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Integrated physiological and agronomic modelling of N capture and use within the plant

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Abstract

Today farmers have several constraints to take into account in managing their crops: (i) competitiveness: productivity must be maintained or increased whereas inputs must be decreased, (ii) the environmental consequences of cultural practices: pesticide and fertilizer use must be decreased, and (iii) product quality must be improved and nitrogen nutrition is an important factor in harvest quality. These new constraints sometimes conflict: maximum yield is often obtained with large amounts of N, increasing the risks of N leaching. The determination of rates and dates for nitrogen application must become more precise in this context. Tools are required for the forecasting of crop requirements, the diagnosis of N deficiencies during the crop cycle and breeding of new adapted varieties. Models and diagnosis indicators have been developed to meet these needs, but those relating to nitrogen are often based on empirical relationships. Moreover, the available models and indicators often fail to account for cultivar-specific responses. The improvement of agronomic tools and the breeding of new varieties adapted to new cropping systems should be based on a thorough understanding of the key metabolic processes involved, and the relative contributions of these processes to yield determination in conditions of fluctuating N supply. For both purposes, more information is required about plant and crop N economy. In this paper, the way in which N absorption and use within the plant and crop, plant responses to deficiencies and excesses of nitrogen are taken into account in major agronomic models is described first. The level of sophistication of the modules comprising these models depends on operational objectives. Secondly, the ways in which the most recent molecular plant physiology findings can, and indeed should, be integrated into models at the crop and crop cycle levels are described. The potential value of this approach for improving current agronomic models and diagnostic tools, and for breeding more efficient varieties is also discussed.

Key words: Agronomic models, nitrogen, plant physiology.

Introduction

In the last few years, agriculture has had to adapt to new constraints and respond to new challenges with major implications for the management of nitrogen fertilization. Losses of nitrogen from arable land to the aquatic environment (nitrate leaching) or the atmosphere (gaseous losses) have increased in the last few decades. Higher quality products are now demanded and farmers must attain the new standards defined by the market for their products. For example, the EC has defined maximum nitrate concentrations for fresh vegetables, including lettuce. For wheat, several markets with specific requirements in terms of grain protein content now exist and appropriate agricultural practices are essential if farmers are to produce grains with the desired characteristics. Thus, farmers now have to combine several objectives: achieving optimum yield, limiting production costs to maximize profit, conserving natural

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Abbreviations: HATS, high-affinity transport system; LATS, low-affinity transport system; NUR, nitrogen uptake rate; RGR, relative growth rate; VSP, vegetative storage proteins.

resources by limiting the negative impact of crop production on the environment, and obtaining crop products with the qualities demanded by the market.

Nitrogen fertilization must be managed very precisely: a wheat crop yielding 8 t ha^{-1} involves more than 300 kg ha^{-1} of nitrogen in the agrosystem (Jeuffroy and Meynard, 1997). The leaching of only 20 or 30 kg of nitrogen ha^{-1} is sufficient to increase groundwater nitrate concentration above the threshold for drinking water imposed by the EC (50 mg l^{-1}). A difference in nitrogen absorption of only 20 kg ha^{-1} may result in a decrease in grain protein content of 1% (from 11.5% to 10.5%, for a crop yield of 8.5 t ha^{-1}), preventing the use of the harvested grains for breadmaking. It is thus necessary to manage nitrogen in the agrosystem with a high level of accuracy, within 10%!

However, the soil–crop system is characterized by several factors. One important characteristic is the rapid dynamics of mineral nitrogen availability in the soil, with abrupt variations associated with the application of high rates of mineral fertilizer, the first rains after a long drought, triggering mineralization, and the ploughing of the soil. It is important to control these rapid variations (i) to avoid long periods of nitrogen deficiency for the crop, detrimental for crop yield and grain quality and (ii) to avoid periods of nitrogen excess in the soil, which could lead to nitrate leaching. Another characteristic is the heterogeneity of the crop environment due, for example, to the activity of the soil fauna, the nature of the soil and human intervention. This heterogeneity has consequences for crop functioning and the utilization of soil nitrogen.

Three main groups of tools are already available to help farmers to attain the new conflicting goals: (1) diagnostic tools, which can be used to characterize crop N nutrition status at a given date or stage, making it possible to correct N deficiencies; (2) genotypes, with characteristics more or less adapted to the aims of the farmers (high yield and/or high quality); (3) crop models, that allow the consequences of cultural practices on yield, quality and environment losses to be simulated.

The diagnostic tools are often derived from crop models, or should be used in a supplementary way with them (Meynard *et al.*, 2001). The genotypes used today and in the near future should be chosen according to the cropping systems in which they will be grown. They must be adapted to the environmental conditions determined by management practices. Such adaptation will require the development of tools adapted to simulate variety behaviour in various environments. Similarly, management practices must be adapted to the genotypes used. For the adaptation of both varieties and management practices, agronomic crop models must take genotypic characteristics into account and adapted crop models must be developed to optimize genotypes and agricultural environment characteristics to each other. Finally, in order to enable the farmers to adapt the fertilization strategy to given production and quality objectives and to reduce environmental N losses, taking into account genotypic characteristics, the relevant crop models must simulate the development, growth, grain yield, quality of the crop, and N losses to the environment, according to the fertilization rules applied and to the characteristics of soil and climate that modify the response of the crop to the fertilizer applied. Moreover, the range of validity of these models must cover the main agricultural situations. For each crop model, the structure and complexity of the model and the relationships included in it must be consistent with the objectives. They must also be based on a sound knowledge of crop functioning.

Firstly the main available agronomic models will be described in terms of the nitrogen metabolism functions generally included and their quantification. The various conceptual approaches taken in modelling the nitrogen budget in the different models will be compared. In particular, these approaches will be discussed relative to the aim of the modellers, to the knowledge of crop physiology and to the range of the field crops the model will relate to. Then perspectives for the inclusion of recent knowledge in plant physiology and its potential consequences will be discussed.

Nitrogen, its capture and use in agronomic models

A large number of models of crop growth and development are currently available. However, they are all based on similar conceptual frameworks, in which nutrient availability and crop demand are compared. If demand exceeds supply, there is a deficiency, which later modifies crop growth, thereby reducing the following crop N demand. If more nutrient is available than is required by the crop for maximal growth, the surplus is absorbed by the plant up to a certain limit and stored.

The available models differ markedly in complexity, according to the functional objective for which they were designed. It was therefore decided to limit the description to the processes taken into account and their quantification. The following major submodels are considered (as summarized in Fig. 1) (i) quantification of crop nitrogen demand; (ii) nitrogen absorption and assimilation. These two processes are generally not separated in agronomic models, and no differentiation is made between NH_4^+ and NO_3^-; and (iii) effects of N deficiency on crop growth and development.

Quantification of crop demand

The quantification of crop N demand differs between agronomical models in terms of the type of processes

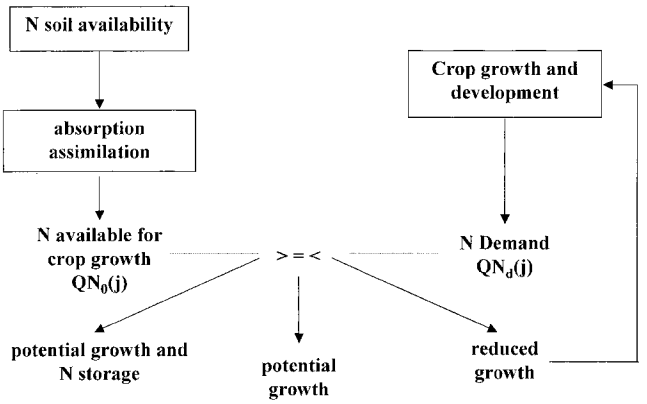

Fig. 1. General framework used in agronomical models.

involved, which may or may not have a sound physiological basis, their degree of complexity and the level of organization considered (i.e. organ, plant or crop level).

