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# Modeling Nitrogen Uptake in Oilseed Rape cv Capitol during a Growth Cycle Using Influx Kinetics of Root Nitrate Transport Systems and Field Experimental Data

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The use of kinetic equations of  $\text{NO}_3^-$  transport systems in oilseed rape (*Brassica napus*), determined by  $^{15}\text{NO}_3^-$  labeling under controlled conditions, combined with experimental field data from the INRA-Châlons rape database were used to model  $\text{NO}_3^-$  uptake during the plant growth cycle. The quantitative effects of different factors such as day/night cycle, ontogenetic stages, root temperature, photosynthetically active radiation, and soil nitrate availability on different components of the constitutive high-affinity transport systems, constitutive low-affinity transport systems, inducible low-affinity transport systems, and inducible high-affinity transport systems of nitrate were then determined to improve the model's predictions. Simulated uptake correlated well with measured values of nitrogen (N) uptake under field conditions for all N fertilization rates tested. Model outputs showed that the high-affinity transport system accounted for about 89% of total  $\text{NO}_3^-$  uptake (18% and 71% for constitutive high-affinity transport systems and inducible high-affinity transport systems, respectively) when no fertilizer was applied. The low-affinity transport system accounted for a minor proportion of total N uptake, and its activity was restricted to the early phase of the growth cycle. However, N fertilization in spring increased the duration of its contribution to total N uptake. Overall, data show that this mechanistic and environmentally regulated approach is a powerful means to simulate total N uptake in the field with the advantage of taking both physiologically regulated processes at the overall plant level and specific nitrate transport system characteristics into account.

Winter oilseed rape (*Brassica napus*) is an important crop in northern Europe because of its varied utilizations (oil and biofuel). However, yields remain highly variable. As a consequence, oilseed rape has been extensively studied to identify key components of yield and to improve them by more effective nitrogen (N) application with the target of reducing environmental impacts such as N leaching and improving N use efficiency for seed filling (Boelcke et al., 1991; Habekotté, 1993; Schjoerring et al., 1995; Sieling and Christen, 1997; Vos and van der Putten, 1997). Many mathematical models have been built to simulate crop growth, development, and yield (BRASNAP-PH, Habekotté, 1997a; and LINTUL BRASNAP, Habekotté, 1997b). Some of these (DAISY, Petersen et al., 1995; and CERES-Rape, Gabrielle et al., 1998) have been devoted mainly to predicting ecological impacts of N losses from winter oilseed rape. When N nutrition has been taken into account, N uptake usually has been based on the balance of demand and supply. In this context, N availability in the soil solution is modeled using mass flow and  $\text{NO}_3^-$  diffusion equations

(CERES-Rape, and DAISY), and N demand is often determined using the critical dilution curve determined by Colnenne et al. (1998) for oilseed rape (CERES-rape). In these models, the root system is considered as a "black box." Measurements of root systems or nutrient uptake by roots are not required. Moreover, the demand/supply balance relies on the assumption that N demand is mainly regulated by shoot biomass production (Gabrielle et al., 1998). However, use of the dilution curve leads to an underestimation of N uptake when soil N concentration is high, suggesting that N uptake at the root level is subject to more complex regulation. This limitation highlights the need to adopt a mechanistic approach to predicting the N uptake by roots from the soil solution and, in a more general sense, to develop a better understanding of the regulation of N uptake as proposed in the review of Jeuffroy et al. (2002). Based on kinetic equations, four main classes of transporter systems might exist in plant roots (constitutive high-affinity transport systems [CHATS], inducible high-affinity transport systems [IHATS], constitutive low-affinity transport systems [CLATS], and inducible low-affinity transport systems [ILATS]) and are now clearly described (Okamoto et al., 2003). As a consequence, an alternative approach for N uptake modeling based on the kinetic equations of these four

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N transporter classes could be used. Such an approach would also provide a means to link and to extend recent knowledge of molecular and physiological characteristics of the 11 nitrate transporter systems (Filleur and Daniel-Vedele, 1999; Vidmar et al., 2000; Cerezo et al., 2001; Filleur et al., 2001; for review, see Forde, 2002; Okamoto et al., 2003). For example, although it is well known that these transport systems operate at different ranges of substrate concentrations (Siddiqi et al., 1990) or react differentially to temperature, there have as yet been no efforts to predict how changes in these two environmental factors will quantitatively affect the relative contribution of each transport system to total N uptake during plant development or at the field scale. In other words, despite numerous state of the art studies on the low-affinity nitrate transport system (Huang et al., 1999; Ono et al., 2000; Fraissier et al., 2001), no study has shown yet how N fertilization rates will modify its contribution, if any, in the field.

To model variation of N uptake in plants under field conditions, regulation of N uptake in roots has been taken into account in agronomic approaches by many authors (Kleemola et al., 1996; for review, see Le Bot et al., 1998). Thus, potential N uptake of a crop is determined by a relationship between the biomass of roots and N uptake rate. Likewise, physiological mechanisms of N transport systems were included in some models to simulate N uptake from N transport system equations. For example, in simulateur multi disciplinaire pour ICS Cultures Standard (STICS) maize (Brisson et al., 1998), N supply was based on the soil N availability and on the activities of the high-affinity transport systems (HATS) and low-affinity transport systems (LATS). Despite the fact that this model was able to run at the field scale, N demand was still defined by the N dilution curve, and kinetic parameters of N uptake (Michaelis-Menten constant [ $K_m$ ] and maximum  $\text{NO}_3^-$  influx [ $I_m$ ]) were assumed to be constant during the growth cycle. Adopting a more complete physiological approach (i.e. understanding the role of the regulation of N transport systems), other models built from experiments conducted under controlled conditions have made it possible to establish relationships between  $\text{NO}_3^-$  influx and its regulation through the negative feedback of endogenous root  $\text{NO}_3^-$  contents on root N uptake in tomato (*Lycopersicon esculentum* Mill.; Cárdenas-Navarro et al., 1998; 1999) and in wheat (*Triticum durum*; Cacco et al., 2002). However, these physiological models cannot be used to predict N uptake under field conditions over the whole growth cycle. In addition, none of these models include effects of environmental variables (such as temperature or radiation) or endogenous variables (such as day/night cycle and ontogeny) known to affect plant growth and N uptake. Moreover, to our knowledge, no physiological model takes into account recent molecular results that indicate that feedback

