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Predicting grain yield and protein content in winter wheat and spring barley using repeated canopy reflectance measurements and partial least squares regression

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SUMMARY

By providing both spatial and temporal information remote sensing may function as an important source of data for site-specific crop management. This technology has been used for nitrogen application strategies to obtain optimum yield and grain quality. Here, the objective was to use early repeated remotely sensed multi-spectral data to predict grain yield and quality for winter wheat (*Triticum aestivum* L.) and spring barley (*Hordeum vulgare* L.). The crops were sown with two different seeding rates and a wide range of nitrogen strategies were applied. Multi-way partial least squares regression (N-PLS) was used to predict grain yield and protein content. The results were compared with unfold-PLS1 and PLS1 using reflectance data from the last measurement day. Both single reflectance wavelengths and selected vegetation indices were used simultaneously. The results reveal that all models can make a good prediction of yield in both crops with unfold-PLS1 and N-PLS as the best. However, estimation of grain protein content at harvest was very poorly determined in barley, as no relation between the reflectance measurements and barley protein content was obtained. The relation between reflectance measurements and protein content was slightly better in wheat, where especially N-PLS improved the prediction of grain protein content. The overall conclusion of the present experiments is that data from repeated measurements of reflectance used in multi-way partial least squares regression before heading improved the prediction of grain yield and protein content in wheat and barley.

INTRODUCTION

For more than a decade scientists have tried to describe and quantify the relation between in-season canopy reflectance and crop plant physiological status (Shull 1929; Mestre 1935). A number of spectral vegetation indices have been proposed, which include individual reflectance factors, linear combination of bands, two-band vegetation indices including normalised difference vegetation indices, soil-adjusted vegetation indices, non-linear indices, perpendicular vegetation indices and derivative indices (Dusek *et al.* 1985; Wiegand *et al.* 1991; Penuelas *et al.* 1994; Chen 1996; Datt 1999; Adams *et al.* 1999; Daughtry *et al.* 2000). These indices have been related to vegetation density or green leaf cover (Dusek *et al.* 1985; Daughtry *et al.* 2000), light use efficiency (Penuelas *et al.* 1994), green leaf area index (Best & Harlan 1985; Dusek *et al.* 1985), photosynthesis rate (Penuelas *et al.* 1994), amount of photosynthetically active tissue (Wiegand *et al.* 1991), chlorophyll and other pigments (Penuelas *et al.* 1994; Datt 1999; Daughtry *et al.* 2000), canopy water balance (Penuelas *et al.* 1994) and chlorosis (Adams *et al.* 1999). Some of these vegetation indices have been very useful for early prediction of grain yield, allowing in-season nutrient adjustments. An ideal vegetation index should be

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highly sensitive to a single vegetation parameter e.g. leaf cover or chlorophyll concentration, but insensitive to soil background, irradiance strength and direction, and phenological stage. None of the spectral vegetation indices can fully meet all these criteria, but they can reduce the noise threshold from the above-mentioned parameters significantly compared with reflectance wavelengths (Jackson *et al.* 1983; Wessman 1990). Canopy physiological properties, and consequently the reflectance spectrum, change according to growth conditions and time of measurement (Filella *et al.* 1995). Consequently, changes of reflectance and vegetation indices observed individually and/or in combination through time give valuable information when trying to model crops grown under variable conditions, because final yield is the function of all conditions or growth factors influencing plant growth over the whole growth period. The result of using several indices, each related to different crop physiological properties, should be a more precise prediction of both grain yield and quality (Hansen *et al.* submitted).

In recent years cereal production in high-input systems has been a major concern in many regions of Europe due to nitrogen leaching to ground and surface water (EC-Council Directive 1991; van Alphen & Stoorvogel 2000). There is a demand for environmentally friendly or more efficient management systems. These systems should have a high net N uptake in order to limit residual soil N after harvest. They should also use N more efficiently in order to maximize yield and ensure that the targeted grain quality is obtained. In wheat there is an economic incentive to produce high protein bread wheat, and in barley there is an economic motivation to produce malting barley with restricted protein content per unit grain dry matter.

Generally, it is important to be able to make an estimation of crop yield. The normalized difference vegetation index (NDVI), calculated on the basis of reflected light from the red and NIR bands, has long been used as an indirect measure of crop yield, but it uses measurements made late in the growth season from late stem elongation (Tucker & Holben 1980; Pinter *et al.* 1981). Recently, NDVIs have been combined with cumulative growing degree days (GDD) and early-season plant N uptake creating yield prediction models in winter wheat (Raun *et al.* 2001; Lukina *et al.* 2001). Repeated spectral measurements accumulating NDVI were useful in predicting grain yield in wheat (Pinter *et al.* 1981; Raun *et al.* 2001) and millet (Rasmussen 1992) compared with a single spectral measurement. However, the extensive use of NDVI for yield prediction does not mean that it is the best index to use. NDVI is used because a lot of reflectance data are available from various satellite systems, vehicle-based systems and handheld systems, all providing red and NIR reflectance measurements. This was confirmed recently, where simple ratios of red and NIR (SR) correlated better to durum wheat yield (Aparicio *et al.* 2000) compared with NDVI and the same was the case for "green" normalized difference index (GNDVI) estimating corn grain yield (Shanahan *et al.* 2001).

