HGCA

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THE EARLY PREDICTION OF BREADMAKING QUALITY OF GRAIN AND ITS IMPROVEMENT THROUGH TARGETED LATE APPLICATION OF NITROGEN FERTILISERS

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by

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Abstract
Grain protein content is important in terms of the market value of the crop, particularly for wheat breadmaking quality, and prediction systems based on this parameter have had varying success. A specific fraction of the grain proteins, known as the high molecular weight glutenin subunits (HMW-G), is believed to be influential in breadmaking quality. A previous study of samples of Hereward grown at one site during a single season had suggested that these components could be exploited as predictive markers for monitoring the crop and applying targeted fertiliser as late foliar urea. This three-year study aimed to:

1. validate and extend these preliminary results by examining additional varieties grown at several sites and over several seasons,
2. develop a simple and reliable test based on the HMW-G protein fraction, or a suitable alternative, which could be used to monitor the crop and predict the final quality in breadmaking wheat.

Growing trials were conducted by Levington Agriculture over three seasons. These involved several sites, selected breadmaking varieties, and a range of ammonium nitrate and late foliar urea treatments. Developing grains were sampled at growth stage (GS) 75, and analysed for HMW-G levels by gel electrophoretic methods, protein content and in later years by scanning near infrared (NIR) reflectance spectroscopy. Harvest samples were subject to the same analyses, in addition to a series of flour quality tests and performance in standard breadmaking processes.

In Year 1 trials, six varieties were grown with a basic fertiliser treatment of 200 kg N/ha of ammonium nitrate with or without the application of 40 kg N/ha of late foliar urea at GS 60 at five sites. With the exception of a minor effect on protein content, quality test results, breadmaking performance and HMW-G measurements generally proved to be inconclusive.

The Year 2 trials featured Hereward and Rialto, grown at four sites, and treated with 0, 150 and 200 kg N/ha applied as ammonium nitrate, plus 0, 25, 50 and 75 kg N/ha applied as foliar urea at GS 65.

In Year 3, Hereward was grown at two sites and Caxton grown at two other sites. Ammonium nitrate application ranged between 120 - 240 kg N/ha, plus a control of 0 kg N/ha. Foliar urea was added at levels of 20 and 40 kg N/ha to a set of plots receiving 160 and 200 kg N/ha of ammonium nitrate. The main conclusions from these trials were:

- In most cases the optimal loaf volumes were achieved with the addition of 20-50 kg N/ha of late foliar urea and 150 kg N/ha of ammonium nitrate.
- Site and variety significantly influenced HMW-G levels. HMW-G levels may be unsuitable as quantitative markers of breadmaking quality as there was no consistent relationship between HMW-G and protein content or loaf volume.
- While the addition of both types of fertiliser influenced the protein content significantly, their effects on other measures of quality were less consistent.
- The protein content of the harvested grain could be predicted well from the NIR spectral data of the immature grain at GS 75, a growth stage at which late-applied foliar urea can be effective in improving wheat quality.

Conclusions and Implications

This study has demonstrated that:

- The non-destructive NIR technique can be applied to predict the final level of grain protein rapidly from measurements of immature grain samples, irrespective of variety.
- NIR could become a reliable and a cost-effective tool to assist the grower in early decision making for effective crop management, which would be beneficial both economically and environmentally.
- Recent developments in NIR technology offer the prospect of portable instruments suitable for field-testing being commercially available in the near future.

Summary

Introduction

The application of nitrogen fertilisers to boost the yield and protein content of wheat crops is widely practised. Research studies have indicated that fertiliser can also be used to boost protein quality and subsequent breadmaking quality. However, such improvements may be dependent on several factors including site, season, environment, variety, amount and timing of fertiliser application. It would be beneficial to growers to have a reliable means of determining the expected final crop quality using measurements made on growing plants. This would assist in early decision making for effective crop management. By identifying those crops that benefit most from additional nitrogen (N) fertiliser treatments, unnecessary N applications could be avoided and the farmer's return maximised.

Grain protein content is important in terms of the market value of the crop, particularly for breadmaking quality of wheat. However, prediction systems based on determining end protein content from the nitrogen content in growing wheat tissue have had varying success. The commonly used methods for measuring plant nitrogen content have been the Kjeldahl and Dumas techniques, both of which are time consuming and too costly for assessing developing crops that are grown for commercial purposes in this country. Near infrared (NIR) reflectance spectroscopy is currently being used for measuring nitrogen and fructan levels in shoots, as part of an affordable laboratory-based tissue testing service that is offered in most wheat growing states in Australia.

A specific fraction of the grain proteins known as the high molecular weight glutenin subunits (HMW-G) determine the elastic properties of dough and, thus, are believed to be influential in breadmaking quality. A preliminary CCFRA study funded by the HGCA (Project No. 0008/1/94) had monitored the formation of glutenin and gliadin proteins in developing grains. It was observed that, in samples of the leading breadmaking variety Hereward, grown with nitrogen fertiliser treatments ranging from 0-240 kg N/ha, the proportion of the protein expressed as HMW-G increased proportionally with increasing fertiliser. Furthermore, the level of HMW-G detected at 31 days after anthesis was related to the breadmaking quality of the harvested grain. This is around the latest growth stage (GS) at which late-applied foliar urea may be effective in improving breadmaking performance. These findings suggested that HMW-G components could be exploited as predictive markers for monitoring the crop and judging the need to apply targeted fertiliser as late foliar urea.

A three-year study was undertaken to validate and extend these earlier results obtained with Hereward grown at one site during a single season, through examining additional wheat varieties, grown at several sites and over different seasons. The project sought to:

1. establish the threshold levels of foliar urea and ammonium nitrate that are effective in improving wheat quality,
2. establish the relationship between HMW-G in grain at GS 75 and breadmaking quality,
3. develop a rapid assay based on HMW-G, or a suitable alternative indicator, as predictive test which may be adapted for field use.

In addition, the potential of NIR for predicting the protein content of mature wheat from developing grain was evaluated. NIR is regarded as being a rapid, accurate and reliable technique and is used for the non-destructive analysis of a wide range of food materials. In the UK it has been demonstrated to be a valuable tool in the assessment of developing crops such as peas and other vegetables. It is reported that it is currently being used in Australia for the monitoring and prediction of the end quality of wheat, oats, barley and rice. Whole plant samples of the growing crop are dried and sent to laboratories for NIR testing to monitor nitrogen and starch levels. This information is then used to advise farmers of the nutritional status of their crop and assist them in making appropriate decisions in applying nitrogen fertilisers. A similar approach may be possible for assessing the quality of UK wheat, and therefore, there is a need for evaluating the efficacy of the NIR method. At present, NIR instrumentation appropriate for wheat protein analysis is not available in a readily portable form, and

samples are processed and measured in laboratories. Looking ahead, recent developments in NIR technology offer the prospect of more portable or hand-held instruments suitable for field-testing.

The aim of this study was to validate and extend the previous findings, through the examination of additional varieties and growing sites, and to monitor and manipulate HMW-G levels within grain. Depending on the outcome, the next stage was to develop a simple and reliable test for the HMW-G protein fraction, or develop an alternative method such as NIR, which could be used to monitor the crop and allow targeted late nitrogen fertiliser to improve the quality of breadmaking wheat. This approach could help to bring about more effective use of nitrogen fertilisers, with the aim of reducing overall levels of nitrogen fertiliser application in the production of breadmaking wheat in the UK.

Methods

Growing trials were conducted by Levington Agriculture over three seasons, involving several sites, selected breadmaking varieties, and a range of ammonium nitrate and late foliar urea treatments.

Year 1 (1995/6)

Trials conducted with 6 breadmaking varieties Hereward, Soissons, Rialto, Caxton, Cadenza and Spark grown at 5 sites within the UK (Devon, 2 in Lincolnshire, Suffolk and Cambridgeshire). All plots received 160 kg N/ha of ammonium nitrate, and two rates of foliar urea (0 and 40 kg N/ha) at around GS 60. A randomised plot system was employed in which the treatments were replicated four times.

Year 2 (1996/7)

Hereward and Rialto were grown at 5 sites that were different from those used in the first year. Site **1** (Devon), sites **2** and **3** (Lincolnshire), sites **4** and **5** (Suffolk). Ammonium nitrate was applied at rates of 0, 150 and 200 kg N/ha as main dressings, and late foliar urea applied at rates of 0, 25, 50 and 75 kg N/ha at GS 65. The 12 treatments were replicated three times for each variety.

Year 3 (1997/8)

Levington Agriculture supplied material from existing trials, commissioned by Hydro Agri (UK) Ltd., for assessing nitrogen fertiliser input and predicting grain protein content. Nitrogen levels were measured by Levington Agriculture, in developing plant tissue at GS 30 and GS 60, and in harvested grain, using the Kjeldahl method. Hereward was grown at site **6** in Oxfordshire and **8** in Yorkshire. Caxton was grown at site **7** in Lincolnshire and **9** in Suffolk. Ammonium nitrate was applied at rates of 0, 120, 140, 160, 180, 200 and 240 kg N/ha. In addition, foliar urea was added at two levels of 20 and 40 kg N/ha at GS 65 to a set of plots receiving 160 and 200 kg N/ha of ammonium nitrate. The 11 treatments were replicated four times.

In all trials, 10 ears of developing grain from each plot were sampled at GS 75 and dispatched in dry ice to CCFRA. These were stored at -20° C before being freeze-dried. The middle section (~ one third) of each ear was de-husked and milled using a Bühler Miag mill. Replicate samples were combined to produce bulked samples and then analysed for protein content, HMW-G levels by gel electrophoresis followed by densitometry. The amount of each sample applied to the electrophoresis gel was adjusted for protein content to ensure uniform loading. A highly sensitive colloidal Coomassie Blue stain was used, which is reported to produce better detection of the HMW-G subunits than the conventional stain used in the previous study. Quantification of the HMW-G levels by gel densitometry was achieved by the inclusion of a step to calibrate the optical density of the resolved bands. This reduced the influence of gel-to-gel variations in the measurements. HMW-G content was expressed as the percentage of total protein extracted and resolved as bands within each lane of the electrophoresis gels. In the last two years, samples from selected sites were measured by scanning NIR spectroscopy using a Foss NIRSystems 6500 instrument.

Representative samples of the harvested grain were subject to the analyses described above. The remaining grain was milled using a Bühler Laboratory Mill and the following quality tests were performed on the flour: Hagberg Falling Number; protein and moisture contents (by NIR); water

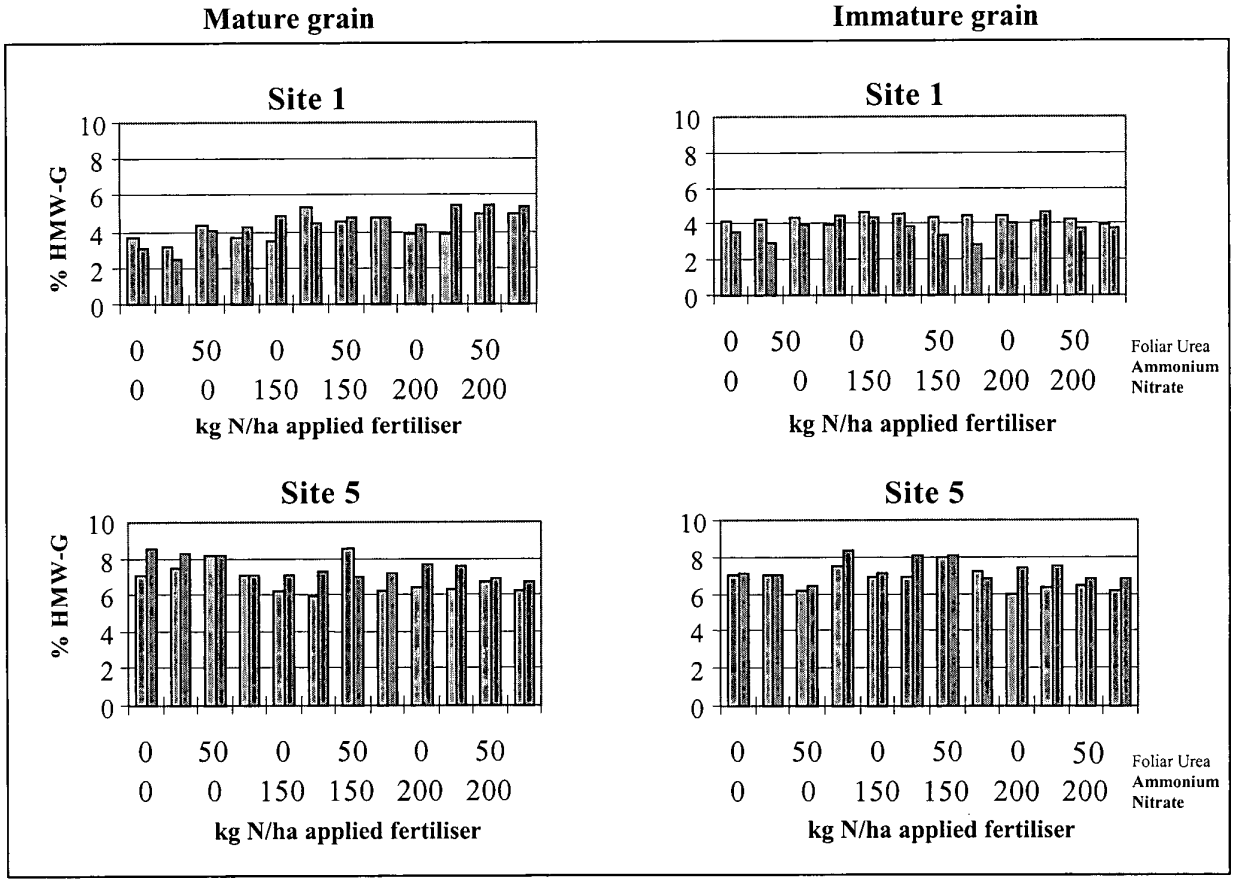
absorption (Farinograph, 600 line); starch damage; gel protein weight and elastic modulus (G'). Breadmaking performance was assessed by a standard Chorleywood Bread Process, and additionally by the slower speed Spiral mix method in Year 2 and 3 trials. The SDS sedimentation test was carried out on samples from Year 1 and 2 trials.

Results

In the Year 1 trials, the application of 40 kg N/ha of foliar urea generally had a small positive effect on protein content (an average increase of 0.3 %) and gel protein weight (an average increase of 4.6 %). The most consistent increase in protein content across all sites was found with samples of Spark. The effects of this level of fertiliser treatment on crop yield and the other flour quality parameters, including HMW-G content and CBP loaf volume, were inconclusive.

In the Year 2 trials, in most cases, maximum loaf volume was achieved with the addition of 20-50 kg N/ha of late foliar urea and 150 kg N/ha of ammonium nitrate, as assessed by the Spiral mix method. Higher levels of rainfall at sites **1**, **2** and **3** in July/August produced some grain sprouting and very low Hagberg Falling Number values, particularly with Hereward. Generally, there was an increase in the protein content in response to increasing levels of both ammonium nitrate and foliar urea treatments. The rate of increase in protein content diminished at the higher levels of fertiliser addition. Site and variety largely influenced the response in protein content to the various treatments with both types of fertiliser. Ammonium nitrate addition had a positive effect on the following quality tests: gel protein G', SDS sedimentation volume test and baking performance by the Spiral mix method (but not CBP).

Figure A. Effects of fertiliser and site on HMW-G levels in Hereward (▩) and Rialto (■) in 1996/7



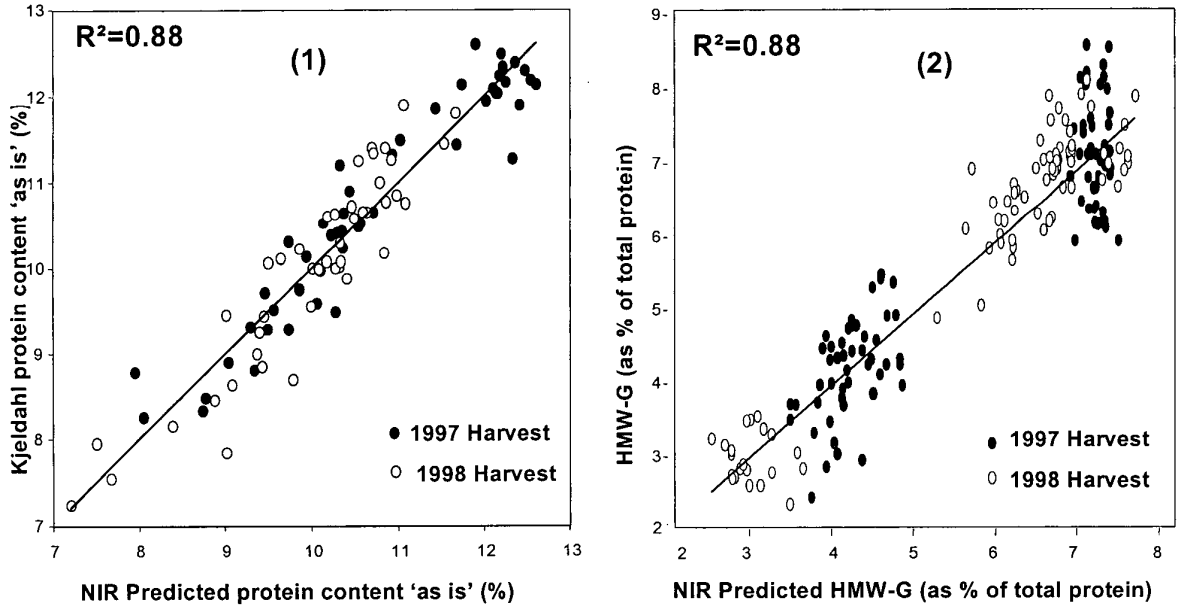
Of the two fertilisers, only foliar urea showed a small but significant (at the 5 % level) effect on HMW-G levels. A predominant site effect was observed, together with evidence of a varietal effect on the % HMW-G data.

The effect of site on % HMW-G levels is illustrated in Figure A. The % HMW-G values of mature and immature grain samples of both varieties are lower at site **1** than at site **5.** The overall correlation between immature and mature HMW-G levels for each variety was good when all the sites were considered. This suggested that the % HMW-G levels in immature grain samples could be reflecting the % HMW-G levels in corresponding mature samples.

