CHAPTER 15

SIMULATION ANALYSIS OF PHYSIOLOGICAL TRAITS TO IMPROVE YIELD, NITROGEN USE EFFICIENCY AND GRAIN PROTEIN CONCENTRATION IN WHEAT

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Abstract. The pressure of economic cost and environmental constraints dictates that farmers must optimize the use of nitrogen fertilizer. Industrial uses of new wheat varieties require specific and stable grain protein concentration, which needs accurate estimation of N demand during the crop cycle. Thus breeding for high N use efficiency (NUE) and yield, whilst maintaining high grain protein concentration, is of high priority for cereal geneticists. Here, the wheat simulation model *SiriusQuality*1 was used to analyse the effect of variation in physiological traits on wheat NUE, grain protein composition and concentration under variable climate and conventional and limited N supply conditions. Twenty-three of the 53 parameters of *SiriusQuality*1 were selected for sensitivity analysis based on a literature survey – four parameters were related to phenology and canopy development, seven to crop C assimilation and partitioning, eight to crop N uptake and assimilation, and four to grain development and C and N accumulation. Variations in weather and N treatments induced larger variations in NUE than most of the physiological traits considered. The simulations suggest that a single physiological trait is unlikely to break the negative correlation between the grain protein concentration and yield over a wide range of sites and seasons, especially under low N input environments. Increasing the N storage capacity of the leaves and stem and the allocation of N to non-structural proteins appeared as the more promising strategy to breaking the negative correlation between grain yield and protein concentration.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the major European arable crop with a total annual production of 212 Mt of grains. Nitrogen fertilization is an important component of

wheat production and quality, and over 35% of the total annual N-fertilizer applications in the UK and France are given to wheat crops. These fertilizers have a direct economic cost to growers, but are also responsible for environmental impacts on groundwater quality through N leaching. The use of fossil fuels for their manufacture and application, plus N_2O emissions associated to denitrification, all potentially contribute to global warming. More efficient use of N fertilizers by wheat crops is thus particularly important.

Minimizing environmental impacts of applied N inputs requires N-efficient crops with lower fertilizer-N requirements. Crop N use efficiency (NUE) has been defined in different ways in the literature, depending on the objectives (Moll et al. 1982; Peng and Bouman in press). Here, our interest is to consider the productivity of N, regardless of its origin (added N fertilizer, inorganic soil N, organic soil N mineralized during the growing period), in order to define genetic traits that improve N utilization and minimize N losses. Therefore, we define NUE as the ratio of grain yield to total available soil N during the crop growth cycle. Using this definition, NUE can be decomposed into two components: the efficiency of apparent N uptake from the soil (NUpE, i.e., the ratio of crop N to the total amount of available N) and the utilization efficiency (NUtE, i.e., the ratio of yield to crop N content).

Proteins are the most important components of wheat grains governing end-use quality (Weegels et al. 1996), and variations in both protein concentration and storage protein composition significantly modify flour end-use quality (Wrigley et al. 1998; Lafiandra et al. 1999; Branlard et al. 2001). Storage proteins are divided into two broad fractions, which are the main contributors to the rheological and bread-making properties of wheat flour (Shewry and Halford 2002). Glutenins are mainly responsible for visco-elastic properties, and gliadins are important in conferring extensibility to dough (Branlard et al. 2001). The glutenin:gliadin ratio is a measure of molecular-weight distribution or protein size, and determines the balance between dough viscosity and elasticity independently of total protein concentration, and therefore affects dough rheological behaviour (Uthayakumaran et al. 1999).

Genetic improvement for traits such as yield or grain protein concentration is complicated. First, the complications arise because the traits result from several linked processes. Second, it is difficult to select for traits that are sensitive to environmental variations and show significant genotype × environment interactions. Quantitative-trait loci (QTLs) for grain protein concentration usually explain less than 10% of the observed variations and show low environmental stability (Blanco et al. 2002). One possibility for overcoming this difficulty is to link crop simulation models with genetic analysis. Simulation models relate a trait to various processes subjected to a range of environmental conditions, with parameters independent of the environment and characteristic of a genotype. Thus, simulation models specifically describe traits × traits and traits × environment × management interactions. Until recently, there have been only tentative relationships between these parameters and genotypes, and until very recently gene-based approaches to modelling have not received much attention (White and Hoogenboom 2003; Wollenweber et al. 2005). Simulation models often used empirical response curves (e.g., N dilution curve) and failed to link model parameters with physiological traits.

Current advances in the understanding of N uptake and redistribution and of wheat phenology allow us to model these processes more mechanistically, and it is now possible to link model parameters with physiological traits (Martre et al. 2006). In theory, such links allow the possibility of associating these model parameters with loci or genes (Yin et al. 2003; Quilot et al. 2005).

In the present study, we used the wheat simulation model *SiriusQuality*1 (Martre et al. 2006) to analyse the effect of different physiological traits on NUE, grain protein composition and concentration under variable climate and conventional and limited N supply conditions. Two sites where considered (Clermont-Ferrand, France and Rothamsted, UK). The effect of the climate at these two sites was assessed by running the model with over 30 years of observed climate data. Selection of physiological traits to increase NUE may involve antagonistic criteria, such as high grain yield, protein concentration and low N fertilizer use. Therefore, the effects of the identified physiological traits on the relationship between grain yield and protein concentration were investigated.

MATERIAL AND METHODS

The SiriusQuality1 *wheat simulation model*

The model used in this study is based on Sirius (Jamieson et al. 1998). It consists of sub-models that describe phenological and canopy development, biomass and N accumulation and partitioning, including responses to shortages in the supply of soil water and N. Canopy development is simulated as a series of leaf layers associated with individual main-stem leaves, and tiller production is simulated through the potential size of any layer (Lawless et al. 2005). The canopy intercepts light and uses it to produce biomass at an efficiency (radiation use efficiency, RUE) calculated from temperature, CO_2 concentration, water stress and the ratio of diffuse to direct radiation (Jamieson et al. 2000). The canopy radiation extinction coefficient, K, is assumed to be independent of N and water shortages (Robertson and Giunta 1994). The RUE in Sirius is also independent of N supply because a major assumption is that the specific leaf N concentration (SLN, N content expressed per unit green area) is constant (Grindlay 1997; Jamieson and Semenov 2000). Hence, shortage of N limits leaf area, and thus light interception, rather than RUE. Transfer of dry matter (DM) and N to grain after anthesis, and partitioning of grain N between gliadins and glutenins have been described in detail (Martre et al. 2006), as well as calculations of phenological development, evapotranspiration, soil water and N distributions (Jamieson et al. 1998). Sirius has been calibrated and evaluated for several modern wheat cultivars and tested in many environments and climates, including conditions of climate change (Jamieson et al. 1998; 2000; Jamieson and Semenov 2000; Martre et al. 2006).