Yield and protein content are two important key factors for bread wheat production and marketing (Jenner *et al.* 1991) as well as for barley production for malt and feed (Bertholdsson 1999). Protein concentration is known to influence the bread-making quality of wheat (Finney & Barmore 1948; Johansson *et al.* 2001). The protein concentration is determined in wheat by the genetic background, but also, to a large extent, by environmental factors such as nitrogen, water access and temperature conditions (MacDonald 1992; Johansson & Svensson 1998; Johansson *et al.* 2001). In barley used for malt, the grain protein content should be lower than 11·5% (Bertholdsson 1999). This may be difficult as the protein content is influenced by cultivation practices and by environmental factors such as availability of nitrogen and stress situations caused by drought (Bertholdsson & Stoy 1995; Eagles *et al.* 1995; Birch *et al.* 1997).

Prediction of grain protein for the prospective wheat and barley harvest would, therefore, be of value to farmers when deciding if the field should be divided into different management zones in order to harvest and deliver the targeted qualities. Grain yield and quality can however be influenced by late season fertilizer and fungicide application (Gooding *et al.* 1991; Birch *et al.* 1997; Gooding *et al.* 1976*b*; Bertholdsson 1999), but the net profit for the farmer depends on application costs, yield response and crop value. There is therefore a need to predict grain quality during the growing season to improve decision-making concerning management practice.

Our approach here is to use all available data in the prediction model and not, as previously done, to reduce the information level by calculating a single index or summarize index values when repeated measurements have been done over time. Therefore, this work uses all available reflectance wavelengths, and also calculates indices presented earlier in the literature and assembles them into one data structure of variables (X). The indices were included because it has been concluded previously that non-linear transformations can provide additional information. However, this implies that the methods used for prediction analysis can handle multi-variate data structures with high covariance and redundancy. Partial least square regression models are ideal for that purpose (Martens & Næs 1989).

When including time as an additional dimension, the dataset becomes three-way, which cannot be handled in the prediction models normally used. One may be tempted to analyse three-way data after aggregating over one of the three ways (sample, variable or measuring time), or by analysing all two-way datasets contained in the three-way data set separately. However, it should be noted that such approaches do not offer an explicit description of the three-way interaction in the data. Hence, they may lead to conclusions that are at best incomplete.

Techniques for handling three-way data sets have been developed in the last few decades. N-PLS is a potential prediction model, which uses latent structures for making predictions of dependent variables within empirical three-way data sets. The strength of N-PLS is that it summarizes all latent information from a large N-way dataset of object variables (X) and relates it to a dependent variable (y) using a relatively low number of parameters, which makes the prediction more robust.

This paper uses N-PLS for prediction of grain yield and protein content using repeated measures of canopy reflectance during vegetative growth in winter wheat and spring barley. The results are compared with a bilinear PLS1 analysis using the reflectance data from the last date of measurement (single-PLS1) and a bilinear PLS1 using the unfolded three-way data cube (unfold-PLS1). Both reflectance of various wavelengths and indices previously presented in the literature are used in the analysis to improve the prediction ability. Further, an assessment is made of whether there is the potential to predict grain yield and quality early enough for the farmers to alter crop management.

MATERIALS AND METHODS

Two separate experiments, one in winter wheat (*Triticum aestivum* L. cv. Ritmo) and one in spring barley (*Hordeum vulgare* L. cv. Alexis) were carried out in 1999/2000 at the Danish Institute of Agricultural Science, Foulum (56° 29′ N, 9° 34′ E). The experiments were placed on two different fields approximately 500 m apart. The soil type was the same sandy loam for both experiments, containing 10% clay and 2% total carbon. The distribution of the plots was in both cases designed as a two-factor split-plot design with three randomized blocks. The sowing date was 20 September 1999 in wheat and 10 April 2000 in barley. The row distance was 12·5 cm, and the direction of the rows was east–west. Sufficient PKS-fertilizer was supplied in early April at the start of growth to avoid deficiency and efficient weed and fungus management was achieved using approved pesticides. Irrigation was performed immediately after nitrogen application treatments, applying 10 mm of ground water to ensure immediate effect. The weather conditions did not reach the extremes of the long-term average and severe drought was observed.