regulations are controlled by N products derived from nitrate assimilation ( $\text{NH}_4^+$  and amino acids) instead of directly from nitrate (Touraine et al., 2001; for review, see Forde, 2002).

It is well known that the maximum  $\text{NO}_3^-$  uptake capacity of roots, i.e.  $I_m$  expressed in micromoles per hour per gram, is not constant but varies with plant species and genotypes (Rodgers and Barneix, 1988), age (Wild and Breeze, 1981), and growth conditions (Jackson et al., 1972; Hallmark and Huffaker, 1978; Lee, 1982). Therefore,  $I_m$  is not the absolute maximum uptake capacity per root unit but, rather, the maximum net inflow under the given growth conditions, plant age, and history. Little is known about the magnitude of the  $I_m$  of nitrate uptake and its changes as a function of growth conditions in rape.

The aim of our experiments was to determine the effect of several environmental factors (low root temperature and photosynthetically active radiation [PAR]) and endogenous factors (day/night cycle and ontogenetic stages) on HATS and LATS activities. Regulation of these transport systems by these factors, which was formalized in a model, made it possible to simulate a regulated nitrate uptake capacity by plants. Moreover, N uptake by a rape crop during the growth cycle was simulated using field experimental data and compared with observed exported N by a rape crop under field conditions. Finally, the mechanistic model made it possible to evaluate by different simulations: (a) the contribution of each  $\text{NO}_3^-$  transport system to N uptake when N fertilizer was applied at different levels; (b) the impact of each tested variable on N uptake by the crop during the growth cycle; and (c) on the basis of a sensitivity analysis, parameters for which model outputs were sensitive.

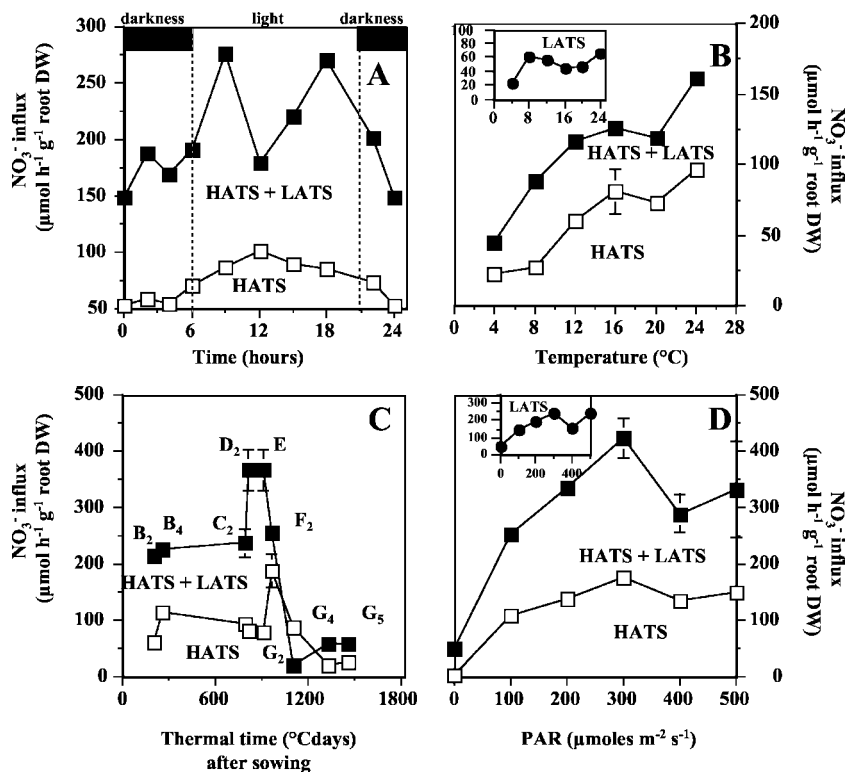
## RESULTS

### Effect of Environmental and Endogenous Factors on $\text{NO}_3^-$ Influx

#### *Effect of Light/Darkness Cycle (Experiment 1)*

$\text{NO}_3^-$  influx displayed a marked diurnal rhythmicity as shown by minimal and maximal values reported in Figure 1A. From the start of the light period (6 AM), HATS activity increased about 1.5-fold to reach a maximum value (about  $100 \mu\text{mol NO}_3^- \text{ h}^{-1} \text{ g}^{-1}$  root dry weight) at 12 AM. HATS influx then decreased progressively until the end of the light period and attained a similar value to that observed at the start of the light period ( $70 \mu\text{mol NO}_3^- \text{ h}^{-1} \text{ g}^{-1}$  root dry weight). During the dark period, HATS influx decreased to a nearly constant value of about  $50 \mu\text{mol NO}_3^- \text{ h}^{-1} \text{ g}^{-1}$  root dry weight. The pattern of HATS + LATS influx exhibited two peaks (about  $266 \mu\text{mol NO}_3^- \text{ h}^{-1} \text{ g}^{-1}$  root dry weight) at 9 AM and 6 PM. The average influx by HATS + LATS was about  $170 \mu\text{mol NO}_3^- \text{ h}^{-1} \text{ g}^{-1}$  root dry weight during the dark period. It can be noted that HATS + LATS

