lines. This difference may appear unacceptable from the point of view of

potential commercialisation of the CCP. However, it needs to be borne in mind that the parentage of the CCP investigated in this study included some relatively old varieties with relatively low yield potential. There is therefore a dilution effect of the more recently released higher-yielding parents of the CCP, leading to a decreased yield potential in the populations.

Accordingly, when the CCP were compared with all monocultures of their respective parents in a previous study, the CCP were not out-yielded by these pure lines, but achieved a (marginally) larger grain yield than the monocultures. These contrasting results indicate that the yield effects seen in the present study are mainly the consequence of a comparatively strong impact of parentage of the CCP. This confirms the importance of the choice of parents for generating CCP (Döring et al., 2011b). In addition, our findings suggest that the size of the yield gain achieved through conventional pedigree breeding over the last few decades is likely to be larger than the yield-increasing effects of genetic diversity, when older varieties are included as parents of the CCP. Therefore, future CCP could be generated with a stronger reliance on high-yield parents; alternatively, the existing CCP could be subjected to a process of (artificial) selection to enrich the population and increase its yield potential.

On the other hand, however, the older varieties which were included in the creation of the CCP certainly contributed to the genetic diversity of the population, and they also clearly contributed to the CCPs' relatively high protein content. In fact, grain protein content was consistently higher in the CCP than in the pure lines (Table 30, page 80). Thus, if future CCP are based on higher-yielding parents, potential trade-offs with grain protein content would then have to be considered carefully.

In addition, the CCP showed a number of advantages over the pure lines during the growing season. In the context of organic and low-input agriculture the most relevant of these advantages is the high leaf area index, which was shown in this study to be consistently larger than in the pure lines (by around 10%; Table 24, page 73). Also, CCP were considerably taller than the pure lines and tended to produce a higher amount of straw mass (Figure 14, page 72). Taking both results into account, it can therefore be concluded that the competitive ability of the wheat crop against weeds was higher in the CCP than in the pedigree varieties, making the CCP particularly suitable to organic agriculture where herbicides are not applied. This is also in line with the finding that yields in the organic system did not significantly differ between CCP and pure lines.

On the other hand, however, infections with foliar pathogens such *Septoria tritici* were found to be significantly higher in the CCP than in the pure lines (Table 25, page 73); this is in contrast with findings reported elsewhere that genetic diversity decreases incidence and severity of fungal diseases in wheat (e.g. Finckh & Wolfe 1998). However, studies showing such effects follow a more systematic approach in that they compare variety mixtures with their component monocultures. For the comparison studied here, specific resistance present in the pure lines may explain the observed result, but further research would be needed to investigate whether this was indeed the case. A further, more comprehensive assessment of disease restriction in the CCP relative to pure lines is presented in WP4, which showed that the

YQCCP and YQ mix were consistently amongst those entries that showed the lowest degree of yield reduction in response to fungal diseases.

Increased plant height as seen in the CCP in this study has been predicted to be associated with increased lodging and potentially with subsequent yield reductions; while lodging was indeed observed to be higher in the CCP than in the pure lines, lodging was not severe and did not impact on grain yield.

One of the advantages of CCP over pure lines that has previously been suggested, is its higher stability of grain yield (e.g. Döring et al., 2011a). With the present study we show that CCP are indeed characterised by higher yield stability than the tested pure line varieties (Table 29, page 78). This is in line with results from a previous research project, which showed that CCP are more stable than the monocultures of the corresponding parents (Döring et al., in preparation). Higher yield stability was observed for a number of stability parameters based on both static and dynamic concepts of yield stability. In addition, the CCP_{ms} showed the highest stability of the protein content among the entries across most stability parameters; for four out of the six parameters, the pure lines were the least stable.

These findings may be a result of a combination of compensation, complementation and potentially also facilitation effects, but data obtained in WP1 does not allow us to tease apart the mechanism underlying the higher yield stability of the CCP. However, our data also show a strong compensative ability within the pure lines; e.g. effects of reduced head density on grain yield were buffered by compensating effects of later yield components.

An alternative way of increasing genetic diversity of a wheat crop in the field is the use of variety mixtures. In this study we trialled complex variety mixes which were equivalent to the CCP in terms of the genetic starting point, i.e. the parent varieties used to generate the mixtures and CCP, but the CCP exhibited much greater genetic diversity than the mixes. Despite this difference in genetic diversity between the CCP and the mixtures, both performed equally for most parameters trialled in this WP, e.g. no differences between CCP and mixes were observed for grain yield, grain protein content (with the exception of the Y parent group under organic management), protein yield, as well as a range of pre-harvest parameters (leaf area index, plant height, fungal diseases). This means that both CCP and (complex) mixtures are more or less equivalent with regard to their agronomic performance. Preference for CCP or for mixtures is therefore likely to follow other criteria than those based on agronomic performance.

Adaptation trials

In the additional adaptation trials conducted as part of this WP, no effects of adaptation were observed for the CCP or mixtures. Material grown at its home site did not show any significant advantages in terms of grain yields (

Table **34**, page 82) or other parameters (

Table **33**, page 82) over material that had been grown under different conditions for the past few generations.

This result, in particular from the cross-over trial, is unexpected since management conditions contrasted strongly between the sites (organic vs. conventional cropping systems), and both CCP and mixtures were exposed to separate growing conditions for a relatively long time (7 generations). The findings of the two adaptation trials, especially the cross over trial, suggest that factors which were shared by all four sites (e.g. weather fluctuations over the trial years), might have counteracted any adaptation to the site-specific factor associated with cropping management and soil conditions.

3.4.2 Genotypic evolution in adaptation of CCP (WP2)

There were no clear signs of differences in selection pressure resulting in changes in allelic composition between sites or agronomy regimes. However, a few loci did show significant changes in allele frequency over time.

Natural selection and domestication

Principal component analysis and F-statistics showed that no differential selection between the sites could be observed Figure 22, page 87). F-statistics and the analysis of change of allele frequencies over generations indicated that the magnitude and direction of changes of allelic composition was of equal magnitude at each site (Figure 23, page 89). These observations suggest that an adaptation process over time did take place, but that it may not have affected the populations differently at the four sites, but instead occurred in the same direction at all sites.

This finding could be due to several reasons. First, environmental conditions may have been relatively similar at the four sites; more specifically, environmental variation within sites (i.e. year-to-year variation) may have been much greater than environmental differences between sites. Thus, consistency of environmental differences between sites may have been too small in relation to the effects of weather and other year-specific conditions.

Second, the markers selected for the analysis may not have been appropriate for detecting differentiation between the sites. This is particularly likely since the number of markers applied was relatively small. Thus, while no significant adaptation to site conditions was observed in this study, it cannot be excluded that undetected adaptation did indeed take place.

Third, processes within the populations (such as general competition among plants) may have been in place in the same way at all four sites and may have dominated the evolution of the CCP. In fact, the general phenotypic effects of the alleles of the 7 loci that showed to have undergone some form of

selection are an increase in plant height over time and delay in ear emergence. The effects on grain properties like number, weight and shape are rather ambiguous.

The general increase in plant height and the observation that the two loci that have undergone the greatest change of allele frequencies were genes coding for height, suggests that plant height has been a major driving force in the evolutionary process of the CCP. It has been selected for the wild type allele at both genes for dwarfing characteristics, RhtB1 and RhtD1. This observation is confirmed by the observation of Raquin et al. (2008). They showed the increase of RhtB1 in an experimental population of winter wheat from a frequency of 0.66 in the initial generation to near-complete extinction of the dwarfing allele after 17 generations. The dwarfing allele at both loci were of major importance during the Green Revolution (Borlaug, 1983). It can hypothesized that selection acted towards wild type and thus against 'domestication alleles'. The selection for height can be explained by competition for light between the different genotypes.

The trend towards later ear emergence and thus later flowering is related to the selection for the wild type alleles at the genes PpdB1L5 and PpdD1. The mutated allele at both genes cause insensitivity to photoperiod (day length neutrality) and have also been important during the Green Revolution (Borlaug, 1983). Photoperiod insensitivity allowed for a wider regional spread of wheat cultivation world-wide due to earlier flowering and thus avoid stress through high temperatures during grain development (Kato & Yokoyama, 1992). It has been shown that the wild ancestors of wheat are sensitive to photoperiod and carry the wild type alleles at both the Ppd genes (Thomas & Vince-Prue, 1997). Thus, also for the two Ppd genes, selection seems to have happened towards wild type and against alleles that are supposed to be important modern agricultural production. The selection for the wild type form can also be hypothesized for the X1B1R marker. Introgression of the translocation from rye into wheat was done by breeders and mostly originates from the rye variety Petkus (Schneider & Molnár-Láng, 2009). It has been selected against the translocation, even though it is assumed that the introgression confers increased resistance and should thus also lead to improved fitness.