The most simple formalisms link crop growth to fertilization regime (Overman *et al.*, 1995) or to time, expressed in days or in time units (cumulative degree-days) from sowing (Addiscott and Whitmore, 1987). These very simple models do not account for nitrogen availability in the soil or for crop responses to possible deficiency at low levels of fertilization. In addition, although very robust in the conditions in which they were developed, these models cannot be extrapolated to other environmental conditions.

In other more recent and complex models, crop nitrogen demand is quantified on the basis of single-plant nitrogen concentrations, varying between maximal %N (%N_{max}), which corresponds to the maximum amount of N that can be accumulated by its constitutive organs or the plant itself, and minimal %N (%N_{min}), below which plant cannot survive. These single values are generally obtained in field trials with contrasting fertilization regimes.

Other models refine the expression of crop demand by adding a 'critical' value for N concentration between %N_{max} and %N_{min}. This 'critical' value (%N_{crit}) corresponds to the minimum N concentration permitting maximal crop growth. Thus, crops grown in conditions in which actual %N is below %N_{crit} suffer N deficiency and reduced growth, whereas some of the N taken up by crops grown in conditions with actual %N above %N_{crit} is stored. Critical N concentration can be used to estimate crop minimal N requirement for maximal growth as the product of biomass produced with the corresponding %N_{crit} (Ulrich, 1952). This concept of %N_{crit} is used to estimate the crop demand in many models, including CERES-Maize (Jones and Kiniry, 1986), CERES-Rape (Gabrielle *et al.*, 1998*a*), DAISY (Hansen *et al.*, 1991), CROPSYST (Stockle and Nelson, 1996), and Azodyn (Jeuffroy and Recous, 1999). %N_{crit} has also been used to diagnose N deficiency (Lemaire and Gastal, 1997). If actual N concentration is lower than the corresponding %N_{crit}, crop N uptake is insufficient for maximal growth.

The values of %N_{min}, %N_{crit} and %N_{max} change along the crop cycle. The three curves (%N_{min}, %N_{crit} and %N_{max}) are defined differently and often empirically in models. All decrease with time, expressed as days or time units (cumulative degree-days) in DAISY, as growth stages in AFRCWHEAT2 (Porter, 1993) and CROPSIM-Wheat (Hunt and Pararajasingham, 1995), as fractions of the crop cycle in EPIC (Williams *et al.*, 1989) or even as increases in shoot biomass in CROPSYST or Azodyn.

The empiricism of the representation of crop %N changes with time results in poor description of crops supplied with different regimes of N fertilization. Stockle and Debaeke compared actual %N (%N_{act}) and corresponding crop %N values calculated in AFRCWHEAT2, EPIC, DAISY, and CROPSYST for wheat (*Triticum aestivum* L.) field crops supplied well or inadequately with N (Fig. 2) (Stockle and Debaeke, 1997). The relevance of calculations of crop %N values in each model can be established by separating field positions according to their N nutrition: the %N of the well-fertilized fields must be between corresponding %N_{crit} and %N_{max}, and that for N-deficient crops between %N_{min} and %N_{crit}, with no points above %N_{max} or below %N_{min}. Three out of four models did not meet these expectations. AFRCWHEAT2 and DAISY underestimated all three crop %N curves as almost all the points corresponding to fields well supplied with N were above %N_{max}, whereas the %N of N-deficient crops was above %N_{crit}. EPIC offered a better classification of field crop situations and CROPSYST was the most appropriate model for the description of situations. The %N of field trials well supplied with N was between %N_{crit} and %N_{max} and the %N of deficient crops was between %N_{min} and %N_{crit}. Calculation of the curves in this model is based on aerial biomass, in contrast to the other models.

The relevance of the CROPSYST model for describing N supply regimes results from the sounder ecophysiological basis of crop %N estimation in this model, linked to crop shoot biomass rather than time or growth stage. This approach is based on recent work on the response to competition of crops in dense stands (Lemaire and Gastal, 1997). These authors identified two growth phases of plants according to their level of competition. The first phase corresponds to young plants either not yet competing or in only slight competition for resources. In this phase, N uptake and increase in mass or leaf area (Grindlay, 1997) are closely related. Many authors have found a linear relationship between relative growth

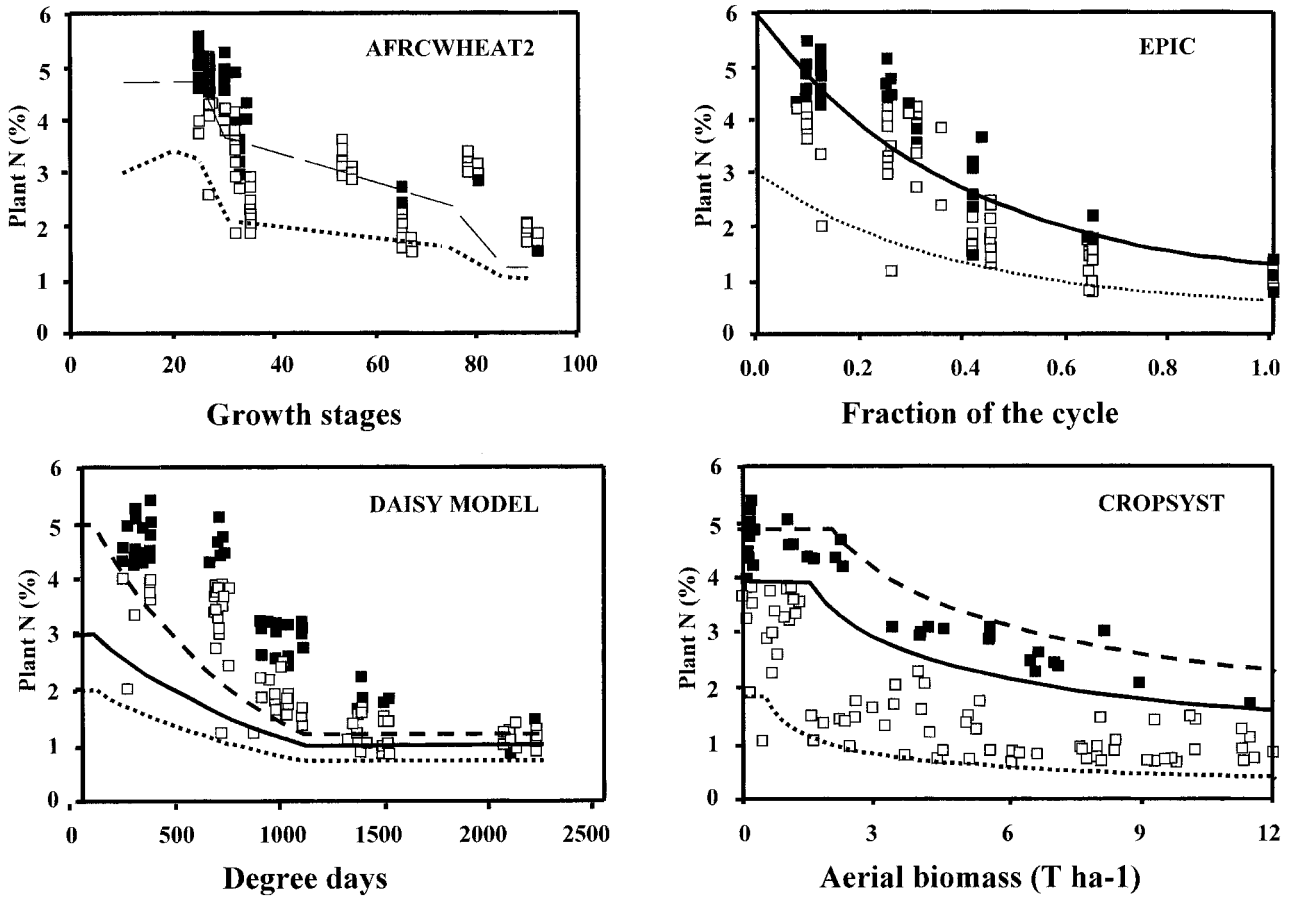

Fig. 2. Comparison of four models for estimating characteristic plant N content concentration curves with data from N-limited (open squares) and non N-limited plots (closed squares) of winter wheat cultivar Soissons grown in Auzeville, France in 1992 and 1993. $\%N_{max}$, dashed lines; $\%N_{crit}$, solid lines; $\%N_{min}$, dotted lines. (Redrawn from Stockle and Debaeke, 1997.)

