# Modelling protein content and composition in wheat

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### Abstract

Protein accumulation in wheat is subject to both genetic and environmental influences. Recent work strongly suggests that both forms of influence are regulated by the sources of N within the plant, rather than demands by the grain. We have extended Sirius, an existing model of wheat that includes N uptake and redistribution within the plant, to predict the accumulation of broad fractions of protein types within the grain. Genetic control of grain protein content is through the manipulation of the capacity a major vegetative N-pool. Environment and management determine whether or not the pool can be filled, and hence how much plant N is available for translocation. Partitioning rules for N among structural and major storage proteins determine grain protein composition. The model was tested using data from experiments in France and New Zealand, both from the field and semi-controlled environments.

### Media summary

Collaborating New Zealand and European scientists have developed a robust system for predicting the effects of genetic and environmental factors on wheat quality.

### **Key Words**

Nitrogen uptake, structural and storage proteins, wheat.

#### Introduction

Protein composition is a key component of the end-use value for wheat grain. Although the qualitative composition of the grain is genetically determined, the quantitative composition is significantly modified by the growing conditions, and there are significant genotype ? environment interactions (e.g. Zu and Khan, 2001). Recently published work on nitrogen uptake and redistribution by wheat plants (Jamieson & Semenov, 2000) and accumulation of grain protein (Martre et al., 2003) has shown that the last process is dominated by N supply from vegetation to grain. The analysis strongly suggests that modelling must concentrate on defining the genetic variation in the size of pools of N that are available for translocation to the grain, and how these interact with grain growth duration to determine grain protein content. In this paper we used the wheat model Sirius to investigate how varying the capacity of one of the two main vegetative pools of translocatable N affects grain protein content at harvest, and to test the extension of Sirius developed by Martre et al. (2003) with several cultivars.

Sirius 2000 is the current release of the model described by Jamieson & Semenov (2000). Sirius has predicted grain protein content accurately over a substantial range for several cultivars in widely varying conditions (Jamieson & Semenov, 2000; Martre et al, 2003). It allocates the N taken up before anthesis into three main pools. These are a "green" pool that contains N at a constant specific concentration per unit green area ( $1.5 \text{ g/m}^2$ ), stem pools that contain structural (unrecoverable) N at 0.003 kg N / kg (DM), and labile N with a storage capacity of 0.012 kg N / kg (DM). After anthesis, all non-structural N (i.e.,

"green" and labile N pools) is available for translocation to grain, and is moved at a constant rate in thermal time to be in the grain at physiological maturity in unstressed conditions. Available soil N is used in preference to N from premature senescence of green area. Hence potential final grain N concentration can be varied only by varying either the duration of grain filling (so that grain mass is lowered for the same N content) or by increasing the size of the N pools available for translocation to the grain.

# Methods

### Model development

In Sirius 2000, the capacity of the labile N pool was fixed at 0.012 kg N / kg (DM), assuming upper ( $N_m$ ) and lower limits of stem N concentration of 0.015 and 0.003 kg N / kg (DM). To investigate the effects of varying N pool sizes on final grain N concentration, a further "genetic" control parameter was added to the cultivar description in Sirius and, with other parameters held constant, was used to vary  $N_m$  between limits of 0.010 – 0.017 kg N / kg (DM). This corresponded to grain protein contents in benign growing conditions from approximately 8 – 14% at 14% grain moisture content.

Partitioning of N among major protein classes was as described by Martre et al. (2003). The main hypotheses of this model are: (1) the accumulation of structural/metabolic N is sink-driven and is a function of temperature; (2) the accumulation of storage N is supply limited; (3) the allocation of structural/metabolic N between albumin-globulin and amphiphilic protein fractions and the allocation of storage N between gliadin and glutenin fractions during grain growth is constant.

In addition to simulations to match experimental treatments, a set of simulations were run using weather and soil data for Lincoln, New Zealand. Chosen cultivars were Belfield, a bread wheat with no vernalisation response, and Claire, a European feed wheat.. The same sowing date (8 May 2003) was used for both.  $N_m$  values were varied from 0.010 to 0.017 kg N / kg (DM), representing a range of types with similar phenology but differing capacity to produce protein. N inputs were high, irrigation was either supplied or not, and deep and shallow soils were used in the simulations.