Implications for evolutionary plant breeding

The major motivation of the idea of evolutionary plant breeding is that through natural selection mixed crop populations are established that are well adapted to local environmental conditions and farming practices and still produce a good yield (Döring et al., 2011a). However, in this study no specific adaptation to either farm or site could be detected, albeit with a limited number of markers. The major pattern that is revealed is that selection has acted against the mutant alleles at 5 genes that have been of major importance during the Green Revolution. Selection has thus acted towards a more wild type form. The major force of selection that was identified is selection for height, which is most probably due to competition between the plants for light. According to the theory of the trade-off between vegetative and reproductive growth, increased height means greater investment in vegetative growth and thus a decrease in harvest ratio and yield. The main obstacle is the trade-off between natural selection and

human demands. Whereas the former selects for *individual* performance through competition, the latter demands *population* performance. An increase in individual fitness does not necessarily lead to an increase in population performance (Weiner 2003).

The decrease of alleles in frequency that have been important for the establishment of modern varieties shows that natural selection has acted against important domestication types. Based on the observation, that the major pattern of selection is due to competition and selection for wild-type alleles, an improved method for evolutionary breeding is suggested: if populations would be created that are fixed for alleles that confer major domestication traits like dwarf type and photoperiod insensitivity, less selection could take place at these traits. Plants would not waste resources on competitive behaviour and selection would probably be more pronounced towards prevailing local growing conditions.

As mentioned before, the analysis was only based on a limited set of markers that did only capture a small region of the wheat genome. Loci that have undergone selection could be positioned on regions that were not investigated. Additionally, phenotypic characteristics like resistance and tolerance were not scored. Such traits could be crucial to local adaptation. A proper assessment of differentiation of the 4 populations over time and between sites would necessitate a trial where different generations from different sites are grown together at several sites to be able to make valid comparisons.

3.4.3 Genetic and phenotypic contributions of individual parent varieties to CCP (WP3)

To investigate phenotypic characteristics of the evolutionary process, allele-substitution effects were calculated from the estimated allele effects of the alleles that have increased in frequency (Table 39, page 92). The general pattern that can be discovered when looking at the trends over all 7 loci that were identified as undergoing selection is an increase in number of tillers per plant, plant height, grain number, grain weight and a change towards later ear emergence and a more upright growth habit. However, when the effects of each locus are investigated separately, the pattern becomes much more complex (see Table 39). Effects on the grain properties area, length and width were too small for any sensible interpretation.

The loci that were identified as associating with dwarf growth and have been very important in the Green Revolution in reducing harvest ratio and thus generally leading to a substantial increase in yield (Peng et al., 1999) show to have a oppositional effect on phenotypic traits. In both loci, selection has acted against the dwarfing alleles, which can be identified by the known genotypes from the parental lines. The allele that has increased in frequency of the locus *RhtD1* that has been under strongest selection, has the strongest increasing effect on plant height, reduces grain number and grain weight with no substantial change on thousand grain weight. Growth habit also tends to be associated with the selected allele towards a more upright position. *RhtB1*, which is an orthologue copy of *RhtD1*, seems to be counteracting *RhtD1* by an increase of grain weight and grain number and towards a more prostrate growth habit.

The two loci that are known to be associated with photoperiod response and thus associated with ear emergence (Beales et al., 2007) also show their effect on ear emergence in the association analysis. At both loci it was selected for the allele that codes for sensitivity to photoperiod. The allele that is increased in frequency of *PpdD1* has the strongest effect towards later ear emergence and also greatest effect on number of tillers per plant among the 7 loci. *PpdB1L5*, which is an orthologue copy of *PpdD1*, has an effect towards later emergence similar to *RhtB1*, but a slight decreasing effect on plant height.

The marker *X1B1R*, which is assumed to show the presence/absence of the 1B1R translocation from rye (Zeller et al., 1973), only showed association with growth habit, where it also exhibits the strongest effect towards more upright growth among the 7 loci. It has been selected against the translocation. It has been found that the translocation confers increased resistance to several diseases and adaptation to low moisture conditions (Fluch et al., 2012). The marker *BS03916* that has been included in order to cover a genomic region previously identified to be associated with height and ear emergence (Griffiths et al., 2009; 2012) shows a pattern different to the other loci. The allele that has increased in frequency confers a decrease in plant height.

The only locus that has been identified as being selected differentially between sites as well through *Fc*, simulation tests and F-statistics was *PpdB1L5*. Also, *BS03916* has been identified with higher *Fc* values in only one out of two populations. Both these loci have a reducing effect on plant height.

To summarise the observations, two different potential patterns could be identified: (1) If the phenotypic effects of the locus that has undergone strongest selection are considered the phenotypic characteristics of adaptation are: increased plant height, reduced grain weight and number, and later ear emergence; however, (2) if the sum of phenotypic effects of all 7 loci are considered the pattern of phenotypic characteristics also shows increased plant height, coupled with increased biomass and later ear emergence, but towards increased grain weight and number.

Implications for evolutionary plant breeding

The general increase in plant height and the observation, that the two loci that have undergone the greatest change of allele frequencies were genes coding for height, suggests that plant height has been the driving force in the evolutionary process. It has been selected for the wild type allele at both genes for dwarfing characteristics, *RhtB1* and *RhtD1*. This observation is confirmed by the observation of Raquin et al. (2008). They showed the increase of *RhtB1* in an experimental population of winter wheat from a frequency of 0.66 in the initial generation to near-complete extinction of the dwarfing allele after 17 generations. The dwarfing alleles at both loci were of major importance during the Green Revolution (Borlaug 1983). It can hypothesised that selection acted towards wild type and thus against ’domestication alleles’. The selection for height can be explained by competition for light between the different genotypes.

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3.4.4 Disease resistance and yield stability of CCP (WP4)

The untreated trials highlighted varieties at the greatest risk of drastic yield loss associated with poor disease resistance. The ranking of varietal performance in untreated trials was heavily influenced by septoria leaf blotch, for which Timber and Gatsby display good resistance. For varieties at higher risk of disease associated yield reductions, a grower will need to consistently monitor the crop and regional disease patterns to ensure that the crop receives the correct fungicide applications at the correct timing in order to protect it from any potential disease epidemics. This is particularly true for Oakley which has a high yield potential, displayed under treated trials (even in 2013 it is 106% of the controls: cereals.ahdb.org.uk), but has poor resistance to septoria (AHDB rating = 5 out of 9). Oakley's resistance to yellow rust dropped from a rating of 9 in 2008-09 to 2 in 2012-13 which led to the variety being dropped from recommended list trials in 2009-10. Despite high susceptibility to yellow rust in 2009 Oakley made up 12% of the certificated seed area in the UK, its popularity due to its high yield potential but also its resistance to orange wheat blossom midge. Oakley represents a high risk variety that remains appealing to many farmers because of the potentially huge returns provided it receives the proper agronomy.

The YQCCP and YQ-mix were consistently amongst those varieties that showed the least reduction in yield in response to disease. They also rated similar to those varieties that had the greatest yield tolerance in response to a reduction of their green leaf area by disease. However, they were also typically amongst the lowest yielding, indicating a low yield potential. This makes them appealing to farmers who have timeliness issues regarding fungicide sprays, or are moving towards being organic or at least less dependent upon high levels of fungicide inputs. Yields for the YQCCP and the YQ-mix appeared to be the most stable, which may appeal to farmers wanting insurance against dramatic yield losses associated with high disease pressure.

The CCP approach to breeding shows potential for future use in both organic and conventional systems if new populations are generated from modern varieties to enable them to achieve yields more comparable to modern elite lines. The high levels of genetic diversity contained within CCP increases the environmental stress buffering capacity of the crop which leads to an increase in ecological stability through processes such as compensation and complementation (Wolfe 1985; Finckh and Wolfe 1998; Mundt 2002). Favourable plant-plant interactions lead to increased stability of the system providing insurance against environmentally induced substantial yield reductions (Yachi and Loreau 1999; for review see Tilman 1996). Application of appropriate levels of crop diversity may buffer against both known and unknown stresses leading to increased yield stability over space and time (Finckh et al., 2000; Creissen, 2013). Together with the results from the current study, this indicates that crop diversity may be capable of replacing high levels of chemical inputs commonly used to buffer against environmental stress in conventional systems.

3.4.5 Participatory farm trials of CCP (WP5)

Pre-harvest parameters

The main difference between pure lines and CCP, consistently observed for the three different CCP across environments was greater plant height in the CCP (Figure 28, page 107), and an associated tendency of increased lodging, though the extent of lodging was generally small and not relevant for grain yield in the YQCCP (Figure 29, page 108).

Principally, greater plant height in the CCP can be seen as a result of two main properties of the CCP. The first is the genetic make-up of the parents; specifically, several parents used for creating the CCP were relatively tall varieties. Second, genetic diversity within the CCP coupled with natural selection may lead to a decrease of the frequency of *Rht* genotypes (i.e those with reduced height) in the population, as observed in WP2 and WP3. Thus, as competition for light within the genetically diverse plant population favours tall phenotypes, it would be expected that mean plant height in the CCP may increase over time.

However, over the (relatively short) time period of this study, such an increase was not observed, suggesting that at this point in time equilibrium may already have been reached, or that other factors may have masked an increase of plant height over time. Greater plant height in cereals has been associated with a better ability of the crop to compete with weeds. However, in this study, weed cover was not significantly affected by the CCP, though this parameter was measured before booting, so that competition effects of increased plant height cannot be assessed in this case.