Sites, N treatments, weather and cultivars

For this analysis, we selected two European sites with contrasting climates: Clermont-Ferrand, France (45°47' N, 3°10' E, 329 m elevation) and Rothamsted, UK (51°49' N, 0°21' W, 128 m elevation). Simulations were carried out over 32 and 40 years of daily weather records for Clermont-Ferrand and Rothamsted, respectively. We considered the same soil at both sites, with a rooting depth of 1.0 m, a plant-available water-holding capacity of 160 mm, 8 Mg ha^{-1} of organic N and 20 kg N ha^{-1} of inorganic N at sowing. Two N treatments were considered: a non-limiting N treatment (high N, HN) and a limiting N treatment (low N, LN). Nitrogen was applied as split dressings at the development stages described by the Zadoks scale (Decimal Codes (DC), Zadoks et al. 1974); in the HN treatment, three dressings of 50 kg N ha^{-1} at DC 21, 32 and 39, and one time 100 kg N ha^{-1} at DC 30. In the LN treatment two dressings of 40 kg N ha^{-1} were applied at DC 31 and 37.

In order to assess specifically the effects of climate, N fertilization and physiological traits on grain yield, NUE and protein concentration, only one set of cultivar-specific parameters, for the French winter bread-wheat cultivar Thésée, was used (Martre et al. 2006). For each year, the sowing date was held at November 1 for Clermont-Ferrand and October 10 for Rothamsted.

Observed weather for simulations consisted of 32 years for Clermont-Ferrand and 40 years for Rothamsted. Monthly average maximum daily temperature is 1.1 to 4.4 °C higher at Clermont than at Rothamsted all year around (data not shown). Monthly average minimum daily temperature is 0.5 to 2.6 °C higher at Clermont-Ferrand than at Rothamsted from March till October, but is ca. 1 °C lower at Clermont-Ferrand than at Rothamsted from November till February. Monthly total solar radiations show similar variations during the year at both sites, with Clermont-Ferrand receiving on average 80 MJ m^{-2} month^{-1} more solar radiation than Rothamsted. Mean annual cumulated rainfall is 18% lower at Clermont-Ferrand than at Rothamsted (587 vs. 693 mm y^{-1}), and autumn and winter are drier in Clermont-Ferrand, whereas the spring and the summer are drier at Rothamsted.

PARAMETERS OF PHYSIOLOGICAL TRAITS

Below are physiological traits that were examined for improving wheat yield, NUpE and/or NUtE, and grain protein concentration. These parameters are defined in Table 1. They were modified independently in the model by + and –30% of their default values in 10% increments. This range of variations probably encompasses the genetic variability that could be expected for these different traits in wheat (Table 1).

Phenology and canopy development

Modifying the duration of crop photosynthesis and its timing in relation to seasonal variations of resource availability may have significant effects on DM and N yields (Akkaya et al. 2006; Richards 2000). Past increases in wheat yield have been associated with shortening the duration of vegetative development phases (Calderini et al. 1997; Donmez et al. 2001). The rate at which the leaves appear, determined by

Table 1. *Symbols, definitions, and units of the most important parameters of the SiriusQuality1 wheat simulation model*

Parameter	Definition	Default value	Unit	Nb of cultivars	Min–max	References
				Genetic variability		
Phenology and canopy development						
P	Phyllochron	97	°Cday	4	78–110	Asseng et al. (2002)
				20[a]	93–119	Mosaad et al. (1995)
				8[b]	91–101	Giunta et al. (2001)
$P_{\text{flag}}^{\text{anth}}$	number of P between flag leaf ligule appearance and anthesis	3	phyllochron			
$\alpha_{\max}$	maximum potential size of biggest leaf layer (including the ear)	60	$cm^2 \, mainstem^{-1}$	8	22.1–39.7[c]	Shearman et al. (2005)
				8	34.0–50.3[c]	Fischer et al. (1998)
				10	41.3–87.6[d]	Motzo and Giunta (2002)
P_{sen}	fraction of P for leaf senescence	1.8	phyllochron			
Crop C assimilation and partitioning						
K	radiation extinction coefficient, based on intercepted PAR	0.45	dimensionless	3	0.63–0.76	Yunusa et al. (1993)
				5	0.36–0.57[e]	Green (1989)
				3[f]	0.48–0.78	Miralles and Slafer (1997)
				7	0.37–0.46[g]	Calderini et al. (1997)
				8	0.45–0.70	Shearman et al. (2005)
				3	0.66–0.76	Muurinen and Peltonen-Sainio (2006)
RUE	radiation use efficiency, based on PAR	2.46	$g \, DM \, MJ^{-1}$	3	1.5–2.4	Yunusa et al. (1993)
				3[f]	2.27–2.79[h]	Miralles and Slafer (1997)
				7	1.96–2.5[h]	Calderini et al. (1997)
				8	2.33–2.64	Shearman et al. (2005)
				3	1.69–2.04	Muurinen and Peltonen-Sainio (2006)
SLW	specific leaf mass	45	$g \, DM \, m^{-2}$	8	40.8–54.0	Shearman et al. (2005)
				8	45.1–53.9[g]	Fischer et al. (1998)
				4[i]	32.2–45.0	Duggan et al. (2005)

Table 1 (cont.)

Table 1 (cont.)