The samples from sites **1** and **5** were selected for examination by scanning NIR spectroscopy. Analysis of the NIR spectral data revealed the influences of maturity and as well as site. The mature grain data were clearly distinct from that of the immature grain. The influence of fertiliser treatment on the spectra of immature and mature sample sets was also observed, but to a lesser extent. More importantly, the protein content of the harvested grain could be predicted from the NIR spectral data of the immature grain, taken at GS 75 from two selected sites. A relationship between the NIR spectra and the HMW-G levels of both immature and mature grain samples was also observed, indicating that this NIR technique was capable of discriminating important functional proteins in wheat.

In Year 3 both types of fertiliser, as well as site and variety affected grain protein content. Effects on the other quality tests were less conclusive. HMW-G levels were only marginally influenced by foliar urea. There was no evidence of a relationship between HMW-G levels in mature wheat (expressed as a percentage of total protein) and any of the quality tests, except for protein content. The reasons for the observed relationship between HMW-G and protein content in Year 3 trials, but not in Year 2 trials, are not clear. Differences in trial design between the two sample sets may have introduced distinct site and variety effects and interactions, and the apparent relationship between protein content and HMW-G levels in Year 3 may be casual.

Figure B. NIR calibrations for (1) mature wheat protein using spectra from immature wheat and for (2) HMW-G content of immature and mature wheat. The line of best fit is shown in each plot.



Analysis of the NIR spectral data from all four sites in Year 3 was consistent with those obtained for Year 2. These were treated as a combined data set, from which it was possible to:

1) discriminate between samples on the basis of their maturity and growing locations,
2) predict the mature grain protein content from developing grain samples [Figure B(1)]
3) predict a range of quality parameters (gliadin and HMW-G content, gel protein G′ and water absorption values) in mature wheat.

Interestingly, a good relationship between the NIR spectra and the % HMW-G was observed, and this supports the validity of the HMW-G values determined using the improved gel electrophoresis and densitometry procedures. This relationship was independent of the total protein content within the samples, and may be reflecting functional properties of both immature and mature wheat. Further work on the spectral characteristics related to biochemical components such as the glutenins would be useful in developing NIR calibrations for predicting quality more accurately, and would facilitate the transfer of calibrations to simpler filter type instruments.

Relevant results from a parallel study conducted by Levington Agriculture on behalf of Hydro Agri (UK) Ltd were made available to complement those of this project. This 3 year study sought to examine:

1) the relationship between late foliar urea application and grain protein content,
2) the possibility of early prediction of grain protein content.

Material from Hydro Agri trials provided the samples for Year 3 of the CCFRA study. It was concluded that, where the main application was 200 kg N/ha of ammonium nitrate, the addition of 40 kg N/ha of late foliar urea was associated with an increase of about 0.1 % in grain nitrogen content. Results from immature samples taken at GS 30 indicated that this stage might be too early to provide a representative picture of the nitrogen content of the final crop. A better correlation was found between the N content of the whole plant at GS 60, measured by the Kjeldahl method, and the final nitrogen content at harvest.

This observation supports the independently obtained result where the final protein content in harvested grain was predicted by NIR analysis of immature grain at GS 75. Therefore, it should be possible to develop a monitoring system for predicting mature grain protein content from samples of grain taken in early June. This information would help growers to decide whether or not to apply a late treatment of foliar urea to their crop. The nitrogen content of wheat has commonly been measured using the Kjeldahl method, but is currently being replaced by the Dumas method. Both are chemical based methods that are relatively time-consuming, require specialised equipment and facilities for testing sample moisture content to report results on a fixed moisture basis. The NIR technique has the advantage of speed and reliability, and the ability to determine a number of parameters simultaneously.

Conclusions

The results from trials, conducted over three years, involving several growing sites and wheat varieties showed that the application of ammonium nitrate and foliar urea fertilisers influenced protein content in all varieties. Effects on the other measures of breadmaking quality were less consistent and therefore, less conclusive.

HMW-G levels, measured as a percentage of the total protein extracted from each sample, were found to be unsuitable to be used on their own as quantitative markers for breadmaking quality. No evidence of any significant and consistent relationship between HMW-G levels and protein content or loaf volume was found.

The protein content of the harvested grain could be predicted well from the NIR spectral data of immature grains sampled at GS 75, irrespective of variety. The NIR technique has the potential to be adapted as a reliable test for the rapid assessment of the nitrogen fertiliser needs of developing wheat crops, grown in the UK.

Implications

This study has clearly demonstrated the feasibility of applying NIR technology to provide a rapid predictive test for home-grown breadmaking wheat. However, there is a need for further work to develop NIR calibrations for predicting grain protein content more accurately by studying larger sample sets, and other factors such as the time of sampling immature grains and environmental influences. A fast and reliable test would allow the farmer to determine the plant's nutrient status and make more informed judgements about the final crop quality and assist in decision making regarding the necessity for late foliar urea application. Reduced fertiliser usage would result in reduced soil residues leaching into water supplies and thus have an environmental impact. Applying extra nitrogen fertiliser only where it was needed would benefit the grower financially, and assist in meeting marketing specifications. The maintenance and improvement of the quality of breadmaking wheat is of great importance to breeders, farmers, and end-users. Achieving target specifications is also of paramount importance if the UK is to be competitive in overseas markets.

Abbreviations used in this report

AN	Ammonium nitrate
ANOVA	Analysis of variance
CBP	Chorleywood Bread Process
CCFRA	Campden & Chorleywood Food Research Association
CE	Capillary Electrophoresis
CV	Canonical Variate
CVA	Canonical Variates Analysis
DM	Dry matter
FU	Foliar urea
G′	Elastic modulus of Gel Protein
GS	Growth stage
ha	Hectare
HFN	Hagberg Falling Number
HMW-G	High molecular weight glutenin subunit
LMW-G	Low molecular weight glutenin subunit
HPLC	High performance liquid chromatography
ICC	International Association for Cereal Science and Technology
LSD	Least significant differences
LV	Loaf volume
MPLSR	Modified partial least squares regression
MSC	Multiplicative scatter correction
MW	Molecular weight
M_r	Relative or apparent molecular weight determined by SDS-PAGE
N	Nitrogen
nabim	National Association of British & Irish Millers
NIR	Near infrared
Ns	Not significant
Pa	Pascal
PAGE	Polyacrylamide gel electrophoresis
PC	Principal component
PCA	Principal component analysis
PLS	Partial least squares
PLSR	Partial least squares regression
SDS	Sodium dodecyl sulphate
SEC	Standard error of calibration
SECV	Standard error of cross validation
SE-HPLC	Size exclusion - high performance liquid chromatography
SEP	Standard error of prediction
SMLR	Stepwise multiple linear regression
1/R	Reciprocal of reflectance (NIR measurement)
t	tonne
W.Ab.	Water absorption

1. Effect of Nitrogen fertilisers on wheat quality and HMW-G levels

DG Bhandari

1.1 Abstract

High molecular weight glutenin subunits (HMW-G) are closely related to breadmaking quality. A number of studies have suggested that the amount of HMW-G, as well as the composition, influence breadmaking properties. A previous HGCA-funded study had indicated that the quantity of HMW-G detected in Hereward 31 days after anthesis (GS 80) might be related to its breadmaking quality. This present study sought to validate the preliminary findings through the examination of additional varieties grown at several sites and over different seasons. Trials involving Hereward, Rialto and Caxton were conducted over two years with varying levels of ammonium nitrate and late foliar urea fertilisers. A good relationship was found between HMW-G levels in developing and in mature wheat samples of all three varieties. However, there was no consistent evidence of changes in the levels of HMW-G, relative to total protein, in response to ammonium nitrate application. The addition of late foliar urea produced an overall small, but significant, effect on HMW-G levels. Levels of HMW-G, in either immature or mature grain could not be correlated with a range of quality measures, including protein content and breadmaking performance.

1.2 Introduction

It is now well established that gluten proteins are responsible for the visco-elastic properties of bread dough. In particular, it is the high molecular weight glutenin subunits (HMW-G) that form a network of large interlinked polymers and determine the elastic properties of dough. Hexaploid bread wheats all feature six HMW-G subunit genes, occurring as pairs on the group 1 chromosomes of the A, B and D genomes, and of which only three, four or five subunits are expressed (Shewry and Tatham, 1990).

This variation in gene expression can influence breadmaking quality in two principal ways. The first is through qualitative differences in the structures and properties of HMW-G subunit proteins. The second is through quantitative differences in the HMW-G protein levels and hence, in the amount of elastic HMW polymers. Each expressed HMW-G subunit is thought to account for about 2 % of the total grain protein, and theoretically, can range from about 6 % to 10 %, depending on subunit composition, normally between three to five subunits. Reconstitution (Khan *et al.,* 1991) and gene transformation (Barro *et al.,* 1997) studies indicate that the presence of higher levels of HMW-G proteins within wheat flour results in improved functional properties such as dough elasticity.

The effect of nitrogen fertiliser addition to growing wheat has been the subject of numerous studies (Dampney *et al.*, 1995; Triboi and Branlard, 1990; Jia *et al.,* 1996). Generally, when extra nitrogen fertiliser is applied at an early stage of crop growth (around GS 32) in the form of ammonium nitrate prills, an increase in yield results. Later application, during the "milky ripe" development stage, using foliar-applied liquid urea can result in an increase in protein content, and in some instances a subsequent improvement in breadmaking performance. The precise timing of the application and the type and amount of fertiliser used may be important for them to be effective in agronomic and economic terms (Dampney *et al.*, 1995).

There are a number of varying reports about the extent to which nitrogen fertilisers influence the quantities and proportions of the storage (gliadin and glutenin) and other endosperm (albumin and globulin) proteins in wheat flour (Kolster *et al.*, 1991; Peltonen and Vitanen, 1994; Weiser and Seilmeier, 1998). The reasons for some of the contradictory reports may, in part, be due to the methods used by the different groups. SDS-PAGE followed by gel densitometry is the most widely used method (Payne *et al.,* 1987; Kolster *et al.,* 1991; Hou and Ng, 1995). Reverse phase (RP)- and size exclusion (SE)-HPLC are also extensively used for analysing protein fractions in wheat flour

(Sutton 1991; Singh *et al.*, 1991; Gupta *et al.*, 1993). More recently, capillary electrophoresis has also been employed in the analysis of wheat proteins (Bean *et al.*, 1998; Sutton and Bietz, 1997; Weegels *et al.*, 1995). Different methods of extracting the protein fractions employed by the various research groups can also contribute to the incompatibility of the results.

An earlier study funded by the HGCA (Project No. 0008/1/94 - Pritchard and Bhandari, 1995) had sought to examine the effect of timing and quantity of nitrogen fertiliser addition on the expression of proteins that are functional to breadmaking, during the growth period between anthesis and maturity. The formation of glutenin and gliadin proteins was monitored by SDS-PAGE in samples of the leading breadmaking variety Hereward, grown with different fertiliser treatments. The amount of HMW-G was found to increase proportionally with increasing fertiliser nitrogen. Furthermore, the level of HMW-G detected at 31 days after anthesis was closely related to the breadmaking quality of the harvested grain.

The initial aim of this study was the validation and progression of the previous findings, through the examination of additional varieties and growing sites, and to monitor and manipulate HMW-G levels within grain. Depending on the outcome, the next stage was to develop a simple and reliable test for the HMW-G protein fraction, or a suitable alternative method, which could be used to monitor the crop and apply targeted late nitrogen fertiliser to improve the quality of breadmaking wheat. This approach could facilitate the effective use of nitrogen fertilisers, and thereby reduce their overall usage in the production of breadmaking wheat in the UK.

1.3 Materials and methods

Growing trials were conducted by Levington Agriculture over three seasons, involving several sites, selected breadmaking varieties, and a range of ammonium nitrate and late foliar urea treatments.

1.3.1 1995/96 (Year 1) Trials

Trials conducted with 6 breadmaking varieties Hereward, Soissons, Rialto, Caxton, Cadenza and Spark grown at 5 sites within the UK (Devon, 2 in Lincolnshire, Suffolk and Cambridgeshire). Levington Agriculture selected sites on the basis of them having low basal nitrogen levels. All plots received 160 kg N/ha of ammonium nitrate, and two rates of foliar urea (0 and 40 kg N/ha) at around GS 60, in accordance with standard farming practice. A randomised plot system was employed in which the treatments were replicated four times, giving 240 samples.

Immature wheat caryopses were sampled at around GS 75. Ten ears of each sample per plot were sent frozen to CCFRA where they were stored at -20°C. The samples were freeze-dried using an Edwards bulk freeze dryer, and then stored at room temperature. The dried grains were removed from the ears using a Wintersteiger laboratory threshing machine, ground with a Stenvert hammer mill, and then stored in desiccators at room temperature.

At harvest, 2 kg grain per plot was sent to CCFRA where each set of four replicates were combined to provide a single bulk sample and stored at 10°C.

1.3.2 1996/97 (Year 2) Trials

Hereward and Rialto were grown at five sites covering a variety of soil types, and spread throughout the main wheat growing regions of England in 1996/7. These were - (**1**) Greendale, Devon; (**2**) Mere, Lincolnshire; (**3**) Bassingham, Lincolnshire; (**4**) Great Livermere, Suffolk; (**5**) Henley, Suffolk. Levington Agriculture selected the sites on the basis of their low basal soil nitrogen levels. Soil types varied from clay to sandy clay loam.

The following fertiliser treatments were applied:

Treatment Number	Ammonium Nitrate kg N/ha	Foliar Urea kg N/ha	Total kg N/ha
1.	0	0	0
2.	0	25	25
3.	0	50	50
4.	0	75	75
5.	150	25	175
6.	150	50	150
7.	150	50	200
8.	150	75	225
9.	200	0	200
10.	200	25	225
11.	200	50	250
12.	200	75	275

Ammonium nitrate: an early dressing of 40 kg N/ha was applied at GS 23 - 30, and the main dressings of 150 and 200 kg N/ha applied at GS 30 - 31.

Foliar urea: 0, 25, 50 and 75 kg N/ha was applied at GS 65.

These treatments were factorially combined to give (3 x 4) = 12 treatments, replicated three times for both varieties to give 72 randomised plots per trial, and 360 samples in total. The plots were 2 x 9 m, with a total trial area of 0.13 ha. Immature wheat caryopses were sampled at around GS 75. Ten ears of each sample per plot were sent frozen to CCFRA where they were stored at -20°C. The samples were freeze-dried using an Edwards bulk freeze dryer, and then stored at room temperature. The middle third of each dried ear was removed, and grains were separated from the husks, initially by hand, and later by agitation with a strong jet of air within a small chamber. The latter method proved to be very effective and rapid.

At harvest, about 2 kg grain from each plot was sent to CCFRA. Each set of 3 replicates was combined, to give 120 x 6 kg samples, and stored at 10°C. Samples from site **3** (Bassingham, Lincolnshire) have been omitted from all analyses due to sampling problems.

1.3.3 1997/98 (Year 3) Trials

Material for Year 3 was obtained by permission from Hydro Agri, from existing trials. These trials, conducted by Levington Agriculture, sought to assess the suitability of the PrecisioN-Tester chlorophyll meter for predicting nitrogen fertiliser input for achieving high grain protein content.

Hereward was grown at trial sites- (**6**) Milton, Oxfordshire, and at (**8**) Skerne, Yorkshire. Caxton was grown at trial sites - (**7**) Mere, Lincolnshire, and (**9**) Livermere, Suffolk.
The fertiliser treatments for each variety were:

Treatment Number	Ammonium Nitrate kg N/ha	Foliar Urea kg N/ha	Total N Fertiliser kg N/ha
1.	0	0	0
2.	120	0	120
3.	140	0	140
4.	160	0	160
5.	180	0	180
6.	200	0	200
7.	240	0	240
8.	160	20	180
9.	160	40	200
10.	200	20	220
11.	200	40	240

Ammonium nitrate: early dressing of 40 kg N/ha was applied at GS 23 - 30, and the main dressings of 120 - 240 kg N/ha applied at GS 30 – 31.

Foliar urea: 0, 20, and 40 kg N/ha applied at GS 65

The treatments were replicated four times to give 44 randomised plots per trial, and 176 samples in total. The plots were 3 x 15 m, with a total trial area of 0.198 ha. Immature wheat caryopses were sampled at around GS 75 as described for Year 2 material. Each set of the four replicates of the harvest material was combined, to give 44 x 8 kg samples, and stored at 10°C.