Yield and quality performance

On average, the YQCCP yielded around 10% less than the common control variety Claire (Figure 30, page 109). To a large degree this yield difference is likely to reflect the parentage of the YQCCP, which was created by crossing 12 high-quality parents and 9 high-yield parents, whereas Claire is classified as a high-yield type. Similar considerations apply to the comparison between YCCP and the high-yield farm-specific control varieties (YOwn). For the QCCP however, yield differences in comparison to the pure line controls were small and not significant (Figure 31, page 110), despite the inclusion of relatively old parents in the QCCP; as before, this may also be seen as a result of the identity of farmer chosen varieties. From this point of view, the highest potential for practical use of CCP is seen in the high-quality QCCP, though consistently low HFN values in the CCP mean that there are some quality limitations.

Stability of performance

In contrast to expectations, and to results obtained in WP1, stability of yield and of other parameters was not significantly higher in the in the CCP than in the pure lines in the field trials of this WP. There are several possible reasons for this result. One is that the pure line variety Claire already shows a high buffering capacity (compared to other pure lines), which may be a reason why this variety has become popular among UK farmers. Another reason may be that functional diversity in the population, to respond to specific stresses that occurred in the on-farm trials was actually not higher than in the pure lines tested. Further, the analysis shows that contrary to expectations, the YQCCP was not consistently better than Claire in low yield environments.

Adaptation trial

As in WP1, and WP2, the WP5 adaptation trial showed no consistent effect that would suggest adaptation to site or management conditions. In the case of the on-farm adaptation trial, the observed results may have been a consequence of differences in seed quality. Reduced seed quality of the North-CON YQCCP may have had a bigger effect on grain yield than adaption to site conditions, as the North-CON YQCCP was also lower in grain yield at the North-CON site than the organic YQCCP from the South. Also, the time of three generations may have been too short for adaptation to take place. Especially in relation to the environmental fluctuations over years within each site, there may not have been a consistent direction of environmental factors influencing the CCP.

3.4.6 Performance of CCP with additional parent material (WP6)

This study showed that the inclusion of crosses from an additional parent to a CCP did not significantly improve the yield or grain quality characteristics of the population. In this study the addition of new

genetic material into the CCP, through crosses with varieties predicted to increase yield (Pegassos) or quality (Xi19), had no significant impact on the performance of the CCP.

Grain yield and protein content differed between sites; however there were few significant differences between entries within each site (Figure 38 and Figure 39, page 119). Only the YCCP and the QCCP differed significantly in terms of protein content (% dry matter), but not grain yield. Evidence suggests that there may be some negative impacts on crop performance associated with the additional parents. There were indications that the addition of Pegassos to the YCCP, and Xi19+Pegassos to the YQCCP may actually reduce both the average grain yield and protein content at the WAF site, however this difference was not statistically significant.

This finding is supported by other work showing that the yield and performance of variety mixtures cannot be easily predicted by the yield of components grown in monoculture due to competitive interactions between genotypes in a heterogeneous crop (Mundt et al., 1995, Newton et al*.,* 2008). The effect of additional crosses, based on the properties of the parent, would be even less predictable in this case due to the much higher levels of diversity in the populations. Although a more genetically diverse population would possess greater potential adaptability to a wider range of extreme or marginal environmental conditions, choice of too many parent varieties covering too large a range of genetic diversity can lead to a greater proportion of unadapted traits and reduced performance of the CCP. Crossing fewer parental varieties would enable a larger number of F1s to be created and a higher rate of recombination within a smaller but more predictable range of genes available (Döring et al*.,* 2011b). Additionally, as neither Xi19 nor Pegassos was included as pure line trial plots there is also no evidence from this study to demonstrate if the additional parent varieties alone do have superior quality of yield properties than the original populations.

The adaptive ability of the CCP would be better investigated experimentally by recreating the full set of initial crosses that make up the original population, or by using seed from earlier generations of each population as a comparison to later generations. Adaptable crops are likely to show increased stability in unpredictable environments which are predicted to become more frequent due to climate change (Morton, 2007).

3.4.7 The effects of mass selection on CCP (WP7)

The main objectives of this research was to ascertain (1) the usefulness of the mass selection tools; whether they are effective as on-farm grain processing tools to separate higher quality grain, and then (2) if these effects are heritable and can be used as a mass selection breeding tool to improve the quality of CCP. Each of the three mass selection methods is considered.

Colour selection

When post selection data was analysed from all four years, colour selection showed statistically significant increases in grain protein content across the four years of the trials. Colour selection for dark grain was able to increase the grain protein content (% dry basis) by an average of 0.15%. These differences however are not consistent in every year of the trial as the magnitude of selection is variable at different selection events (Figure 40, 121). The results after selection in 2011 are anomalous in that selecting for dark grain decreased protein content by 1.06% compared to unselected grain. It is unclear if this may be due to operator error during selection or whether there are other significant environmentally controlled factors, other than grain colour, affecting protein content. However, in general this experiment confirms the link between dark coloured grain and increased protein, but it debateable as to whether this increase is large enough to be of commercial use to a farmer.

By analysing the grain protein content of the progeny of the selected grain fractions we can test how heritable these selected differences are. Results from the four years showed that there are significant differences at the second harvest (H2) and fourth harvest (H4) in protein content between progeny of the dark and light selected grain. However neither of the light or dark selected grain significantly differed from the unselected, therefore selecting for darker grain did not significantly increase the protein content of the grain above that of the grain before selection.

This significant difference between light and dark grain progeny at H2 could be related to the fact that the selection event immediately prior to this created the greatest differences between the light and dark fractions out of the four years. This would suggest that if there is enough variation in grain colour, and that the selection method is able to separate them with great accuracy, then there could be a greater difference in the protein content of the subsequent progeny. However by selecting only a small fraction of grain the available genetic diversity within the population may be decreased. As the magnitude of mass selection differs year to year there are likely to be inherent factors involved such as the machine operator experience and/or environmentally controlled year to year variations in the range of grain colour in the CCP that is available for selection.

Considering the cumulative effect of repeatedly selecting the darker grain over the four years there does not appear to be an increasing difference observed in the protein content of the progeny of the fractions. Any difference separated by the colour selection quickly reverts back in subsequent generations to being almost insignificantly different to that of the unselected. The trial in the fourth year also included six replicates rather than three to better examine any significant differences between the fractions after four years of selection. There were still only significant differences found between the light and dark fraction but not the unselected control. This means that the quality improvements of increased protein content by colour selection would quickly be lost in subsequent generations of the CCP.

If methods of colour selection were able to improve and increase the protein content of a CCP for subsequent generations this could address the issue of a negative correlation between grain yield and

protein content (Simmonds, 1995). As a CCP may be evolving to include more high yielding genotypes there may be a trend of reducing protein content and a trade-off associated with this trend. However, when considering yield data from the last year of the trial there were no significant differences in yield or TGW between any of the fractions. If there is such a trade-off then choice of parental varieties for creating new populations should consider high protein material (Döring et al., 2011b).

Size selection

The effect of selecting for grain size was measured as the thousand grain weight (TGW). The trials were only carried out over two growing seasons.

To test how useful size selection is at separating the grain into different size fractions the TGW was measured immediately after selection. In the first year the largest fraction the TGW was significantly greater than all the other fractions including the control (Figure 41, page 123). In the second year there were even greater average differences between the fractions with the largest fraction having a TGW of 17.54g greater than the smallest fraction. This suggests that the range of available grain sizes to select is large enough and the selection method is effective at separating grain into significantly larger fractions. This could then be of potential use to the farmer as larger grain will have better initial competitive ability being more likely to develop into a more vigorous plant (Döring et al., 2011a), thus increasing the overall crop yield.

By measuring the TGW of the progeny of the selected size fractions it was possible to test how heritable these differences in grain size are. TGW data from progeny of the grain after the first selection event showed that the large fraction was significantly greater than both the medium and small fractions, however none of the fractions differed significantly from the unselected control. In the second year however there were no significant differences between any of the fractions even though there were consistent trends in the average TGW of the fractions. By combining the data from both years only the large and small fractions significantly differed in TGW so there is still not a significant heritable improvement in TGW by mass selection from the original unselected grain. This study over only two growing seasons would not be enough to examine if there is a cumulative effect of repeated size selection on a population over a greater number of generations.

Because in neither of the two years did the yields differ significantly between the fractions, the small heritable increase in TGW does not seen to translate into higher yields. This could be explained by the suggestion that the morphology of the wheat ear limits available space for increased grain size which would increase yield (Döring et al., 2011b).

Density selection

Similarly to size selection, the effects of selecting for grain density are examined by measuring the TGW. Trials on density selection were also carried out over two years.

To evaluate the usefulness of density selection as a tool for on farm selection to separate fractions of better quality grains, the TGW was measured immediately after selection. The results from the first year showed that the TGW of the heaviest selected fraction was significantly greater than that of the unselected control (Figure 42, page 125). This suggests that the density selection is potentially an effective method for separating grain. However, considering data from the second year, the average difference between the heavy and light fraction was 9.4g which is less than for size selection.