rate (*RGR*) and total plant N uptake (Schenk, 1996). The second phase involves much stronger competition between plants. Two processes combine to decrease the $\%N$ of plants in the canopy during growth. Firstly, the ratio of 'metabolic' (estimated from leaves) to 'structural' (e.g. stems, midribs) tissues decreases during growth. As the plant grows, competition for resources leads to greater N investment in structural tissues, which are less rich in nitrogen than the metabolically active leaves. Secondly, plant nitrogen is redistributed on closure of the canopy. The nitrogen from the shaded parts of the canopy is recycled, providing some of the N required for growth, decreasing N uptake with time. The combination of these two processes accounts for the decrease in crop $\%N$ and the relevance of shoot biomass as the integrative variable driving this decrease.

Empirical studies (Greenwood *et al.*, 1990) have suggested that the amount of N taken up by the canopy (QN, kg N ha^{-1}), in situations with no deficiency and no storage, may be related to aerial biomass (DM, t ha^{-1}) as follows:

$$QN = 10a(DM)^{2/3} \quad (1)$$

Later, studies gave an account of this formalism, based on the stable surface/volume allometry, at least during the vegetative period (Lemaire and Gastal, 1997). Surface and volume may be associated with the metabolic and structural compartments, respectively.

This equation allows $\%N$ to be calculated from DM, in situation of optimal nitrogen nutrition (no deficiency and no storage), as:

$$\%N_{crit} = a(DM)^{-1/3} \quad (2)$$

Many studies have been carried out to determine the parameters of this equation for various crops. The power coefficient is statistically close to $-1/3$ (Table 1). The a coefficient differs only between C_3 and C_4 plants.

The existence and stability of $\%N_{crit}$ open up interesting possibilities for modelling, facilitating evaluation of the minimal N uptake for maximal growth and thus of crop N demand for morphogenesis without N storage, and for diagnosis, because the crop is N-deficient at a given time if its $\%N$ is lower than the $\%N_{crit}$ for its current biomass.

Table 1. *Coefficients* a *and* b *of the relationship* $\%N_{crit} = a(DM)^{-b}$, *where* %N *is the crop N concentration (g* g^{-1}*) and* DM *(t* ha^{-1}*) the aerial dry matter for various crops*

	$\%N_{min}$		$\%N_{max}$		$\%N_{crit}$		
	a	*b*	*a*	*b*	*a*	*b*	
Oilseed rape	2.07	−0.17	6.18	−0.21	4.48	−0.25	(Colnenne *et al.*, 1998)
Pea					5.08	−0.32	(Ney *et al.*, 1997)
Lucerne					5.50	−0.36	(Lemaire and Gastal, 1997)
Wheat	2.10	−0.44	8.50	−0.44	5.29	−0.44	(Justes *et al.*, 1994)
C_3					5.10	−0.34	
Sorghum					3.90	−0.39	(Plénet and Cruz, 1997)
Maize	2.05	−0.56	6.30	−0.42	3.40	−0.37	(Plénet and Cruz, 1997)
C_4					3.65	−0.38	

Coming back to the quantification of crop N demand, different models use $\%N$ concentration in different ways. Some use $\%N_{crit}$ to define the requirements for growth, others use $\%N_{max}$ to account for the storage of nitrogen in conditions of excess. Others use a combination of crop $\%N$ values. In CERES-Maize, for instance, crop N demand is estimated as:

$$D_N = DM \times (\%N_{max} - \%N_{actual}) + \Delta DM \times \%N_{crit} \quad (3)$$

where D_N is the demand for N on a given day, DM is the aerial biomass on the same day, ΔDM the biomass created on that day, $\%N_{crit}$ and $\%N_{max}$ are the N critical and maximal concentrations corresponding to the stage of the crop, and $\%N_{act}$ the actual N concentration. It is assumed that the requirements of the new biomass correspond to $\%N_{crit}$ whereas those of the older plant parts tend towards $\%N_{max}$. This assumption has no sound physiological basis, but allows 'luxury' consumption by the crop when N is in excess. This device makes it possible to take into account the storage of nitrogen in the plant. In previous models, crop demand was quantified at the crop scale. Other models make the system more complex by using the same $\%N$ approach, but at the scale of the organ. The demand of each organ is calculated daily, and crop demand is estimated by the sum of demands for all the organs (Kersebaum and Richter, 1991; Groot and De Willigen, 1991; Aggarwal *et al.*, 1994; Eckersten and Janson, 1991).

Whereas $\%N_{crit}$ has received a great deal of attention in many studies on many crops, $\%N_{min}$ and especially $\%N_{max}$, which are often used in demand quantification, have been studied in much less detail. They are experimentally and empirically determined. For example, the maximal $\%N$ concentrations that can be reached in leaves, stems and roots have been estimated at 1.4, 1.0 and 1.4 times the $\%N_{crit}$, respectively, for winter oilseed rape (Gabrielle *et al.*, 1998*a*), and $\%N_{max}$ has been estimated at 1.2 times the $\%N_{crit}$ for potatoes (Greenwood *et al.*, 1985). Empirically, the $\%N_{max}$ curve is often estimated as the envelope curve of experimental points from field trials (Justes *et al.*, 1994; Colnenne *et al.*, 1998). Thus, different experimental measurements may lead to various parameters for this curve, as shown previously (Devienne-Barret *et al.*, 2000).

Crop demand for growth is thus determined very empirically. Despite progress towards the statistical determination of the minimal N concentration permitting maximal growth, at the scale of the crop, the precise N requirements for morphogenesis (leaf area development, tiller appearance and maintenance) are unknown. This knowledge is essential if the distribution of the N taken up between the various compartments (new growth, metabolic compartment, reserves) and the way in which N uptake and storage are regulated are to be understood.

N uptake

Nitrogen uptake is generally taken into account by the use of three terms in models: (1) N availability in soil, with NO_3^- and NH_4^+ ions being distinguished in some models; (2) the depth and density of roots in the different soil layers; and (3) the capacity of the root to take up these ions, expressed per unit area or length.

How N availability in the soil is simulated will not be described here. Soil submodels differ in complexity according to the processes included and the way in which the spatial organization of the soil is considered. Some models consider soil to be a single reservoir; others distinguish layers or, for the most complex, volume units. In the GOSSYM model (Usda-Ed Gossym-Comax Information Unit, 1993) for cotton, the soil is a matrix with 800 cells (20 in length and 40 in depth), the dimensions of which depend on the gap between rows.