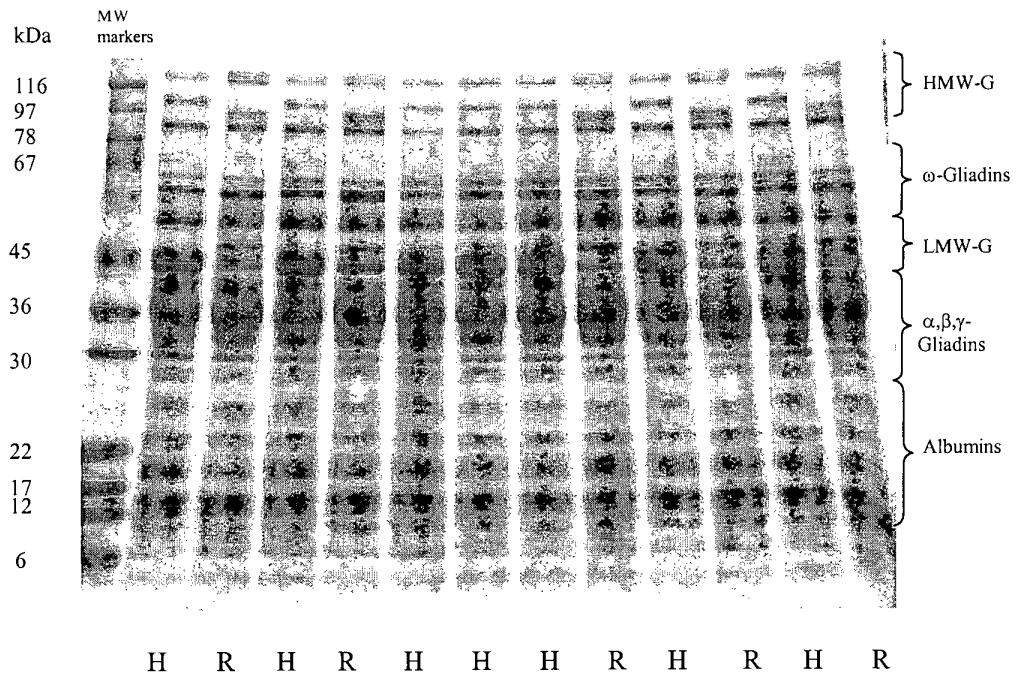
1.3.4 Quality Assessment

The harvest (mature) grain samples were milled to produce white flour using a Bühler Laboratory Mill (MLU 202), together with a Bühler MLU 203 Impact Finisher. Quality assessment of the flour was performed with the following tests: gel-protein test using a Bohlin VOR rheometer (Pritchard and Brock, 1994); Hagberg Falling Number, protein and moisture content by NIR, water absorption and starch damage, all of which are detailed in CCFRA Guideline No.3, updated in 1999. The SDS sedimentation test was carried out on wheat ground using a Falling Number KT mill according to the procedure in CCFRA Guideline No. 3. Breadmaking performance was assessed by standard Chorleywood Bread Process (CBP) and Spiral mix methods. The recipe for both baking methods was (all as a proportion of flour weight): 100 % flour, 2.5 % yeast, 2 % salt, 1 % hard fat, 0.01 % ascorbic acid, fungal *alpha*-amylase supplemented to 40 Farrand units, and water level as indicated by the Farinograph (600 line). The two breadmaking systems differed only in the mixing method used. Dough development is achieved by high speed mixing in the CBP to a work input of 11 watt hours/kg, and slower mixing for a fixed time in the Spiral mix system– 2 minutes slow speed and 6 minutes fast speed. Single dough preparations, producing four x 400 g white loaves each, were carried out on all flour samples. Measurement of loaf volume was performed on both CBP and Spiral mix bread. A subjective crumb score (on a scale from 0-10) was assigned for the CBP bread.

Analysis of the HMW-G subunits in immature and mature grain samples was performed using a SDS-PAGE (polyacrylamide gel electrophoresis), based on a method reported by Laemmli (U.K., Laemmli, 1970) using Ready Gel Cell and Mini-Protean II gel systems, both from BioRad Ltd. Material from Year 1 trials was analysed using BioRad pre-cast gels featuring a 10-20 % polyacrylamide gradient, and stained with conventional Coomassie Blue stain. All the samples from Year 2 and Year 3 trials were analysed with laboratory cast gels (7 x 8 x 0.15 cm) containing a 7.5-25 % polyacrylamide gradient. These gels were run at 150 V for 100 minutes and stained using a colloidal Coomassie Brilliant Blue staining procedure (Neuhoff *et al.,* 1988). The grains were ground using a Bühler Miag mill, and the crude protein content of each immature sample was determined by the Lowry assay (Lowry *et al.,* 1951). A uniform amount of 0.04 mg total extracted protein was applied to each gel lane.

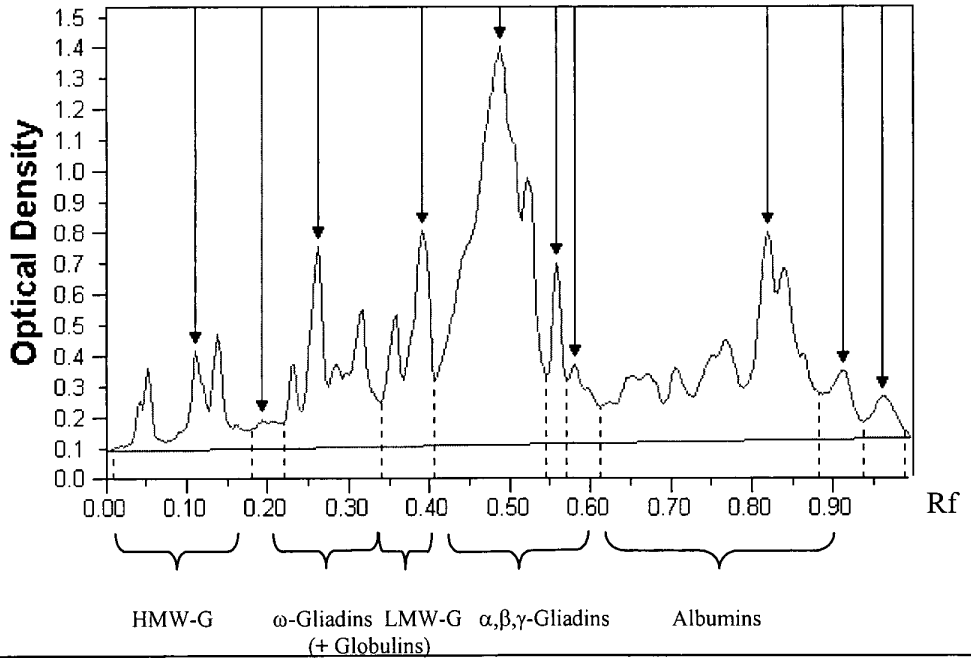
The gels were converted into images using the GDS 8000 system video camera documentation system (UVP Ltd). Densitometric analyses were performed using Phoretix 1D Advanced software (Phoretix International Ltd). A 14-step optical density strip (Kodak Control Scale T-14) was used to calibrate band intensities, and to correct for variations in staining and the image capture process. The bands were assigned as being HMW-G subunits, based on a report by Bénétrix *et al.* (1994) (see figures 1 and 2). The amount of HMW-G reported was as a percentage of the total protein detected in each gel lane.

Figure 1. SDS-PAGE of mature samples of Hereward and Rialto.



Lanes containing Hereward are labelled H, and those containing Rialto are labelled R.

Figure 2. Densitometric profile of a gel lane containing Hereward



1.3.5 Analysis of Quality Data

Analysis of variance (ANOVA) was carried out on the quality data using Minitab 12.2 software. Due to the differences in trial design the two years were treated separately. In Year 2, the general linear model comprised terms for variety, site, variety x site interaction, ammonium nitrate level, foliar urea level, and interaction of ammonium nitrate and foliar urea. Correlations (Pearson) between the variables were calculated separately, pooled within site, for each variety.

In Year 3, two statistical models were fitted:

a) Where no foliar urea had been added, the general linear model had terms for variety, site (within variety), and ammonium nitrate level.

b) Where foliar urea had been added, in addition to ammonium nitrate at 0, 160 and 200 kg N/ha rates, the model had terms for variety, site (within variety), ammonium nitrate level, foliar urea level, and interaction of ammonium nitrate and foliar urea.

Overall (Pearson) correlations between the variables were calculated for both varieties at all sites in Year 3, as there was an insufficient number of samples of each variety to perform separate analyses.

1.4 Results

1.4.1 Quality data

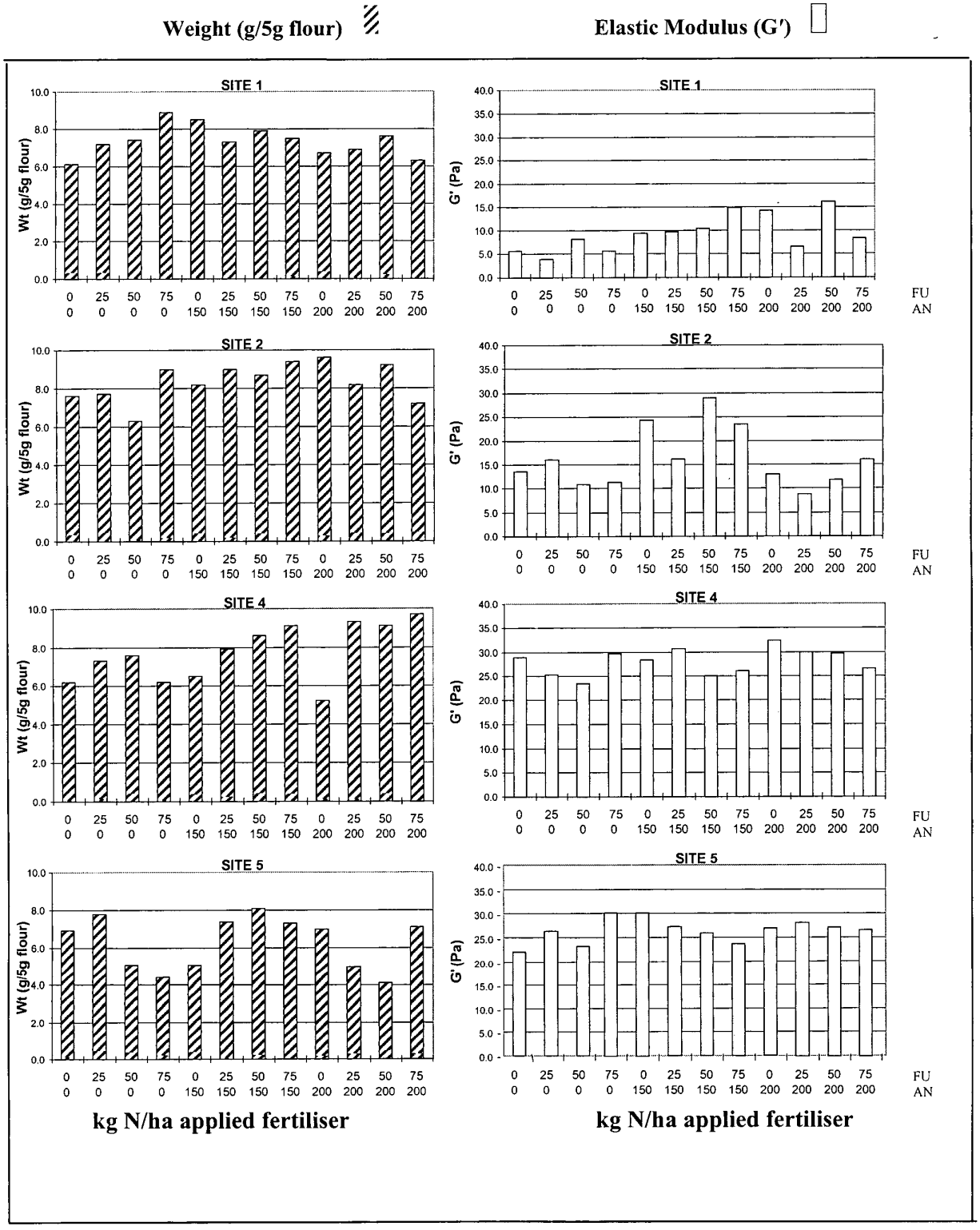
Year 1 trials

The addition of 40 kg N/ha of foliar urea generally had a small positive effect on the protein content of the six varieties at all sites. An average increase of 0.3 % in protein content was obtained. Inspection of the gel protein data showed that, overall, there was no significant effect of foliar urea addition on the elastic modulus (G'), but there was an average increase of 4.6 % in gel protein weight. Minor increases as well as decreases were observed in the remaining quality measures, including CBP loaf volume, and it was concluded that there were no consistent effects over all varieties resulting from this fertiliser treatment. The quality of the pre-cast polyacrylamide gels from BioRad was variable and the sensitivity of the conventional Coomassie Blue stain was considered to be too low to determine the HMW-G levels in immature grain samples accurately. In addition, the application of foliar urea at a rate of 40 kg N/ha had no measurable effect on the % HMW-G levels within the mature samples using these procedures, and therefore, no further analyses were carried out on the Year 1 material.

Year 2 trials

Analysis of the immature grain data showed that both HMW-G levels and protein content were significantly influenced by site (Table 1a). Both types of fertiliser (Table 1a) affected the protein content of immature wheat. Grain yield increased in response to ammonium nitrate addition at all of the sites. The effect of foliar urea on yield was seen at site **1** only. This site had the highest yield values and also the lowest grain protein content. Analysis of the data from all of the sites showed, generally, that the addition of ammonium nitrate had significant effects on the flour protein content (as measured by NIR), SDS sedimentation values, Spiral mix method loaf volumes, but not CBP loaf volumes (Table 1b). The weight of the gel protein and its rheological properties provide measures of the amount and quality of functional protein within the flour. It consists, principally, of glutenin and its weight is largely under genetic control. While the weight of gel protein does not necessarily reflect the baking quality of a flour, the elasticity of this protein fraction provides a means of distinguishing between samples which may underperform in a standard breadmaking process due to weakness or excessive strengths of their proteins. The elastic modulus (G') of gel protein is known to be influenced by site and seasonal variations. Overall, for Year 2, gel protein G' values, and to a lesser extent, weight, appeared to respond to ammonium nitrate and at some sites to foliar urea, but the effects were not consistent (Figures 3 and 4). The G' values of Rialto seemed to show a greater response to increasing fertiliser addition than Hereward. Site **1** featured the lowest G' values, and site **4** had high values for both varieties

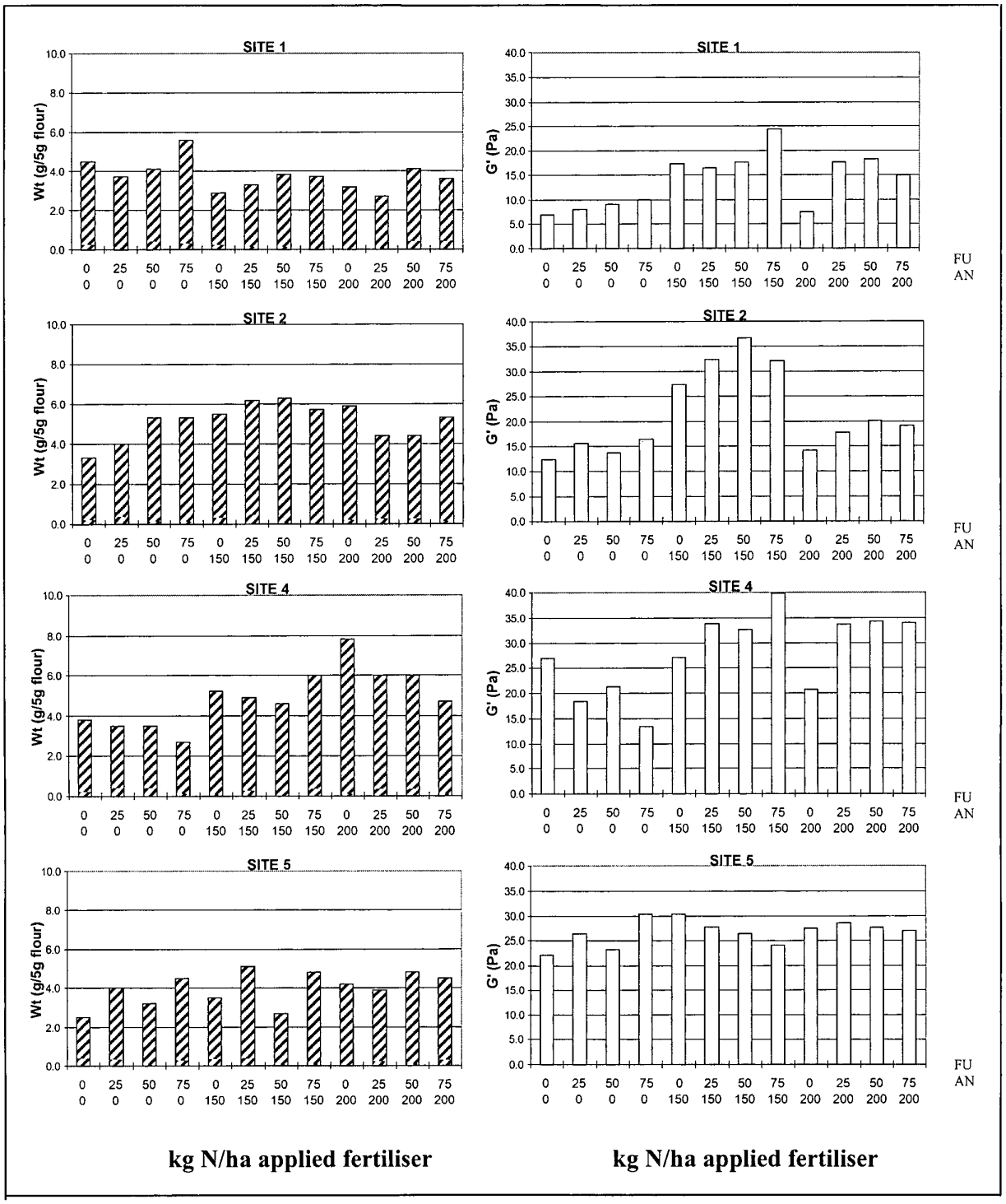
Figure 3. Comparison of Hereward gel protein data in 1996/97



FU – Foliar Urea AN – Ammonium Nitrate

Figure 4. Comparison of Rialto gel protein in 1996/97

Weight (g/5g flour) ▨ **Elastic Modulus (G′)** □



FU – Foliar Urea AN – Ammonium Nitrate

Table 1. Significance test results from ANOVA, 1996/97

a) Immature wheat

Source	HMW-G	(Lowry) Protein
Variety	Ns	Ns
Site	***	***
Variety x site	**	Ns
Ammonium Nitrate (AN)	*	***
Foliar Urea (FU)	Ns	***
AN x FU	Ns	Ns

b) Mature wheat

Source	HMW-G	Protein by NIR	CBP volume	Spiral mix volume	Gel protein weight	Gel protein G'	W.Ab	SDS	HFN
Variety	**	***	***	***	***	**	***	***	***
Site	***	***	***	***	***	***	***	***	***
Variety x site	Ns	Ns	*	Ns	Ns	Ns	*	**	***
Ammonium Nitrate (AN)	Ns	***	Ns	***	**	***	*	***	Ns
Foliar Urea (FU)	*	***	Ns	Ns	Ns	Ns	*	**	Ns
AN x FU	Ns	**	Ns	Ns	Ns	Ns	***	Ns	Ns

(HFN: 2 outliers were omitted) W.Ab. – Water absorption

Ns- not significant, *P<0.05; **P<0.01; ***P<0.001.