The factors included in the wheat experiment comprised two plant densities providing 150 (LD) and 450 (HD) plants/m^2 and 11 strategies of nitrogen application (Table 1). The N applications were performed using two dressings at 5 April (N1) and 2 May (N2).

The factors included in the barley experiment comprised two plant densities providing 150 (LD) and 450 (HD) plants/m^2 and 11 strategies of nitrogen application (Table 1). The N applications were performed using two dressings at 4 May (N1) and 31 May (N2).

Reflectance measurements

Canopy reflectance was measured with a hand-held spectroradiometer fitted with 20·8° field-of-view optics (CropScan MSR87, CropScan Inc., USA). Eight medium broad bands ($\cong$ 10 nm) were used, with centre wavelengths equal to 560, 650, 690, 740, 760, 810, 900 and 970 nm. Both solar irradiation and ground/crop reflectance were detected.

Reflectance measurements were performed in the wheat canopy three times: on 15 May (T1), 22 May (T2) and 30 May (T3), which were growth stages (BBCH) 32, 41 and 51 respectively.

Reflectance measurements were performed in the barley canopy four times: on 22 May (T1), 30 May (T2), 6 June (T3) and 15 June (T4), which were growth stages 28, 30, 33 and 48 respectively.

All data were collected with solar zenith angles less than 40° from 11:00 h to 13:00 h local time (GMT+1). The plants were dry at all times, but the illumination was changing both between measurement days and within each day due to clouds. Four separate measurements were made in each plot representing replicates. The data used in the analysis were the relative reflectances corrected for irradiation, referred to as reflectance data, and selected indices calculated on the basis of the reflectance data.

Harvest procedures and statistical analysis

The plots were harvested with a plot harvester on 23 August 2000 and grain yield (kg/ha) was recorded. Grain subsamples from each plot were analysed for protein content (%) in the laboratory using a near-infrared spectroscopy analyser (Foss Tecator, Infratec 1241). The near-infrared spectroscopy analyser was calibrated and linked to the Danish NIT network (Buchmann *et al.* 2001).

The program 'Proc Mixed' in the software package 'SAS' (SAS Institute, USA) was used for initial statistical analysis of y (response) variables, grain yield and protein content. The initial statistical model included plant density, nitrogen application and their interaction as fixed effects. The blocks were regarded as randomly selected, which implies that whole-plots (plant density) and split-plots were set as random factors. The model was reduced by using -2 REML log likelihood estimates and χ^2 test of significance

Table 1. *Nitrogen strategies for winter wheat and spring barley experiments*

Crop treatment	kg N/ha										
	1	2	3	4	5	6	7	8	9	10	11
Winter wheat											
N1, 5 April	0	40	60	80	120	160	200	0	0	40	40
N2, 2 May	0	0	0	0	0	0	0	120	160	80	120
N1+N2	0	40	60	80	120	160	200	120	160	120	160
Spring barley											
N1, 4 May	0	30	45	60	90	120	150	0	0	30	30
N2, 31 May	0	0	0	0	0	0	0	90	120	60	90
N1+N2	0	30	45	60	90	120	150	90	120	90	120

(0·05% confidence interval). Estimation of the linear combinations of the fixed effects was performed using the reduced final model. Pair-wise tests of difference between the fixed effects were performed using *t*-tests.

Initial pre-treatment and evaluation of reflectance data

Initial outlier control and detection of data with divergent behaviour was performed before averaging the four replicate measurements in each plot. The procedure started with an initial mean centring across reflectance wavelengths by subtracting the average of each variable from each sample, respectively. Principal component analysis (PCA) was carried out on reflectance data from each measurement day. The analysis was carried out in Matlab 6.0 release 12 (Mathworks, USA) using the PLS toolbox 2.0 (Eigenvector Research Inc., USA).

The median of the scores in principal component 1 (PC1) and principal component 2 (PC2) from each of the four measurement replicates within each plot was calculated. If the difference between one of the four scores in PC1 was more than three times the median of the scores in PC1 or the difference between one of the four scores in PC2 was more than three times the median of the scores in PC2, the measurement was regarded as an outlier. By this method 157 out of a total of 792 (20%) measurements in wheat and 273 out of 1056 (26%) in barley were regarded as outliers and left out of further calculation. This outlier detection was done to ensure that one or two outliers did not influence the average of the rest of the measurements. No clear trend or explanation was found to explain the outliers. It could be due to varying growth conditions within the plot. The means of the remaining data, two to four measurements in each plot, were calculated and represent the reflectance data used for analysis.