**Figure 1.** Variations of HATS (□) and both HATS and LATS (■) activities during the light/darkness cycle (A), as a function of root temperature (B), during a growth cycle (C), and as a function of PAR (micromoles per second per meter squared; D) in oilseed rape var Capitol. B and D, insets, LATS activity, obtained by subtracting HATS activity from HATS + LATS activity. Vertical bars =  $\pm$ SD for  $n = 3$  when greater than the symbol.



activity strongly decreased ( $-21\%$ ) during the light/darkness transition (from 6–9 PM) compared with HATS activity ( $-12\%$ ).

#### Effect of Root Temperature (Experiment 2)

The time course of the  $\text{NO}_3^-$  influx of HATS and HATS + LATS showed a similar pattern for the range of tested temperatures (from  $4^\circ\text{C}$ – $24^\circ\text{C}$ ; Fig. 1B). However, LATS influx (obtained by subtracting HATS activity from HATS + LATS activity) was only slightly altered by low root temperature, except at  $4^\circ\text{C}$  (Fig. 1B, inset). In addition, decreasing the root temperature from  $24^\circ\text{C}$  to  $4^\circ\text{C}$  resulted in a strong reduction of HATS activity (from  $100$ – $25 \mu\text{mol NO}_3^- \text{ h}^{-1} \text{ g}^{-1}$  root dry weight).

#### Effect of Ontogeny (Experiment 3)

The time course of the  $\text{NO}_3^-$  influx of HATS and HATS + LATS was followed for different developmental stages (Fig. 1C).  $\text{NO}_3^-$  influx of both transport systems was more or less unchanged from the two-leaf stage (B<sub>2</sub>) to the bolting stage (C<sub>2</sub>; about  $130$  and  $240 \mu\text{mol NO}_3^- \text{ h}^{-1} \text{ g}^{-1}$  root dry weight for HATS and HATS + LATS, respectively). From the bolting stage (C<sub>2</sub>) to the initiation of bud development (E), HATS + LATS activity increased 1.4-fold, whereas HATS activity remained constant (about  $80 \mu\text{mol NO}_3^- \text{ h}^{-1} \text{ g}^{-1}$  root dry weight; Fig. 1C). A drastic increase of HATS influx was observed from the E stage ( $81.27 \mu\text{mol NO}_3^- \text{ h}^{-1} \text{ g}^{-1}$  root dry weight) to

the flowering (F<sub>2</sub>) stage ( $187.71 \mu\text{mol NO}_3^- \text{ h}^{-1} \text{ g}^{-1}$  root dry weight), whereas HATS + LATS influx dropped abruptly from  $366$  to  $250 \mu\text{mol NO}_3^- \text{ h}^{-1} \text{ g}^{-1}$  root dry weight. Both HATS and HATS + LATS activity decreased thereafter to reach a minimal value ( $30 \mu\text{mol NO}_3^- \text{ h}^{-1} \text{ g}^{-1}$  root dry weight).

#### Effect of PAR (Experiment 4)

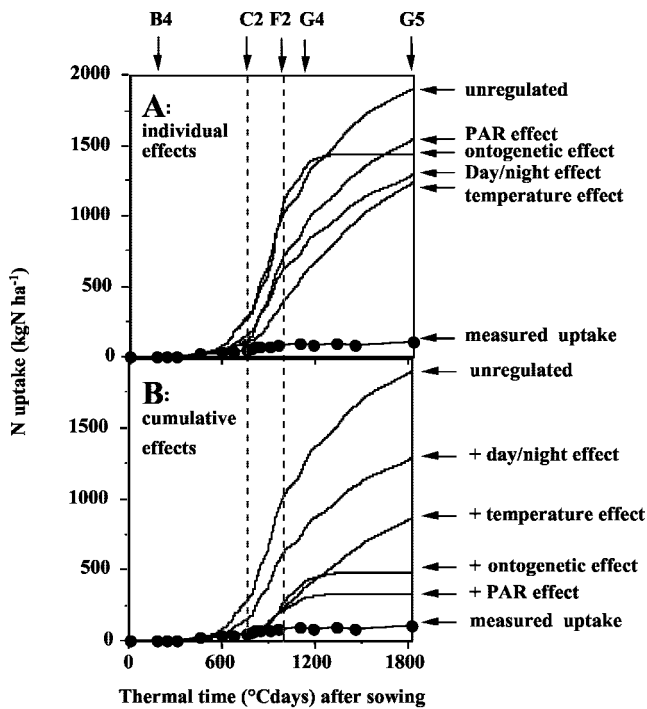
Changes of HATS and HATS + LATS activities were determined in young plants as a function of PAR values ranging from  $0$  to  $500 \mu\text{mol m}^{-2} \text{ s}^{-1}$  (Fig. 1D). Both HATS and HATS + LATS activities increased with PAR up to  $300 \mu\text{mol m}^{-2} \text{ s}^{-1}$  (from  $3.7$ – $177 \mu\text{mol NO}_3^- \text{ h}^{-1} \text{ g}^{-1}$  root dry weight and from  $50$ – $450 \mu\text{mol NO}_3^- \text{ h}^{-1} \text{ g}^{-1}$  root dry weight for HATS and HATS + LATS, respectively). LATS activity followed the same pattern with a saturation point ( $250 \mu\text{mol NO}_3^- \text{ h}^{-1} \text{ g}^{-1}$  root dry weight) at  $300 \mu\text{mol m}^{-2} \text{ s}^{-1}$ . This saturation point value can be related to the light intensity conditions under which seedlings were grown. In this case, it can be assumed that HATS or HATS + LATS activities might be underestimated during the vegetative and preflowering stages when PAR transmitted through the canopy was more important.