The most significant effect of the addition of foliar urea on the quality of both varieties was on the flour protein content (see Table 1b), but this was not reflected in the baking performance by either the Spiral mix method or CBP. The addition of foliar urea also affected in SDS sedimentation and water absorption values. The interaction of the two types of fertiliser was evident only in the protein and water absorption values. Hereward was found to have very low Hagberg Falling Number (HFN) values (<200 s) due to the heavier rainfall at sites **1** and **2**, compared to Rialto. The lowest Falling Number values for Rialto were found at site **2**. These results suggested that Hereward was less resistant to premature sprouting than Rialto, when grown under identical conditions.

Higher loaf volumes for Hereward and Rialto were produced under both baking processes at site **2,** despite low protein content (9.0 - 10.3 %). This appears to be consistent with the lower Hagberg Falling Numbers obtained for the wheat grown at this site (data not shown). However, the crumb score values obtained by the Spiral mix method for Hereward grown at site **2** were only marginally lower than those obtained for this variety at the other sites (data not shown). In most cases the maximum loaf volumes were achieved with the addition of 20-50 kg N/ha of late foliar urea and 150 kg N/ha of ammonium nitrate. Overall, Table 1b shows that within these trials, there were significant effects of site and of variety on all quality parameters.

Year 3 trials

The protein content of the mature wheat and grain yield (data not shown) increased in response to ammonium nitrate addition at all of the sites. Site **7,** involving Caxton, had the lowest protein content of all the sites, in response to the addition of both types of fertiliser (Figure 5). The addition of ammonium nitrate and foliar urea produced inconsistent responses in the protein contents of the immature wheat samples, (Figure 5). This may be due to the relatively lower sensitivity of the Lowry assay used for determining the crude protein content of immature grain samples, compared to the NIR method used for the mature wheat. The overall correlation (r^2) between immature and mature wheat grain protein content is 0.36 (Table 6). This correlation is rather poor, and may be partly reflecting the differences in the accuracy of the two methods of protein determination. The yield at site **8** (Hereward) was very low, with a mean value 2.05 t/ha. This was due waterlogging of

the field in April, and generally poor growth at this trial site. Site **8** also had the lowest Falling Number values. In terms of functional parameters, gel protein G′ values showed significant site and varietal effects. Increases in gel protein G′ values of Hereward in response to ammonium nitrate addition were observed at sites **6** and **8** (Figure 6). This was not so evident for Caxton at sites **7** and **9**. There was no significant effect of foliar urea on yield at any of the sites (data not shown).

Figure 5. Protein content response to fertiliser treatments, 1997/98.

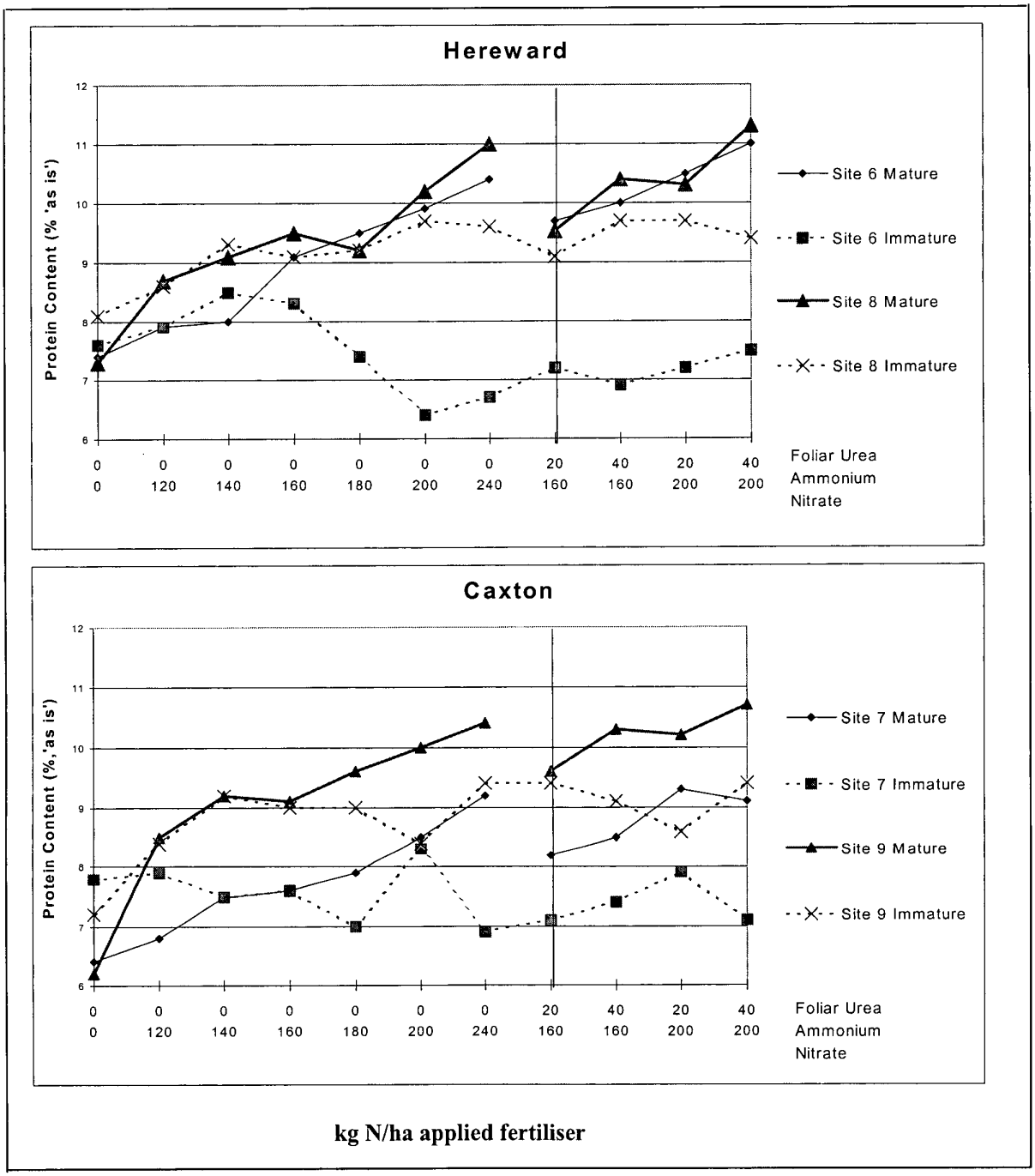
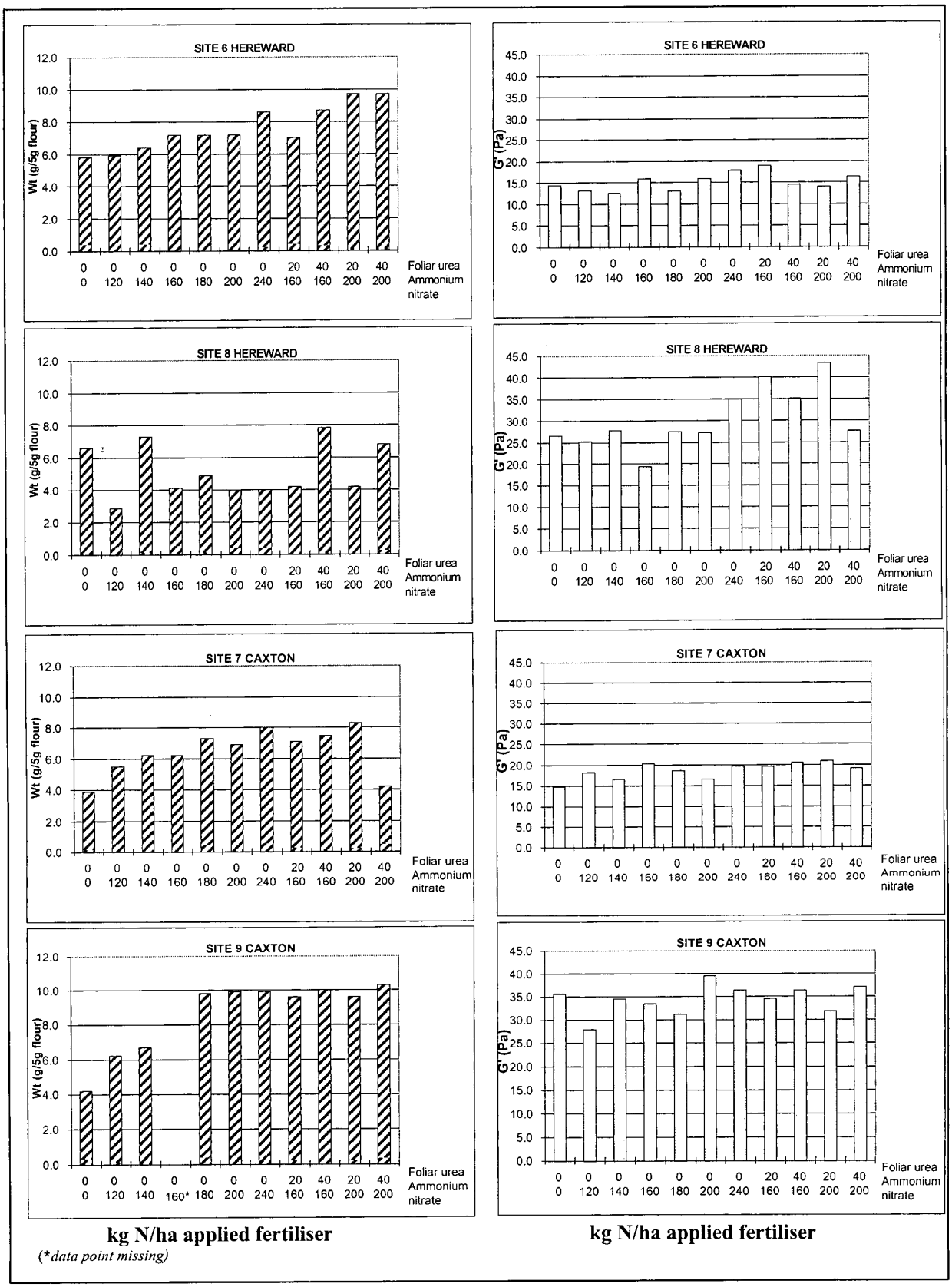


Figure 6. Hereward & Caxton Gel Protein, 1997/98.

Weight (g/5g flour) ▨ **Elastic Modulus (G')** □



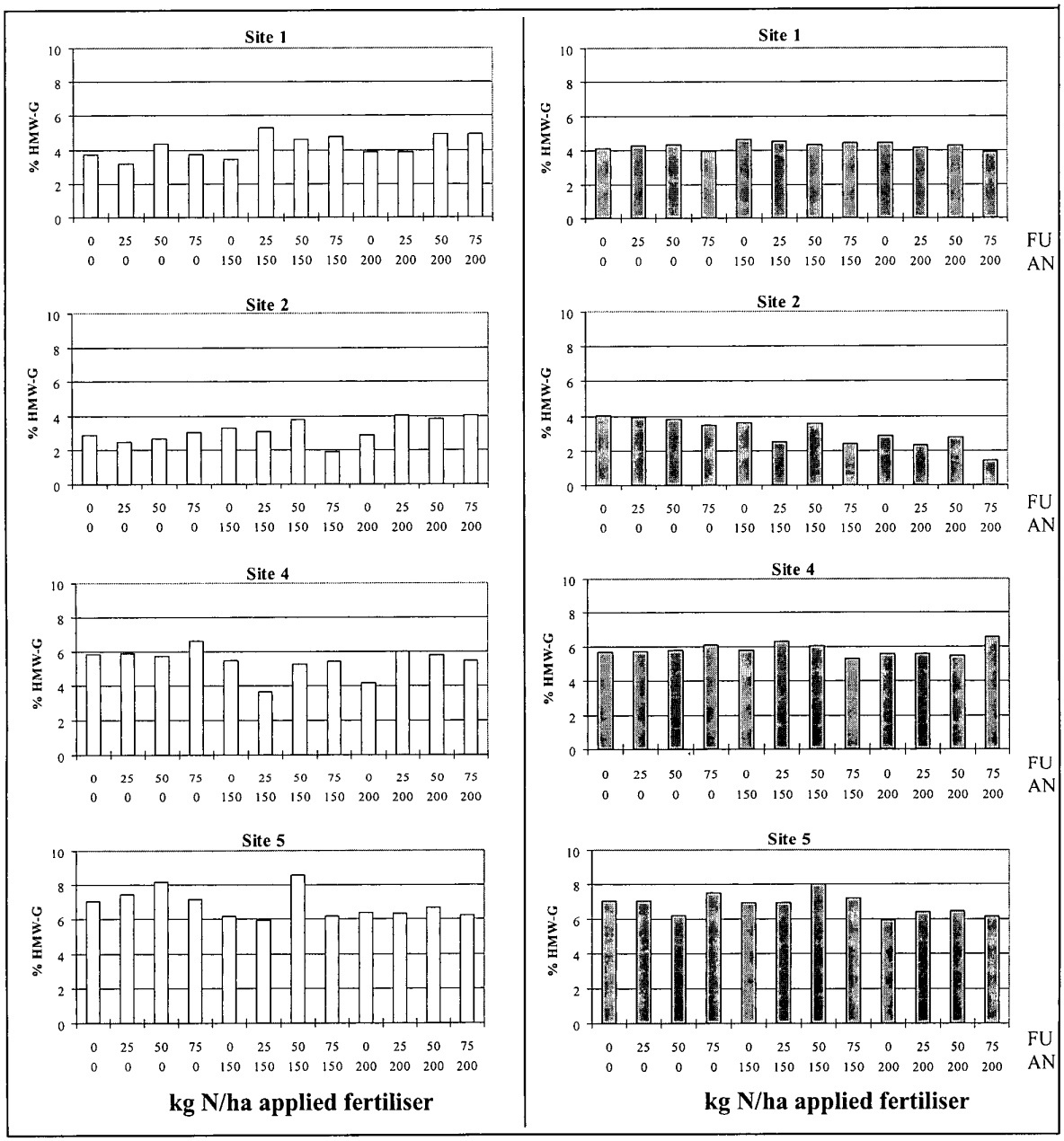
*(*data point missing)*

1.4.2 HMW-G Levels

The use of the laboratory cast SDS-PAGE gels and the highly sensitive colloidal Coomassie Blue staining method improved the detection of the HMW-G protein bands quite markedly compared with the pre-cast gels and the conventional staining procedure. This was particularly noticeable with the immature grain samples where the protein content was low. Statistical analysis of the data show that the addition of ammonium nitrate had no consistent effect on the HMW-G content of mature grain (measured as % total extracted protein) in either Year 2 or in Year 3 (Tables 1, 2 and 3). There was, however, a marginally significant effect (at the 5 % level) of foliar urea treatment on HMW-G levels in all of the trials.

Figure 7a. % HMW-G in mature Hereward, 1996/97

Figure 7b. % HMW-G in immature Hereward, 1996/97



FU – Foliar Urea AN – Ammonium Nitrate

The effect of site on HMW-G levels in mature and immature grain was highly significant in the Year 2 trials (Table 1) and for Year 3 trials involving ammonium nitrate addition only (Table 2). However, in the Year 3 trials involving foliar urea, the effect of site was seen only in the immature wheat (Table 3). Figures 7a and 7b illustrate the effect of site on % HMW-G levels measured in mature and immature samples of Hereward in Year 2. It can be seen from these figures that sites **1** and **2** have lower % HMW-G values than those found at site **4** and **5** in mature Hereward samples (Figure 7a), and this site effect is also featured in the immature samples (Figure 7b). Very similar results were obtained for Rialto (not shown). Sites **1** and **2** had the lowest mean values for HMW-G content in both immature and mature grain samples in the Year 2 trials (Figures 7a and 7b).

Table 2. Significance test results from ANOVA, 1997/98 (Year 2).
Treatments with ammonium nitrate but no foliar urea.

a) Immature wheat

Source	HMW-G	(Lowry) Protein
Variety	*	Ns
Site (within Variety)	***	***
Ammonium Nitrate	Ns	Ns

b) Mature wheat

Source	HMW-G	Protein by NIR	CBP volume	Spiral mix volume	Gel protein weight	Gel protein G'	W.Ab.	HFN
Variety	**	***	Ns	**	Ns	***	**	Ns
Site (within Variety)	***	***	**	**	**	***	**	***
Ammonium Nitrate	Ns	***	Ns	Ns	Ns	Ns	***	***

Ns- not significant, *P<0.05; **P<0.01; ***P<0.001.