Parameter	Definition	Default value	Unit	Genetic variability		References
				Nb of cultivars	Min–max	
D_{eg}	fraction of P_{flag}^{anth} for ear growth	0.25	dimensionless			
μ	fraction of biomass increment allocated to ear during the ear growth period	0.5	dimensionless	6	0.16–0.58	Slafer et al. (1990)
				5	0.28–0.33	Abbate et al. (1998)
σ	Ratio of grains number to ear DM at anthesis	100	grains g^{-1} ear DM	5	62–106	Abbate et al. (1998)
				8	73–129	Shearman et al. (2005)
γ	fraction of crop DM at the end of the endosperm cell division stage remobilized during grain filling	0.25	dimensionless	6	0.13–0.36	Austin et al. (1977)
Crop N uptake and assimilation						
$RVER$	rate of root vertical extension	0.001	m (°Cday)$^{-1}$			
N_{max}^{uptake}	maximum N uptake rate at anthesis	0.4	g N m^{-2} day^{-1}			
k_{sen}	scaling parameter modifying the rate of root N uptake decrease after anthesis	1	dimensionless	-	-	-
$[N]_{max}^{stem}$	maximum stem N concentration	10	mg N g^{-1} DM	3[j]	10–26	Kim and Paulsen (1986)
				21	7.6–14.9	Triboï and Ollier (1991)
				3	6.7–9.5	Ma et al. (1996)
SLN	specific leaf N concentration	1.5	g N m^{-2}			
$[N]_{stru}^{leaf}$	leaf structural N concentration	6	mg N g^{-1} DM	6	3.5–6.9	Halloran (1981)
$[N]_{stru}^{stem}$	stem structural N concentration	3	mg^{-1} N g^{-1} DM	15[b]	1.7–5.1	Desai and Bhatia (1978)
				6	2.1–4.0	Halloran (1981)
β	scaling parameter modifying the rate of crop N remobilization during grain filling	1	dimensionless	-	-	-

Table 1 (cont.)

Table 1 (cont.)

Parameter	Definition	Default value	Unit	Genetic variability		
				Nb of cultivars	Min–max	References
Grain development and C and N accumulation						
D_{gf}	thermal time from anthesis to end of grain filling	750	°Cday	14	600–820	Loss et al. (1989)
				16	717–870	Robert et al. (2001)
				16	719–875	Akkaya et al. (2006)
				194[k]	514–737	Charmet et al. (2005)
D_{cd}	duration of the endosperm cell division stage	250	°Cday	5	176–330	Gleadow et al. (1982)
$\alpha_{N:C}$	grain structural C to N ratio	0.02	$(°Cday)^{-1}$			
k_{cd}	relative rate of accumulation of grain structural C	0.0084	$(°Cday)^{-1}$	11	0.0144–0.0161[l]	Darroch and Baker (1995)

[a] spring wheat cultivars grown under greenhouse conditions with 12 h photoperiod
[b] durum wheat cultivars
[c] flag leaf area
[d] ear plus flag leaf area for awned and awnless isogenic lines of durum wheat
[e] values reported based on intercepted solar radiation and adjusted to intercepted PAR basis by multiplying by 1.3 (Abbate et al. 1998)
[f] tall, semi-dwarf, and dwarf isogenic lines of bread wheat grown under field conditions
[g] not significantly different among cultivars
[h] values reported based on intercepted solar radiation and adjusted to intercepted PAR basis by dividing by 0.48.
[i] pairs of isogenic lines of spring bread wheat differing for the presence of the tillering inhibition gene (*tin*) grown outdoor in tubes
[j] tall, semi-dwarf, and dwarf isogenic lines of winter bread wheat grown in nutrient solutions in a controlled environmental chamber
[k] recombinant inbred lines
[l] use a base temperature of 5 °C

the phyllochron (P), will influence the rate at which the canopy develops and soil N and water are depleted – increasing this rate will shorten the duration of the vegetative development phase. Consistent with P, the duration of the period between the appearance of the flag leaf ligule and anthesis, $P_{\text{flag}}^{\text{anth}}$, was varied in these simulations. Preliminary simulations have shown that vernalization and photoperiod responses have a small effect on the simulated duration from sowing to anthesis or on final leaf number. These parameters were not included in this study.

Increasing the green-area index (GAI) at anthesis may delay the senescence of the canopy and increase the duration of grain filling, and therefore grain yield, especially under limiting N supply (Austin 1999). In the model, the potential size of the culm leaves is scaled based on α_{max}. Changes in α_{max} simulate a change in the leaf area development and the overall potential in the crop GAI at anthesis. Maintaining green leaf area longer, particularly after anthesis, is another mean to increase crop yield, and possibly crop N if N uptake is also maintained (Austin 1999; Triboï and Triboï-Blondel 2002). In *SiriusQuality*1 the ontogenic rate of leaf senescence is constant and is determined by the parameter P_{sen}.

Canopy architecture also becomes important once leaf area index exceeds about three (Reynolds et al. 2000), and has been used to improve light distribution within the canopy and optimize canopy carbon gain (Long et al. 2006). The radiation extinction coefficient (K) represents an integrative measure of the canopy architecture in term of light interception, and is a potential target for increasing RUE. Significant genetic variability for K has been reported for wheat (Abbate et al. 1998), although Shearman et al. (2005) did not find any genetic difference in K for the eight UK wheat cultivars they studied.

The contribution of the stem to grain N content at maturity is close to that of the leaves (Spiertz and De Vos 1983). One possibility for increasing NUpE and grain protein concentration would be to select genotypes with larger stem mass for N storage and subsequent translocation to the grain. Shearman et al. (2005) reported a linear increase of stem (including leaf sheaths) biomass at anthesis with the year of release of UK winter wheat cultivars. An increase in the stem in comparison to the leaf might increase the N storage capacity and slightly decrease the carbon input so that an overall shift to an increase in plant N:C ratio might be achieved. In *SiriusQuality*1, stem DM is calculated as the excess DM after leaf DM has been calculated assuming a fixed specific leaf mass (SLW). SLW is less than its maximum only early in life of the crop, if there is insufficient biomass for leaf tissue of that thickness. Thus decreasing SLW in the model induces an earlier stem growth and increases the stem:leaf ratio at anthesis and the N storage capacity of the stem. In *SiriusQuality*1, the stem includes the ear, and thus changes in stem DM due to SLW variations reflect variations of the biomass of the true stem or/and of the ear.