Consideration of the rooting system differs considerably between agronomic models and, in extreme cases, is not taken into account at all (for instance, AZODYN, Jeuffroy and Recous, 1999; Muchow and Sinclair, 1995; Sinclair and Muchow, 1995). Other models consider only rooting depth, simulated as increasing with time, expressed in days or cumulative degree-days from sowing or emergence. After a certain stage, rooting depth remains constant (CERES-Rape: Gabrielle *et al.*, 1998*a*). Rooting

density is not considered in the CERES-Rape model. By contrast, other models relate the rooting density in each layer to the maximal depth of the layer colonized, by means of an empirical relationship (STICS: Brisson *et al.*, 1998), independent of the water or nitrogen characteristics of the layer, or even crop growth. In more complex models (CERES-Maize or DAISY), root growth is taken into account, and is estimated from the shoot : root ratio. In CERES-Maize, the distribution of root biomass in the various layers is determined and root length is then estimated from root biomass, using empirical coefficients. In this model, the availability of water and nitrogen in the layers influences root distribution.

In some cases, N uptake is driven by crop demand. Absorption is equal to crop demand if the soil nitrogen transport rate at the root surface is higher than the rate required by crop demand. Roots behave as 'zero-sinks', a concept developed earlier (Hoffland *et al.*, 1990). The potential zero-sink uptake is proportional to the mean N concentration of the layer. The proportionality coefficient depends on rooting density, water flux to roots, and the nitrogen diffusion coefficient of the layer, which is itself dependent on water status. In some models (Groot and De Willigen, 1991; Aggarwal *et al.*, 1994; Huwe and Van Der Ploeg, 1991), total crop N demand is divided between layers, according to the contribution of the layer to total crop transpiration. Nitrogen demand and availability in each layer are then compared. N uptake corresponds to the smaller of the two. Thus, if demand is smaller than availability, uptake is equal to demand and if availability is smaller than demand then uptake is equal to availability. Total N uptake is the sum of absorption in each layer. In these models, N uptake is limited only by N availability (and water status in some models) of the layer or by the maximal N concentration possible in the plant. The plant takes up as much nitrogen as possible by the roots. Some authors (Sinclair and Amir, 1992) prefer to assume that N uptake reaches a plateau at high N concentration. According to Sinclair and Amir, maximal N uptake depends on cumulative degree-days from sowing. This model, slightly modified later (Muchow and Sinclair, 1995; Sinclair and Muchow, 1995), simulates N uptake by maize crops in field trials with a good accordance with observations (Muchow and Sinclair, 1995). However, although the description of N uptake is more realistic in this model, its formalism remains quite empirical.

The relationship between soil N concentration and N uptake is assumed to be a Michaelis–Menten function in some models. To increase the realism of their models, some authors have introduced recent knowledge concerning N uptake mechanisms, based on the existence of two absorption domains, according to external NO_3^- concentration [NO_3^-] (STICS). At low [NO_3^-], uptake is controlled by the plant growth permitted by other limiting factors and by [NO_3^-]. At high [NO_3^-], absorption is driven solely by N availability, with the plant storing nitrogen in excess if demand is satisfied. N uptake rate as a function of root area, N_{up}, can be estimated as follows:

$$N_{up} = \{V_{max1}[NO_3^-]/(K_{m1} + [NO_3^-])\} + \{V_{max2}[NO_3^-]/(K_{m2} + [NO_3^-])\} \quad (4)$$

where V_{max1} and V_{max2}, are the maximal uptake rates for the high- and low-affinity transport systems respectively and K_{m1} and K_{m2}, the affinity coefficients of the two systems.

Devienne-Barret *et al.* (Devienne-Barret *et al.*, 2000) estimated parameters for wheat using data published in 1996 (Peuke and Kaiser, 1996): 0.018 μmol cm^{-1} h^{-1} and 0.05 μmol cm^{-1} h^{-1} for V_{max1} and V_{max2}, and 50 μmol l^{-1} and 25 000 μmol l^{-1} for K_{m1} and K_{m2}, respectively. However, these mean values do not give account of the genotypic variability of the N uptake capacities (Oscarson *et al.*, 1995).

To highlight the two domains of uptake and their control, these authors represent N uptake by separating two terms. They consider a nitrogen uptake index, *NUI*, defined as:

$$NUI = U_{act}/U_{crit} \quad (5)$$

where U_{act} (kg NO_3^- ha^{-1} d^{-1}) is actual N uptake and U_{crit} N uptake corresponding to $\%N_{crit}$, as previously described:

$$U_{act} = d(W \times \%N_{act})/dt \quad (6)$$

$$U_{crit} = d(W \times \%N_{crit})/dt \quad (7)$$

where W is crop growth, $\%N_{act}$ and $\%N_{crit}$ are actual and critical N concentrations respectively. $\%N_{crit}$ is calculated from biomass W and equation 2.

NUI corresponds to a N uptake index that satisfies the conditions for both absorption domains, each described formally by a Michaelis–Menten equation:

$$NUI = (NUI_{max1} \times ([NO_3^-] - [NO_3^-]_0)/[[NO_3^-] - [NO_3^-]_0) + K_{m1}]) + (NUI_{max2} \times ([NO_3^-] - [NO_3^-]_0)/[[NO_3^-] - [NO_3^-]_0) + K_{m2}]) \quad (8)$$

where [NO_3^-] is the soil NO_3^- concentration and [NO_3^-]$_0$ the minimal concentration below which absorption is nil. From equation 7, U_{crit} can be calculated as:

$$U_{crit} = d(W \times \%N_{crit})/dt = W d(\%N_{crit})/dt + Nc\, dW/dt \quad (9)$$

As equation 2:

$$\%N_{crit} = aW^{-b}$$
$$d(\%N_{crit})/dt = a(-b)W^{-b-1}\, dW/dt \quad (10)$$

Equation 10 introduced in equation 9 gives:

$$U_{crit} = a(-b)W^{-b}\,dW/dt + aW^{-b}\,dW/dt$$
$$= a(1-b)W^{-b}\,dW/dt$$

And, from equations 5, 8 and 9, equation 11 is obtained:

$$U_{act} = \{a(1-b)W^{-b}\,dW/dt \times (NUI_{max1} \times ([NO_3^-] - [NO_3^-]_0)/([[NO_3^-] - [NO_3^-]_0) + K_{m1])\}$$
$$+ \{NUI_{max2} \times ([NO_3^-] - [NO_3^-]_0)/([[NO_3^-] - [NO_3^-]_0) + K_{m2}]\} \quad (11)$$

Equation 11 separates N uptake into two terms (each in brackets): (i) the first depends on crop growth (W) and soil NO_3^- concentration ($[NO_3^-]$) at low NO_3^- concentration, and (ii) the second depends on $[NO_3^-]$ only, but at high concentration. The second term does not depend on crop growth.

It has been suggested that the first term is associated with the high-affinity transport system (HATS), and is controlled both by crop growth and the availability of nitrate in the soil solution (Devienne-Barret *et al.*, 2000). In this domain, at low N concentration, the N uptake is invested only in morphogenesis. Once the potential growth allowed by external conditions is reached, the plant stores nitrogen. This domain, corresponding to high substrate concentration, is probably related to the low-affinity transport system (LATS), independent of crop growth. Under these hypotheses, morphogenesis plays a central role in the direct or indirect regulation of N uptake. This is coherent with the close relationship between leaf area index and N uptake (Grindlay, 1997). Transport system mutants would be extremely useful tools for investigation of the role of morphogenesis in plant N uptake and storage, and the relationship between carbon (C) and nitrogen (N).

Further clear evidence of the close relationship between C and N is provided by analysis of legume behaviour. Under optimal nitrogen fixation conditions, with low levels of nitrate in the soil solution, pea follows the same $\%N_{crit}$ pattern as C_3 plants (with similar parameters, $a = 5.08$ and $b = -0.32$), as shown in Fig. 3 (Ney *et al.*, 1997). If mutants that do not fix nitrogen (Sagan *et al.*, 1993) are used to analyse the effects of different levels of N supply, high levels of fertilization are found to result in a $\%N$ above the $\%N_{crit}$ curve, whereas N deficiencies were observed in the absence of fertilization. Pea mutants unable to fix nitrogen behaved similarly to C_3 plants that do not fix N, in terms of N uptake. Excess N resulted in N storage rather than additional growth.