### Experimental

Four cultivars of winter wheat, Arche, R?cital, Renan, and Tamaro were studied in Clermont (France). They were grown outside in 2 m<sup>2</sup> containers under non limiting water and N supplies, with day/night air temperatures averaging 19?C/14?C. Starting at anthesis, one container of each cultivar was subjected to water shortage, and received only 20% of the control; another one was transferred under controlled environment closed-top chambers, where day/night temperatures were regulated at 28?C/15?C. These cultivars were also grown in the field with no N fertilisation or with 250 kg N / ha. An intermediate N fertilisation treatment was obtained on a plot where lucerne was previously grown, providing ca. 70 kg?N?/?ha.

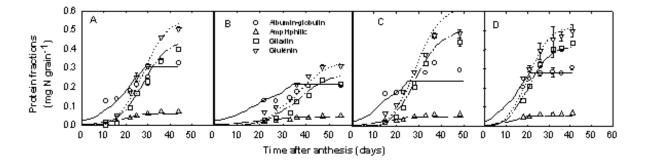
Plants were sampled from anthesis to grain maturity. Grains were ground to wholemeal flour and the protein fractions were sequentially extracted, and their N content was determined using the Kjeldahl method (Tribo? et al., 2003).

In addition we have used results from experiments in Canterbury, New Zealand, from the project described by Armour et al. (2002). Briefly, replicated experiments on five farms had treatments with varying N supplies. Four cultivars were used, two sites with Claire, and one each of Savannah, Regency and Centaur. At harvest grain samples were analysed for grain protein content, and these values were compared with predictions from Sirius 2000 using the original  $N_m$  value of 0.015 kg?N?/?kg?(DM).

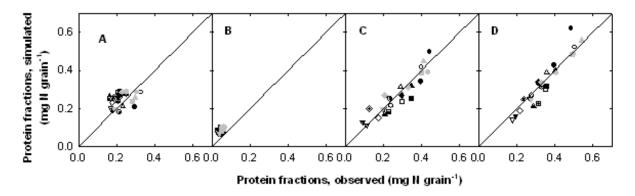
### Results

French Experiments

Under non-limiting water supply and outside temperature, the model gave good simulations of the timing and rates of accumulation of the different protein fractions for the four cultivars (Fig. 1). Similar agreement was observed for the 5 other treatments (data not shown). At maturity, simulated and observed quantities of albumins-globulins were poorly correlated (Fig. 2) but covered a small range compared with other fractions. The amphiphilic proteins represented only 4 to 8% of the total grain proteins and there was a good agreement between observed and simulated quantities of this fraction at maturity. The quantity of storage proteins varied more than 3-fold, and there was a good agreement between simulated and observed quantities of gliadins and glutenins (Fig. 2).



**Figure 1**. Observed (symbols) and simulated (lines) quantity of albumin-globulin (—), amphiphilic (— —), gliadin (—)—), and glutenin (…) protein fractions versus the number of days after anthesis for grain of  $\Sigma$ . *aestivum*. A, Arche; B, Récital; C, Renan; D, Tamaro. Crops were grown in the controlled environment close-top chamber under ambient temperature (19°C/14°C, day/night air temperature) and with non-limited water supply. Experimental data are mean  $\pm 1$  SE (n = 2).



**Figure 2.** Relationship between observed and simulated quantity of protein fractions for mature grain of *T. aestivum* for varying grain growth temperatures, soil water and N supplies (Arche, open symbols; Récital, open symbols with cross; Renan, filled symbols; Tamaro, grey symbols). A, albumin-globulin; B, amphiphilic; C, gliadin; D, glutenin proteins. Solid lines are Y = X. MEP is the root mean square error of prediction.

#### New Zealand experiments and simulations

Over a wide range, there was good agreement between measured and simulated protein concentrations (Fig 3). Even where one group was off line (Centaur, the triangles in Fig. 3), the protein change with N supply was still reflected in the simulations. The following simulations are an attempt to explore the mechanism of cultivar variation.

In well watered conditions, there was strong feedback between achieved yield and  $N_m$ , with high protein achieved at the cost of yield. In water stressed conditions yield was unaffected although grain protein

concentration varied with labile pool size. Achieved grain proteins varied with management and phenology, as well as with  $N_m$ . For instance, at  $N_m = 0.017$  kg N / kg (DM), phenological differences, management and soil variations caused grain protein to vary from 10.9-14.0% (Fig. 4).