### Modeling N Uptake during the Growth Cycle

#### Individual Effects

When total N uptake was only controlled by soil  $\text{NO}_3^-$  concentrations (i.e. unregulated uptake),





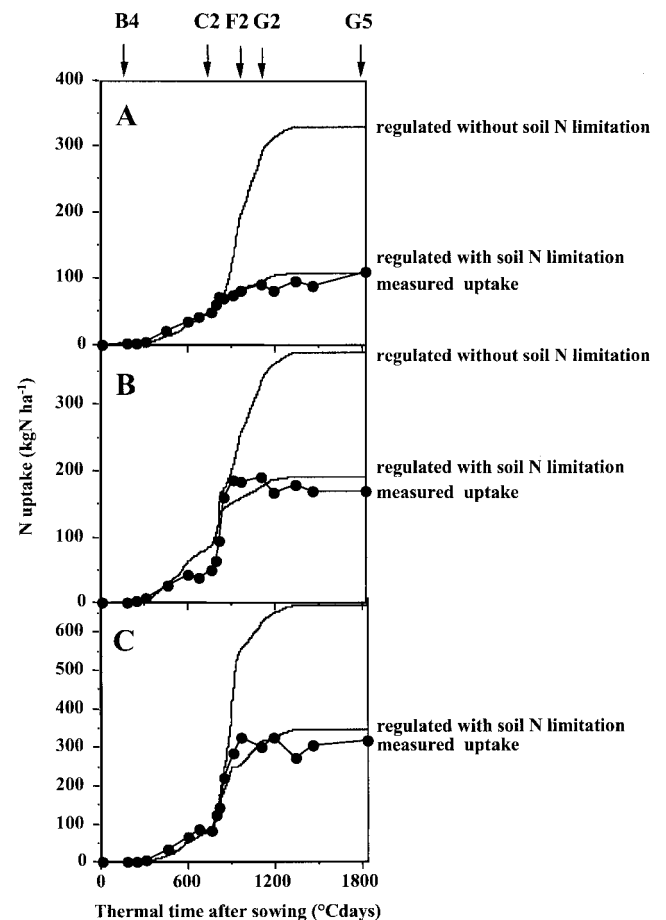
**Figure 2.** Simulation of N uptake by an oilseed rape crop (oilseed rape var Capitol) with no fertilizer when introducing the impact of the light/darkness cycle, root temperature, ontogeny, and PAR effects on NO<sub>3</sub><sup>-</sup> uptake during the growth cycle, either individually or cumulatively.

model outputs were largely overestimated (17 times higher), compared with the measured data (Fig. 2A). Individual introduction of the effect of the environmental and endogenous factors made it possible to quantify the weight of each of them on NO<sub>3</sub><sup>-</sup> uptake. When no fertilization was applied (N0 treatment), temperature, day/night cycle, and PAR factors decreased the amount of the predicted total N uptake during the plant growth cycle by 36%, 32%, and 19%, respectively (Fig. 2A). The effects of these three auxiliary variables were more pronounced during the autumn and winter seasons (from sowing to the C2 stage). Moreover, it was observed that the temperature factor decreased the simulated N uptake (-80%) more than the PAR and light/darkness cycle (-50%) during this period. An ontogenetic effect was only observed at the end of the growth cycle (-24%; Fig. 2A) when plants were at the grain filling stage (G2-G5). Effects of all of these factors on simulated N uptake were not modified by N fertilizer treatments (data not shown).

*Cumulative Effect and the Effect of N Fertilization Levels on NO<sub>3</sub><sup>-</sup> Uptake*

Both light/darkness and temperature effects were responsible for 66% of the overall decrease, emphasizing the major role played by these variables on NO<sub>3</sub><sup>-</sup> uptake by plants when no fertilizer (N0 treatment)

was applied (Fig. 2B). Integration of the four factors in the model reduced the estimation of the total N uptake by rape crop by a factor of 5.8 compared with unregulated uptake (Fig. 2B). Comparison of the amounts of the measured and the predicted N uptake at harvest shows that the simulated N uptake, defined as the regulated uptake without soil N limitation, was still three times higher than the observed regulated uptake with soil nitrate availability (Fig. 2B). When the model was run with inputs from N1 and N2 treatments, outputs showed that the model was responsive to the N application compared with the N0 treatment (Fig. 3). The simulated total N uptake increased by 6% and 21% for N1 and N2 treatments, respectively, when total N uptake was only controlled by soil NO<sub>3</sub><sup>-</sup> concentrations (data not shown). Integration of the four factors decreased the amount of simulated total N uptake by about a factor of 5.5 for N0 and N1 treatments (Fig. 3A and B) and 3.5 for the N2 treatment (Fig. 3C). However, an overestimation of the modeled N uptake after the flowering stage was observed for all N treatments. When