By measuring the TGW of the progeny of the selected density fractions it was possible to test the heritability of these differences in grain density. The results showed that in both years none of the fractions significantly differed in TGW or yield suggesting very little to no heritability of density selected grain. This therefore highlights limited use of density selection as a tool to improve the quality or yield of a population.

Summary

In conclusion, the results of this study show that phenotypic traits of grain such as colour, density and size, which are linked to desirable quality characteristics such as grain protein and TGW, can be selected for using on farm mass selection tools. This means that the diversity of grain colour, size and density is available in populations and the selection methods are able to separate grain into fractions. It is still unclear how useful these separated fractions would be. Whether a premium for a better quality fraction of grain could be achieved or whether increased TGW of seed can give better agronomic performance still needs to be investigated.

However, after re-sowing and harvesting the progeny of these fractions, only the selection methods for grain colour and size showed any consistent difference between the most diverged fractions. There was also rarely any consistent improvement of significant magnitude of the selected fraction over the un-selected control. Therefore, the current selection methods investigated show limited potential as breeding tools to improve the quality or yield of a population. The results suggest these differences in desirable traits have only limited heritability and at relatively small magnitudes, suggesting that mass selection methods need to be improved to be useful as breeding tools for populations.

One reason for the low heritability of these traits may be that, because there is a broad range in both the size and colour of grains within the same wheat ear, the selection method may only be selecting the larger grains from the centre of all the ears or darker grains from the extremities of all the ears rather than selecting for plants that produce larger or darker grains. As there is a link between darkness and higher protein content and that there is a negative link between grain size and protein, then one possible approach would be to select for grain size and colour simultaneously. This could potentially give more

heritable results as it would then be selecting for genotypes whose largest grains in the centre of the ear are also darker because of higher protein content.

3.4.8 Bread-making qualities and micronutrient content of CCP (WP8)

Potential obstacles exist to the use of CCP as milling wheats. Genetic variability for maturation time could cause some plants to be over-ripe by the time others are ready to be harvested, leading to sprouting in a significant proportion of the crop. If so, it might deplete the milling quality by initiating digestion of the long-chain starches (e.g. Ichinose et al. 2001). Initiation of starch digestion is commonly measured by the HFN test, and the effects of low HFN (below 250 seconds) have been shown to include reduced dough water absorption capacity leading to poor consistency and reduced loaf volume (Dowell et al. 2008). It is possible that CCP may have lower average grain protein relative to high-performance pure lines, since with all the novel gene combinations, some plants are likely to have lower protein than their parental pure lines but less likely to have more, since breeders have been effective at identifying and isolating gene combinations generating maximum protein content. Grain and flour protein content are one of the most important predictors of dough quality and loaf volume; while aspects of protein quality such as gluten index or glutenin to gliadin ratio are also important in contributing to loaf outcomes, they have been demonstrated to be highly correlated with total protein content (Dowell et al. 2008).

In addition, it is possible that the addition of 'Yield' parents to the 'Quality' population may have diluted the baking quality of the QCCP. The QCCP is derived solely parent lines bred specifically for the milling and bread-making market, having properties such as high protein content, high hardness and starchy gluten (Wolfe et al., 2006). The parents of the YQCCP, in contrast, included a further 10 lines bred for high yield and targeted at the animal feed market (see 3.2.1), which may have reduced the population's performance relative to the QCCP for baking.

Baking tests

CCP flours were not consistently worse than commercial alternatives as bread-making material (Figure 43, page 127 and Figure 44, page 128). In addition, the yield advantage represented by the YQCCP over the QCCP (see 3.4.1) does not come at the expense of significant quality depletion.

Results suggest that site effects on CCP breadmaking qualities were very strong. It is of interest for farmers in particular to know whether such effects are stronger for CCP than they are for pure line varieties. Ideally, each of the CCP samples tested should be compared against one or more quality pure line controls grown at the same site, and this could potentially be attempted as part of future research activities. Also, more rigorous testing is needed to establish the dynamics of CCP over time. It was beyond the limits of the present study's dataset to conduct proper year-to-year comparisons of the two

CCP and to test whether their performance is depleted or improved, but this is another important aspect of their suitability as commercial prospects.

This research raises further questions about bread quality evaluations and the way they are used to inform wheat breeding work. In the scientific literature, bread quality is generally evaluated on the basis of dough properties such as water absorption, extensibility and mix time in addition to volume and sometimes crumb texture of the baked loaf (e.g. Najafian, 2012; Osman et al*.,* 2012; Różyło & Laskowski, 2011). This reflects an orientation towards the needs of larger-scale producers for a regularised bread-making process and easily measurable results. There has been a lot of research effort invested in elucidating relationships between loaf volume, dough properties and grain parameters (see e.g. Dowell et al*.,* 2008). While many factors are acknowledged to contribute to loaf volume, grain protein tends to have the strongest relationship of all, with the result that loaf volume has become a proxy for bread quality and grain protein content has become the dominant criterion for predicting the bread-making quality of grain.

The use of these conventions seems to have gone largely unquestioned in the literature, which is perhaps surprising, since loaf volume may not be the only or indeed the most valuable way to assess bread quality, with other parameters such as texture and flavour also being important, although with less readily usable measurement conventions available.

In baking tests, there was not a strong correlation between grain protein and loaf height, either in the Chorleywood process or in the sourdough process: the R^2 correlation coefficient for protein and loaf height as measured in all four years at Marriages was 0.10 and that for protein and loaf height as measured in years one through three at Bread Matters (individual sample protein content was not available for year four) was 0.11. This is in contrast to a correlation between grain protein and loaf volume of $R^2 = 0.84$ obtained by Dowell et al. in their 2008 study. It should be noted that CCP flours with relatively low protein, in the range 10%–13%, produced loaves which were considered of adequate height to professional bakers. (Whether or not lower-protein pure line flours would perform in the same way cannot be determined from this dataset.)

Qualitative evaluation results from bakers also violated the conventional three-way association between high grain protein, high loaf volume and high quality. Bakers' most positive quality evaluations often went to samples with low grain protein. For example, WH Marriage & Sons in trial years 2 and 4 singled out low-protein flours (10.3% and 10.8% dry basis, respectively) as being the top amongst a sample set which included flours of more than 15% protein, and the top-selected loaves were also not the tallest. Among the samples tested for the present study, there did not seem to be a need for high protein content to produce loaves that were satisfactory under both industrial and artisanal conditions. This suggests that there may be potential to identify other quality predictors.

These results do, of course, reflect the subjectivity of bread quality assessments. The baking tests were not limited to objectively measurable criteria, and this renders results immediately less consistent. The complexity of consumers' bread quality perceptions is very great (see e.g. Hersleth et al., 2005), and

difficult to capture and to manage in large-scale comparative evaluations. Arguably, finding ways of integrating more of this information into bread-wheat breeding programmes could deliver greatly improved outcomes.

Micronutrients

Although the pure line vs population comparison undertaken in this project is not statistically powerful due to the lack of proper replication and the small sample number, every indication from the results is that the populations do not represent a penalty in terms of mineral nutrition, relative to pure line commercial wheat varieties (Figure 46, page 134). Beyond this, any conclusions about the effects of genetic diversity on mineral accumulation ability of the crop can only be tentative.

A marked tendency emerges from the data for diversified wheat lines to accumulate greater grain mineral concentrations than pure lines, even when samples are compared from within one site, and it may be interesting to undertake further work to explore whether this tendency can be demonstrated more conclusively and to identify the mechanisms at work.

Shewry (2009) reports on three studies where mineral concentration in wheat was found to be negatively correlated with yield due to dilution effects from an expanded endosperm. In the same paper, he also reports on a posited association between straw height and grain mineral concentration: “Although the decrease in the mineral content of modern wheats is partly due to dilution, resulting from increased yield (which was negatively correlated with mineral content), it has been suggested that short-strawed varieties may be intrinsically less efficient at partitioning minerals to the grain compared with the translocation of photosynthate.”

Both of these effects could contribute to the explanation of patterns in the dataset. Firstly, apparent ‘entry’ effects (e.g. YQCCP, Hereward etc.) may also be due to reduced yield of those entries; for example, the trend for CCP to show higher mineral concentration may have been associated with lower per-hectare yields than those shown by the pure-line entries. Secondly, the enhanced mineral content of the CCP relative to the pure lines may be related to the increase in their straw height which has occurred over time (see 3.4.2 and 3.4.3). With neither straw-height nor yield data available for these samples, we are not able to investigate these theories, but they should be included as an element of any further studies to understand the physiological mechanisms at work.

The topic of mineral concentration decreases observed over time in cultivated wheat germplasm has attracted increasing scientific interest in recent years, and multiple studies have been devoted to searches among wheat wild relatives or older wheat lines for genes associated with high mineral concentrations (e.g. Shewry et al*.,* 2011; Fan et al., 2008; Oury et al*.,* 2006). There is strong interest in incorporating such germplasm into high yielding and high quality wheat lines, and a population approach could be a rapid and effective way of doing so.