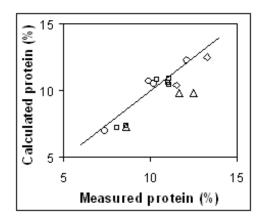


Figure 3. A comparison of measured and simulated grain protein concentrations (14% grain moisture content) for crops grown in Canterbury, New Zealand. Centaur ( $\Delta$ ), Claire ( $\Box$ ), Regency ( $\Diamond$ ) and Savannah ( $\circ$ ).

# Discussion

Although there was very good agreement between observed and simulated storage protein contents across environments and cultivars, it was less good for structural proteins. The lack of correlation here may be due mostly to the small range of variation, but also arises from a lack of response in the model of structural protein accumulation to N fertilisation and post-anthesis water deficit. This may be because there is feedback of N availability on grain demand for structural N, which was not allowed for. Nevertheless, the total content and composition is dominated by the larger fractions, so there is a reduced importance of the errors in structural protein content. The model of Martre et al, (2003) was based on analysis of one variety. The analysis in fig. 2 shows the partitioning rules are similar among the four varieties tested here. Further work comparing more extremely differing cultivars is required to confirm whether this is universal.

Figure 3 provides some evidence of cultivar variation in inherent ability to produce protein. Here we generated this sort of variation by allowing the capacity of one of the main shoot N pools to vary with cultivar. We were able to mimic the sorts of differences observed among cultivars of different end uses. These overlie variations caused by changes in environment and management. Although we have targeted the labile N pool in our simulations, the variation in source strength may involve both major retranslocatable N pools in the shoot. Further experimental work should aim to quantify the pool capacities

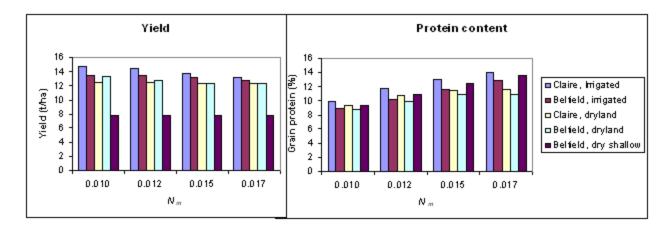


Figure 4. Simulated variation in yield and protein content in response to changes in cultivar, labile N pool capacity and water management.

### Conclusions

The model used here gives a simple mechanistic framework that explains environmental and genotypic effects on grain protein content and composition. Our assumptions that grain protein composition is a direct function of the total quantity of N per grain, and that N partitioning is not affected by the growing conditions appeared to hold for the four cultivars over a significant range of N fertilisation and post-anthesis temperature and water supply. From this study, we suggest that the variations of protein composition for winter wheat are due to different quantity of N per grain.

Recent evidence favours the shoot as the main determinant of grain protein concentration, so research into genetic factors controlling grain protein should concentrate there rather than on the grain itself. We have suggested one avenue of research – quantification of differences in N pool capacities among cultivars – as a good place to start.

#### References

Armour, T, Jamieson, PD and Zyskowski, RF 2002. Testing the Sirius Wheat Calculator. Agronomy New Zealand , 32, 1-6.

Jamieson PD and Semenov MA (2000). Modelling nitrogen uptake and redistribution in wheat. Field Crops Research 68, 21-29

Martre P, Porter, JR, Jamieson PD and Tribo? E (2003) Modeling grain nitrogen accumulation and protein composition to understand the sink/source regulations of nitrogen remobilization for wheat. Plant Physiology, 133, 1959-1967.

Tribo? E, Martre P, Tribo?-Blondel AM (2003) Environmentally-induced changes of protein composition for developing grains of wheat are related to changes in total protein content. Journal of Experimental Botany 54, 1731–1742.

Tribo?, E, Tribo?-Blondel AM (2002) Productivity and grain or seed composition: a new approach to an old problem: invited paper. European Journal of Agronomy 16, 163–186

Zhu J and Khan K (2001) Effects of genotype and environment on glutenin polymers and breadmaking quality. Cereal Chemistry 78, 125–130