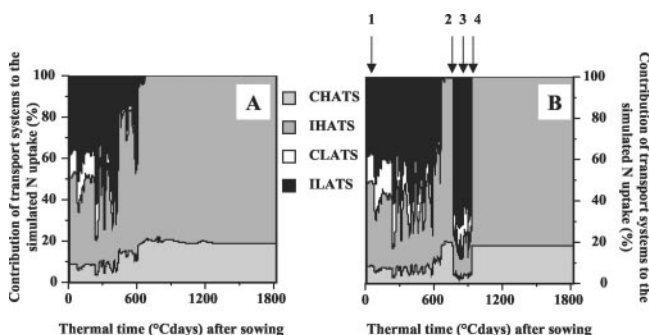


**Figure 3.** Simulation of N uptake by an oilseed rape crop (oilseed rape var Capitol) with or without the N soil limitation during a growth cycle as a function of three N fertilizer levels (A, 0 kg N ha<sup>-1</sup> [treatment N0]; B, 135 kg N ha<sup>-1</sup> [treatment N1]; and C, 273 kg N ha<sup>-1</sup> [treatment N2]).

soil N supply was limited by the soil N available for N uptake, the model more accurately matched the measured data for N0, N1, and N2 (comparison of regulated uptake with and without soil N limitation; Fig. 3). Despite the high potential activity of the N transport system described in the model (i.e. regulated uptake without soil N limitation), soil N supply from the start of the flowering (F2) to the pod filling (G2) stage was not sufficient to meet the N requirement of the plant.

*Simulation of the Relative Contribution of Each Transport System to Total N Uptake*

The realistic modeling of N uptake based on a mechanistic description of root N uptake, regulated by internal and environmental factors, provided a useful means to estimate the putative contribution of each NO<sub>3</sub><sup>-</sup> uptake system to total N nutrition of the plants grown under field conditions (Fig. 4). For the N0 treatment, model outputs showed that the inducible and the constitutive components of HATS were predominantly involved in N uptake (71% and 18% of the simulated total N uptake by IHATS and CHATS, respectively; Fig. 4A). Given that LATS is only operative at nitrate concentrations above 1 mM, which are rarely found in cultivated soil, its contribution to N acquisition by plants was small (about 11%) throughout the growth cycle (Fig. 4A). However, its contribution represented approximately 50% over the first 300°C d after sowing and probably resulted from the increase of NO<sub>3</sub><sup>-</sup> concentrations linked to the mineralization process of organic matter in autumn and the fact that this uptake system is less sensitive to low temperatures relative to HATS (Fig. 1B). Despite the fact that the simulated N taken up by HATS was increased by a high fertilization level (N2), the contributions of IHATS and CHATS were decreased by about 29% and 9%, respectively. On the contrary, high fertilization induced an increase of NO<sub>3</sub><sup>-</sup> uptake by LATS from 12 to 166 kg N ha<sup>-1</sup>



**Figure 4.** Contribution of the constitutive (C) and inducible (I) components of HATS and LATS to the simulated N uptake by oilseed rape var Capitol during the growth cycle as a function of two N fertilization levels (A, 0 kg N ha<sup>-1</sup>; and B, 273 kg N ha<sup>-1</sup>). 1 to 4, Times of N fertilization application for N2 treatment (1, 49 kg N ha<sup>-1</sup>; 2, 78 kg N ha<sup>-1</sup>; 3, 107 kg N ha<sup>-1</sup>; and 4, 39 kg N ha<sup>-1</sup>).

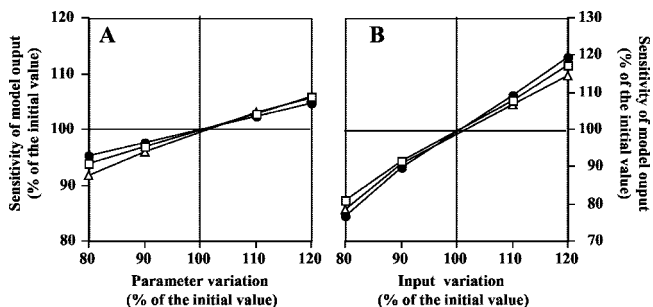
**Table I.** Simulation of total N uptake during the growth cycle of rape by each of the four transporters (CHATS, IHATS, CLATS, and ILATS) and their activity time for an unfertilized rape culture (N0) or one receiving 273 kg N ha<sup>-1</sup> (N2)

Nitrate Transport Systems	N Exported		Duration	
	N0	N2	N0	N2
	kg N ha <sup>-1</sup>		°C d	
IHATS	75	147	1,254	1,254
CHATS	19	32	1,254	1,254
ILATS	10	138	663	863
CLATS	2	28	663	863
Total	106	345	-	-

(Table I). This increase is a consequence of the extended length of time (from 663–863°C d; Table I) of LATS activity and a higher contribution (Fig. 4B) of the LATS components in N uptake.

*Sensitivity Analysis*

A sensitivity analysis was performed to quantify the impact of plant parameters and input variables on N uptake (Fig. 5). Simulations of NO<sub>3</sub><sup>-</sup> influx were conducted by varying the measured value of each parameter between -20% to +20%. For each test, only the value of the studied parameter was modified, whereas the remaining parameters and input variables were maintained at their initial values. Among all the tested parameters, sensitivity analysis results showed that model outputs were only sensitive to variations of the Im of IHATS (Fig. 5A). There was no difference between the N fertilization treatments. Variations of the Im of CHATS, K<sub>m</sub> of IHATS or CHATS, and the slope a of ILATS had no significant effect on the simulated N uptake (data not shown). Concerning environmental factors, N uptake was strongly reduced (about -20%) by a 20% decrease in the temperature variable during the growth cycle, regardless of the N fertilization level (Fig. 5B). Otherwise, varying root biomass involved in N up-



**Figure 5.** Sensitivity analysis of the simulated N uptake by oilseed rape var Capitol with a ±20% variation of the Im IHATS (A) and the root temperature (B) as a function of three N fertilization levels (□, 0 kg N ha<sup>-1</sup> [treatment N0]; ●, 135 kg N ha<sup>-1</sup> [treatment N1]; and △, 273 kg N ha<sup>-1</sup> [treatment N2]).

take (P) by 20% leads to a variation of N uptake of about 12%.