Table 3. Significance test results from ANOVA, 1997/98 (Year 3).
Tests with ammonium nitrate applied at 160 or 200 kg N/ha, and foliar urea at 0, 20 and 40 kg N/ha

a) Immature wheat

Source	HMW-G	(Lowry) Protein
Variety	Ns	Ns
Site (within Variety)	***	***
Ammonium Nitrate (AN)	Ns	Ns
Foliar Urea (FU)	Ns	Ns
AN x FU	Ns	Ns

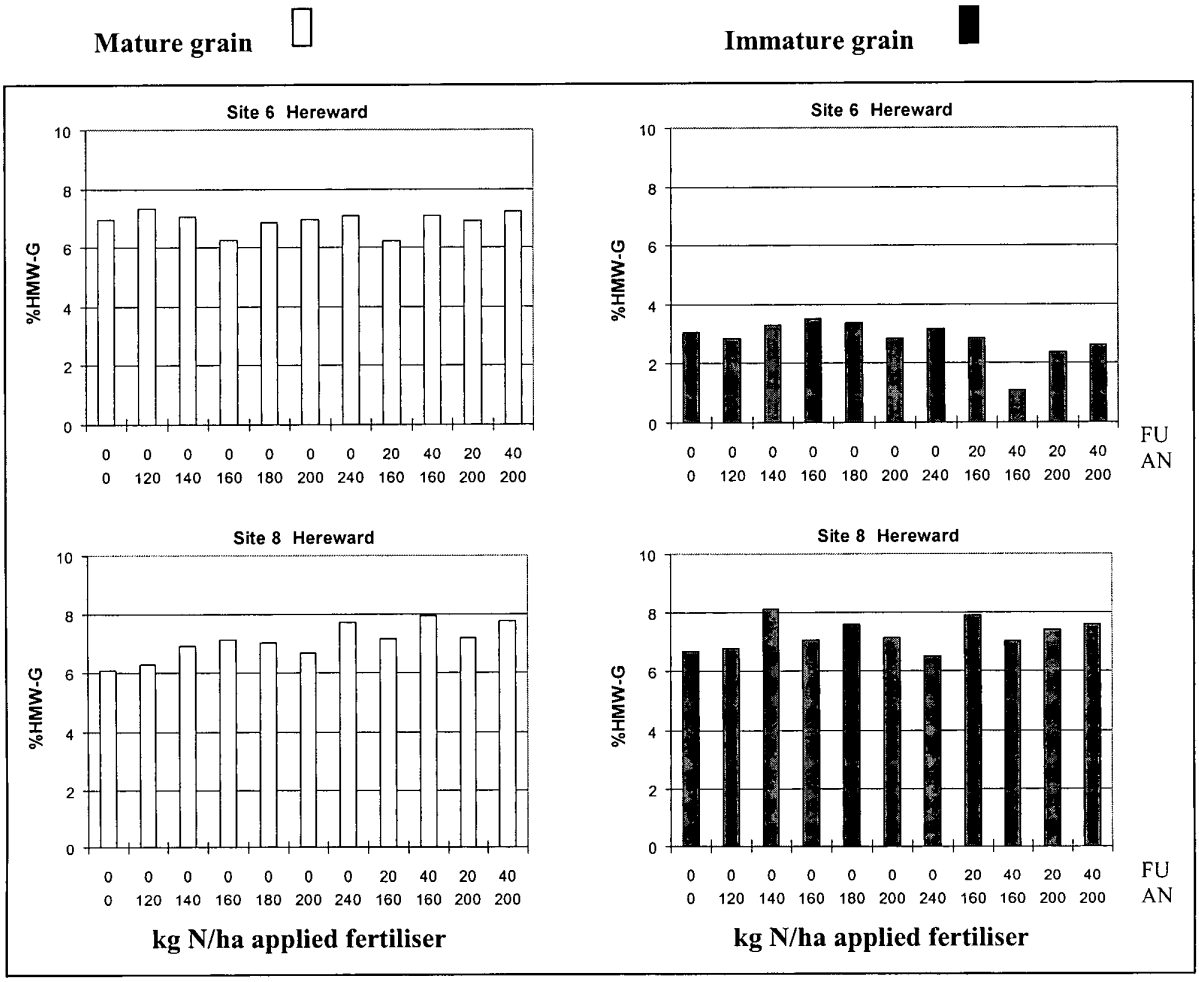
b) Mature wheat

Source	HMW-G	Protein by NIR	CBP volume	Spiral mix volume	Gel protein weight	Gel protein G'	W.Ab.	HFN
Variety	*	***	Ns	Ns	Ns	Ns	*	*
Site (within Variety)	Ns	***	***	**	**	***	***	**
Ammonium Nitrate (AN)	Ns	***	Ns	Ns	Ns	Ns	**	**
Foliar Urea (FU)	*	***	***	Ns	Ns	Ns	**	Ns
AN x FU	Ns	Ns	Ns	Ns	Ns	Ns	NS	Ns

Ns- not significant, *P<0.05; **P<0.01; ***P<0.001.

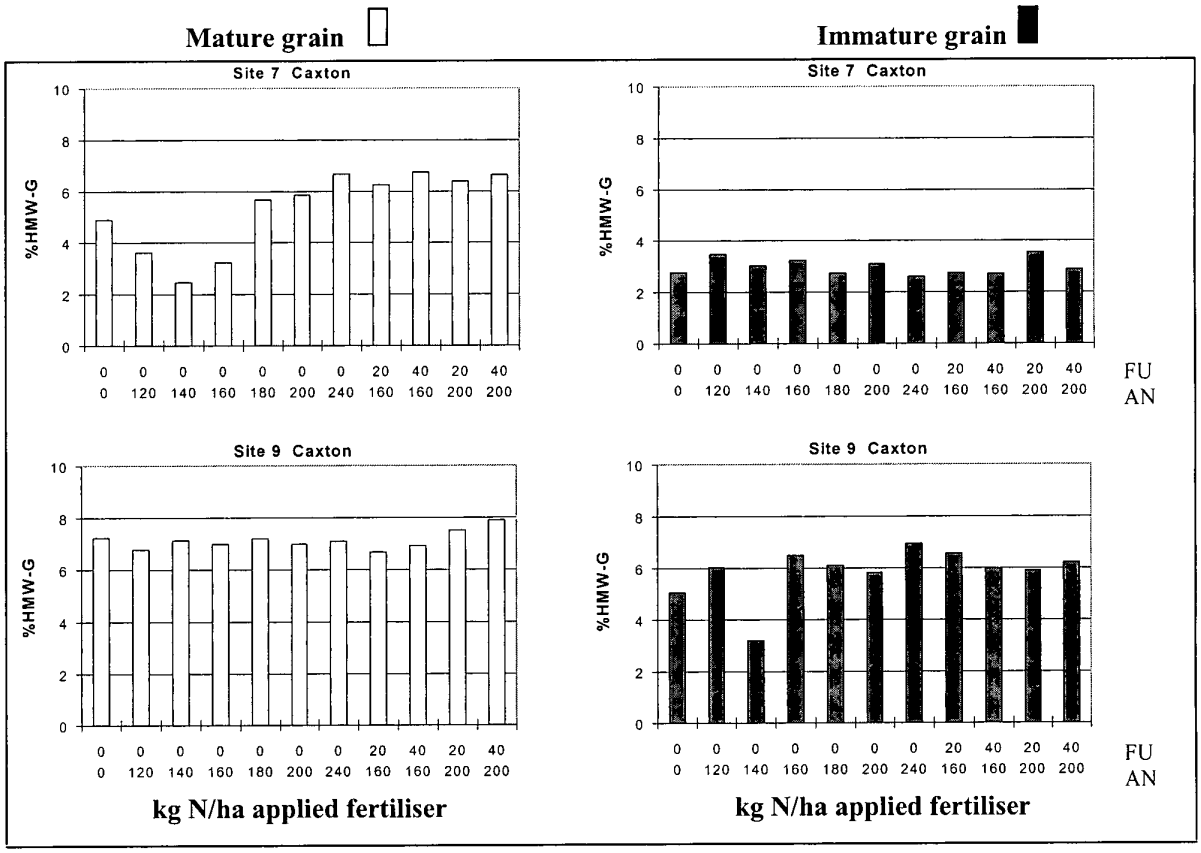
In Year 3, sites **6** and **7** containing Hereward and Caxton respectively registered the lowest % HMW-G levels within this set of immature grain samples (Figure 8a and 8b). However, these low values did not reflect the % HMW-G levels measured in their corresponding mature grain samples (Figures 8a and 8b). One possible explanation for the lower % HMW-G levels present in immature Hereward and Caxton samples, and not in the respective harvested grain at sites **6** and **7**, may be due to imprecise times of sampling of the immature grain. While all immature grain should have been sampled at GS 75, it could be that they were sampled somewhat earlier than the grains at sites **8** and **9**. This view is supported by the observations made of the SDS-PAGE gels (not shown here) which show that the HMW-G subunits in the immature samples taken from sites **6** and **7** were not clearly resolved, unlike those from sites **8** and **9**.

Figure 8a. % HMW-G in Hereward, 1997/98.



FU – Foliar Urea AN – Ammonium Nitrate

Figure 8b. % HMW-G in Caxton, 1997/98.



FU – Foliar Urea AN – Ammonium Nitrate

The relationship between levels of HMW-G in immature and mature grain appears to be complex. The overall correlation (r^2) between the HMW-G content in immature and mature samples for all sites in Year 2 is 0.81 for Rialto, and 0.67 for Hereward. Yet, when examining this relationship within each site, there is little or no correlation (Figures 7 and 9, and Tables 4 and 5). The overall correlation between the HMW-G contents of immature and mature samples of Hereward and Caxton in Year 3 trials was lower than for Year 2 samples (Table 6 and Figure 6).

Table 4. Within site correlation (Pearson) for Hereward, 1996/97 (Year 2).

	Immature (Lowry) Protein	Immature HMW-G	Mature (NIR) Protein	CBP Volume	Spiral Mix Volume	Mature HMW-G	Gel Protein Weight	Gel Protein G'
Immature HMW-G	-0.047							
Mature (NIR) Protein	**0.558**	-0.289						
CBP Volume	0.025	-0.025	-0.147					
Spiral Mix Volume	-0.004	-0.117	-0.015	0.196				
Mature HMW-G	-0.149	-0.074	0.111	0.074	0.122			
Gel Protein Weight	0.040	0.042	0.245	-0.004	-0.041	-0.1		
Gel Protein G'	0.291	0.084	0.27	0.007	0.069	-0.064	-0.022	
W.Ab.	0.271	0.047	**0.588**	-0.271	-0.213	0.134	0.017	0.173
SDS	0.176	-0.352	**0.556**	0.077	0.261	0.287	0.145	0.262
HFN	0.048	0.22	-0.214	**-0.43**	-0.338	-0.287	0.025	-0.192

(Correlations in bold where $p<0.01$)

There was no evidence of a correlation between immature HMW-G levels and any of the measured quality parameters, with the exception of protein content in Year 3. The relationships between HMW-G levels and protein contents of respective samples of immature and mature Hereward and Rialto were poor in Year 2. However, in Year 3 with Hereward and Caxton samples, these relationships were significant (Tables 4, 5 and 6). This suggests that an increase in the overall protein content due to fertiliser treatment does not necessarily result in a concomitant increase in the HMW-G content of the selected varieties.

Table 5. Within site correlations (Pearson) for Rialto, 1996/97.

	Immature (Lowry) Protein	Immature HMW-G	Mature (NIR) Protein	CBP Volume	Spiral Mix Volume	Mature HMW-G	Gel Protein Weight	Gel Protein G'
Immature HMW-G	0.069							
Mature (NIR) Protein	0.371	0.057						
CBP Volume	0.281	-0.096	-0.057					
Spiral Mix Volume	0.126	-0.123	**0.505**	0.348				
Mature HMW-G	0.187	-0.177	-0.019	0.059	-0.043			
Gel Protein Weight	0.116	0.096	**0.45**	-0.025	0.364	-0.153		
Gel Protein G'	**0.493**	-0.057	**0.585**	0.046	**0.394**	-0.028	**0.402**	
W.Ab.	0.054	0.33	0.232	-0.352	-0.095	-0.127	0.01	-0.033
SDS	0.22	-0.177	**0.696**	0.178	**0.558**	0.078	0.297	0.321
HFN	0.249	0.23	**0.473**	-0.064	**0.484**	-0.195	0.363	0.345

(Correlations in bold where $p<0.01$)

Table 6. Overall correlations (Pearson) for both varieties from 1997/98.

	Immature (Lowry) Protein	Immature HMW-G	Mature (NIR) Protein	CBP Volume	Spiral Mix Volume	Mature HMW-G	Gel Protein Weight	Gel Protein G'	W.Ab.	HFN
Immature HMW-G	**0.839**									
Mature (NIR)Protein	0.375	0.323								
CBP Volume	**-0.561**	**-0.603**	-0.363							
Spiral Mix Volume	-0.371	**-0.426**	0.315	**0.528**						
Mature HMW-G	0.349	0.369	**0.614**	-0.29	0.05					
Gel Protein Weight	-0.076	-0.154	**0.436**	0.072	0.312	0.214				
Gel Protein G'	**0.711**	**0.77**	0.354	**-0.65**	**-0.598**	**0.393**	0.077			
W.Ab.	**0.48**	0.338	**0.739**	**-0.546**	-0.099	**0.389**	**0.481**	**0.588**		
HFN	-0.129	-0.231	**0.612**	0.135	**0.419**	**0.403**	**0.67**	0.069	**0.6**	
(Kjeldahl) Mature Protein	**0.406**	0.333	**0.977**	-0.389	0.252	**0.596**	**0.506**	**0.42**	**0.827**	**0.678**

(Correlations in bold where $p<0.01$)

It may be concluded that the final HMW-G content cannot be reliably predicted from immature samples, and that site appears to be a major factor in the expression of these proteins. Sampling time may also be important. All immature samples were taken at GS 75, but inspection of SDS-PAGE images revealed that at sites **4**, **5** (Year 2), **8** and **9** (Year 3), the total protein patterns looked similar to those of mature samples. At sites **1**, **2** (Year 2), **6** and **7** (Year 3) the SDS-PAGE images of these proteins were diffuse, and the glutenin subunits possessing the highest molecular weights were not detected, indicating that these grains were sampled at an earlier growth stage. This finding suggests

that the sampling time-point at all eight sites was not equivalent in terms of protein development, and a more objective method of judging grain development, such as moisture content, is needed.

1.5 Conclusions

Results from trials, conducted over three seasons, involving several growing sites and varieties showed that while the addition of ammonium nitrate and foliar urea influenced grain protein content significantly, the effects of both fertilisers on other standard measures of quality were less consistent.

HMW-G levels, measured as a proportion of total protein extracted, were found to be unsuitable as potential quantitative markers of breadmaking quality, as there was no evidence of any significant and consistent relationship between % HMW-G and protein content or loaf volume.

These findings do not substantiate the results of the previous preliminary study conducted with Hereward grown at a single site in one season (HGCA Project No. 0008/1/94 - Pritchard and Bhandari, 1995). The current study has highlighted the dominant effects of site and season and to some extent the influence of variety on breadmaking quality. The absence of any consistent effects of fertiliser treatment on HMW-G levels in this study, in contrast to the earlier report, is partly due to the way the actual values were determined and expressed. In the preliminary study, the amount of HMW-G was reported as g of HMW-G/100g of flour, which a product of the protein content x relative amount of HMW-G. Here, the HMW-G was presented as a percentage of the total amount of protein extracted by the Laemmli buffer. Treatment of the current data in a similar manner to the previous study would produce an increase in the total amount of HMW-G expressed in response to fertiliser treatment. However, protein content would be the predominant parameter.

Despite the use of a different gel electrophoresis system, featuring more sensitive staining and more accurate densitometric methods, measuring relatively small changes in % HMW-G levels is quite challenging. A uniform amount of material was applied to each gel lane for electrophoresis, to eliminate variations in densitometric analyses arising from uneven sample loading. The inclusion of such steps is regarded to be important to obtain accurate quantitative measurements of wheat proteins resolved by the SDS-PAGE (Fullington *et al.*, 1980; Kolster *et al.*, 1991). The table below gives the comparison of the results obtained in this study for the % HMW-G in the three varieties, grown under varying environmental conditions, with the approximate theoretical values for full expression of each subunit (assuming ~2 % of total protein per subunit). The fact that the measured values are within the maximum expected values gives confidence in the validity of the methodology employed in determining HMW-G levels in this study.

HMW-G subunit composition and Glu score data for the varieties used in this study.

	Subunits			Glu	Maximum	Actually
	1A	1B/1R	1D	Score	Expected %	Measured %
Hereward	-	7+9 (1B)	3+12	5	~8.0	3.0 – 8.5
Caxton	-	17+18 (1B)	2+12	6	~8.0	2.5 – 7.9
Rialto	1	17+18 (1R)	5+10	5	~10.0	2.5 – 8.5

1.6 References

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2. The measurement of immature and mature wheat quality by NIR spectroscopy

SJ Millar and CNG Scotter

2.1 Abstract

Near infrared (NIR) spectroscopy of ground immature and mature wheat samples was used to discriminate between samples on the basis of maturity and growing location, to predict a range of quality parameters for both sample sets and to predict mature grain protein content from the immature grain samples. Using multivariate statistical techniques, samples were clearly grouped on the basis of maturity and site although the latter appeared more successful when groups of immature or mature samples only were examined. Four parameters related to flour quality (HPLC peak 3, HMW-G content, Gel Protein G′ and Farinograph water absorption) could be predicted satisfactorily by NIR in addition to established measurements such as protein and moisture content. The sample set was also split, such that NIR spectra from the immature samples were related to the protein contents of the corresponding mature samples. A calibration was developed which predicted the mature protein content from immature samples with acceptable accuracy for samples from two harvest years (1997 and 1998).

2.2 Introduction

Near infrared (NIR) spectroscopy is now widely applied to the measurement of cereal and cereal product composition. In the UK, a significant proportion of the rapid assessment of wheat lots is performed on ground samples using NIR measurement of protein, moisture and grain hardness. To perform such assessments, the NIR results must be calibrated against the appropriate reference method such as protein by the Dumas combustion or Kjeldahl methods or moisture by oven drying. In general, the technique can predict such parameters with a high degree of accuracy as the relevant regions of the spectrum show reasonably clear changes with, for example, changes in moisture content. In addition to the above parameters, a number of additional calibrations against more functional properties of wheat and flour have also been attempted by a number of workers. Typically for parameters such as flour water absorption and SDS sedimentation volume, the spectral differences observed are much more subtle, and accurate calibrations are more difficult to achieve.

Protein measurement of immature wheat

The measurement of wheat protein by NIR historically has been the most important application (Osborne *et al.*, 1993). Accurate calibrations are in widespread usage world wide at grain intake and in breeding trials. Recently, the technique has been applied to the assessment of wheat plants during development to predict both the yield and the final protein content of the grain (McGrath *et al.*, 1995; Batten *et al.*, 1993). Such measures may then be used to decide on the nutritional status of the crop and the need (if any) for subsequent fertiliser application.