The agronomic models described above display a number of imperfections with respect to N uptake. In particular, they do not adequately take into account the rooting system. No quantified relationships between root morphogenesis and environmental conditions have been identified, particularly with respect to the N concentration surrounding roots, despite the well-known morphogenetic effect of nitrate. The capacity of parts of the root system well supplied with soil N to compensate for low N availability around the rest of the roots is unclear. Many split root experiments have been carried out. Such studies have shown an up-regulation of uptake in NO_3^--fed roots, but have provided no accurate quantitative description of this regulation. An understanding of this regulation should be appreciated in the future, particularly in more extensive cropping systems, which may involve greater heterogeneity in the spatial distribution of N in the soil. Furthermore, models usually consider the rooting system as a whole. However, the proportion of the rooting system actually involved in N uptake may be very small. For example, it was shown that only 11% and 3.5% of the root biomass was involved in N uptake in conditions of N deficiency and optimal N supply, respectively, in experimental conditions (Robinson *et al.*, 1991). Similarly, changes in N uptake capacity with root age have not been quantified, despite evidence that such changes do occur and may be high (Schenk, 1996). Finally, models do not generally account for varietal differences.

Effects of N nutrition on crop functioning

Indicators of nitrogen deficiency. If less nitrogen is available in the soil than is required by the crop, nitrogen absorption is limited and the crop experiences a nitrogen deficiency. Most models calculate a factor reflecting the N status of the crop, and use this factor to quantify the effect of N status on other variables. Different factors are used in different models, depending on the reference curves used, as described in the first part of this review ($\%N_{min}$, $\%N_{max}$, $\%N_{crit}$), but the method of calculation is similar in all cases. For example, the following factors are defined in the following models:

AFRCWHEAT2

$$F = (N_{act} - N_{min})/(N_{max} - N_{min})$$

CERES Maize

$$F = 1 - (N_{crit} - N_{act})/(N_{crit} - N_{min})$$

DAISY

$$F = (N_{act} - N_{min})/(N_{crit} - N_{min}),$$

STICS, AZODYN, CERES-Rape

$$F = N_{act}/N_{crit}$$

The values for N_{min}, N_{max} and N_{crit} are estimated based on crop stage, time since sowing or crop biomass.

Some other models are quite different. SUCROS (Van Keulen and Seligman, 1987), for example, estimates

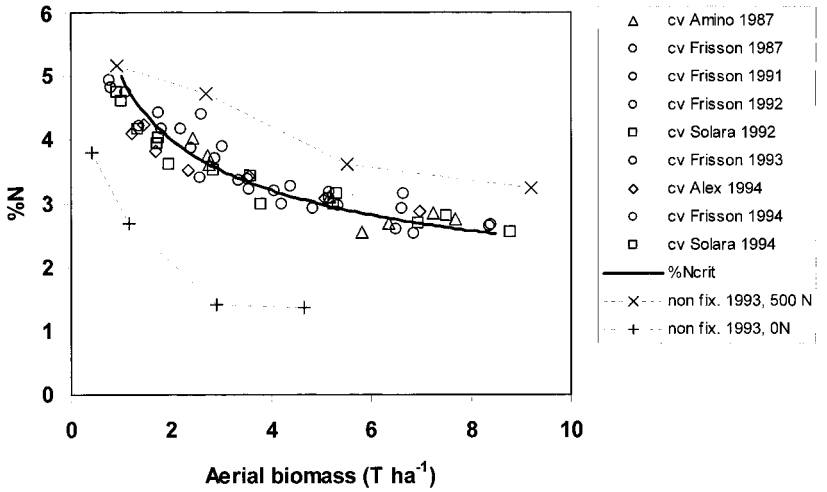

Fig. 3. Nitrogen content and aerial biomass for fixating or not pea crops (solid line represents critical N curve, and dashed line pea crop fertilized with 250 kg N at sowing and flowering). (Redrawn from Ney *et al.*, 1997.)

various indicators according to the process affected by nitrogen shortage.

Consequences of nitrogen deficiency for root and shoot morphogenesis and senescence. The functions modified by N deficiency may differ between models. In most models, organ morphogenesis is affected, but in a highly variable way. All models simulate a reduction of leaf area, but they use different methods. The most simple and frequently used method is a reduction proportional to the stress factor. In STICS, or in a recent version of the CERES-Rape model (Gabrielle *et al.*, 1998*b*), the maximum value of leaf area index is multiplied by the nitrogen nutrition index ($\%N_{act}/\%N_{crit}$) of the crop, according to the relationship proposed earlier (Bélanger *et al.*, 1992) for tall fescue swards (Fig. 4).

According to the model of (Sinclair and Amir, 1992), when N nutrition becomes inadequate, the increase in leaf area index is reduced to maintain a minimum specific leaf N content (g N m^{-2} leaf) and a minimum specific stem N content (g N m^{-2} stem). In SUCROS, leaf expansion rate depends on current leaf nitrogen content, compared with the minimum and maximum values of nitrogen concentration in the leaves. Other organs are sometimes affected by N deficiency. For example, in AFRCWHEAT2, the time-course of changes in leaf area is simulated from the description of each organ involved. In this model, N deficiency reduces the duration of tiller production, increases the mortality rate of tillers between double ridge stage and anthesis, reduces leaf expansion rate and the maximum rate of tiller production, and increases leaf senescence. The reduction of grain number according to stress factors is also sometimes considered (Ney *et al.*, 1997; Jeuffroy and Bouchard, 1999).

The consideration of senescence and its parametrization differ widely between agronomic models. Two

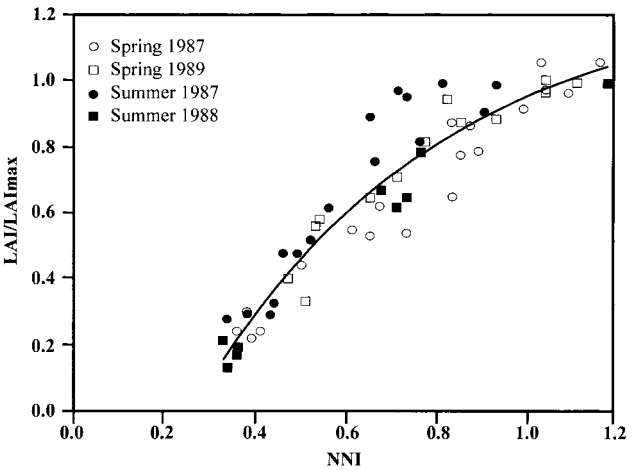

Fig. 4. Relationship between relative leaf area index LAI (LAI/LAI_{max}) and nitrogen nutrition index NNI ($\%N_{act}/\%N_{crit}$). (Redrawn from Belanger *et al.*, 1992.)

processes may be taken into account. (i) Under conditions of prolonged nitrogen stress, some of the nitrogen in older tissues is mobilized and translocated to new growing organs, in some cases resulting in the death of the older tissue. This is the case in SUCROS, in which the mortality rate of leaves is related to the ratio of mean nitrogen concentration in the leaves to structural nitrogen concentration. The same process occurs in stems, leading to death of the leaf sheaths. (ii) During grain filling, the transfer of nitrogen from the leaves to the seeds results in both a decrease in leaf area index and a decrease in specific leaf nitrogen content, reducing radiation use efficiency. N deficiency accelerates N remobilization from leaves to grains (Sinclair and Amir, 1992).