3.4.9 Acceptability of CCP for malting, distilling and animal feed (WP9)

Malting

Conventional management systems led to higher grain nitrogen content, but the organic management system resulted in a final malting product with a higher soluble nitrogen ratio (Table 50, page 136). The latter is a consequence of the organic grain having similar total soluble nitrogen to the conventionally grown grain, but lower total nitrogen levels, which Crisp Malting report is a tendency often seen in the malting of organic cereals. Overall, there were very few significant differences detected in comparisons between years, sites and populations with respect to the parameters measured by micro-malting tests. In addition, there are acceptable standard deviations (operational tolerances) associated with each of the methods used to determine total nitrogen (±0.028), total soluble nitrogen (±0.025), extract (±1.5) and diastatic power (±7.5) which should be taken into consideration when interpreting statistical significance in the analyses presented; clearly the impact will be most critical when significance levels are close to borderline.

In summary, these results do not show any trends which suggest that the wheat CCP offer something 'new' to the malting industry. In fact, in terms of overall values for key malting parameters, the results indicate they have limited potential for use in malting. However, these particular populations were not developed specifically with malting as an end-use target; hence the inclusion of a number of high protein parents. The concept of populations could still be of value in developing a malting grain that would have an advantage over pure-lines of high genetic diversity to buffer against increasingly extreme seasonal weather fluctuations.

Distilling

The variables tested by SWRI are used to evaluate the suitability of grain for distilling purposes because they are all related to the final alcohol yield, which is the main outcome of interest. The alcohol yield of the grain tested was not significantly affected by crop management system or growing site. In fact, the only factor that had an impact on the alcohol yield was the population type, with the YCCP giving a higher yield than the YQCCP. As explained in the results section, this is most probably due to the higher nitrogen levels in the YQCCP (and consequently lower starch levels), which are inversely related to alcohol yield (Brosnan et al., 1999). However, results obtained under laboratory conditions using small samples can often fail to show statistical significance compared to industrial processes where the effects of massive scale-up operations apply; nevertheless due to the relatively low alcohol yield from the populations compared to pure line wheat varieties already commercially available and because, in addition, the site and agricultural system had no effect on this outcome, it was concluded that these

particular populations do not show potential for use in distilling. For this reason the distilling tests were not carried forward into the project after year 1 (2007-08).

Animal Feed

The frequency of the 1R chromosome decreased in advanced generations of both the YCCP and YQCCP compared to the starting frequencies, and this could be observed as continuing in the majority of the populations from the F_7 to F_8. This suggested strong selection against this chromosome segment. The reasons for this decrease are not clear. They could be due to increased disease susceptibility as a result of the virulence of new isolates of yellow rust and brown rust to the resistance genes known to be carried on the 1R arm. Against this argument is the fact that the decrease occurred at both organic (unsprayed) and conventional (sprayed) paired sites. Unfortunately, detailed disease data are not available to test this hypothesis.

In the YQCCP samples, the frequency of the 1R arm was usually less than 10% of the seed. At this frequency, the arm is unlikely to impart any significant detrimental effect on quality due to the dilution effect of the 1BS arm. Therefore, these results suggest that the use of the seed of the YQCCP for bread making and the YCCP for animal feed will not be impaired by the low frequency of the rye genes.

3.5 Conclusions – Challenges of evolutionary plant breeding

Seed health

Using healthy, vital seed is essential for all farming systems, but low-input and organic farming are particularly vulnerable to the consequences of low seed vitality. Seed infected with seed-borne diseases can produce healthy plants if treated with fungicides, but the availability of effective seed treatments is limited for organic farmers. Since conventional cereal breeding has given low priority to seed-borne diseases (Matanguihan et al. 2011), resistant and agronomically appropriate genotypes are difficult to obtain. In addition, seed health is particularly important for evolutionary plant breeding (Döring et al. 2011b, Howlett et al. 2011). This is because farm-saving of seed over many plant generations is instrumental in this breeding approach, so that it is vulnerable to seed-borne diseases building up over time (this is one of the key reasons for crop rotation as a management tool). As a consequence, all agro-ecological breeding activities, but in particular breeding approaches relying on farm-saving of seed, need to ensure high seed vitality and would benefit hugely from more durable resistance and effective organic treatments against seed-borne diseases. Conversely, evolutionary breeding can be used as a tool to identify germplasm with high field resistance to seed borne diseases (Steffan et al., 2013). A seed-borne

disease with high relevance to organic agriculture is dwarf bunt, which has become more widespread in recent years (Huss & Buerstmayr, 2011; Voit & Killermann, 2011), but which is more difficult to control than common bunt on which most research and breeding has focused so far. No winter wheat cultivars with acceptable dwarf bunt resistance are currently available for organic wheat production in Europe. Dwarf bunt resistance is usually found in old cultivars or landraces. A further aspect with relevance to the central issue of seed health is the development of advanced non-destructive diagnostic tools. Recent investigations indicate that multispectral techniques have the potential to identify infected from non-infected seeds and viable seeds can be identified from non-viable seeds by the use of near-infrared spectroscopy (NIRS; Peiris et al., 2009).

Multiple stresses

As mentioned above, varieties are needed that can cope with multiple, and (increasingly) variable stresses. While evolutionary plant breeding offers a potential solution, as genetic diversity within the crop populations buffers against such stresses, it is currently unknown how much, at what spatial level, and what kind of diversity is needed in relation to specialist approaches (such as varietal drought resistance), for building resilient cropping systems that can withstand multiple and variable stresses. One approach is the use of evolutionary plant breeding to increase the tolerance of variability through exposure of genetically diverse populations to highly variable conditions.

Funding and regulations

Across Europe new approaches are being developed for breeding varieties better adapted to organic and low-input conditions. In many cases, such varieties have higher levels of genetic diversity than currently allowed, and often do not meet the current European legal DUS standards (Finckh, 2007). An initial hurdle therefore lies in the current legal framework of plant breeding. The development of pure line breeding in the context of the industrial agriculture revolution over the last hundred years has led to a system which, although it may be regarded as highly productive, is also highly restrictive and expensive in terms of resources, as noted above. In its original intention the system was set up to protect the farmer from unscrupulous breeders and seed merchants. Currently, however, it can be regarded as a system to protect breeders and seed merchants from the farmer and change, and, as such, inhibits the agroecological approach to crop production.

As the variety laws stand at present, populations of wheat and other self-pollinated crops cannot be legally traded; currently, the only legal possibility would be to limit the use of populations to closed contract deals involving an end-user. Ironically, the use and trading of variety mixtures is permitted in the EU – the seed lot simply requires a label declaring the varieties used in the mixture. Unfortunately, a seed lot of a population, even though it could be labelled in a similar way, is not permitted. Very recently

however, legislative space has been created by allowing a ‘temporary experiment’ on the trade of CCP in Europe. The objective is to determine whether an agreed protocol for sale and distribution of populations, based largely on the availability of a transparent database of origin and previous use of the populations, will be sufficient to protect both farmers and breeders from exploitation. The system used would retain the current protocols for ensuring healthy seed.

A more radical approach would be to follow the Open Access system currently used in software development (Kloppenburg, 2005). This would eliminate the charging involved in the system. Anyone involved in population production would have to accept that the material that they produced would be freely available (other than seed production costs) to anyone who wanted to exploit it further. If the population approach and its potential applications do prove to be biologically sound and desirable in a time of climate change and resource scarcity, then a lack of action over the law could lead rather quickly to an anarchical approach, which may not be satisfactory, for anybody.

In addition, further adjustments to the current registration processes for organic varieties are necessary (Bocci & Chable, 2008). In particular, the breeding sector is hesitant to invest in breeding for organic and low-input agriculture, the main reason being the relatively small market (Döring et al., 2013) Crucial questions are therefore how to develop bridges between the breeding sector and the respective food sectors; how to ensure that collaborations continue to thrive after being established; and to find options for sustainable financing models for agroecological approaches to plant breeding. Although coordinated activities have started, current activities in agroecological plant breeding are still fragmented and the agroecological breeding sector is under-developed, showing structural weaknesses. Better transnational coordination is therefore needed to exchange breeding material, to harmonize evaluation tests, and to exchange experiences and results.

3.6 Output and dissemination record

3.6.1 Publications

Dissemination articles

Döring, T. F. (2010): *Weatherproofing Wheat*. In: Elements. Science for a sustainable planet. Food and Farming Edition. London UK: Defra, p. 6-7.

Döring, T. F., Pearce, H. (2012): Baking quality of genetically diverse wheat populations. *The Organic Research Centre Bulletin* 109: 9.

Döring, T. F., Smith, J., Wolfe, M. (2010): The audacity of the obvious. *The Organic Research Centre Bulletin* 100: 6-7.

Döring, T. F., Wolfe, M. S. (2009): Stabilising wheat yields – can genetic diversity increase reliability of wheat performance? *The Arable Group Research Bulletin* 22: 10-11.

Howlett, S., Döring, T. F., Winkler, L., Wolfe, M. (2010): Breeding biodiversity. *The Organic Research Centre Bulletin* 103: 6-7.

Howlett, S., Pearce, H., Winkler, L., Döring, T. F. (2011): Battling with bunt. *The Organic Research Centre Bulletin* 107: 4-5.

Knapp, S., Griffiths, S., Snape, J., Döring, T. F., Wolfe, M., Jones, H. (2012): Do wheat populations adapt to organic farming? *The Organic Research Centre Bulletin* 110: 11.