The partial least square regression models can operate with a high degree of redundancy, and for strengthening the predictive power of the X (independent) variables, ten indices used in the literature were calculated on the basis of the initial pre-treated reflectance data. These indices were by nature closely related to the eight wavelengths, but it was believed that additional information would be provided for the analysis, because it has been observed that non-linear transformations can provide new information. The equations used for calculating the indices are shown in Table 2.

Auto-scaling before partial least squares regression

It is necessary to pre-treat both the reflectance data and the dependent variable before actual PLS data analysis and modelling (Harshman & Lundy 1984). In this case, the data were auto-scaled, which means that data were mean centred and scaled. Mean centring across all samples was performed for each measurement date separately by subtracting the average of each variable from each sample, respectively. Mean centring will remove possible differences in offsets between the different variables and at different occasions thereby focusing on the variation between the samples. To avoid differences in magnitude between the variables from dominating, scaling within all variables was performed. The scaling factor was the inverse of the standard deviation (1/Sdev) of each variable.

Partial least squares regression

Partial least squares regression (PLS1) was used for creating models between the auto-scaled spectral variables X and a dependent variable (y). All first-order data can be used in the algorithm, and it is ideal to use on data where several or multiple dependent variables have been determined for each sample/plot even if the variables show colinearity. The data set was arranged in a matrix containing the spectral variables X and a vector containing the dependent variable (y). Therefore, the dimension of X in single-PLS1 for both wheat and barley using the data from

Table 2. *The 18 variables used throughout the modelling of grain yield and grain protein content. The variables consist of eight separate measured wavelengths and ten indices calculated on the basis of these wavelengths*

Name	Wavelengths/Index equation	Reference
Separate wavelengths	R560*, R650, R690, R740, R760, R810, R900 and R970	
DVI	R810 − R690	Jordan (1969)
NDVI	$\frac{(R810-R690)}{(R810+R690)}$	Rouse *et al.* (1974)
GNDVI	$\frac{(R810-R560)}{(R810+R560)}$	Gitelson & Merzlyak (1996)
GRVI	$\frac{(R690-R560)}{(R690+R560)}$	Tucker (1979)
SAVI	$\frac{(R810-R690)\times(1+L)}{(R810+R690+L)}$	Huete (1988)
MSAVI2	$\frac{1}{2}[2(R810+1)-\sqrt{(2R810+1)^2-8(R810-R690)}]$	Qi *et al.* (1994)]
RDVI	$\sqrt{NDVI\times DVI}$	Roujean & Breon (2001)
TVI	$0{\cdot}5(130(R810-R560)-210(R690-R560))$	Broge & Leblanc (2001)
REIP	$700+40\times\frac{(DVI/2)-R740}{(R760-R740)}$	Guyot *et al.* (1988)
D-chl-ab	$\frac{(R760-R740)/2}{R560}$	Gitelson & Merzlyak (1996)

* R560 indicates that it is the reflectance ratio for the band with centre wavelength at 560 nm. Similar symbols are used for the other separate wavelengths.

the last measurement day is 66×18. The numbers indicate the 66 samples and the 18 selected variables constructed from reflectance of various wavelengths and calculated indices (Table 2). The PLS1 algorithm is based on the 'non-linear iterative projections by altering least-squares' (NIPALS) algorithm. The basis of the PLS1 algorithm will not be explained in this paper, but further information can be found in Hoskuldson (1988) and Kvalheim (1987).

The present data set can also be set up to have three dimensions. By regarding the data set as three-dimensional the analysis can be extended and possibly better prediction models can be developed. There are several possible three-dimensional or three-way calibration methods. Among the most common are unfolding of the three-way data cube into a normal two-way data matrix (unfold-PLS1) using standard bilinear methods (PCR, PLS1), multi-linear PLS, trilinear PARAFAC (parallel factor analysis) or modifications of the so-called Tucker models.

It was decided to use the relatively new method N-PLS (Bro 1996) and compare the results of this model to bilinear single-PLS1 (see above) and unfold-PLS1. The unfold-PLS1 was chosen because it resembles the N-PLS to some extent. However, the data are analysed as if they were two-dimensional, which makes them difficult to interpret and not ideal for these kinds of data, unlike N-PLS.

The variables have been determined at three different dates in wheat and four different dates in barley. Therefore, the unfolded matrices X used in the unfold-PLS have the dimensions 66×54 for wheat and similarly for barley, where the matrix dimension was 66×72. The data in N-PLS have to be arranged in a cube. The three dimensions of the cube for wheat were $66\times18\times3$ and for barley were $66\times18\times4$ in the 1st, 2nd and 3rd direction, respectively. Here again, the experimental plots are listed in the first direction or mode. There are 1–66 plots in total and each of the 66 observations in direction/mode one is represented by 18 variables (reflectance wavelengths and indices), which is mode two. The variables have been measured three and four times for wheat and barley, respectively. The data from each measurement day were stacked to give the third mode. The algorithm is superior to unfolding methods, primarily owing to a stabilization of the decomposition. This stabilization potentially gives increased interpretability and better predictions. The algorithm is fast compared with, for example, PARAFAC, because it consists of solving eigenvalue

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problems. Further information about the algorithm and use of N-PLS can be found in Bro (1996), Bro & Heimdal (1996) and Andersson & Bro (2000).