NIR measurement of wheat quality

Due to the importance of the visco-elastic properties of the gluten protein fraction in breadmaking, a number of workers have investigated ways in which NIR could be used to assess the protein quality of wheat samples in a rapid manner. Early attempts to calibrate against SDS sedimentation volume using Stepwise Multiple Linear Regression (SMLR) (Osborne, 1984) were deemed unsuccessful as the prediction obtained by NIR was not significantly better than that which could be achieved by the use of protein content alone. Since this work, advances in computing power have seen the introduction of more powerful statistical techniques to the analysis of NIR data and multivariate techniques such as Principal Components Regression (PCR) and Partial Least Squares Regression (PLSR) and have become commonplace. Rather than limiting the regression to a small number of wavelengths, a number of spectral patterns covering the full wavelength range are used. Delwiche and Weaver (1994) used PLSR to predict dough and bread properties from NIR spectra of flour. Of

the parameters measured, only water absorption was modelled with any degree of accuracy. Similar results for water absorption have also been reported by Williams (1996). More recently, work using spectra from whole wheat kernels has shown that satisfactory predictions of wet gluten content and Mixograph times could be achieved for Canadian wheats from the 1995 and 1996 harvests (Pawlinsky and Williams, 1998). This latter work is significant in showing the benefits of including wheat samples from more than one harvest year in such calibrations (the value of this for protein and moisture calibrations has long been accepted). Mixograph results have also been predicted from NIR spectra of flour (Delwiche *et al.*, 1998), again using samples emanating from two harvest years. This work also included reports of acceptable NIR calibrations for size exclusion high performance liquid chromatography (SE-HPLC) glutenin and gliadin contents, as well as SDS sedimentation volume. These workers demonstrated that, although crude protein content was an important factor, the performance of the calibrations did not wholly rely on this constituent. Thus NIR measurements were shown to have inherent value in the measurement of flour breadmaking quality.

2.3 Materials and methods

2.3.1 Quality measurements

The details of the locations and fertiliser treatments used in the Year 2 and Year 3 trials, together with the various quality tests performed, are given in Section 1. For the purpose of this study, the immature and mature samples from sites **1** and **5** (both Year 2) and samples **6**, **7**, **8** and **9** (all Year 3) were measured by NIR. In addition, SE-HPLC analyses of the sample from sites **1** and **5** were carried out at IACR, Long Ashton, Bristol. The ground wheat samples were used to extract and fractionate proteins on the basis of size according to the method of Batey *et al.* (1991). The SDS-extracted proteins were resolved by SE-HPLC into three main fractions, termed peaks 1, 2 and 3. Peak 1 corresponds mainly to high M_r (relative molecular weight) glutenin polymers, peak 2 to a mixture of medium M_r polymers and monomers, and peak 3 to mainly monomers with some low M_r polymers (Zhao *et al.*, 1999).

2.3.2 NIR measurements

Instrumentation

The samples were scanned in a Foss NIRSystems 6500 monochromator in the wavelength range 400-2500nm. The NIR spectral data were acquired as the log reciprocal of reflectance (Log 1/R) using NSAS software and analysed using WINISI software (Infrasoft International Inc., Port Matilda, USA).

Sample Presentation

All the wheat samples studied were ground using a Bühler-Miag mill. Mature wheat samples were presented in a standard Pacific Scientific Inc. sample cup, while the immature freeze-dried ground samples were presented using a space-restricting ring to reduce sample volume. The sample cups were held against the optical window by a spring-loaded plate.

Replication

Three separate sub-samples were packed and presented to the instrument for each main sample. The sub-sample spectra were averaged to provide the basic data for further analysis.

2.3.3 Data pre-treatment

For full wavelength data, it is normal and advisable to employ a range of mathematical pre-treatments to optimise the spectral data for quantifying specific sample characteristics. Based on regression analysis, various pre-treatments and combinations of pre-treatments were assessed for their performance.

2.3.4 Discriminant functions and regression analysis

Principal Components Analysis (PCA)

Principal Components (PCs) were derived from the basic spectral data. These functions reduce the spectral data (700 points) to a much smaller number of PCs that retain the information contained in the original NIR data. The PCs can then be used to locate the samples in, for example 3D space. This exhibits special properties based on inter-sample distances within the space, such that samples can be selected to optimise calibration performance.

Canonical Variates Analysis (CVA)

CVA is a discriminant technique with a long pedigree in statistical analysis. It is a powerful technique that is capable of discriminating between groups of samples, in this case, based on the differences between sample NIR spectra. The Canonical Variates (CVs) are generated from PCs, which have been produced from the basic spectral data. The CVs are co-ordinates within which the samples are plotted in a similar way to PC plots. The analysis is termed 'supervised', because the samples are labelled prior to analysis, in this case by site. For this work, only a calibration analysis was possible since there were too few samples to test the calibration model.

Stepwise Multiple Linear Regression (SMLR)

Regression is the statistical technique whereby a linear mathematical model is constructed to relate two sets of data. The regression analysis allows statistical estimates of differences between the NIR estimate of the variable and the reference measurement of the same variable. SMLR generates an equation of the type noted in (i). The equation can be used to plot a regression line using the NIR estimated value and the matching reference value for each sample.

$$\hat{Y} = a + b_1x_1 + b_2x_2 + b_3x_3 + \ldots\ldots\ldots\ldots + b_px_p \qquad (i)$$

$\hat{Y}$ = the NIR estimate of the reference variable, protein in wheat for example

a = the intercept term derived by the regression analysis

$b_1..b_p$ = coefficients derived by the regression analysis

$x_1..x_p$ = NIR instrument responses (Log 1/R) at specific wavelengths chosen by the regression analysis

Modified Partial Least Squares Regression (MPLSR)

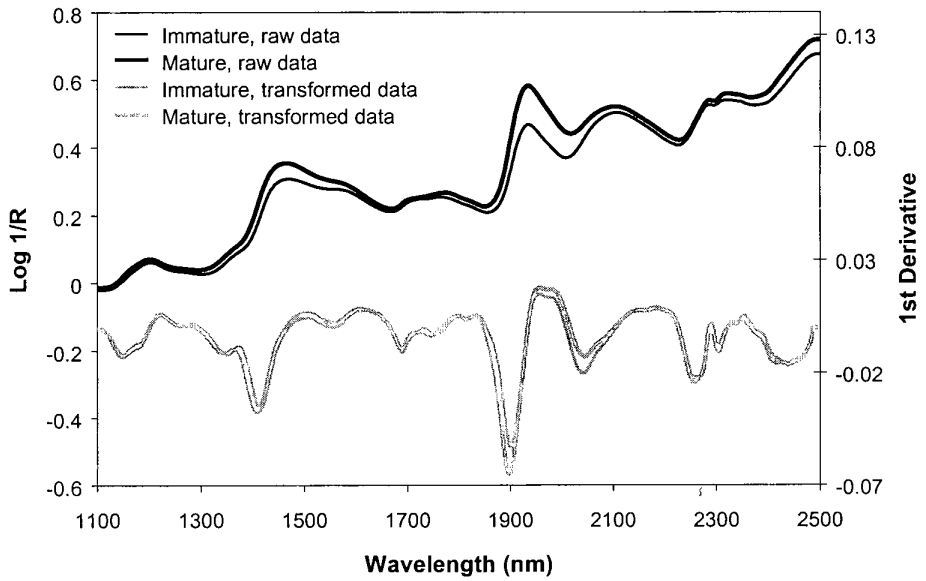
Partial Least Squares Regression (PLSR) is a multivariate technique in which both the reference and NIR spectral data are used to produce new variables somewhat similar to PC's, i.e. the spectral data are reduced to a small number of PLS factors, with minimal loss of spectral information. The factors are then regressed against the reference data to give a linear equation similar to equation (i). Modified PLSR (MPLSR) is an adaptation of PLSR used within the WINISI software.

2.4 Results

2.4.1 Discriminant functions

PCA was performed on the raw and transformed[1] spectral data. An example of each type of spectrum is given in Figure 9.

Figure 9. Raw and transformed NIR spectra (mean spectra for mature and immature samples)



The differences between the spectra for the immature and mature wheats are sufficient for these samples to be clearly grouped in the PCA plot (Figure 10). Differences between sites within the immature and mature groups are also apparent (Figure 11). This was further investigated using CVA of the NIR spectra as related to site. The sites for each harvest year were coded individually for the purposes of the analyses as follows: sites **1** and **5** in Year 2 and Year 3 as sites **6-9** (see Section 1 for full details).

[1] First derivative with datapoint gap of 4nm, datapoint smooth of 4nm and no secondary smooth (1,4,4,1); Multiplicative Scatter Correction (MSC).

Figure 10. Principal Components Analysis for all samples using transformed data – differences due to maturity

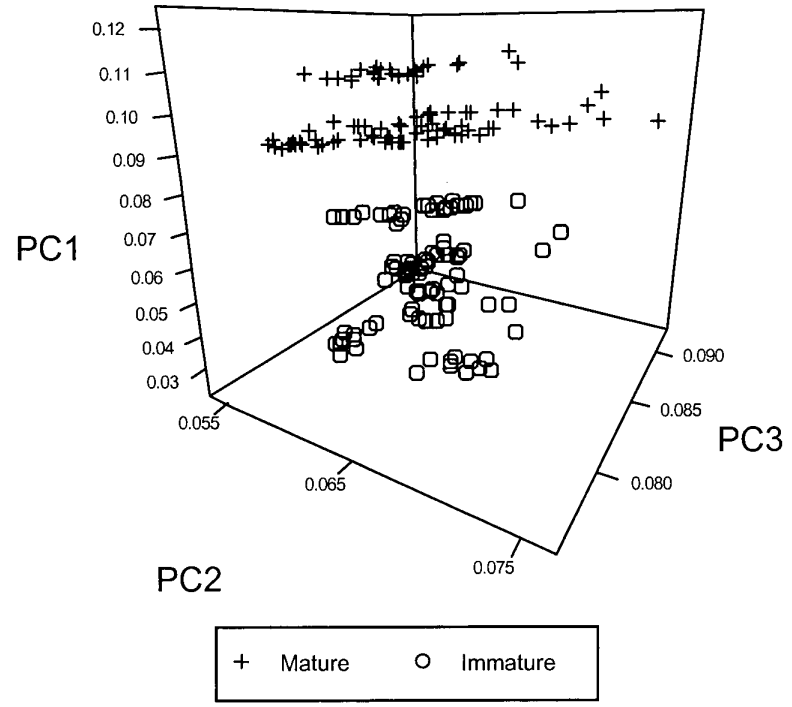
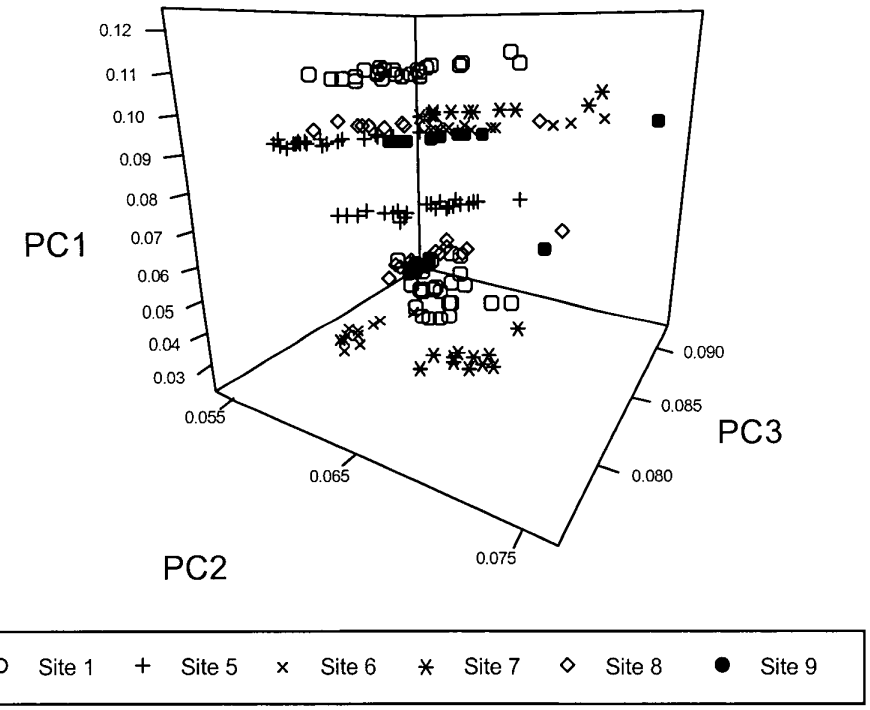


Figure 11. Principal Components Analysis for all samples using transformed data – differences due to site

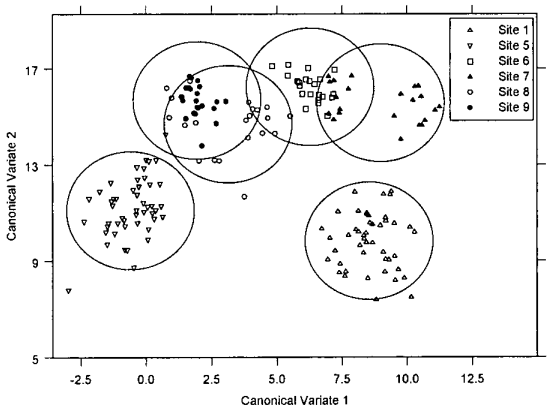


When all the samples were included in the analysis, some separation of the samples according to site was noted (Figure 12) for both the raw (12a) and the transformed data (12b). The ellipses around the

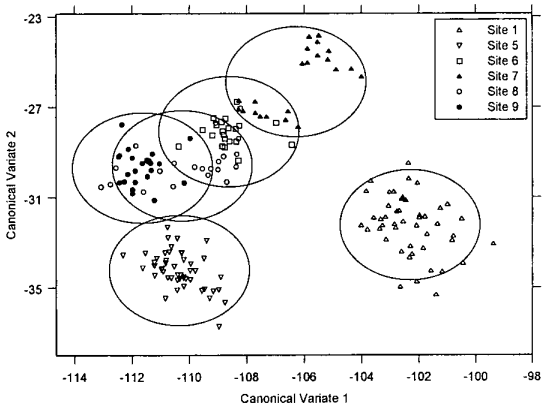
groups of data points are 95 % confidence limits, and where there is no overlap, then these groups are significantly different ($p<0.05$). The ellipses in Figure 14b appear to look like circles, but this only due to the scaling effects on this plot.

Figure 12. Canonical Variates Analysis on all wheat samples. Score plots of canonical variate 1 versus canonical variate 2.

a. Raw spectral data



b. Transformed spectral data

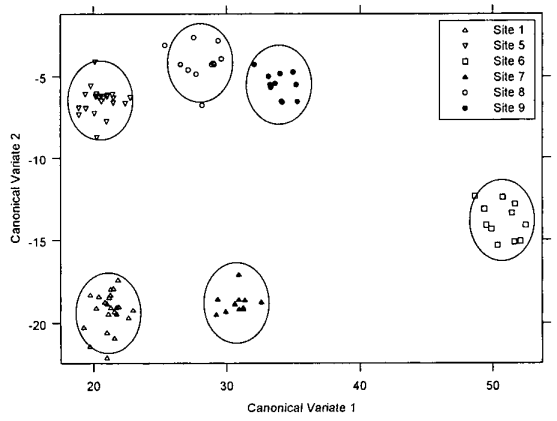


When the analysis was restricted to immature raw and transformed data (Figure 13a and b) or mature raw and transformed data (Figure 14a and b), however, the differences between sites became clearer especially in the case of the immature samples.

It was noted that the immature samples appeared to differ in maturity between sites (Section 1.4.2) and it is possible that this was responsible for the clear differences between sites observed. For the mature samples, site **1** is clearly separated from the other sites according to CV1. It has been noted that this site had some problems with high *alpha*-amylase activity as shown by the low Falling Number noted for the Hereward samples and this may be the cause of the differences observed. The results for site **5** differ from those from the remaining sites due to differences in CV2 although the separation from the other sites is less marked than that for site **1**. The differences observed between sites are also apparent when all the samples are included in the CVA, but the spread of samples within each group is such that the distinction is less marked. For the immature samples, sites **5**, **8** and **9** seem to be more closely grouped, although there seems to be no obvious explanation for this.

Figure 13. Canonical Variates Analysis on immature wheat samples. Score plots of canonical variate 1 versus canonical variate 2.

a. Raw spectral data



b. Transformed spectral data

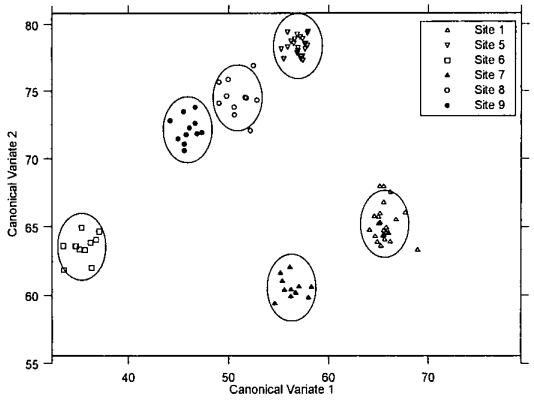
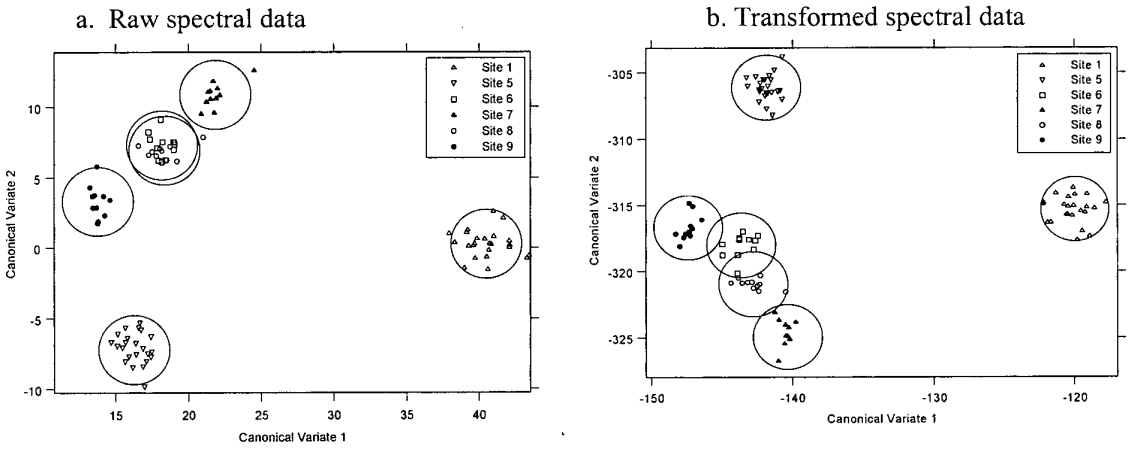


Figure 14. Canonical Variates Analysis on mature wheat samples. Score plots of canonical variate 1 versus canonical variate 2.