Winkler, L. (2011): The Heritage Loaf. *The Organic Research Centre Bulletin* 107: 6.

Scientific publications

Döring, T. F., Crowley, O., Wolfe, M. S. (2011): Against the grain. *Organic Farming* 107: 42-43.

Döring, T. F., Knapp, S., Kovacs, G., Wolfe, M. S., Murphy, K. (2011): Evolutionary plant breeding in cereals – into a new era. *Sustainability* 3: 1944-1971.

Book chapters

Döring T. F., Pautasso, M., Finckh, M. R., Wolfe, M. S. (2012): Pest and disease management in organic farming: implications and inspirations for plant breeding. In: Organic Crop Breeding, edited by Lammerts van Bueren, E., Myers, J. Chichester, UK: Wiley.

Conference proceedings

Crowley, O., et al. (2013): Is mass selection a tool to improve quality in winter wheat composite cross populations? International Symposium on Evolutionary Breeding in Cereals. Aston University, Birmingham – 21 January 2013.

Döring, T. F. (2010): Genetic, ecological and economic approaches to improve yield reliability in cereals. Organic Producer Conference. 8–9. Jan. 2010, Harper Adams College, UK.

Döring, T., Grosse, M., Wolfe, M. (2010): Growing together – evolution of plant height in wheat composite cross populations. In: Breeding for resilience: a strategy for organic and low-input farming sytems? Edited by Goldringer, I., Lammerts van Bueren, E. Paris, France: Eucarpia 2nd Conference of the Organic and Low-Input Agriculture Section. 1-3 December 2010, p 35.

Döring, T. F., Howlett, S. A., Fradgley, N., Winkler, L. R., Wolfe, M. S. (2013): Agronomic performance of mixtures of pure lines and composite cross populations. EUCARPIA Breeding for Nutrient Efficiency - Joint Meeting of EUCARPIA Section Organic & Low-Input Agriculture and EU NUE-CROPS Project, 24-26. September 2013, University of Göttingen, Germany, p. 70.

Döring TF, Wolfe M, Jones H, Pearce H, Zhan J (2010): *Breeding for resilience in wheat - Nature's choice.* In: Goldringer I, Lammerts van Bueren E (Eds.): Breeding for resilience: a strategy for organic and low-input farming sytems? Edited by Goldringer, I., Lammerts van Bueren, E. Paris, France: Eucarpia 2nd Conference of the Organic and Low-Input Agriculture Section. 1-3 December 2010, p 45-48.

Knapp, S., et al. (2013): Genetic analysis of evolving winter wheat populations reveals reversion to wild type. International Symposium on Evolutionary Breeding in Cereals. Aston University, Birmingham – 21 January 2013.

Winkler, L.R., et al. (2013): Baking quality of two winter wheat CCPs in the UK. International Symposium on Evolutionary Breeding in Cereals. Aston University, Birmingham - 21 January 2013.

Wolfe, M., Boyd, H. E., Clarke, S., Haigh, Z. E. L. and Jones, H. (2008): *Wheat populations: stability in an increasingly unstable environment.* In: Aspects of Applied Biology 88, Effects of Climate Change on Plants: Implications for Agriculture, Rothamsted Research, Harpenden, Herts, 12-13 November 2008, p.61-68.

Wolfe, M., Döring, T. F. (2010): *Steps towards an ecological future.* In: Goldringer I, Lammerts van Bueren E (Eds.): Breeding for resilience: a strategy for organic and low-input farming sytems? Edited by Goldringer, I., Lammerts van Bueren, E. Paris, France: Eucarpia 2nd Conference of the Organic and Low-Input Agriculture Section. 1-3 December 2010, p 38-41.

Wolfe, M.S., et al. (2013): Adaptive winter wheat populations in the UK: selected results. In: Döring TF, Howlett S, Winkler LR, Wolfe MS (Eds.): International Symposium on Evolutionary Breeding in Cereals. Aston University, Birmingham – 21 January 2013.

3.6.2 Events

Date	Audience	Location	Title	KT activities	Partners responsible
12-13/11/08	Researchers	Hertfordshire	Association of Applied Biologists meeting on the 'Effects of Climate Change on Plants: Implications for Agriculture'	Introduced the Wheat Breeding LINK project to international researchers.	ORC
6/09	Farmers	Norfolk	NIAB TAG conference / training events	Wheat Breeding LINK introduced with demonstration plots, as part of presentation on the latest agricultural research.	NIAB TAG
10-11/06/09	Farmers	Hertfordshire	Cereals	Presenting Wheat Breeding LINK to farmers with demonstration plots.	ORC
18/06/09	Public	Suffolk	Wakelyns open day 2009	Wheat Breeding LINK introduced with demonstration plots, as part of presentation on the latest agricultural research.	ORC
30/06/09	Farmers	Berkshire	ORC Arable Day	Discussion of new research findings: Wheat breeding and wheat agronomy.	ORC
8-9/01/10	Farmers	Gloucestershire	ORC Producer Conference	Workshop held on genetic, ecological and economic approaches to improve yield reliability in cereals.	ORC
15/06/10	Public	Suffolk	Wakelyns open day 2010	Wheat Breeding LINK was presented and the trial plots shown to attendees.	ORC
06/10	Farmers	Norfolk	NIAB TAG conference / training events	Wheat Breeding LINK introduced with demonstration plots, as part of presentation on the latest agricultural research.	NIAB TAG
16/07/10	Public	Berkshire	ORC open day 2010	Visit to the ORC Wheat Breeding LINK trials on Sheepdrove Organic Farm.	ORC

1-3/12/10	Researchers	Paris, France	Eucarpia 2nd Conference of the Organic and Low-Input Agriculture Section	Presentation of research on Wheat Breeding LINK to international researchers.	ORC
22/06/11	Public	Suffolk	Wakelyns open day 2010	Wheat Breeding LINK was presented and the trial plots shown to attendees.	ORC
06/11	Farmers	Norfolk	NIAB TAG conference / training events	Wheat Breeding LINK introduced with demonstration plots, as part of presentation on the latest agricultural research.	NIAB TAG
Spring/Summer 2008,9,10,11 & 12	Farmers & Advisors	Norfolk	Various NIAB TAG events	Field studies.	NIAB TAG
Autumn 2008,9,10,11 & 12	Agronomists	Norfolk	NIAB TAG agronomist meetings	Data presentation.	NIAB TAG

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4 APPENDIX

4.1 Appendix A: Plant protection products applied at Metfield in WP1

Date	Product	Function	App. rate	unit
12.09.2007	Alpha Triflu---alin EC	Grass and BL weed control	2	lt/ha
12.09.2007	Crystal	Blackgrass and BL weeds	4	lt/ha
02.11.2007	Omex liquid Manganese	Trace element	3	lt/ha
02.11.2007	Permasect c	Aphid	0.25	lt/ha
22.02.2008	Atlantis WG	Blackgrass/ryegrass	0.4	kg/ha
22.02.2008	Biopower	Wetter	1	lt/ha
18.03.2008	Ally Max 5x	BL weeds	30	gms/ha
20.03.2008	BASF 3C CHLORMEQUAT 720	Growth regulator	1.25	lt/ha
20.03.2008	Bravo 500	Disease control	1	lt/ha
20.03.2008	Manganese sulphate	Nutrient	3	lt/ha
20.03.2008	Moddus	Growth regulator	0.15	lt/ha
20.03.2008	Venture	Disease control	0.75	lt/ha
04.05.2008	Amistar Opti	T2 Disease control	1	lt/ha
04.05.2008	Nionic 90	?	0.06	lt/ha
04.05.2008	Opus	Disease control	0.5	lt/ha
04.05.2008	Terpal	Growth regulator	1.25	lt/ha
21.05.2008	Firefly	Disease control	0.75	lt/ha
21.05.2008	Hallmark with zeon tech	Pest control	0.05	lt/ha
22.09.2008	Alpha Triflu---alin EC	Grass and BL weed control	2	lt/ha
22.09.2008	Crystal	Blackgrass and BL weeds	4	lt/ha
17.11.2008	Omex liquid Manganese	Trace element	3	lt/ha
17.11.2008	Permasect c	Aphid	0.25	lt/ha
08.03.2009	Atlantis WG	Blackgrass/ryegrass	0.4	kh/ha
08.03.2009	Biopower	Wetter	1	lt/ha
25.03.2009	BASF 3C CHLORMEQUAT 720	Growth regulator	2.25	lt/ha
25.03.2009	Bravo 500	Disease control	1	lt/ha
25.03.2009	Omex liquid Manganese	Nutrient	3	lt/ha
25.03.2009	Totem	eye spot disease	0.75	lt/ha
04.05.2009	Amistar Opti	T2 Disease control	1	lt/ha
04.05.2009	Proline	Disease control	0.5	lt/ha
04.05.2009	Tern	Mildew	0.5	lt/ha
04.05.2009	Tomahawk	Cleavers	0.5	lt/ha
04.05.2009	Activator 90	Adjuvant	0.05	lt/ha
04.05.2009	Terpal	Growth regulator	0.75	lt/ha
20.05.2009	Firefly	Disease control	1	lt/ha
20.05.2009	Hallmark with zeon tech	Pest control	0.05	lt/ha
03.09.2009	Crystal	Blackgrass and BL weeds	4	lt/ha
29.10.2009	Omex liquid Manganese	Trace element	3	lt/ha
29.10.2009	Permasect c	Aphid	0.25	lt/ha