## DISCUSSION

The aim of these experiments was to build a mechanistic N uptake model based only on the functioning (kinetic equations) and the regulation of nitrate transport systems (HATS and LATS). In contrast to other N uptake models (Brisson et al., 1998), N demand in our model was not estimated by an N dilution curve but by the effects of endogenous and exogenous factors on  $\text{NO}_3^-$  uptake.

### Effect of Endogenous and Environmental Factors on $\text{NO}_3^-$ Influx

Our results have shown that  $\text{NO}_3^-$  influx of HATS and HATS + LATS decreased by approximately 1.5-fold between the light and dark periods (Fig. 1A). These results are in agreement with those of Delhon et al. (1995) who reported a 45% to 50% decrease of  $\text{NO}_3^-$  influx at the end of the dark period relative to the  $\text{NO}_3^-$  influx measured during the light period in soybean (*Glycine max*). Moreover, our results demonstrated that HATS activity reached a plateau (Fig. 1D) when the net assimilation of  $\text{CO}_2$  was at a maximum (data not shown). This result suggests that HATS and HATS + LATS influx could be regulated by the current photosynthetic activity and supports the hypothesis that the effect of light on  $\text{NO}_3^-$  influx is mediated by the availability of photosynthetates in roots. In soybean,  $\text{NO}_3^-$  influx decreased sharply (-75%) in response to a reduction in the ambient  $\text{CO}_2$  concentration, the shading of shoots (-45%), and by the girdling of the stem (inhibiting the phloemic flux from the shoot to the root, -85%). Conversely, the supply of sucrose in the nutrient solution during these treatments prevented these decreases (Delhon et al., 1996). This regulation may act, at least partly, at the molecular level. It also has been demonstrated that genes (*AtNRT1.1* and *AtNRT2.1*) encoding nitrate transporters (HATS and LATS) in Arabidopsis were positively regulated by Suc supply (Lam et al., 1996) and light (Lejay et al., 1999). Moreover, Lejay et al. (1999) and Matt et al. (2001) suggested that diurnal decrease of *NRT2* expression could result from changes in the root carbohydrate content in tobacco (*Nicotiana tabacum*) and in Arabidopsis.

$\text{NO}_3^-$  influx during the growth cycle showed an increase of N uptake from the bolting (C2) to the flowering (F2) stage because HATS + LATS is mainly involved until bud development (E) and HATS from the E to the F2 stage (Fig. 1C). This strong increase of N uptake can be related to an increase in N demand due to the growth of the stem and the leaves during the bolting period. A similar up-regulation of total N uptake preceding the development of reproductive tissues was described previously in barley (*Hordeum*

*vulgare*; Mattson et al., 1992) and Arabidopsis (Nazoa et al., 2003). The transition between the vegetative and the reproductive phase is characterized by a drastic decrease of HATS and HATS + LATS activities. Rossato et al. (2001) have already shown, using  $^{15}\text{N}$  labeling in oilseed rape grown under controlled conditions, that there was an inhibition of N uptake at flowering and that the seed filling was mainly achieved by remobilization of N from the leaves and the stem. Our results were in agreement with these conclusions and demonstrated that the near cessation of N uptake is due to a drastic reduction of HATS and HATS + LATS activities in oilseed rape. As a consequence, the drop in the N uptake at flowering could not be only explained by a water stress as proposed by Merrien et al. (1988). This decrease of HATS and HATS + LATS activities could result from a modification in the allocation of N and C from shoot to root. For example, carbon partitioned between vegetative organs during the vegetative period was preferentially allocated to the newly appearing sink tissues during the reproductive period as it has already been reported by many authors (Quilléré and Tribou-Blondel, 1988; Rode, 1988). Nazoa et al. (2003) also have reported that *AtNRT2.1* was developmentally controlled and was down-regulated from flowering to pods bearing plants.

Cooper and Clarkson (1989) also suggested that cycling of the free amino acid pool between the shoot and the root could regulate  $\text{NO}_3^-$  uptake. Increased cycling of the free amino acid pool resulting from an increase of the proteolysis in senescing foliar tissues, especially at flowering, could repress HATS and LATS activities. It is well known that  $\text{NO}_3^-$  uptake is feedback regulated by free amino acid compounds in many species at physiological and molecular levels (Forde and Clarkson, 1999; Zhuo et al., 1999). For example, Vidmar et al. (2000) demonstrated that treatment of root tissue with an inhibitor of Glu synthase led to an increase of root Gln concentration and a concomitant decrease of the *HvNRT2* transcript level of 97% in barley.