The spectral patterns associated with the CVs (Figures 15-17) tend to show peaks and troughs in the regions associated with hydroxyl groups (~1445nm and ~1930nm) for the raw or un-transformed data. The patterns associated with the transformed data are much more complex and difficult to interpret although there is still some evidence of peaks and troughs in these regions. In the case of the immature samples there is some evidence of a 'shoulder' at 2100nm (indicated by the arrow in Figure 16) in the loading associated with CV1 which would be consistent with differences in starch concentration for these samples. This is of interest in light of the observed differences in maturity between them as the relative proportions of protein and starch in the grains may be expected to change during grain development.

Figure 15. Canonical Variates Loading Plots for all samples

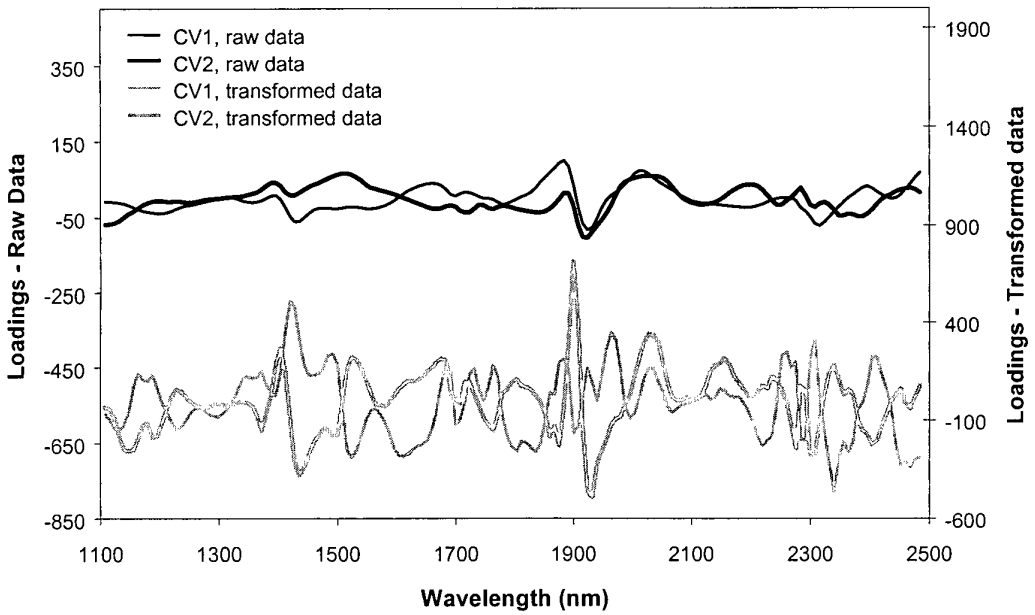


Figure 16. Canonical Variates Loading Plots for the immature samples

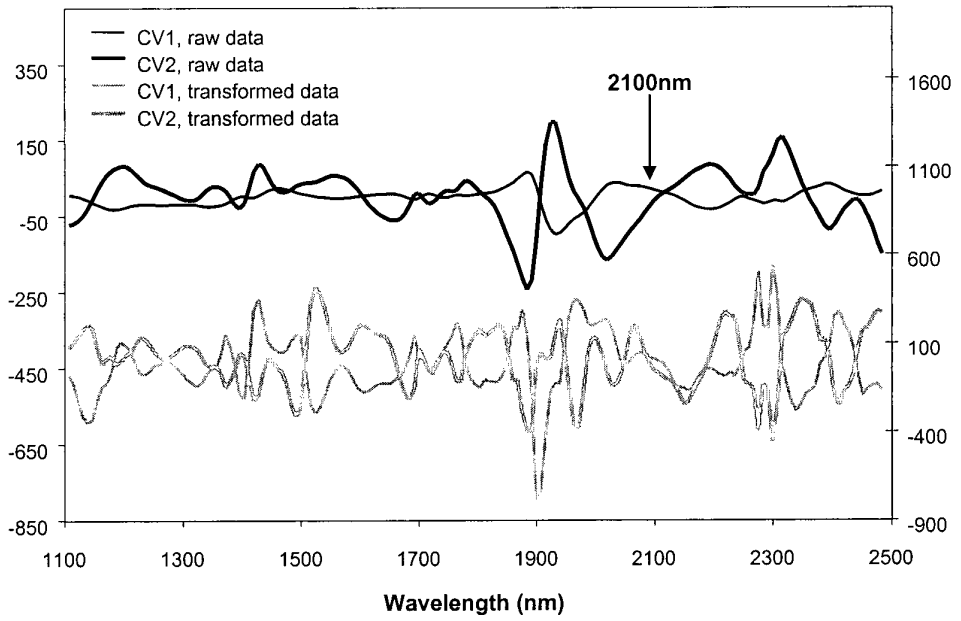
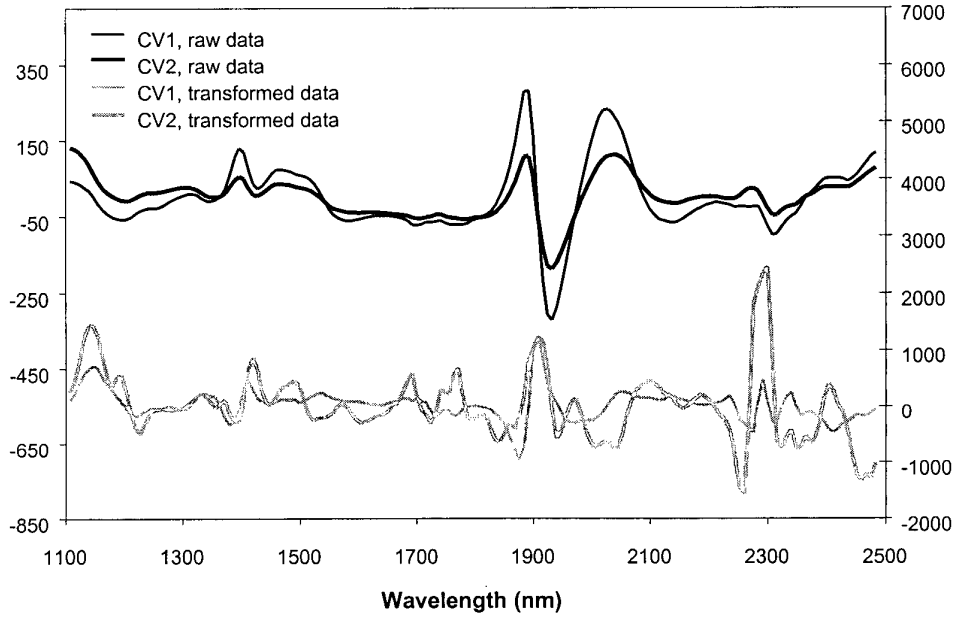


Figure 17. Canonical Variates Loading Plots for the mature samples



2.4.2 NIR calibration against measures of grain quality for immature and mature wheat

To establish the best protocol, calibrations for protein and moisture (on the understanding that these are easily modelled by NIR) were derived using a number of data pre-treatments and calibration procedures (Table 7). The best results for Kjeldahl protein were obtained using MSC and a first derivative transform of the Log 1/R data in the range 1108-2492nm. MPLSR was used and gave a Standard Error of Cross Validation (SECV) of 0.178, which compares well with previous results (Osborne, 1983). For oven moisture the best results were again obtained using MSC on a first

derivative transform of the Log 1/R data in the range 1108-2492nm. In this case, however, SMLR out-performed MPLSR giving a Standard Error of Calibration (SEC) of 0.061, which again compared favourably with previous work (Osborne *et al.*, 1982). Both MPLSR and SMLR were then applied to the remaining constituents using the best data pre-treatment.

Table 7. Effect of NIR data pre-treatment on regression results (optimum highlighted)

Constituent	Data pre-treatment†		SMLR		MPLSR		
			R^2	SEC	R^2	SEC	SECV
Kjeldahl Protein	1,4,4,1	No MSC	0.980	0.183	0.982	0.173	0.208
Kjeldahl Protein	1,4,4,1	MSC	0.988	0.140	0.990	0.126	0.178
Kjeldahl Protein	2,8,6,1	No MSC	0.981	0.176	0.989	0.137	0.200
Kjeldahl Protein	2,8,6,1	MSC	0.988	0.140	0.989	0.135	0.185
Oven Moisture	1,4,4,1	No MSC	0.998	0.082	0.997	0.087	0.140
Oven Moisture	1,4,4,1	MSC	0.999	0.061	0.998	0.074	0.137
Oven Moisture	2,8,6,1	No MSC	0.998	0.080	0.996	0.101	0.151
Oven Moisture	2,8,6,1	MSC	0.999	0.062	0.998	0.078	0.141

† 1,4,4,1 – First derivative with datapoint gap of 4nm, datapoint smooth of 4nm and no secondary smooth

2,8,6,1 – Second derivative with datapoint gap of 8nm, datapoint smooth of 6nm and no secondary smoothing

MSC – Multiplicative Scatter Correction

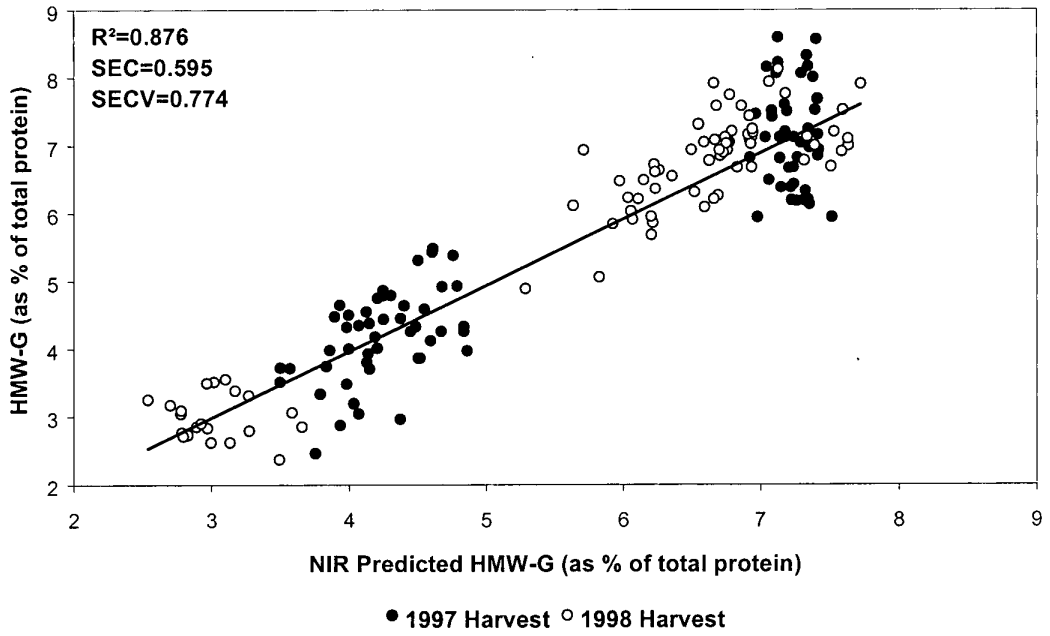
The results for the remaining constituents are summarised in Table 8 below. In each case, samples having standardised residuals of 3.0 or greater were removed. In no case were more than 5 samples removed as outliers. The results indicate that only HPLC peak 3 (a large proportion of which consists of the gliadin proteins), HMW-G, Gel Protein G′ and Farinograph water absorption could be predicted with R^2 of greater than 0.8. This figure is at the lower limit of useful prediction and represents a level of confidence that may be useful in screening or early prediction work.

Table 8. NIR Regression results for Years 2 and 3

Constituent	SMLR		MPLSR	
	R^2	SEC	R^2	SECV
Loaf volume	0.535	40.242	0.490	36.574
HPLC Peaks 1+2	0.416	3.846	0.185	4.542
HPLC Peak 3	0.887	1.477	0.845	1.728
HMW-G	0.814	0.73	0.876	0.595
Gel Protein weight	0.400	1.612	0.736	1.068
Gel Protein G′	0.696	5.444	0.803	4.394
Water absorption	0.809	1.529	0.831	1.438

Preliminary investigation indicated that some of these relationships owed much to the difference between immature and mature samples. The results for HMW-G using MPLSR (Figure 18) were encouraging, however, as maturity appeared to be less of a factor.

Figure 18. NIR calibration for HMW-G using the 1997 and 1998 harvest samples (Modified Partial Least Squares Regression)



It is known that protein quantity (amongst other factors) has a significant effect on breadmaking quality and so the HMW-G results have been expressed as a percentage of the protein extract loaded onto the gels to account for this. It is clear from the results in Table 7 and from previous work that NIR can be used to estimate sample protein content with great accuracy. To ensure that the calibrations derived for the functional parameters in Table 8 were not due to secondary correlations with protein, the calibration exercise was repeated using Kjeldahl protein as a predictor rather than the NIR data (Table 9). For those samples where Kjeldahl data had not been gathered, the Lowry results were transformed using the following equation derived using those samples where both determinations had been performed (n=48):

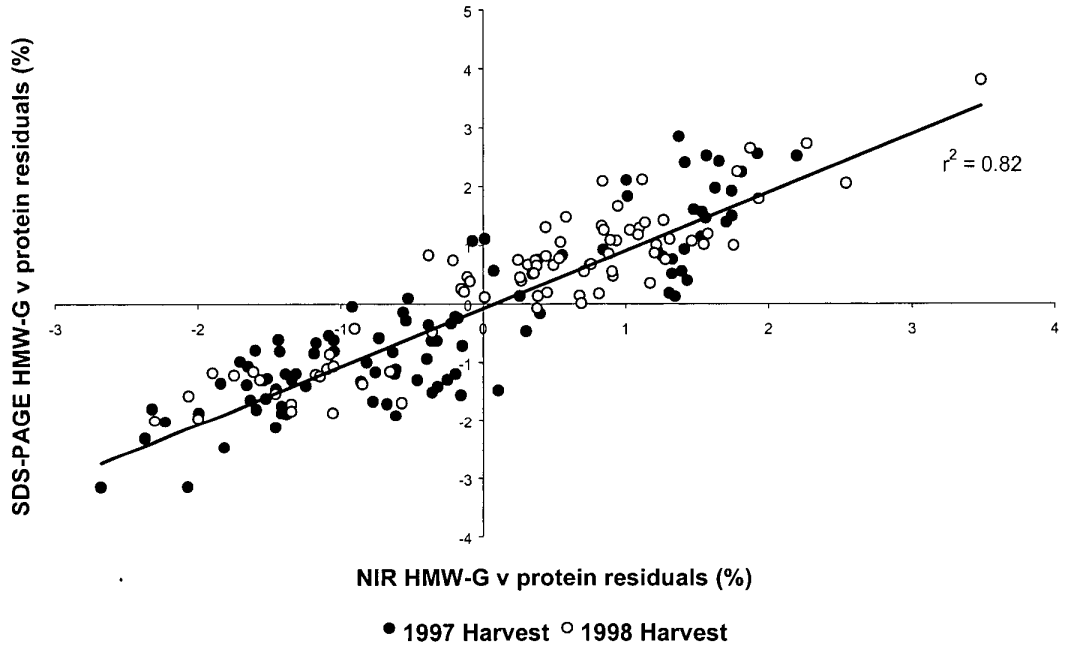
$$\text{Kjeldahl (\%)} = [\text{Lowry (\%)} \times 1.1] + 0.047 \qquad \text{(ii)}$$

Table 9. Regression results, using crude protein for the 1997 and 1998 harvest years

Constituent	r^2	Standard Error
Loaf volume	0.058	57.32
HPLC Peaks 1+2	0.036	6.083
HPLC Peak 3	0.000	6.074
HMW-G	0.366	1.389
Gel Protein weight	0.004	2.077
Gel Protein G'	0.286	8.335
Water absorption	0.464	2.560

These results indicate that NIR was a much better predictor than crude protein alone. To confirm that the HMW-G results were truly independent of protein content for both the SDS-PAGE and NIR measurements, the partial correlation (adjusted for protein content) was calculated (Fearn, 1999). This involves plotting the residuals (observed-predicted values) of SDS-PAGE HMW-G v Kjeldahl against the residuals of NIR HMW-G v Kjeldahl (Figure 19).

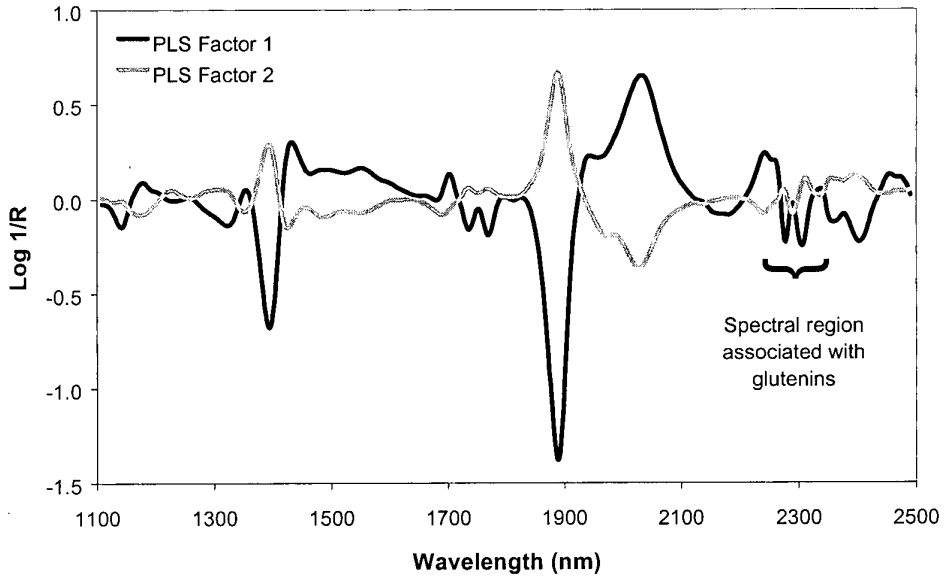
Figure 19. Partial Correlation between SDS-PAGE HMW-G and NIR predicted HMW-G corrected for Kjeldahl protein content



This shows that there is an underlying relationship between the NIR spectra and the HMW-G content (expressed as a percentage of protein loaded onto the gels) which is unaffected by overall crude protein content. From this it may be concluded that there is a fundamental relationship between the NIR spectra and the functional properties of both immature and mature wheat. To understand what areas of the spectrum are important for the performance of a particular calibration, either the wavelengths used (SMLR) or the spectral patterns derived (PCR and PLSR) should be examined. In

the case of PLSR, these patterns are not wholly dependent on spectral information, relying also on the reference data. The spectral loadings associated with the PLS factors can, therefore, be difficult to interpret. Nevertheless, other workers have shown their relevance in understanding the relationships between particular parameters of interest and underlying spectroscopic properties (Williams, 1996). Five factors were used to produce the calibration for HMW-G and the first two of these are reproduced below (Figure 20).