In principle, all three methods use component projection successively to find latent structures. The first principal component (PC) includes the maximum possible variation in X relevant to describe the variation y. The second PC was found in the same way by including the second highest variation. Visual inspection of score-plots and residual validation variance plots was used to find the optimal number of PCs avoiding over fitting. In most cases this procedure can reduce the number of correlated variables to a few independent variables here as principal components. The final model predicting $\hat{y}_i$ had the following form (Eqn 1):

$$\hat{y}_i = b_0 + b_1 t_{1i} + b_2 t_{2i} + \cdots + b_n t_{ni} \quad (1)$$

where t_{1i} to t_{ni} are the scores from principal component (PC) 1 to n for variable i. The scores were calculated from auto-scaled X data. By linear regression of t versus y in the calibration iteration process the regression coefficient b_n was calculated. The centred mean b_0 has to be added to get $\hat{y}_i$ in the right proportion due to the initial centring of y.

Model validation

The models used in this experiment were cross-validated. As for the statistical analysis, the blocks in each experiment were regarded as a random selection from a larger population and therefore treated as new independent samples. Data from one block were successively left out one at a time (test set) and a model was built by using the remaining two blocks (calibration set). The model created was used to predict the dependent variables in the test set. Therefore, the results were not a simple fit of data, but a true prediction. In all prediction models D.F. was the size of the test set = 22. Root mean square error of prediction was calculated (Eqn 2):

$$\text{RMSEP} = \sqrt{\frac{\Sigma_{i=1}^{n}(\hat{y}_i - y_i)^2}{n}} \quad (2)$$

RMSEP expresses the average error to be expected associated with the future predictions, where y_i expresses the measured value and $\hat{y}_i$ expresses the predicted value in n samples. Three RMSEP values were calculated for each test model and root mean square error of cross-validation (RMSECV) were calculated (Eqn 3):

$$\text{RMSECV} = \sqrt{\frac{\Sigma_{n=1}^{N}\text{RMSEP}_n^2}{N}} \quad (3)$$

where N is the number of RMSEP, which in this experiment is three. RMSECV is calculated to give the greatest RMSEP the highest weight, compared with an average of the RMSEPs from the cross-validation. In this paper $2 \times$ RMSECV is given as the percentile of the range between the minimum and maximum value of the dependent variable.

The analysis was carried out in Matlab 6.0 release 12 (Mathworks, USA) using the PLS toolbox 2.0 (Eigenvector Research Inc., USA) for single-PLS and unfold-PLS. The N-way toolbox 1.04 was used in the N-PLS analysis (Andersson & Bro 2000).

RESULTS

Treatment effects on grain yield and protein content

Nitrogen status was the major factor affecting yields and protein content in wheat and barley (Table 3). The temporal distribution of nitrogen also influenced, in particular, protein content in winter wheat. The results indicate that the conditions of this study were favourable for evaluating the potential of canopy reflectance data for determining the N status of winter wheat and spring barley.

There were significant increases in wheat grain yield with increasing N fertilization (Table 3), ranging from approximately 4 tonnes/ha in the 0–0 N strategy to 10 tonnes/ha in the 200–0 N strategy ($P < 0{\cdot}001$), whereas no significant differences were observed between same levels of nitrogen applied at different strategies.

Late application of nitrogen (N2) had a significant positive influence on protein content. No significant ($P > 0{\cdot}05$) difference in yield was observed between the two seeding rates. However, both nitrogen application and plant density influenced grain protein content significantly. On average, protein contents were 10·2% in LD plots and 9·7% in HD plots. There was no significant interaction between nitrogen strategy and plant density.

Barley grain yield and protein content are summarized in Table 3. The grain yield in spring barley was strongly dependent on the different nitrogen rates as well as different nitrogen application strategies. The lowest yield (3 tonnes/ha) was obtained with the 0–0 N application strategy and highest yield (6 tonnes/ha) was achieved with the 150–0 strategy. Thus, application of only late nitrogen (0–90 and 0–120) gave significantly lower yields than early applications. As in wheat no significant differences in yield were observed due to different plant densities. Grain protein content was weakly influenced by N-application strategies as well as plant density. On average protein contents were 11·0% in LD plots and 10·6% in HD plots. No significant interaction between nitrogen strategy and plant density (NS) was observed.