In the MecaNiCAL model (Tabourel-Tayot and Gastal, 1998*a*, *b*), the nitrogen content of shoots is

reduced in nitrogen-deficient plants. This model includes a shoot structural protein content variable, which depends on organic nitrogen substrate concentration, whereas root structural composition is not affected by nitrogen deficiency in the plant.

Consequences of N deficiency for assimilate production. Two methods are commonly used in models to quantify the consequences of N deficiency for assimilate production. The first involves a reduction of radiation use efficiency as a function of the stress factor (STICS, AZODYN, DAISY, for example). The second is more mechanistic: photosynthesis is reduced as a function of specific leaf nitrogen concentration, which is estimated daily (CERES-Rape; Sinclair and Amir, 1992; Van Keulen and Seligman, 1987; MecaNiCAL: Tabourel-Tayot and Gastal, 1998*a*, *b*).

Consequences of N deficiency for assimilate partitioning. Most models ignore the effects of N status on assimilate partitioning. They often do not include variation in the shoot : root ratio, despite the fact that it appears as a mechanism of adaptation to the constraints associated with a shortage of resources in the soil. Some models try to fill this gap, but in a very empirical way. In AFRCWHEAT2, one of the four factors indicating nitrogen deficiency increases the partitioning of dry matter to roots and increases the pool of dry matter that can be translocated and is available for grain filling. In SUCROS, nitrogen shortage favours the growth of roots at the expense of aerial material, resulting in lower shoot : root ratios in conditions of suboptimal nitrogen supply. Partitioning between the aerial organs also changes, with a lower proportion allocated to leaves under suboptimal nitrogen nutrition conditions.

N storage and translocation to grains. In the SUCROS model, when leaves die, some of their nitrogen can be mobilized to other tissues where demand is not satisfied. The rate of decrease of leaf nitrogen content depends on the difference between the total N concentration of the leaf and the concentration of residual non-remobilizable nitrogen in leaf blade tissue. The amount of unavailable nitrogen in the leaves, stems and roots depends on developmental stage. When tillers die, only this residual level of N remains in the dead stem, the rest being translocated to the living leaf and stem tissue of the plant. During grain filling, the rate of N depletion from the vegetative parts of the plant is fairly constant as long as the nitrogen concentration in the tissue is above a threshold value. This may be due to a constant rate of withdrawal of amino acids from the turnover pool.

In the model described earlier (Sinclair and Amir, 1992), the proportion of nitrogen transferred from leaves to seeds every day during seed filling is equal to the proportion of total plant nitrogen in the leaves at anthesis, which corresponds to the beginning of the period of nitrogen translocation. Under suboptimal nitrogen nutrition conditions, if the rate of nitrogen transfer to seeds does not allow seeds to maintain a minimum nitrogen content of 1.5%, additional nitrogen is transferred to achieve that level, leading to more rapid leaf area loss, a reduction in crop radiation use efficiency, and earlier maturity.

In the model described by Jeuffroy and Devienne for wheat (Jeuffroy *et al*., 2000) and that described by Lhuillier-Soundele *et al*. for pea (Lhuillier-Soundele *et al*., 1999*a*, *b*), the amount of N translocated each day depends on the amount of vegetative N that is still available for the grains and on the time since flowering, expressed in degree-days. This makes it possible to take the effect of temperature on protease activity into account in a simple way (Herzog, 1982).

The various available agronomic models are very similar as they use the same processes to model the N budget in crops. However, they differ in terms of the level of detail of the simulation. They could be improved, qualitatively at least, by the incorporation of information concerning the processes involved. Recent findings could be used, but few of the potential relationships have been quantified. For example, recent detailed qualitative results concerning the effect of nitrogen on the shoot : root ratio and plant morphogenesis (Jeuffroy and Sebillotte, 1997; Gastal *et al*., 1992) are available, but few quantitative data exist. The relationships included in the models tend to be empirical.

However, the most important differences between models concern the nitrogen nutrition of the crop. Regulation occurs both at the level of the plant and the material flux itself. However, the limits to N absorption are simulated differently in the various models analysed here. In some, nitrogen uptake by the crop is regulated by crop demand. Provided that the supply of N in the environment is not limiting (this criterion being assessed differently in the different models), and crop N is not maximal, the plant can continue to accumulate nitrogen. If there is less nitrogen available in the environment than is required by the crop, then nitrogen demand for the next model time step decreases. In other models, N uptake is limited by the absorption capacity per unit area of the roots. These models, which more closely resemble reality, involve one or two curves tending towards asymptotes. However, the regulation of nitrogen assimilation in the plant is not taken into account. Possible future conceptual improvements in models are described, based on the notion that mechanisms may differ according to the status of the crop with respect to critical nitrogen content (Devienne-Barret *et al*., 2000). Absorption may be driven by crop requirements if nitrogen is limiting, and by N supply if nitrogen is in excess. These two processes may correspond to different N transport systems. In other

models, regulation is provided by the range of validity of the model itself: for example, in the model of Sinclair and Amir, there is no equation limiting N absorption, but N absorption is nonetheless limited as the model was established and used in frequent conditions of water stress during the crop cycle, which itself limits crop N uptake (Sinclair and Amir, 1992). Finally, in more mechanistic models (Thornley and Johnson, 1990), absorption is regulated by the C and N assimilate content of the crop. Quantitative knowledge, based on biological reality, is thus necessary to improve model performance.

The need to integrate new molecular and physiological knowledge into mechanical models

Although a wide diversity of agronomic models is available, there is a real need for mechanical modelling integrating the most recent advances in plant physiology and molecular biology, particularly concerning N and C metabolism, to generate a more operational modelling approach. For example, tremendous progress has been made through the use of curves describing the changes in maximal and critical N concentrations in shoots as a function of biomass production. This has made it possible to simulate N uptake, taking into account the activity of putative transporters (Devienne-Barret *et al.*, 2000). Although agronomically robust, this methodology is subject to various limitations. In particular, it does not allow simulation of the high level of complexity typical of metabolic pathways and their regulation. For example, the agronomic models described here do not predict the partitioning of the N taken up by the plant (i.e. its direct use to sustain growth and its transient storage). However, such mechanisms are fundamental to understanding plant productivity and the effects of N fertilization. Regulation at different levels (transporters, root growth, allocation) is not taken into account explicitly, but is statistically down-regulated, for example, by the higher limits of critical or maximal curves describing changes in N concentration in the shoots. It is not possible to summarize here all recent progress towards the identification, characterization and regulation of mineral N transport in roots (for reviews see Forde and Clarkson, 1999; Touraine *et al.*, 2001; Von Wiren *et al.*, 2001). Instead, the aim is to determine how this knowledge can be integrated in mechanical models and, if this is not possible, to identify alternative approaches that could be used, for putative regulation in particular.