In rape grown under field conditions, PAR transmitted through the canopy to 50 cm above ground level (approximate base of inflorescence) was reduced by 76%, 93%, and 94% at early flowering, late flowering, and pod filling stages, respectively (Chapman et al., 1984). Mendham et al. (1981) also have reported similar results. These results were confirmed using an apetalous flower line (Rao et al., 1991). This line allowed 30% more short-wave radiation to reach the base of the inflorescence, leaves at this level having a longer life span. Therefore, it was assumed that the lack of light was probably an important cause of initiation of senescence of lower leaves (Chapman et al., 1984; Daniels et al., 1986). Moreover, Rossato et al. (2002) suggested that decrease of N uptake could be a result of methyl jasmonate (a phytohormone) production in senescing



tissues during flowering. Thus, a foliar application of methyl jasmonate during the vegetative period induced leaf senescence and was associated with a drastic decrease of  $\text{NO}_3^-$  uptake.  $\text{NO}_3^-$  uptake may also be inhibited by an external supply of methyl jasmonate to roots. From these combined results, it can be hypothesized that  $\text{NO}_3^-$  uptake by ontogenetic and PAR effects takes indirectly into account the relationships between N assimilation and C fixation during the growth cycle. However, the nature of the signal molecules (carbohydrates, amino acid compounds, and phytohormones) that control  $\text{NO}_3^-$  uptake remains to be elucidated, a task that will require the application of physiological and molecular approaches around the flowering period.

To study the effect of root temperature on  $\text{NO}_3^-$  influx, measurements of  $\text{NO}_3^-$  influx were conducted after a 1.5-h period of acclimation of the root system to the tested temperatures. As a consequence, it can be suggested that the modification of  $\text{NO}_3^-$  influx by HATS and HATS + LATS was only due to a change in the activity of  $\text{NO}_3^-$  transport systems. A consistent decrease of HATS activity was observed when root temperature decreased (from 24°C–4°C; Fig. 1B). These results are in agreement with previous studies on oilseed rape (Macduff et al., 1987; Lainé et al., 1993). For example, Lainé et al. (1993) reported that cooling the roots from 25°C to 9°C for 5 d, with shoot temperature maintained at 25°C, resulted in a 62% decrease of maximum net nitrate uptake, whereas  $K_m$  was unaltered. The response of HATS to the application of low root temperatures may be explained by the putative enzymatic nature of the HATS, which suggests a subsequent ATP driven mechanism as proposed by Rao and Rains (1976) and Glass et al. (1992). LATS is less sensitive to a decrease of root temperature, suggesting that it might be an ionic canal through which  $\text{NO}_3^-$  enters showing a low sensitivity to cold temperatures and to metabolic inhibitors (Glass et al., 1990).

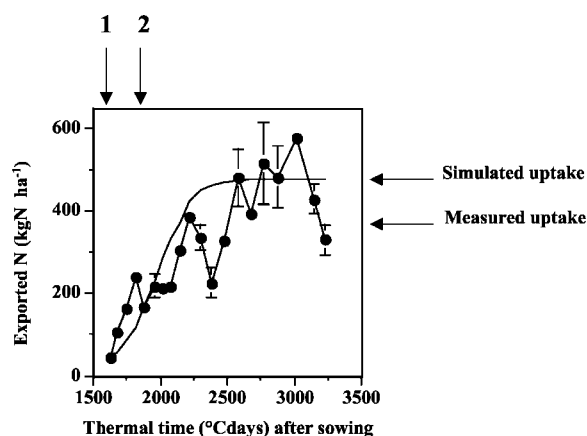
### Modeling of N Uptake by an Oilseed Rape Crop

The model satisfactorily simulates the N uptake by an oilseed rape crop, regardless of the N fertilizer application. Our original mechanistic approach was based on the functioning of  $\text{NO}_3^-$  transport systems (kinetic equations) regulated by soil nitrate concentrations, auxiliary variables defined as endogenous and exogenous factors (light/darkness and ontogenetic cycles, temperature, and PAR), and also soil  $\text{NO}_3^-$  limitation. The physiological description of effects of these factors on  $\text{NO}_3^-$  influx has been taken into account in the model by using response curves. With this approach, we can predict  $\text{NO}_3^-$  influx variations induced by endogenous factors under given environmental conditions. For example, from a physiological point of view, description of influx response curves during light/darkness and ontogenetic cycles

of the  $\text{NO}_3^-$  transport systems (HATS and HATS + LATS) takes into account their internal regulations, regardless of their complexity and the putative signals involved (amino acids, organic acids, and sugars; for review, see Forde, 2002). It follows that both conditions are relevant to focus on some specific up- and down-regulations. From the modeling point of view, integration of light/darkness and ontogenetic response curves permits alteration of the time scale from hours to a growth cycle. This change of time scale was essential to determine  $\text{NO}_3^-$  uptake at the crop level. The effects of environmental factors (temperature and PAR) were then integrated by a multiplying approach to simulate  $\text{NO}_3^-$  uptake variations induced by climatic conditions in the field. However, it must be stressed that this integrated approach might lead to an overestimation of the effects of endogenous and exogenous factors on  $\text{NO}_3^-$  uptake during the growth cycle. This approach was adopted due to difficulties in determining experimentally the interactions and combined effects of these factors on HATS and LATS activities.

The simulation of  $\text{NO}_3^-$  uptake by the model showed that predicted exported N is slightly underestimated from the sowing to the bolting stage (C2) for N0 (0 kg N ha<sup>-1</sup>) and N2 (273 kg N ha<sup>-1</sup>) fertilization during the autumn-winter period (Fig. 3, A and B). This underestimation related to the assumption that influx rates of HATS and LATS were null when root temperature was under 4°C. In the same period, an overestimation of simulated N uptake was observed after an application of 135 kg N ha<sup>-1</sup> (N1 treatment; Fig. 3B). This could result from the fact that the model was running with root biomass data of the N2 treatment because no data on root length density corresponding to the N1 treatment were available in the oilseed database. From bolting (C2) to flowering (F2), the pattern of N exported was correctly simulated and increased with the increasing N fertilization levels (Fig. 3). This result could be explained by the model responses to N fertilization levels, mainly due to increase of the LATS activity and its duration of functioning (Fig. 4). At the beginning of flowering, simulated exported N estimated after the introduction of auxiliary variables (N uptake regulated by N demand) markedly increased when soil  $\text{NO}_3^-$  limitation was not considered (Fig. 3). The regulation of N demand by soil  $\text{NO}_3^-$  stock (see "Materials and Methods") allowed satisfactory simulation of N exportation by a rape crop during this period. From flowering to the harvest, simulated N uptake was over-predicted. On the one hand, the difference between simulated and observed data could be linked to a significant fall of seeds to the soil at the end of the growth cycle, which led to an underestimation of the observed N exported by the crop. It is well known that pod opening at seed maturity is sensitive to mechanical actions (for example, wind or agricultural apparatus used for the har-