Past gains in crop yield have often been associated with increases of grain number per ground area unit (Reynolds et al. 1999; Donmez et al. 2001; Shearman et al. 2005). However, this relationship may reflect more the adjustment of the sink size to the capacity of the source to fill them, than a causal relationship (Sinclair and Jamieson 2006). Although, within the Sirius framework, accumulation of grain DM and N is calculated independently of ear growth and grain number (Jamieson et al.

1998), in *SiriusQuality*1 grain number is a coupling variable between DM and N supplies, defined at the crop scale, and the grain demand for structural/metabolic DM and N, defined at the grain scale (Martre et al. 2006). Thus, in *SiriusQuality*1 grain DM and N are not fully independent of ear growth and grain number. Grain number per unit area is also needed to partition storage protein between gliadin and glutenin fractions (Martre et al. 2003). Grain number is computed as in ARCWHEAT1 (Weir et al. 1984), where it is calculated from the ear mass at anthesis assuming a constant number of grains per unit ear dry mass (σ). Ear biomass is assumed to accumulate during a fraction D_{eg} of the thermal time between the appearance of the flag leaf ligule and anthesis. During that period of time, a fraction μ of the biomass accumulated each day is allocated to the ear. These parameters have been considered here, because the allocation of biomass to the ear may have feedback effects on crop biomass and N. Genetic variability for these parameters has been reported, especially for σ, which has been associated with genetic gain in yield in Argentina (Abbate et al. 1998). Ear to stem DM ratio at anthesis has also increased in modern cultivars (Yunusa et al. 1993). In the model, this ratio is determined by μ and D_{eg}.

Finally, increasing the fraction of pre-anthesis crop DM remobilized during grain filling (γ) may also be a way to increase the DM harvest index and thus crop NUE. However, this trait may have a negligible effect on N dynamics, and may thus contribute to grain N dilution.

Crop N uptake and assimilation

A critical question regarding the increase of N accumulation by wheat plants is the role of roots in limiting N uptake. Are there critical features in the roots that are constraining the rate of N uptake by crops? While physiological research indicates that roots have a very high capacity for N uptake (Oscarson et al. 1995), in whole-plant studies there appears to be a maximum uptake by cereal crops of 0.5 to 1.0 g N m^{-2} d^{-1} (Spiertz and Ellen 1978; Sinclair and Amir 1992; Asseng et al. 2002). Is N uptake constrained by shoot activity and overall plant growth rates, or are there processes in the roots that can be altered to increase N uptake? In *SiriusQuality*1, before anthesis crop N uptake is driven by the potential expansion of green area to maintain a constant SLN, and is limited only by the capacity of the stem to store accumulated N (Martre et al. 2006), but under limiting N supply it can also be limited by the capacity of the roots to explore deep soil layers. In the model, roots are characterized by their vertical extension in the soil profile. They are assumed to extend downward at a constant rate (RVER) until they reach the soil-dependent maximum depth or anthesis, whichever occurs first (Porter 1993). Here shoot demand was modified by changing phenological and canopy development as well as vegetative storage parameters. The root capacity to explore deep soil layers was changed through RVER.

The higher grain protein concentration of high grain protein concentration isogenic lines of durum wheat results partly from increased N uptake during grain filling (Kade et al. 2005). Similarly, increased grain N content in wheat hybrids compared with their parents (Oury et al. 1995), or in maize and sorghum stay-green

mutants (Rajcan and Tollenaar 1999; Borrell et al. 2001) is largely due to increased N uptake during grain filling. Maintenance of root activity after anthesis has been suggested to extend the C and N supply period as well as overcoming the trade-off between N remobilization and senescence (Richards 2000). In *SiriusQuality*1, after anthesis root N uptake is co-limited by the capacity of the stem to store N and by the activity of the roots, which decreases linearly with accumulated thermal time after anthesis to reach zero at the unconstrained end of grain filling (Martre et al. 2006). In this study, the potential maximum rate of N uptake (N_{max}^{uptake}) and the storage capacity of the stem ($[N]_{max}^{stem}$) were modified, and a parameter (K_{sen}) was introduced to scale the rate of root activity decrease during grain filling.

Increasing the N storage capacity of the crop may allow N to be taken from the soil more quickly, and therefore reducing potential N losses by leaching or denitrification. If this extra N can then be transferred to the grains, increasing N storage capacity might increase both NUE and grain protein concentration. In these simulations the storage capacity of the crop was modified through $[N]_{max}^{stem}$ and SLN. Alternative hypotheses regarding the relationship between RUE and SLN were implemented in the model: (1) RUE is independent of SLN (the assumption used in the Sirius framework), and (2) RUE depends on SLN according to the relationship given by Sinclair and Amir (1992). The former hypothesis assumes that the efficiency of the Rubisco carboxylase reaction can be engineered so that it is increased as the Rubisco concentration per unit of leaf area is decreased (Long et al. 2006).

In general, N harvest index, the ratio of grain N to total shoot N, decreases with increasing N supply (Ugalde 1993). Increase of N remobilization efficiency during grain filling, may increase both NUE and grain N content in wheat (Kichey et al. 2006), maize (Gallais and Hirel 2004) or barley (Mickelson et al. 2003). Here, increase of N remobilization efficiency was simulated by modifying the structural N concentrations of leaf ($[N]_{stru}^{leaf}$) and stem ($[N]_{stru}^{stem}$). In *SiriusQuality*1, N is supplied to grain assuming that all non-structural shoot N is available for transfer to grain. At the end of the cell division phase, the daily flux of N transferred to grain is set daily so that all of the non-structural crop N would be transferred by the unconstrained end of grain filling (Martre et al. 2006). The importance of the rate of N remobilization during linear grain filling was assessed by introducing a scaling parameter (β) to modify proportionally the rate of N remobilization.

Grain development and C and N accumulation

Increasing the length of the grain-filling period has been also suggested as a putative trait for increasing grain yield in wheat (Evans and Fischer 1999), and low, but significant, genetic variability has been reported for this trait (Robert et al. 2001; Charmet et al. 2005). The potential size and storage capacity of the grain are determined during the initial phase of endosperm cell division. Increase of the growth rate or/and duration of this phase is another proposed trait to increase grain yield and NUE. These traits were manipulated in the simulations by changing the

duration of grain filling (from anthesis to end of grain filling, D_{gf}), the duration of the cell division phase (D_{cd}), the potential relative rate of accumulation of structural C (k_{cd}), and the grain structural N:C ratio ($\alpha_{N:C}$). It is worth noting, that in *SiriusQuality*1, modifying D_{cd} or D_{gf} also modifies the rate of DM and N remobilization after the end of cell division phase, which are scaled based on ($D_{gf} - D_{cd}$). Parameters affecting the partitioning of grain protein have not been considered here, because they have no effects on grain yield, protein concentration or crop NUE.