Estimated v. *measured grain yield*

The three different multi-variate calibration methods were able to predict yield in both wheat and barley,

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Table 3. *Mean grain yield and protein content in response to N-strategies and plant density for winter wheat* (a) *and spring barley* (b)

(*a*) Winter wheat	Yield tonnes/ha		Protein dry matter (%)		(*b*) Spring barley	Yield tonnes/ha		Protein dry matter (%)	
N-strategy (NS)					N-strategy (NS)				
0–0	4·51 a		10·0 c		0–0	3·12 a		10·0 a	
40–0	6·22 b		9·0 a		30–0	4·55 b		9·9 a	
60–0	7·21 c		9·0 a		45–0	4·87 c		10·0 a	
80–0	7·65 c		8·9 a		60–0	5·08 c		10·0 a	
120–0	8·88 d		9·1 a		90–0	5·64 d		9·9 a	
160–0	9·70 e		10·4 c		120–0	5·82 de		9·9 a	
200–0	9·94 e		11·1 d		150–0	6·05 e		11·1 b	
0–120	8·63 d		10·1 c		0–90	4·70 bc		10·5 a	
0–160	9·37 e		11·5 e		0–120	4·90 c		11·1 b	
40–80	8·85 d		9·6 b		30–60	5·38 cd		9·9 a	
40–120	9·58 e		10·5 c		30–90	5·71 d		10·8 a	
Plant density (PD)					Plant density (PD)				
150 (LD)	8·18 a		10·2 a		150 (LD)	5·59 a		11·0 a	
450 (HD)	8·29 a		9·7 b		450 (HD)	5·83 a		10·6 b	
Model information					Model information				
	S.E.	D.F.	S.E.	D.F.		S.E.	D.F	S.E.	D.F.
NS	0·16	9·21	0·17	7·91	NS	0·16	10·9	0·34	46·9
PD	0·15	5·07	0·13	3·40	PD	0·20	6·0	0·17	6·34
NS × PD	0·21	20·5	0·20	15·2	Na × PD	0·23	10·9	0·47	62·7

* Test of significance between treatments using *t*-test. The limit of significance was set to $P > 0·01$ and identical letters indicate no significance while divergent letters indicate significant difference.
† Standard error (S.E.) and degrees of freedom (D.F.) for the full model with NS, PD and NS × PD as fixed effects and blocks as random effects using least square means in proc mixed in SAS.

Table 4. *Summary of the cross-validation of single-PLS1, unfold-PLS1 and N-PLS1. Three segments represented by the experimental blocks were successively used as test set data*

	Method	PCs	Correlation (D.F. = 22)	RMSECV	Range	% of range
Winter wheat						
Yield	Single-PLS1	3	0·953	0·034	6·42	1·1
	Unfold-PLS1	3	0·966	0·027		0·9
	N-PLS1	3	0·967	0·108		3·4
Protein	Single-PLS1	3	0·672	0·792	3·9	40·6
	Unfold-PLS1	2	0·697	0·739		37·9
	N-PLS1	3	0·754	0·405		20·8
Spring barley						
Yield	Single-PLS1	3	0·930	0·043	4·25	2·0
	Unfold-PLS1	4	0·972	0·037		1·7
	N-PLS1	3	0·968	0·068		3·2
Protein	Single-PLS1	2	0·242	1·078	3·6	59·9
	Unfold-PLS1	1	0·447	1·072		59·6
	N-PLS1	1	0·457	0·812		45·1

irrespective of the different nitrogen application strategies. The two three-way methods, unfold-PLS1 and N-PLS, performed slightly better compared with single-PLS1 (Table 4).

The correlation between measured and predicted yields were > 0·96 using the two methods. This indicates that the growth history measured spectrally had a positive effect on the predictability of yield. The root mean squared error of cross-validation (RMSECV) was greater using N-PLS (wheat, 0·108 and barley, 0·068) compared with unfold-PLS1 (wheat, 0·027 and barley, 0·037) for predicting yield.