Figure 20. Modified Partial Least Squares Regression Factors 1 and 2 for the HMW-G NIR calibration

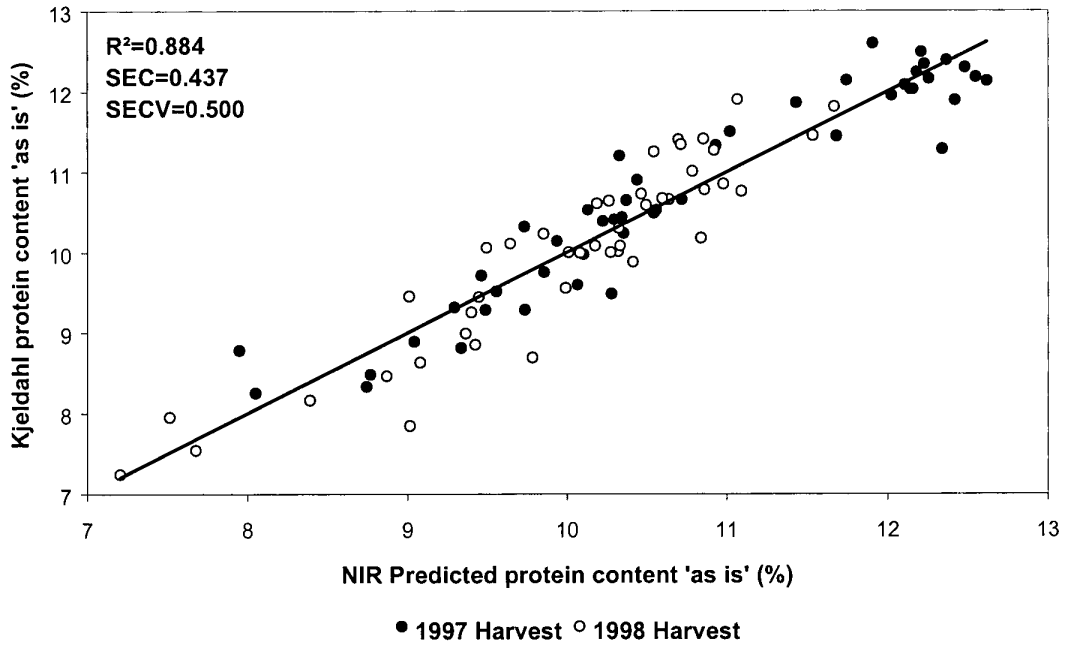


All of the spectral loadings had a number of peaks/troughs in the region 2250 - 2350nm as indicated in Figure 20 for factors 1 and 2. This area is often associated with lipids (Osborne *et al.*, 1993) but recently workers have shown that both gliadins and glutenins have specific absorbances in this region (Wesley *et al.*, 1999). This is encouraging, as NIR calibrations based on an understanding of the spectral and biochemical properties of the samples in question are preferable to those where the statistical analysis has been treated as a 'black box', to give results with no indication of how they have been derived. Other workers have also reported this region to be associated with measures of flour functionality (Brian Osborne, private communication) and it is clear that further work should concentrate on a better understanding of the spectral properties of the gluten proteins and how these change with varying protein quality and quantity.

2.4.3 Predicting mature grain protein content from NIR spectra of immature grain

To demonstrate the potential of NIR to assist in making agronomic decisions, NIR spectra of the immature samples were used to develop prediction equations for mature grain protein. Due to the relatively small number of samples, separation of the data into separate calibration and prediction sets was deemed impractical. The MPLSR method used, however, estimated the prediction error using cross validation producing the statistic SECV, which is a better estimate of future predictive performance than SEC. Both harvest Years 2 and 3 were included and an overall bias between the mean protein contents for these years (1997 = 10.72 %; 1998 = 10.00 %) was observed (Figure 21). When developing NIR calibrations, it is important to include samples from a range of growing sites, harvest years and varieties as well as a good range of the parameter of interest to ensure robust calibrations. The inclusion of all the samples studied goes some way toward this, although the calibration would be further improved by the addition of more samples.

Figure 21. NIR calibration for mature wheat protein content (Kjeldahl) using immature wheat spectra (Modified Partial Least Squares Regression)



ICC Recommendation No. 202 (ICC, 1986) for the measurement of wheat protein indicates that the Standard Error of Prediction (SEP) between the NIR and reference methods should be no greater than 0.3 %. Although sophisticated research instruments may allow this figure to be further reduced, in routine testing situations it represents an acceptable degree of accuracy. It is clear that predicting mature grain protein from immature spectra introduces a greater degree of inaccuracy. The SECV is 0.5 indicating that the SEP would be approximately twice that defined by the ICC. However, the demands on the calibration are greater given the differing stages of development in the immature samples. In addition, there are a number of external factors that will affect the final stages of grain development which cannot be accounted for at the time of NIR measurement. These results show that the NIR technique may be applied to the measurement of immature grains to indicate the probable level of protein that may be expected in the final grain.

2.5 Conclusions

Analyses of the quality and NIR spectral data of immature and mature wheat samples of Hereward, Rialto and Caxton showed that the NIR technique was able to:

1) discriminate between samples on the basis of their maturity and growing locations
2) predict the harvested grain protein content from developing grain samples taken at GS 75, irrespective of variety
3) predict a range of quality parameters (gliadin and HMW-G content, gel protein G′ and water absorption values).

These findings suggest that NIR technology has a potential role in rapidly assessing the nitrogen fertiliser needs of home-grown wheat crops.

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3. Prediction of nitrogen (N) content from whole plant at GS 60

IR Richards and DG Bhandari.
This section contains selected information from the more detailed report prepared by Levington Agriculture for Hydro Agri (UK) who have given permission for this trial study to be discussed.

3.1 Introduction

High grain protein contents are required for breadmaking wheat. These are usually achieved by increasing the application of total fertiliser nitrogen (N) by around 25 kg N/ha above the amount needed for optimum yield. If this additional N is applied as foliar urea, there may be an opportunity to measure N levels in growing wheat plants before such an application in order to predict whether extra N is needed and, if so, how much. This Hydro Agri (UK) commissioned project aimed to provide evidence on two key aspects:

- Can grain protein content be predicted early enough in the crop's growth for corrective action to be taken?

- What is the relationship between late foliar urea and grain protein content?

Trials were carried out in 1996 and 1997 when 3 trials were conducted. This report discusses trials carried out in 1998. The design of trial was similar in the two years.

3.2 Materials and methods

Four trials were conducted and corresponded to the sites referred to as sites **6**, **7**, **8** and **9** in Sections 1 and 2 of this report. Ammonium nitrate was added as the main dressing in April, and foliar urea was applied as a late dressing in June.

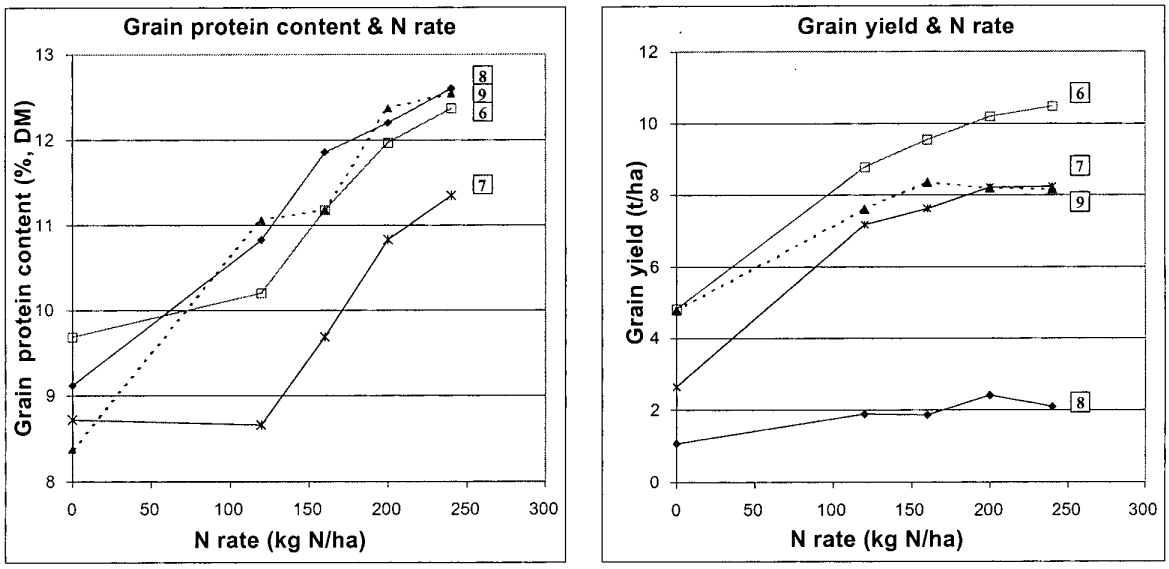
Treatment Number	Ammonium Nitrate kg N/ha	Foliar Urea kg N/ha	Total kg N/ha
1.	0	0	0
2.	120	0	120
3.	140	0	140
4.	160	0	160
5.	180	0	180
6.	200	0	200
7.	240	0	240
8.	160	20	180
9.	160	40	200
10.	200	20	220
11.	200	40	240

The following determinations were made in all the trials: flag leaf and whole crop (all the plant material above ground) N contents at GS 30 and GS 60; grain yield, grain N content and grain N yield. The N content was determined using the Kjeldahl method, and expressed on a dry matter basis (dm). Statistical analysis was performed by the ANOVA (analysis of variance) method.

3.3 Results

Generally, there were significant responses to rate of N in both grain protein content and grain yield (Figure 22). An exception occurred at site **8** where yield showed a poor response due to generally poor growth. This site suffered waterlogging in April. However, even on this, site, there were responses to N in terms of grain N content (Figure 22, where N content has been converted to protein content).

Figure 21. Relationships of N rate (total nitrogen fertiliser) with harvest grain % protein content and yield at 4 sites.



The N contents of flag leaf and whole crop measured at GS 60 are shown in Figure 23. Both the flag leaf and whole crop show an increase in N content in response to nitrogen fertiliser addition. Interestingly, the poor growth at site **8** was reflected in the flag leaf N content, but less so in the whole crop N content (Figure 23). The data for flag leaf and whole crop measurements taken at GS 30 were less conclusive as they could not be related very well with the harvest crop N content, and have not been included here.

Figure 23. Relationships of N rate (total nitrogen fertiliser) with flag leaf and whole crop % protein content at GS 60 at 4 sites.

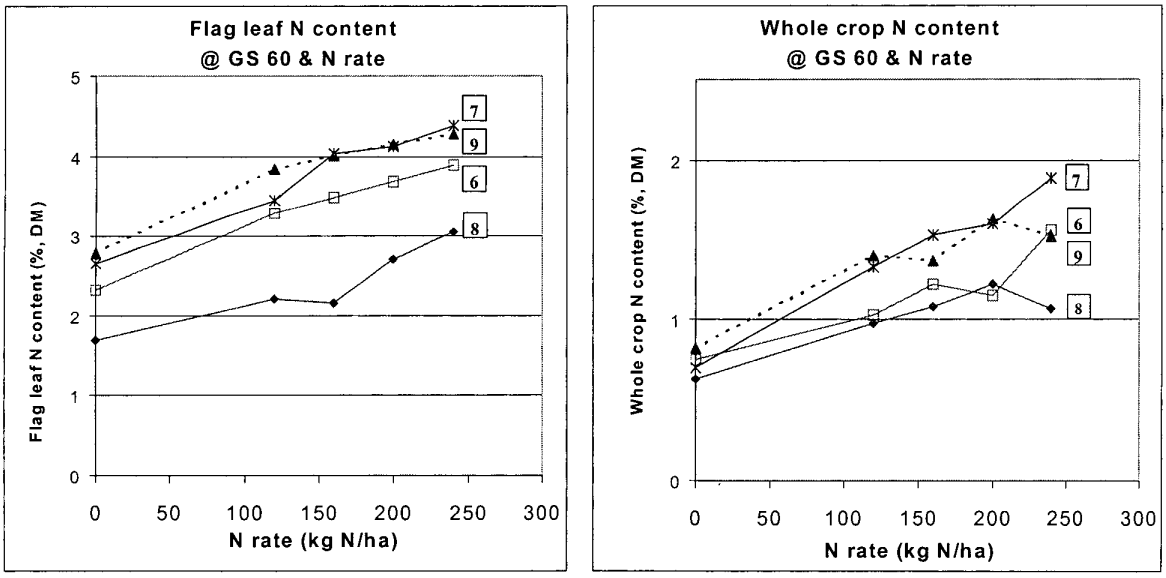
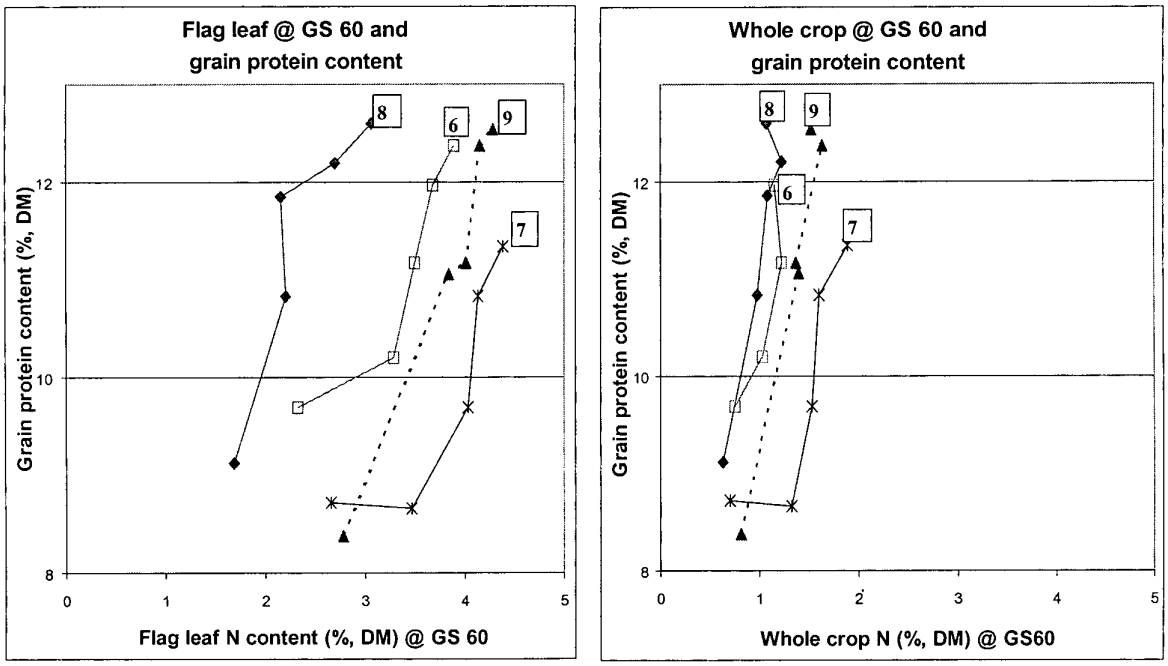


Figure 24. Relationship between grain protein content and flag leaf or whole crop N at GS 60



When the data from all the trials from the 3 years were considered, it was found that the variation among trials was somewhat less in the relationship between whole crop N content at GS 60 and grain protein content than the corresponding one with flag leaf N content. Figure 24 shows the results for sites **6**, **7**, **8**, and **9**, where the range for N content in flag leaf is greater than for whole crop. Using data from all trials over the three years, this relationship for the whole crop was described by r^2 =0.42. This correlation is not as good as that found with the NIR calibration for grain protein content using immature wheat sampled at GS 75 (where r^2 = 0.88). However, as the data used in each type of predictive model were derived from largely different sets of trials, it is not possible to make a direct comparison of the correlation values.

Analysis of the data indicates that, for a grain N content of 1.5 % N (equivalent to 8.6 % protein) whole crop N content should be around 0.48 % N on a dry matter basis at GS 60.

Where the main application N rate was 200 kg N/ha, the effect of foliar urea on grain N content was consistent with that found in earlier work, that is, 40kg N/ha as urea is associated with an increase of around 0.1 % in grain N content (0.57 % protein content). The reason for the smaller effect (0.02-0.03 % in grain N content) where the main application was 160 kg N/ha is not clear. Foliar urea had no significant effect on grain yield, so dilution of grain N by additional yield was not the reason.

Economic optimum N (fertiliser application) rates were calculated for the 4 trials using quadratic regression functions to relate grain yield and N rate, with an assumed cost of £0.29/kg N and value of £75/t for grain.

Trial	Calculated economic optimum N (kg N/ha)	Yield at optimum (t/ha)
6	245	10.55
7	202	8.19
8	165	2.05
9	180	8.2

3.4 Conclusions

Results for 4 trials in one year cannot be regarded as definitive. However, some tentative conclusions can be drawn. The results, including those from the two previous years, suggest that it could be possible to develop a predictive system by analysing whole crop N content at GS 60, although a better correlation is obtained by NIR measurements of grain at GS 75. Both these growth stages would be early enough for farmers to intervene, as necessary, and further studies are required to develop optimal sampling protocols and more robust predictive models from a larger sample set. It is also possible that an advisory system could be based on whole crop N content at GS 30, but more data are needed to confirm this. While Flag leaf N content measurements taken at GS 30 were consistent with those taken at GS 60, they did not correlate with the harvest crop N content, and therefore, are not useful for predicting end protein content of the grain.

4 Acknowledgements

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