RESULTS AND DISCUSSION

Effects of climate, sites and N treatments

Variations in NUE and yield due to climate were substantial (Figure 1A). NUE was closely related to grain yield variations, because total available soil N during the cropping period was fairly constant. The range of NUE variations was similar for both N treatments, whereas for yield it was two times higher for HN than for LN. In good agreement with experimental results (Le Gouis et al. 2000), for most of the years, NUE was higher for LN than HN treatments. NUtE was largely independent of N supply, and as reported by Dhugga and Waines (1989), NUtE became more important than NUpE in determining NUE as N supply increased.

   Average yield and grain protein concentration were ca. 35% lower for LN than for HN treatments (Table 2). Grain yield was negatively correlated with grain protein concentration for both N treatments ($r = -0.51$ for both LN and HN; Figure 1B). In good agreement with observed data (Triboï et al. 2006), N deficit lowered both yield and grain protein concentration, but the negative genetic correlation was conserved and, although not statistically significant, the slope was steeper (more negative) for HN than for LN treatments.
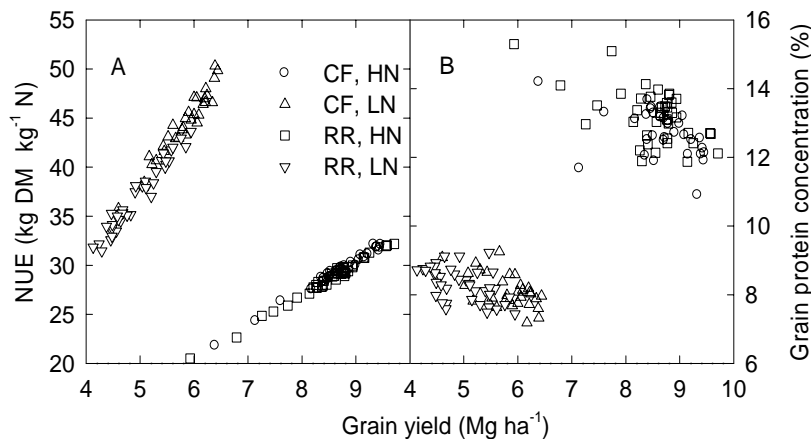
Figure 1. *Simulated N use efficiency* (**A**) *and grain protein concentration* (**B**) *versus grain yield at high (HN) low (LN) N supplies for 32 and 40 years at Clermont-Ferrand (CF) and Rothamsted (RR), respectively*

Although the weather was significantly different at the two sites, using the same cultivar and soil for HN treatments average yield was not significantly different (Table 2). Under these conditions, NUpE and NUtE, and grain protein concentration and composition were also similar at the two sites. As expected, N harvest index and NUE components were higher for HN than LN (Table 2). At low N supply, average yield was 0.8 Mg ha^{-1} higher at Clermont-Ferrand than at Rothamsted. For the LN treatment, NUE was also higher (18%) at Clermont-Ferrand than at Rothamsted, but average grain protein concentration and composition were similar at both sites.

However, the simulated gliadin and glutenin percentages and the glutenin:gliadin ratio showed an inter-year variability two times higher at Rothamsted than at Clermont-Ferrand (Table 2). In *SiriusQuality*1, grain protein composition is determined at the individual-grain level, whereas total yield and grain protein content are determined at the crop level. The higher variability of grain protein composition at Rothamsted reflects the higher variability of single grain size and protein content at this site compared with Clermont-Ferrand.

Table 2. Average simulated grain yield, N harvest index, N use efficiencies, grain protein concentration, gliadin and glutenin percentages, and glutenin:gliadin ratio at low and high N supplies for 32 and 40 years at Clermont-Ferrand and Rothamsted, respectively. Numbers in parenthesis are the coefficients of variation (%)

	Clermont-Ferrand		Rothamsted	
	Low N	High N	Low N	High N
Grain yield (Mg ha^{-1})	5.80 (7.9)	8.67 (7.7)	4.97 (10.0)	8.51 (8.4)
N harvest index (g g^{-1})	0.68 (3.6)	0.78 (1.6)	0.64 (4.4)	0.78 (2.2)
NUpE (kg N kg^{-1} N)	0.92 (3.6)	0.84 (6.2)	0.84 (5.1)	0.85 (5.6)
NUtE (kg DM kg^{-1} N)	48.0 (6.2)	35.1 (6.1)	44.2 (5.8)	33.7 (7.3)
NUE (kg DM kg^{-1} N)	44.3 (7.7)	29.4 (7.4)	37.1 (9.2)	28.6 (8.1)
Grain protein concentration (%)	8.11 (6)	12.72 (5.3)	8.30 (5.9)	13.21 (5.8)
Gliadins (% of total grain N)	11.5 (28.8)	27.7 (8.5)	7.2 (54.7)	27.0 (11.2)
Glutenins (% of total grain N)	28.8 (16.6)	45.5 (3.1)	21.2 (34.7)	44.8 (4.3)
Glutenin:gliadin ratio	2.56 (12.8)	1.64 (5.4)	3.23 (26.9)	1.66 (7.0)

Effects of physiological traits

As for the effect of climate variability and N treatments, the different physiological traits had fairly similar effects at both sites and only the results for Clermont-Ferrand are reported here.

At low N supply, none of the parameters had a significant effect (i.e., > 5%) on N uptake efficiency (NUpE; Table 3), because crop N uptake was primarily limited by soil N availability.