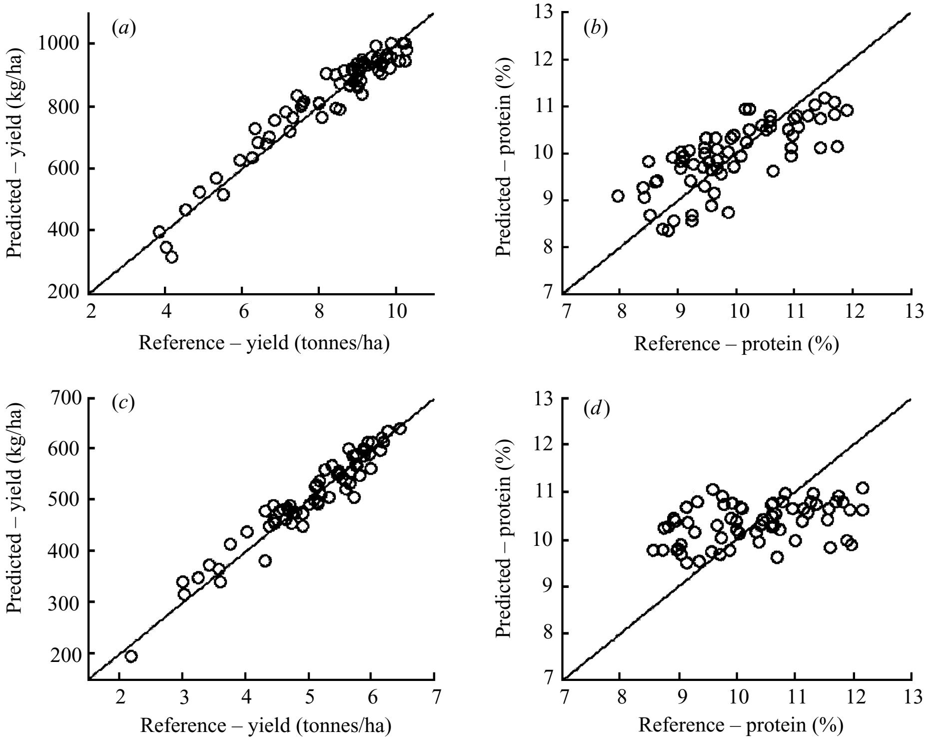

Fig. 1. Relation between predicted and reference values using N-PLS for wheat yield (*a*), wheat protein content (*b*), barley yield (*c*) and barley protein content (*d*). The line of equality is indicated. The total number (*n*) of samples in each plot is 66. No y samples were detected as outliers.

Estimated v. *measured grain protein content*

Using a single spectral measurement just before heading in wheat and barley was not adequate for predicting grain protein content. Single-PLS cannot predict protein content at the time measured in barley ($r = 0{\cdot}24$, D.F. $= 22$). It is slightly better in wheat ($r = 0{\cdot}67$, D.F. $= 22$).

Using three-way methods did improve the prediction, especially in wheat ($r = 0{\cdot}75$), between measured and predicted. However, the RMSECV is still quite high when it was compared with the range between the lowest and highest measured values. The RMSECV was still 21 % of the total range, but it is a clear improvement compared with single-PLS (41 % of total range).

N-PLS models

An overall indication of the N-PLS model ability to predict yield and protein is shown in Fig. 1. Clearly, grain yield in both crops was estimated well, while protein content was estimated less successfully. The overall parameters used for prediction of y are presented in Table 5. The parameters in Eqn 1 can be used to predict new samples.

Table 5. *Parameters used to construct the final models of N-PLS according to Eqn 1 for predicting grain yield and protein content in winter wheat and spring barley*

Crop variable (y)	N-PLS model parameters b_0	b_1	b_2	b_3
Wheat				
Grain yield (kg/ha)	8·23	1·29	11·5	51·5
Protein content (%)	9·94	−0·05	−0·19	0·09
Barley				
Grain yield (kg/ha)	5·07	−0·23	0·81	2·99
Protein content (%)	10·33	0·14		

DISCUSSION

In this work PLS1, unfold-PLS1 and N-PLS were compared as tools to predict grain yield and protein content. Multi-variate calibration models have been used before in other research areas to predict a number of different variables using a multivariate data structure. The methods have not been used within remote sensing in agriculture, but it is apparent from our work that these models can contribute to the development of robust prediction models of, for example, yield and grain protein content.

The repeated in-season spectral measurements, in the time range from early stem elongation until heading, have been used in the prediction of grain yield and protein content. Treatments applied to the crop were intended to create a wide range of biomass, growth rates and chlorophyll concentrations in the wheat and barley crops. The in-season variation measured spectrally approximately 2–3 months before harvest was expected to provide adequate information to predict grain yield and protein content.

The spectral data contained enough extractable information for the models to predict yield over a very broad range, regardless of plant density and nitrogen strategy, even when nitrogen was applied inside the time span of measurement. The positive yield response to nitrogen under sufficient water supply is evident and well known (Olesen *et al*. 2000). The differences in plant density had no significant effect on yield either in wheat or barley. This is due to the ability to compensate by increasing the number of fertile tillers and produce larger kernels (Darwinkel 1978).