Factors determining root N uptake

Total root N uptake can be considered to result from potential uptake activity (density per unit area of root, internal regulation as a function of environment), soil N availability and root growth. The root growth factor suffers from the empiricism that is used in models. It has recently been shown that root growth and morphology (Zhang and Forde, 2000) are affected by nitrate, which may either trigger local growth or have a more systemic effect, leading to the reduction of lateral root growth. However, root architecture and growth remain difficult to predict from current knowledge, given the heterogeneity of the soil in terms of water content and chemical characteristics. However, several studies with split root systems (Lainé *et al.*, 1995, 1998) have shown that local increases in root N uptake compensate for local nitrate deficiency. However, this local stimulation of uptake probably has a threshold corresponding to the maximum value of increase in influx rate for each transporter. Thus, root growth is also an efficient adaptation for the plant, particularly if less mobile ions, such as ammonium, are considered. As for many other plant nutrient transporters, putative genes encoding nitrate transporters have been identified (Forde and Clarkson, 1999; Touraine *et al.*, 2001). Several are substrate inducible, and it is widely accepted that the concomitant presence of ammonium ions exerts a direct or indirect feedback effect. As nitrogen concentration within plant tissues are generally maintained within a narrow range and studies have been performed under a wide range of conditions (effects of temperature, relationships between *RGR* and *NUR*, up-regulation of N uptake following N deprivation), it is generally thought that nitrate transporter activity is finely regulated by the N plant demand. This plant N demand has been defined as the amount of N required to sustain growth under a given set of environmental conditions. This definition is, however, far too restricted and instantaneous to match reality. The analysis of critical curves (from their lower or higher limit) and increasing knowledge concerning the role of N reserves (nitrate, amino acids and proteins) in plants (Ourry *et al.*, 2001), may provide a more dynamic view (Fig. 5). Plant N demand may therefore be defined as the amount of N needed to sustain the growth of plants at

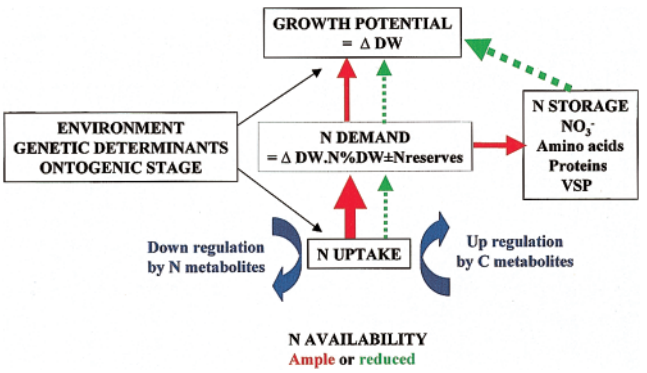

Fig. 5. Plant N demand defined from growth potential and N reserves status.

a given developmental stage and in instantaneous environmental conditions, with large or small amounts of N available from the mobilization of reserves, or required for their replenishment. This approach would therefore be able to take into account the history of a plant that had previously been subjected to N deprivation or had received an ample supply of N. Nevertheless, this approach, which is intended to quantify instantaneous plant N demand, is not devoid of difficulties; changes in the N concentration of biomass are too approximate, even if they can show general trends and seem operational (Lemaire and Meynard, 1997), and the quantification of reserve compounds remains difficult. Stored nitrogenous compounds comprise a wide spectrum from nitrates to polypeptides and their tissue distributions are even more diverse (from perennial tissues such as roots, stems or trunks storing vegetative storage proteins, VSP, to senescing leaves from which most of the reduced N can be mobilized). Little is known about the regulation of N reserves, even for specific compounds such as VSP (Ourry *et al.*, 2001). There may be specificities linked to the plant growth cycle and ecological characteristics of a given species, and it is therefore too early to propose a mathematical description of such regulation using functionally significant parameters.

Can we model the regulation of N uptake, and for what purposes?

The physiological regulation of root N uptake has been studied for several decades. More recent molecular studies have suggested that root N uptake regulation may involve phloem-derived compounds. Prime candidates for involvement include amino acids, organic acids and simple sugars such as sucrose and glucose. The possible effects of phytohormones are still a matter of debate. The transduction pathways for such signals have not been elucidated and interactions between them, which are highly probable, have not been described. In addition, this type of regulation has been studied over very short time scales, compatible with the measurement of influx rates by isotopic labelling over min or h and with the quantification of putative transcripts encoding N transporters. It is therefore very difficult to describe this regulation and to extrapolate data to the scale of the growth cycle. However, alternative ways of integrating more global and long-term regulation (from nycthemeral to ontogenic changes) into mechanistic modelling have been tested. This approach, recently used by Faure (Fig. 6), calculates plant uptake rates as the sum of two Michaelis (inducible and constitutive high-affinity transport systems, IHATS and CHATS) and two linear functions (describing non-saturable inducible and constitutive low-affinity transport systems, ILATS and CLATS) (Faure, 2000). The activities of these putative transporters are then down-regulated to simulate nitrate uptake by *Brassica napus* L. plants, at the scale of the growth cycle. Rather than down-regulating N uptake due to the effects of N and C metabolites, for which no mathematical description is available, changes in the regulation of uptake during the nycthemeral cycle and during ontogeny were mathematically described. The functions obtained were used to down-regulate nitrate uptake by reducing V_{max} for the two high-affinity transporters (CHATS and IHATS) and the slope for each low-affinity transporter (CLATS and LATS). This method facilitates a scale transition from influx rate measurements over minutes or hours, to the nycthemeral cycle (from hours to days) and finally to the growth cycle (from days to weeks). The specific effect of temperature, which affects HATS more strongly than LATS, was taken into account with a Q_{10} effect. The oilseed rape database (Gosse *et al.*, 1999) can be used to feed the model with variables such as nitrate concentration in the soil at various depths, root biomass and its distribution within the soil, and temperature. The obtained simulations were close to experimentally measured N exportation in field conditions for different levels of N fertilization. Therefore, the main up-regulating (organic acids, sugars) and down-regulating (e.g. amino acids) factors governing the uptake capacities of the four physiologically characterized nitrate transporters, which may interact, can be integrated into models by considering changes in N uptake resulting from (i) plant development (ontogenic variations), (ii) photoperiod (nycthemeral effects), (iii) temperatures, and (iv) substrate availability. The more immediate outputs of this model (Faure, 2000) show, for example, that the two LATS are not heavily involved in total N uptake, their contribution being restricted to the upper layers of the soil and to periods in which soil nitrate concentrations are rather high (autumn or during the first 2 weeks after N fertilization). These results clearly show that uptake rate increases during the bolting phase and then strongly decreases immediately after flowering, whatever the uptake system. A similar down-regulation of total uptake preceding the development of reproductive tissue was previously described in *Hordeum vulgare* (Mattson *et al.*, 1992). Thus, it is necessary to identify precisely which factors or compounds are responsible for this post-flowering repression of nitrate transporter activity (phytohormones, reduction in photosynthetic rates and, therefore, a reduction in the availability of carbon skeletons). It is also necessary to increase current understanding of the way in which reproductive tissues are filled with N (N reserve mobilization, N recycling from rapidly senescing leaves). Experiments are currently underway (Malagoli *et al.*, personal communication) to quantify N allocation as a function of morphogenesis in oilseed rape plants. The findings will be incorporated