Under non-limiting N conditions, reducing the duration of the vegetative phase of the crop cycle, either through the rate of leaf appearance and canopy development (P) or after the canopy has reached its maximum size ($P_{\text{flag}}^{\text{anth}}$), increased NUpE

Table 3. *Average simulated changes in NUpE and NutE in response to a 30% increase or decrease of parameters values for 32 years at Clermont-Ferrand at low and high N supplies*

| Parameter	Changes in yield (%)				Changes in NUpE (%)				Changes in NUtE (%)			
	Low N		High N		Low N		High N		Low N		High N	
	−30	+30	−30	+30	−30	+30	−30	+30	−30	+30	−30	+30
Phenology and canopy development												
P	0.8	-11.7	-11.5	1.7	-2.4	1.9	-21.2	12.5	7.0	-15.2	11.3	-10.6
P_{flag}^{anth}	11.5	-10.0	1.0	-0.5	-1.6	1.3	-7.1	6.9	13.8	-11.3	9.3	-7.2
α_{max}	2.0	-1.3	-3.8	0.2	0.0	0.0	-14.0	9.2	2.0	-1.3	12.5	-8.2
P_{sen}	-0.6	0.6	-0.3	0.2	0.0	0.0	0.1	0.1	-0.6	0.6	-0.4	0.1
Crop C assimilation and partitioning												
K	-8.6	6.0	-8.7	4.4	0.5	-0.5	-5.9	3.7	-8.4	5.9	-2.5	0.6
RUE	-15.9	-0.6	-29.5	26.6	-1.2	2.2	-16.2	15.5	-15.7	-0.8	-15.7	9.9
SLW	1.6	-1.3	0.3	-0.5	-0.1	0.1	6.5	-6.3	1.6	-1.3	-5.8	6.3
D_{eg}	-3.6	3.8	-0.1	0.2	0.3	-0.2	0.2	-0.2	-3.6	3.8	-0.3	0.4
μ	3.7	-10.3	0.1	-0.3	-0.7	1.0	-0.7	0.5	11.2	-10.2	1.2	-0.6
σ	11.2	-10.3	0.7	-0.3	-0.7	1.0	-0.7	0.5	11.2	-10.3	1.3	-0.6
γ	-9.4	9.2	-9.0	8.9	0.0	0.0	0.0	0.0	-9.4	9.2	-9.1	9.0
Crop N uptake and assimilation												
RVER	0.6	0.0	0.1	-0.1	-0.1	0.1	0.1	-0.2	0.8	-0.1	0.1	0.1
N_{max}^{uptake}	-0.1	0.1	-1.1	1.8	-0.2	0.1	-7.3	2.2	0.6	0.0	4.4	-0.4
K_{sen}	0.0	0.0	-0.6	1.4	0.0	0.0	-0.2	0.1	0.0	0.0	-1.9	1.3
$[N]_{max}^{stem}$	0.1	0.0	-2.6	1.5	-0.1	0.0	-9.5	9.0	0.1	0.1	7.7	-6.9
SLN (con. RUE)	4.9	-6.4	1.8	-3.9	-0.7	0.5	-17.8	12.9	8.0	-6.6	24.9	-14.8
SLN (var. RUE)	0.8	-4.2	-7.8	3.8	-2.3	0.7	-27.1	18.1	-0.4	-4.4	18.4	-11.9
$[N]_{stru}^{leaf}$	5.0	-5.5	0.7	-0.8	-0.3	0.3	-0.1	0.1	5.0	-5.5	0.7	-0.8
$[N]_{stru}^{stem}$	3.3	-4.1	-1.2	1.1	-0.2	0.2	0.3	-0.3	3.4	-4.2	-1.4	1.4
β	8.2	-17.1	8.2	-17.7	-0.3	0.9	-0.8	0.5	31.9	-17.0	30.8	-17.7
Grain development and C and N accumulation												
D_{gf}	-18.7	20.1	-24.3	24.0	2.3	-0.3	1.2	-1.1	-18.5	20.1	-24.2	23.9
D_{cd}	19.9	-22.9	9.0	-7.4	-0.7	1.3	-0.2	0.3	20.0	-22.8	9.0	-7.4
$\alpha_{N:C}$	6.8	-3.7	4.9	-2.7	0.1	0.0	0.0	0.0	6.8	-3.7	4.9	-2.7
k_{cd}	16.0	-24.3	0.9	-1.3	-1.0	4.4	-0.3	0.4	16.0	-24.3	1.0	-1.4

(Table 3), but this possible effect was overwritten by the reduction of NUtE, and overall had no effect on NUE and yield. However, grain N and protein concentration increased by 5 to 7% in response to a 30% increase of P or P_{flag}^{anth}. In contrast, under

limiting N conditions, increasing these two parameters by 30% produced a 10 to 15% decrease in mean NUtE, NUE, yield, and grain protein concentration.

The potential size of the culm leaves (α_{max}) had no significant effect on NUpE, and its effect on NUtE depended on N supply (Table 3). For both N treatments neither α_{max} nor P_{sen} had a significant effect on yield, NUE or grain protein concentration. Surprisingly, P_{sen} had no effect on both NUpE and NUtE.

The effect of K on yield and NUE was largely independent of N supply, and mean yield and NUE increased by 4 to 6% in response to a 30% increase of K. Increasing RUE by 30% benefited (+27%) mean yield and NUE only at high N supply. None of these two parameters was able to sustain grain protein concentration as yield increased. However, under low N supply cultivars with a low K or RUE had similar yield but a higher grain N and therefore grain protein concentration compared with cultivars with a high K value (Figure 2).

For both N treatments, SLW had a small effect on yield and NUE, but for the HN treatment, mean grain N increased by 8% in response to a 30% decrease in SLW, which resulted in an increase of mean grain protein concentration from 12.7% to 13.6% (Figure 2).
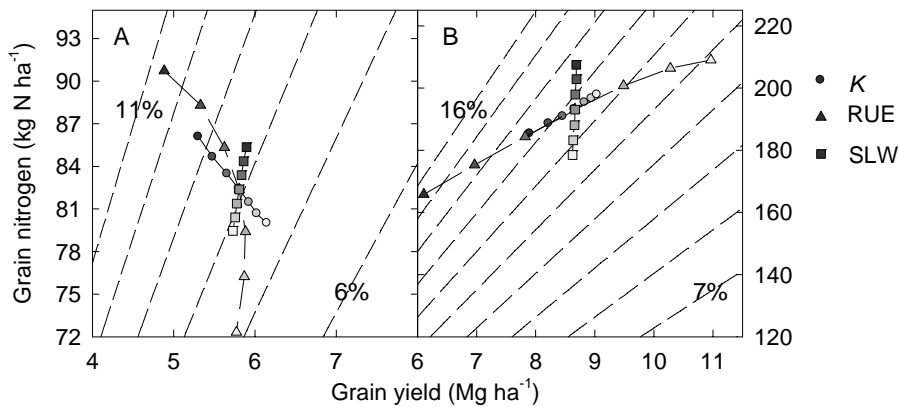
Figure 2. Grain N versus yield in response to variations of K, RUE and SLW for 32 years at Clermont-Ferrand at low (**A**) *and high* (**B**) *N supplies. The grey intensity of the symbols decreases as the value of the parameters increases by 10% increments from –30% to +30% of their default value. Dashed lines are grain protein concentration isopleths in 1% increments*