23.03.2010	Ally Max 5x	BL weeds	30	gms/ha
23.03.2010	Atlantis WG	Blackgrass/ryegrass	0.4	kg/ha
23.03.2010	Biopower	Wetter	1	lt/ha
08.04.2010	BASF 3C CHLORMEQUAT 720	Growth regulator	2.25	lt/ha
08.04.2010	Bravo 500	Disease control	1	lt/ha
08.04.2010	Omex liquid Manganese	Nutrient	3	lt/ha
08.04.2010	Tracker	eyespot disease	0.8	lt/ha
03.05.2010	Activator 90	Adjuvant	0.05	lt/ha
03.05.2010	Amistar Opti	T2 Disease control	1	lt/ha
03.05.2010	Instinct	Mildew	0.4	lt/ha
03.05.2010	Opus	Disease control	0.7	lt/ha
03.05.2010	Terpal	Growth regulator	0.75	lt/ha
26.05.2010	Firefly	Disease control	1	lt/ha
26.05.2010	Hallmark with zeon tech	Pest control	0.05	lt/ha

4.2 Appendix B: Baking methods

Trial Year 1 (harvest year 2008)			
Bakery	Flour	Recipe	Methodology
WH Marriage & Sons Ltd.	Wholemeal roller-milled	Flour 100%; Improver 0.5%; Salt 1.3%; Fungal alpha amylase 0.04%; Yeast 3%; Water as required.	The test bake method was a **standard 10 minute no-time dough process**. All mixing was carried out on planetary mixers. The flour, 1kg, was mixed for 1 minute slow speed and 7 minutes 2nd speed, Rested for 10 minutes, the dough is then scaled at 460g, moulded round and then rested for a further 10 minutes before being moulded into 1lb bread tins. The doughs were proved to the height of 12.0cm or for 60 minutes, whichever came first. The final dough was baked in deck ovens at 230˚C for 20 minutes or as required.
Shipton Mill	White roller-milled	Flour 100%; salt 2%; yeast 2%; water 58-62%.	**30 minute bulk fermentation**. 30mins bulk fermentation was followed by 15 minutes intermediate proof and 35 minutes final proof.
Bread Matters	Wholemeal stone-milled	All samples were tested twice using the following recipes: 1. 8-hour dough • Sample flour, 1000g • Organic dried yeast (Agrano), 4g • Water, 675g (approx) • Salt, 8g 2. 20-hour dough with 16-hour sponge • Sponge: Sample flour 450g; Organic dried yeast (Agrano), 4g; Water, 350ml. • Dough: Sponge 800g; Sample flour 550g; Water 300g (approx); Salt 8g.	**Long fermentation (two methods per sample).** Doughs were mixed on a Sammic variable speed planetary mixer, fermented at ambient temperature, divided and moulded by hand and finally proved in a rack under cloth covers again at ambient temperature (approx 21°C). The loaves were baked in a brick oven at 230°C. **1. 8-hour dough** •Dissolve dried yeast in some of the water. Mix dough for 2 minutes on slow speed (Sammic 1), then 5 minutes on medium (Sammic 4). Bulk ferment for 5 hours, with knock-back after 4 hours. Scale at 500 g. Mould. Prove for 60-80 minutes at ambient (20°C). Bake at 230°C for 20 minutes. **2. 20-hour dough with 16-hour sponge** • Sponge: Dissolve dried yeast in some of the water. Mix sponge dough for one minute by hand. Ferment for 16 hours at ambient (20°C). • Dough: Add flour and salt to sponge and sufficient water to make the dough. Mix at slow speed for 2 minutes and medium for 4 minutes. Bulk ferment for 2 hours. Scale at 500 g. Mould. Prove for 60-90 minutes at ambient (20°C). Bake at 230°C for 20 minutes.

Trial Year 2 (harvest year 2009)			
Bakery	Flour	Recipe	Methodology
WH Marriage & Sons Ltd.	White roller-milled	Flour 100%; Improver 0.5%; Salt 1.3%; Fungal alpha amylase 0.04%; Yeast 3%; Water as required.	The test bake method was a **standard 10 minute no-time dough process**. All mixing was carried out on planetary mixers. The flour, 1kg, was mixed for 1 minute slow speed and 7 minutes 2nd speed, Rested for 10 minutes, the dough is then scaled at 460g, moulded round and then rested for a further 10 minutes before being moulded into 1lb bread tins. The doughs were proved to the height of 12.0cm or for 60 minutes, whichever came first. The final dough was baked in deck ovens at 230˚C for 20 minutes or as required.
Shipton Mill	White roller-milled	Flour 100%; salt 2%; yeast 2%; water 58-62%.	**30 minute bulk fermentation**. 30mins bulk fermentation was followed by 15 minutes intermediate proof and 35 minutes final proof.
Bread Matters	Wholemeal stone-ground	Leaven refreshment: 130g sample flour, 100g starter/old leaven (Bread Matters Standard), 70ml (approx) water. Final dough: 300g refreshed leaven (from above), 400g sample flour, 300ml (approx) water, 8g salt.	**Sourdough long fermentation.** For the leaven refreshment, mix for one minute or until thoroughly combined. No gluten development is necessary at this stage. Dough temperature 25°C. Ferment for 4 hours at 22°-25°C. Note: normally an additional amount of leaven is produced at the refreshment stage to initiate the next batch of dough. The quantities given here are the exact amounts required to produce one large loaf. For the final dough, make a dough with the flour, water and salt. Adjust the water to produce a markedly soft dough (hydration rate approx 70%). Mix at slow speed for 2 minutes and medium for 6 minutes. Add refreshed leaven and mix for another 2 minutes at medium speed. Scale at 930 g. Mould. Dry prove in baskets for 4-5 hours at ambient (20°-25°C). Tip onto peel, slash and place on oven sole. Bake at 220°-230°C for 25-35 minutes.
Panary	Wholemeal stone-ground	2.5kg flour, 1750ml water, 2% yeast, 2% salt.	Dough: Cool (22°C). Fermentation: 2 hours. Bake time and temp: 25mins, 220°C.
Letheringsett Watermills	Wholemeal stone-ground	680g flour; 0.5tsp salt; 1 tsp quick dried yeast; 1 tsp sugar; 2 tsp olive oil; 430ml tepid water.	Put all the dried ingredients into a bowl and mix well. Make a well in the centre of the mixture and add the water and olive oil. Bring mixture together and work until the dough leaves the bowl clean. Turn out onto floured work top and knead until it becomes elastic and develops a slight shine. Return to bowl and cover put aside in a warm draught free place until the dough has doubled in size. Turn out of bowl onto floured work top and knead. Shape dough as required. Cover and put aside until doubled in size. Put into pre-heated oven at 220 degrees for 10 mins. Reduce heat to 190 degrees and continue to cook for a further 25mins or until loaf sounds hollow when tapped on its bottom.

Trial Year 3 (harvest year 2009)			
Bakery	Flour	Recipe	Methodology
WH Marriage & Sons	White roller-milled	100% flour (1kg), 1.3% salt, 0.014% fungal alpha amylase, 2.5% yeast, water as required	**Standard 60-minute bulk fermentation.** All mixing were carried out on VMI spiral mixers. The flour was mixed for 2 minutes 1st speed and 6 minutes 2nd speed, and then rested for 60 minutes. The dough was scaled at 460grams, moulded into 1lb bread tins and placed in the prover with the humidity set at 85%-87% at a temperature of 35ºC -37ºC. The doughs were proved to a height of 12.0cm then placed in the deck oven at 230˚C for 20 minutes.
Bread Matters	Wholemeal stone-ground	Leaven refreshment: 130g sample flour, 100g starter/old leaven (Bread Matters Standard), 70ml (approx) water. Final dough: 300g refreshed leaven (from above), 400g sample flour, 300ml (approx) water, 8g salt.	**Sourdough long fermentation.** Doughs were mixed using a Sammic variable speed planetary mixer. For the leaven refreshment, mix for one minute or until thoroughly combined. No gluten development is necessary at this stage. Dough temperature 25°C. Ferment for 4 hours at 22°-25°C. Note: normally an additional amount of leaven is produced at the refreshment stage to initiate the next batch of dough. The quantities given here are the exact amounts required to produce one large loaf. For the final dough, make a dough with the flour, water and salt. Adjust the water to produce a markedly soft dough (hydration rate approx 70%). Mix at slow speed for 2 minutes and medium for 6 minutes. Add refreshed leaven and mix for another 2 minutes at medium speed. Scale at 930 g. Mould by hand. Dry prove in tins for 4-5 hours at ambient (20°-25°C). Tip onto peel, slash and place on oven sole. Bake at 220°-230°C for 25-35 minutes.
Panary	Wholemeal stone-ground	2.5kg flour, 1750ml water, 2% yeast, 2% salt.	Dough: Cool (22°C). Fermentation: 2 hours. Bake time and temp: 25mins, 220°C.
Letheringsett Watermills	Wholemeal stone-ground	680g flour; 0.5tsp salt; 1 tsp quick dried yeast; 1 tsp sugar; 2 tsp olive oil; 430ml tepid water.	Put all the dried ingredients into a bowl and mix well. Make a well in the centre of the mixture and add the water and olive oil. Bring mixture together and work until the dough leaves the bowl clean. Turn out onto floured work top and knead until it becomes elastic and develops a slight shine. Return to bowl and cover put aside in a warm draught free place until the dough has doubled in size. Turn out of bowl onto floured work top and knead. Shape dough as required. Cover and put aside until doubled in size. Put into pre-heated oven at 220 degrees for 10 mins. Reduce heat to 190 degrees and continue to cook for a further 25mins or until loaf sounds hollow when tapped on its bottom.