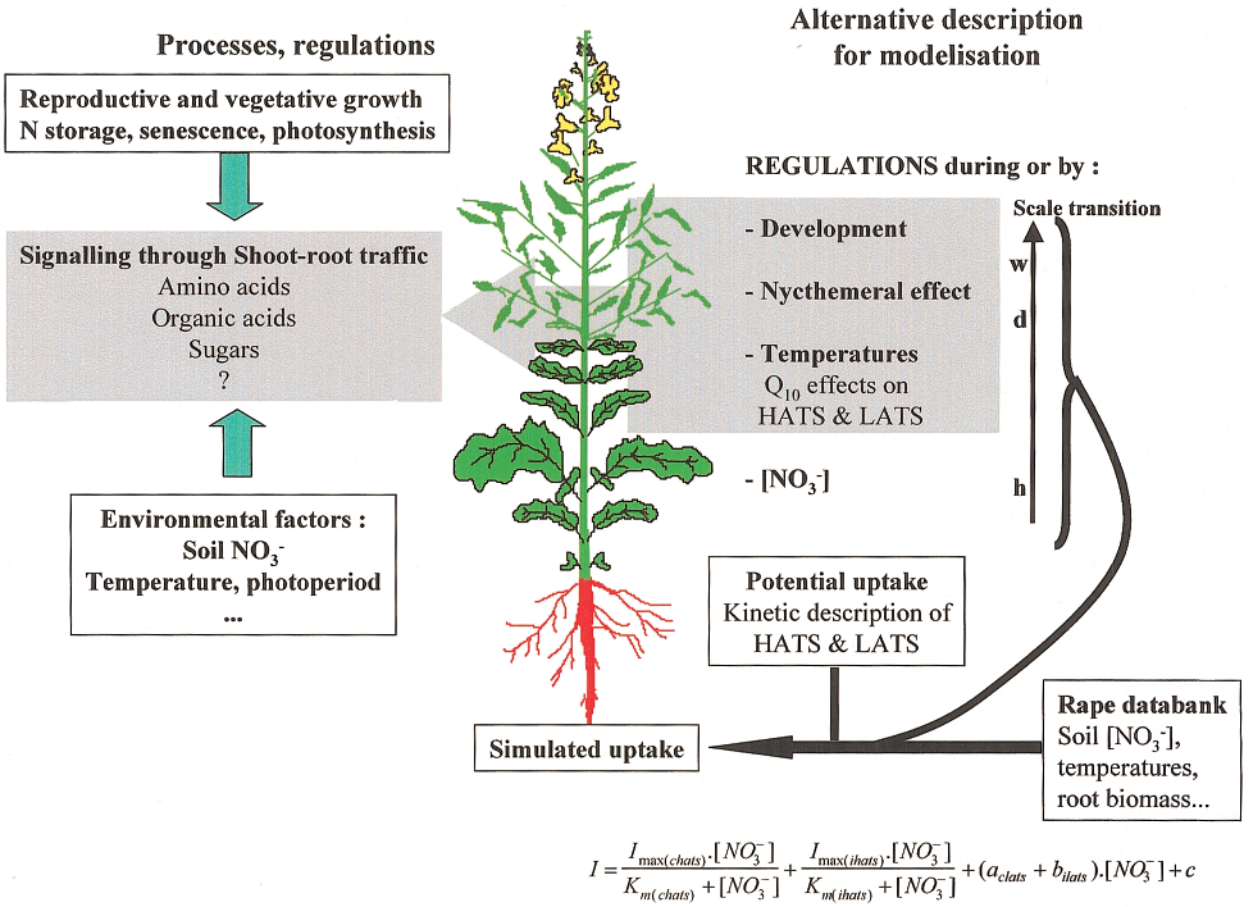

Fig. 6. Simplified representation of processes and putative regulations taken into account in the mechanical modelling of nitrate uptake during the growth cycle of *Brassica napus* L. (as proposed by Faure, 2000).

into the previous model (Faure, 2000), to simulate grain filling with nitrogen, according to the origin of the nitrogen, from current N uptake or from potential N storage pools.

Other authors have proposed more mechanistic models of N uptake including various degrees of functional regulation. Cardenas-Navarro *et al.*, for example, showed that net uptake of nitrate in hydroponically grown tomato was negatively correlated with plant nitrate content (Cardenas-Navarro *et al.*, 1999). They satisfactorily simulated net nitrate uptake during a nycthemeral cycle using down-regulation by plant nitrate concentration, which directly or indirectly indicated the N status of the plant. However, the regulation taken into account had little functional significance as, for instance, nitrate is not translocated from the phloem and cannot be considered physiologically as a means of down-regulating root activity by shoot status. Another limitation of this approach is the cultivation of plants in hydroponic conditions. Plants grown in such conditions have access to potentially large nitrate pools, which is not often the case for field-grown plants. This restricts the use of tissue nitrate concentrations as an indicator of plant N status.

Carbon and nitrogen metabolism have also recently been modelled mathematically (Bijlsma and Lambers, 2000; Bijlsma *et al.*, 2000). This model takes into account a number of factors, including the interaction between nitrate and ammonium uptake. The aim of the model was to evaluate the carbon cost of nitrate and ammonium uptake (linked for each ion to the functioning of a saturable transporter mediating influx and taking into account an efflux system) and assimilation for various species with different ecological requirements. The complex system of equations obtained was driven by ammonium and nitrate availability and by irradiance. Regulation functions were also included to take into account the down- and up-regulation of N uptake by N and C metabolites, respectively. However, this approach required a large number of parameters (more than 40), a proportion of which were derived from previous studies. This illustrates one of the limitations of such mechanistic modelling, which integrates a high level of complexity, but requires parameters that may be largely specific (thereby limiting the validity of the model for a large range of species) and difficult to assess experimentally. The prediction of root nitrate uptake by modelling has

also been achieved by another method (Buysse *et al.*, 1996), by considering root density per unit volume of soil and by the kinetic description of uptake linked to the N status of the plant. In the kinetic study, N status was evaluated as plant nitrate reduction capacity (the amount of reduced N affecting the growth potential and shoot : root distribution of dry matter), whereas the modulation of uptake was determined by the order of magnitude of the cytoplasmic nitrate pool.

This analysis, although limited, provides an overall picture of the various approaches that have been used for the mechanistic modelling of nitrate uptake in plants. New findings concerning physiology or functional genomics will undoubtedly provide new insight, not only into mechanisms but also into regulatory processes. Nevertheless, to move from a qualitative description to a more quantitative simulation of regulation that could be verified experimentally, new information concerning not only very general processes, but also their potential regulatory interactions, is required. (i) The determinism of root growth (root fraction really involved in uptake), affected not only by environmental heterogeneity (physical, hydric, and mineral, such as the NH_4^+/NO_3^- ratio) but also by the C : N status of the plant (shoot : root ratio), needs to be better defined. (ii) Although the putative candidates involved in regulation of N uptake have been the subject of numerous studies at the physiological and molecular levels, little is known about their interactions. This lack of knowledge may account for the frequently conflicting results obtained. This raises the question of co-regulatory processes in C and N metabolism. It also demonstrates the need for pertinent indicators of C : N status, which would clearly be of value for further agronomic modelling and for genotypic or QTL analysis. (iii) The study of environmental constraints, incorporating new information, would increase present understanding, making it more dynamic. This would require the inclusion not only of abiotic factors, but also of interactions between organisms (intra or interspecific competition for resources). (iv) As stressed in the introduction, the nutrient history of the plant in terms of deprivation or excess has major consequences for the instantaneous capacity to regulate N uptake. Several N pools, with buffering capacity, are known to exist. More precise studies are required to determine how storage capacity and mobilization interact with root uptake.

Perspectives

The challenge for the future should be to succeed in connecting operational agronomic models with mechanistic findings on crop physiology. Indeed, models need to be based on known processes to account for reality as much as possible, to widen their range of validity, to account for the behaviour of various genotypes, and to develop reliable diagnostic tools. There is a need for models that are to be used in fields, for operational diagnostic tools, and for guidance in the choice of genotypes based on the environmental conditions in which they are the best adapted. Towards these aims, the tools which are to be developed must be simple to use and robust. The link between models and new findings on plant physiology comes up against the problem of scale. Often, studies aiming at understanding plant processes, and regulations in particular, are driven at the scale of plant, or even of an organ itself, and over a timescale of several minutes or hours, whereas a crop model must account for the functioning of the whole crop at the level of the whole growth cycle. It is not easy to link these two highly different scales. The other main problem concerns the parameters. The more mechanistic models, based on new findings concerning physiology, include a lot of quantitative relationships with a large number of parameters, some of them difficult to assess experimentally. By contrast, the adaptation of the models for various environmental conditions or genotypes requires the specific parameters to be estimated easily. Thus, efforts must come, on the one hand, from physiologists to propose robust quantitative relationships, with easily measured parameters and a well characterized domain of validity. But efforts must also come on the other hand from agronomists to include new results on plant physiology in their models, or to explain the empirical relationships observed by results on plant physiology. The two approaches must converge to better N use efficiency, reduction of N lost to the environment, and improvement of product quality.

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