Plant breeding has reduced the grain protein concentration of barley and wheat progressively within the last century, especially with the newer cultivars. Reduction in grain protein concentration is not associated with lower protein amount per grain basis, but rather with an increase in amount of non-structural carbohydrate per grain (Bulman & Smith 1993).

The models used seem to predict yield well, but the approach of each model is quite different. Single-PLS is very dependent on the measurement date. In general, earlier measurements contained less information about yield compared with later measurements, e.g. taken from stem elongation until ripening (Aparicio *et al*. 2000). We chose to use our last measurement, because it should contain the most information about crop development, and because, in these investigations, the early measurements did not contain information resulting from the second nitrogen application (Shanahan *et al*. 2001).

Unfold-PLS uses the same PLS1 calibration model, but the unfolded data matrix was used with all measured data in the same data matrix (see Materials and Methods). It did not increase the prediction power significantly, but it increased, by definition, the number of parameters used in the model very drastically, to produce a complex model with low interpretability (Bro 1996). N-PLS compared with unfold-PLS is much simpler. If models describe the data equally well, one should choose the simplest one in order to keep the model robust against over-fitting of parameters and to aid interpretability. The price for increased simplicity is loss of fit, because of more severe constraints, and this was observed here when predicting yield in wheat and barley (Table 5).

The quality of wheat and barley is important, because the producers have an economic incentive based on, for example, protein concentration. In general wheat producers aim at high protein content in order to harvest bread wheat (Jenner *et al*. 1991) otherwise it is regarded as feed wheat for animals. In contrast, malting provides an economic incentive to produce spring barley for malt, based on a limited but not low protein content (Bertholdsson 1999).

It is much more difficult to predict grain protein content based solely on in-season spectral measurements. Plant density had a negative significant influence on grain protein in both wheat and barley. The HD crop invested more nitrogen on leaves and stems during growth compared with LD crops (data not shown). The nitrogen left at grain filling was less and the remobilization from the other organs to the head did not compensate for the relative lack of nitrogen in HD crops compared with LD crops.

The highest supply of nitrogen at N1 raised the protein content significantly compared with other treatments supplied at N1 only for both wheat and barley. However, later application of nitrogen at N2 proved less effective than application at N1. This was true for both yield and protein content in wheat, but the yield decreased significantly at N2 in barley compared with N1 application. This is related to the amount of nitrogen left at grain filling. Late application of nitrogen simply provides more nitrogen to the protein creation at grain filling in wheat compared with early nitrogen application. This is supported by work done in winter wheat and barley (Gooding & Davies 1991; Bulman & Smith 1993; Gooding *et al*. 1997*a*).

All models except the N-PLS model in wheat predicted protein content very poorly. The in-season reflectance measurements did not provide enough information to make a good prediction model. The process of grain filling relies on plant-available nitrogen and on translocated nitrogen from other organs, but takes place later than the actual spectral measurements. The spectral measurements seem to be closely related to nitrogen application. This should provide a good prediction of yield but a poor estimate of protein content, at least in barley, because yield responds greatly to nitrogen while protein content is related to events after measurement. However, the N-PLS model predicts protein content quite closely ($r = 0{\cdot}75$, D.F. $= 22$) and reduced the RMSECV by 50% compared with unfold-PLS. This indicates that N-PLS could be developed further and used on other datasets which ideally would include spectral measurements closer to harvest and data from several years as a fourth dimension.

Variable rate applications of nitrogen, based on spectral measurements in winter wheat, can be performed before heading, in Northern Europe (Jørgensen & Jørgensen 2001), and no effect on protein was observed compared to uniform application. The present results suggest that the use of repeated measurements can be incorporated into a model to support such nitrogen application strategy in winter wheat. The model would permit grain protein optimization through a second variable rate application just before heading.

The relation of crop status at a given stage of growth with yield at a later stage of growth may be affected by external factors operating from the time of crop scanning until the time at which the grain is harvested. Therefore the indication of final grain yield and protein content can usually only be established as a yield and protein range.

In conclusion, this paper shows how grain yield and protein content in spring barley and winter wheat can be predicted using canopy reflectance measurement data. The proposed method employs multi-way partial least square regression and can be applied with response functions other than those considered in this paper. Large variation in grain yield and protein content can be predicted by canopy reflectance measurement data. Site-specific predictions could be made known to growers to enable late-season nitrogen application in the milky-ripe growth stage in order to modify grain protein content.

Further development of the model could include consideration of other field characteristics such as the yield of the previous crop, soil organic matter and texture and temperature, but collection and implementation could be time consuming compared with canopy reflectance measurement in the actual crop. A major improvement of the models to predict final grain yield and protein content could probably also be achieved by introducing complementary canopy reflectance measurement data later in the growing season. This would however reduce the possibility to improve grain protein content and yield by late application of nitrogen if wanted.

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