For HN treatment, the parameters related to ear growth (D_{eg} and μ) and grain number to ear DM ratio (σ) had no significant effect on yield and NUE components (Table 3). However, under limiting N supply, increasing μ or σ by 30% reduced mean yield and NUtE by ca. 10%. These parameters had no significant effect on crop or grain N dynamics. The fraction of crop DM remobilized during grain filling (γ) had no effect on grain N either (data not shown). However, a 30% increase in γ

increased mean yield and NUE (NUtE) by 9% independently of N supply (Table 3), because of a higher DM harvest index, which increased from 0.39 to 0.43 and from 0.48 to 0.52 for LN and HN, respectively.
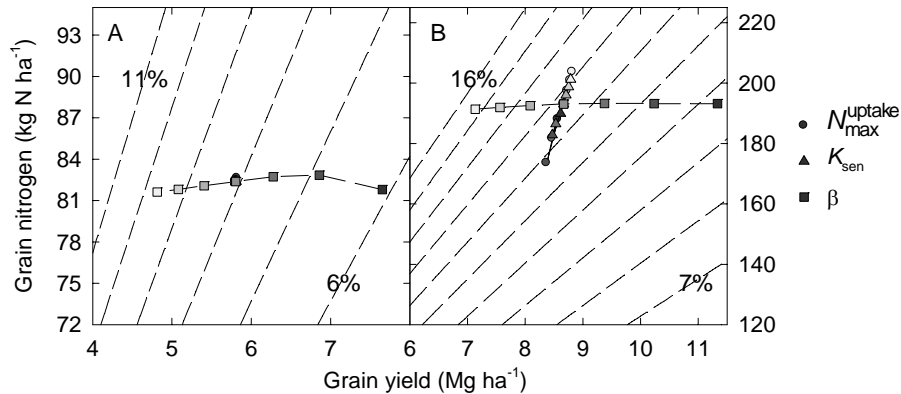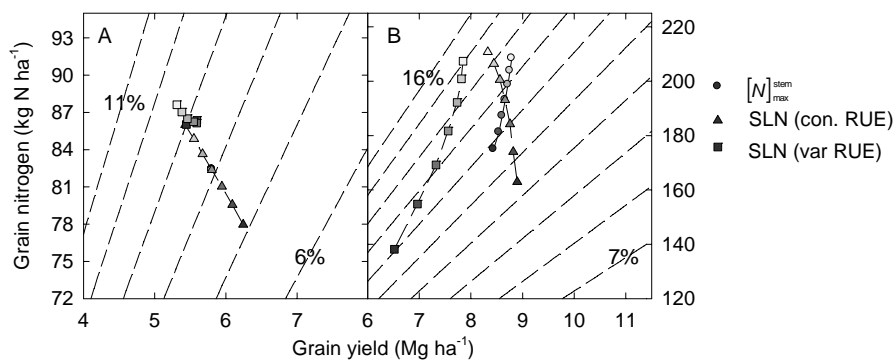

Figure 3. *Grain N versus yield in response to variations of* N_{max}^{uptake}, K$_{sen}$ *and* β *for 32 years at Clermont-Ferrand at low* **(A)** *and high* **(B)** *N supplies. The grey intensity of the symbols decreases as the value of the parameters increases by 10% increments from –30% to +30% of their default value. Dashed lines are grain protein concentration isopleths in 1% increment*

For both N treatments, RVER had no effect on mean NUE components, grain yield or N. Similarly changes in potential maximum rate of root N uptake at anthesis (N_{max}^{uptake}) or in the rate of root senescence (K_{sen}) during grain filling had no significant effect on mean NUE or yield (Table 3). However, for HN treatment, both N_{max}^{uptake} and K_{sen} were positively associated with grain N and protein concentration (Figure 3). In contrast with earlier suggestions (Richards 2000), delaying leaf senescence by reducing the rate of N remobilization during grain filling (b) had no effect on final grain N content, but allowed the crop to assimilate more C; therefore, grain yield and NUE increased, but grain protein concentration decreased (Figure 3). Interestingly, lowering of the rate of N remobilization (β) by 30% increased mean NUtE by ca. 31% at both N supplies (Table 3). Increasing the efficiency of N remobilization during grain filling through changes in $[N]_{stru}^{leaf}$ and $[N]_{stru}^{stem}$ had small effects on yield and NUE (Table 3). For LN, decreasing $[N]_{stru}^{leaf}$ and $[N]_{stru}^{stem}$ by 30% produced a 3 to 8% increase in mean grain yield and N, and therefore yield was increased while grain protein concentration was maintained. For HN, decreasing $[N]_{stru}^{leaf}$ and $[N]_{stru}^{stem}$ by 30% had no significant effect on grain yield, but increased mean grain protein concentration from 12.7% to 13.0% and 13.3%, respectively.

Under non-limiting N supply, increasing $[N]_{max}^{stem}$ and SLN by 30% increased mean NUpE between 9% and 18%, but this was partly cancelled by a decrease (between 7 and 15%) of NUtE (Table 3). Overall, the improvement in yield and

NUE due to N storage capacity of the crop was surprisingly low (between 1 and 5%); but for HN treatment, leaf and stem storage capacity had large effects on grain protein concentration (Figure 4). The same conclusions were reached in terms of yield and NUE improvement with the two hypotheses regarding the relationship between SLN and RUE. However, assuming that RUE increases non-linearly with SLN (Sinclair and Amir 1992), which is the most likely hypothesis, under non-limiting N supply, increasing SLN by 30% resulted in a 4% and 11% increase of grain yield and protein concentration, respectively (Figure 4).