Trial Year 4 (harvest year 2012)			
Bakery	Flour	Recipe	Method
WH Marriage & Sons Ltd	White roller-milled	100% flour (1kg), 1.3% salt, 2.5% yeast, 2.0% fat, water as required	**Standard 60-minute bulk fermentation.** All mixing were carried out on VMI spiral mixers. The flour was mixed for 2 minutes 1st speed and 6 minutes 2nd speed, and then rested for 60 minutes. The dough was scaled at 465 grams, moulded into 1lb bread tins and placed in the prover with the humidity set at 85%-87% at a temperature of 35ºC -37ºC. The doughs were proved to a height of 12.0cm then placed in the deck oven at 230˚C for 21 minutes.
Bread Matters	Wholemeal stone-ground	Leaven refreshment: 200g sample flour, 150g starter/old leaven (Bread Matters Standard), 105ml (approx) water. Final dough: 450g refreshed leaven (from above), 600g sample flour, 450ml (approx) water, 12g salt.	**Sourdough long fermentation.** Doughs were mixed using a Sammic variable speed planetary mixer. For the leaven refreshment, mix for one minute or until thoroughly combined. No gluten development is necessary at this stage. Dough temperature 25°C. Ferment for 4 hours at 22°-25°C. Note: normally an additional amount of leaven is produced at the refreshment stage to initiate the next batch of dough. The quantities given here are the exact amounts required to produce one large loaf. For the final dough, combine the flour, water and salt. Adjust the water to produce a markedly soft dough (hydration rate approx 70%). Mix at slow speed for 2 minutes and medium for 6 minutes. Add refreshed leaven and mix for another 2 minutes at medium speed. Scale at 500 g and mould (two loaves were moulded and placed in tins with the third being placed in a round proving basket). Dry prove for 4-5 hours at ambient (18-20°C). Bake at 220°-220°C for 25-35 minutes.
Panary	Wholemeal stone-ground	1500g flour, 900 ml water, 2% compressed yeast, 1.8% salt, water as required.	Steady hand kneading occurs inside a vessel until the water absorption is complete, no more additions required. Water additions stop when it feels right for wholemeal, and the dough is still tight enough for a cob loaf to support itself. A fold occurs inside the vessel after approx 10 minutes, then it is left for its bulk proof- approx 1½ hours. (Less than that if dough finishes warm, up to 2½ hours if it is cold). Finishing dough temp - 22 C to 26C. It is divided, then given an intermediate rest for 10-15 minutes. Final moulding occurs into small tins, with the rest made into cobs. Final proof occurs in at ambient temperature, in a cool room. Average overall time to the oven is 3 to 4 hours.
Wee Boulangerie	Wholemeal stone-ground	37% starter culture (baker's own white flour), 37% sample flour, 36% water, 2% salt.	The dough is made of the same weight of sourdough (which is a 50/50 mix of water and white flour) and sample flour, with water added so that the total water weight is 70% of the total flour weight (wholemeal + white). The mixing time has been identical for all batches. I noted the dough temperature and the various temperatures affecting the fermentation - on average the first fermentation was 3h without knock back and the

			second 1h30, but in general I tried to get the same level of fermentation and this is a subjective thing. The baking time has been 45 minutes at between 240 and 250 celcius, with initial steam.

4.3 Appendix C: Methods Of Malt Analysis.

Extract (Bruce Johnson, Crisp Malt Ltd)

The methods detailed below are those published in the Institute of Brewing's 'Methods of Analysis' (1997) and the European Brewery Convention's Analytica-EBC V (1998) together with other methods commonly used within the industry.

A. Hot Water Extract

In the IOB procedure, 50 g of coarsely milled malt (Buhler-Miag Disc Mill, Setting 0.7 mm), pre-heated at 65°C, is extracted with 360 ml pre-heated water for 1 hour at 65°C mash temperature in a stainless steel beaker with either continuous stirring in a purpose-built bath or manual stirring at 10 min intervals. The mash is cooled to 20°C and made up to 450 g, mixed and filtered. The specific gravity of the wort is measured at 20°C and the hot water extract (Lo/kg) is calculated.

HWE (as is, 0.7 mm) = 8.733G/SG

Where SG=Specific Gravity of laboratory wort at 20°C; G=Excess gravity=1000 (SG - 1)

HWE (dry basis) = HWE (as is) x 100/(100 - % malt moisture)

The same principles and similar equipment is used in the Analytica-EBC procedure, but with the following changes in practical conditions:

Mill Setting: 0.2 mm

Mashing Water : 200 ml

Temperature programme: 45°C for 30 min when the mash is raised to 70°C at 1° C/min for 25 min and then maintained for 1 hour after the addition of 100 ml water

Stirring: continuous stirring is mandatory

By means of tables, the extract is calculated as % of malt to the dry basis.

B. Moisture

Both IOB and EBC methods are essentially the same. The malt is milled (0.2 mm) and dried in a standardised oven (105-107°C) for 3 hours. The % moisture is calculated from the loss in weight.

C. Diastatic Power

The diastatic power is a measure of all starch-degrading enzymes present in the malt. The IOB and EBC have both adopted a "harmonised" approach to the determination of diastatic power.

Malt enzymes are extracted in distilled water at 40°C. A standard starch substrate is hydrolysed by the enzyme extract and the reducing sugars formed by amylolytic action are estimated iodometrically. The result is expressed as grams of maltose per 100 grams of malt. The result is expressed as °Windisch - Kolbach or °IOB. An approximate relationship between the two is:

$$°\text{IOB} = \frac{\text{DP (WK)} + 16}{3.5}$$

D. Total Nitrogen

The IOB and EBC have recently accepted the Dumas combustion method as acceptable for the analysis of ground malt and barley. The IOB have designated the procedure as its reference method whereas the EBC have included the Dumas method as an alternative to the Kjeldahl method. The Kjeldahl process remains as a recommended procedure for both the IOB and EBC.

Dumas Procedure

A sample of the ground malt is combusted in the presence of oxygen at about 1000 degrees C to give oxides of nitrogen which are then catalytically reduced to nitrogen. Other products of combustion are removed by selective adsorption, or separated from elemental nitrogen on a chromatographic column. The nitrogen gas is measured by a thermal conductivity detector and the nitrogen content is calculated from the detector response having been calibrated by measuring the response given by an organic

compound of known nitrogen content. Automated combustion analysers for nitrogen are available which utilise either helium or carbon dioxide as the carrier gas.

Kjeldahl Procedure

A weighed sample of milled malt is digested in sulphuric acid to convert all nitrogenous constituents to ammonium sulphate, from which ammonia is then liberated by distillation from an alkaline solution. The ammonia is measured and the result is expressed as a percentage of nitrogen in the dry malt.

E. Total Soluble Nitrogen

The Kjeldahl procedure is recommended by both the IOB and EBC for the determination of soluble nitrogen in worts and beer. The IOB however, have approved the Dumas method as an alternative to the above and the EBC are about to publish the findings of a collaborative trial. In addition, the EBC have published a spectrophotometric method for the determination of TSN directly on wort by measuring the absorbance at two wavelengths. Laboratory wort is produced in accordance with IOB/EBC requirements and results are expressed as a percentage in the dry malt.

F. Nitrogen Index (Soluble Nitrogen Ratio)

This gives the percentage of degradation and solubilisation of protein after malting and mashing. For IOB it is known as the Soluble Nitrogen Ratio (SNR) and for EBC, it is known as the Kolbach Index (KI).

Nitrogen index= 100 TSN/TN

Abbreviations

Populations:

CCP:	Composite Cross Population
QCCP:	Quality Composite Cross Population
YCCP:	Yield Composite Cross Population
YQCCP:	Yield-Quality Composite Cross Population

Experimental sites:

MET:	Metfield Conventional Farm
MOR:	Morley Conventional Farm
SOF:	Sheepdrove Organic Farm
WAF:	Wakelyns Agroforestry, Suffolk

Other abbreviations:

ANOVA:	Analysis of variance
CV:	Coefficient of variation
GLA:	Green Leaf Area
HFN:	Hagburg Falling Number
HSD:	Honest Significant Difference
LAI:	Leaf Area Index
NSPs:	Non-Starch Polysaccharides
PCA:	Principal Component Analysis
POLAR:	Power Law Residuals
proteinDB:	% dry weight of protein
TGW:	Thousand Grain Weight