Figure 4. *Grain N versus yield in response to variations of* $[N]_{max}^{stem}$ *and SLN for 32 years at Clermont-Ferrand at low* **(A)** *and high* **(B)** *N supplies. The grey intensity of the symbols decreases as the value of the parameters increases by 10% increments from –30% to +30% of their default value. Dashed lines are grain protein concentration isopleths in 1% increment*

The parameters related to grain development (D_{gf} and D_{cd}) and structural N and C accumulation ($\alpha_{N:C}$ and k_{cd}) had no significant effect on NUpE (Table 3). Rather they modified C and N grain demand, and therefore NUtE. Increasing D_{gf} by 30% produced a 20 to 24% increase in yield and NUE, independently of N treatments; D_{gf} had a much lower effect (less than 7%) on grain N, and grain protein concentration decreased as D_{gf} increased. Similar results were obtained with D_{cd}, but its effects on yield, NUE and grain protein concentration were larger under limited N supply (data not shown). The ratio of structural grain N to C ($\alpha_{N:C}$) had no effect on grain N (data not shown). For the two N treatments a decrease in $\alpha_{N:C}$ of 30% resulted in an increase in mean yield and NUE of 7% (Table 3). Under low N supply, the grain demand for structural C (k_{cd}), had a symmetrical effect on yield and NUE and grain N compared with D_{gf}. Under non-limiting N supply, k_{cd} had no effects on grain yield and NUE, and slightly increased (+3%) grain N when increased by 30%.

CONCLUSION

Crop simulation models provide the best approach for integrating our understanding of complex plant processes as influenced by weather, soil and management. As such

they provide a powerful tool in guiding the direction of future research by providing quantitative predictions and highlighting gaps in knowledge. The effect of changing a single plant characteristic on crop performance can be determined in a field experiment assuming that suitable plant material is available. But the crop response will also depend on weather and environmental conditions. So determining how a range of plant characteristics, either individually or in combination, will affect crop performance under a range of growing conditions becomes a daunting practical task.

Some cautions are required when using crop simulation models, since the ability of a model to predict subtle traits × environment × management interactions depends on the simplifications and assumptions made in the model (Boote et al. 2001). On the other hand, simulation models allow us to focus on the most important aspects of the physiology of the crop. Simulation models also show complex interactions that are not intuitive. For example, in this study delaying leaf senescence by reducing the rate of N remobilization during grain filling (β) had a large effect on C assimilation and final yield but had no effect on final grain N content, and thus resulted in a large decrease of grain protein concentration. This result is in good agreement with experimental results, where stay-green mutants for durum wheat had a higher NUE than the wild type but grain N content was the same for the mutants and the wild type, and therefore the grain protein concentration was lower for the mutants than for the wild type (Spano et al. 2003).

Increasing the N storage capacity of the leaves and stem and the allocation of N to non-structural proteins appeared as the more promising strategy to break the negative correlation between grain yield and protein concentration. Moreover, it may also reduce the risk of N losses by leaching, denitrification and volatilization. The analysis of 21 genotypes of bread wheat revealed a significant genetic variability of stem N storage capacity, ranging from 7.6 to 14.9 mg N g^{-1} DM (Triboï and Ollier 1991). In *SiriusQuality*1, the default value for $[N]_{max}^{stem}$ lies in the middle of this range; we could thus expect a larger increase of $[N]_{max}^{stem}$ than what has been considered here. In good agreement with our results, a recent survey of UK winter-wheat cultivars revealed a positive association between grain yield and stem N concentration (Shearman et al. 2005). We are not aware of any study reporting the observed range of genetic variability for wheat canopy SLN.

Martre et al. (2006) showed that in *SiriusQuality*1, in most cases the end of grain filling occurred before D_{gf}, and was triggered by leaf senescence, which was driven by N remobilization and not by the ontogenic rate of leaf senescence (P_{sen}). Thus, we expected that increasing root N uptake after anthesis would delay N remobilization and leaf senescence. However, increasing the rate of post-anthesis root N uptake had not effect on N remobilization and grain-filling duration or grain yield, but it promoted grain N content and protein concentration. Overall, crop N accumulation appeared to be more limited by the shoot demand and its capacity to store newly assimilated N temporarily, than by the capacity of the roots to extract N from the soil.

Intuitively, one can consider that increasing RUE might be more important under limiting N conditions, where the leaf area and the canopy duration are reduced. However, our results suggest that for low-N-input environments, it might be more

interesting to select cultivars with low RUE. Under low N, RUE had a relative small effect on grain yield, but a decrease in RUE by 30% increased mean grain protein concentration from 8.1% to 10.6%.

From our simulations it clearly appears that the importance of a physiological trait for crop breeding is largely dependent on the objectives in terms of quality. Crop N requirement and NUE cannot be used independently to identify favourable traits. For example, the effects of P and $P_{\text{flag}}^{\text{anth}}$ on NUE were similar and depended largely on N supply, but only P allowed breaking the negative correlation between grain yield and protein concentration for both N treatments.

In agreement with theoretical and experimental considerations, plants exhibiting a similar capacity of growth show a low genetic variability for NUtE among C_3 species (Greenwood et al. 1991). In this study, changes in NUtE were closely correlated with changes in grain yield, and none of the physiological traits considered had an effect on NUtE except the trivial effect of plant mass *per se*. More surprisingly, under low N conditions, none of the parameters had a significant effect on NUpE. This may be due to a severe N shortage, but it may also point at a lack of understanding of root system development and functioning under limiting N supply (Jamieson and Ewert 1999).

In this study, variations in weather and N treatments induced larger variations in NUE than most of the physiological traits considered. These simulations suggest that a single physiological trait is unlikely to break the negative correlation between the grain protein concentration and yield over a wide range of sites and seasons, especially under low-N-input environments. In a recent study, using the APSIM–NWHEAT simulation model Asseng and Milroy (2006) reached the same conclusion. Much of the genetic gain in complex traits such as yield or grain protein concentration is believed to be due to the accumulation of genes with small additive effects (Blanco et al. 2002). Similarly, our simulation results support the idea that breaking the negative relationship between grain yield and protein concentration will require pyramiding physiological traits, and with opposite effects for some of them.

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