**Figure 6.** Simulation of N uptake by an oilseed rape crop (oilseed rape var Capitol) from bolting to harvest with a different data set collected from an experiment conducted under field conditions. Oilseed rape was sown on a clayey loamy soil in autumn at Hérouvillette, France (49°10' north, 00°27' west). N fertilizer was applied at the start of the bolting stage. 1 and 2, Times of N fertilization application (1, 75 kg N ha<sup>-1</sup>; and 2, 150 kg N ha<sup>-1</sup>).

vest). On the other hand, the difficulty of precisely determining the date of flowering could also lead to an overestimation of N exported. A delay of 1 month was often observed under field conditions between the beginning and the full flowering.

Model outputs showed that HATS was dominating N uptake over the whole growth cycle, even if its

participation decreased with the increase of N fertilization level (Fig. 4). When soil NO<sub>3</sub><sup>-</sup> limitation was not taken into account, the final amount of potential simulated N uptake by the HATS was not modified by the N fertilization level (data not shown). Soil nitrate concentrations were always above 100 μM, which implies that HATS operates always at its I<sub>m</sub> over the whole growth cycle. As a consequence, with or without N fertilization, corrected influx of HATS (see "Materials and Methods") already operates at its maximum required influx to satisfy the N demand at a given developmental stage under particular environmental conditions. This conclusion is in close agreement with results from the lettuce nitrate uptake model of Steingrobe and Schenk (1997). Moreover, it has been shown that N uptake by HATS was limited by the soil N supply when soil N limitation was included (Fig. 4). Conversely, the part of N uptake by LATS during the growth cycle is small (Table I). However, its contribution is strongly increased by N fertilization. The low activity of LATS can be explained by a soil NO<sub>3</sub><sup>-</sup> concentration, often under the threshold of the NO<sub>3</sub><sup>-</sup> concentration of LATS activity (1 mM). This value is attained either at the beginning of the growth cycle (mineralization peak) or when N fertilizer is applied. Thus, the low amount of N uptake by LATS is a consequence of a restricted period of activity. Model outputs showed that the model was responsive to N fertilization mainly via an increase in the contribution of LATS.

**Table II.** Plant and soil parameters used for N uptake model calculations

Parameters	Units	Values
Plant parameters		
I <sub>m</sub> CHATS	μmoles NO <sub>3</sub> <sup>-</sup> h <sup>-1</sup> g <sup>-1</sup> root dry wt	27
I <sub>m</sub> IHATS	μmoles NO <sub>3</sub> <sup>-</sup> h <sup>-1</sup> g <sup>-1</sup> root dry wt	135
K <sub>m</sub> CHATS	μM	16
K <sub>m</sub> IHATS	μM	85
a CHATS + CLATS	L h <sup>-1</sup> g <sup>-1</sup> root dry wt	0.007
a IHATS + ILATS	L h <sup>-1</sup> g <sup>-1</sup> root dry wt	0.01
b CHATS + CLATS	μmoles NO <sub>3</sub> <sup>-</sup> h <sup>-1</sup> g <sup>-1</sup> root dry wt	19.54
b IHATS + ILATS	μmoles NO <sub>3</sub> <sup>-</sup> h <sup>-1</sup> g <sup>-1</sup> root dry wt	136.8
P (ratio lateral root dry wt to total root dry wt)	%	43
C2 (rosette stage)	°C d	793
D2 (visible inflorescences)	°C d	813
E (extension of floral peduncles)	°C d	904
F2 (flowering)	°C d	959
G2 (beginning of pod filling)	°C d	1,096
G4 (end of pod filling)	°C d	1,324
Si value	μmoles NO <sub>3</sub> <sup>-</sup> h <sup>-1</sup> g <sup>-1</sup> root dry wt	
for Day/night cycle		
HATS		102
HATS + LATS		180
for Temperature		
HATS		72
HATS + LATS		118
for Ontogeny		
HATS		100
HATS + LATS		234
for PAR		
HATS		177
HATS + LATS		424
Soil parameters		
Temperature below which no uptake occurs	°C	4

**Table III.** Adjusted equations describing variations of influx for HATS or HATS + LATS as a function of time within the light/darkness cycle, root temperature, developmental stage, and PAR

Details of the developmental stages (C2, D2, E, G2, and G4) are found in Table I. t, Time of day;  $\theta$ , root temperature ( $^{\circ}\text{C}$ ); d, thermal time ( $^{\circ}\text{C d}$ ) after sowing; PAR ( $\mu\text{moles s}^{-1} \text{m}^{-2}$ ).

Effects	Equations	
	HATS	HATS+LATS
Light/darkness cycle	$P_1(t) = -4.44 \cdot 10^{-5} \times t^6 + 0.00304 \times t^5 - 0.0758 \times t^4 + 0.799 \times t^3 - 3.1516 \times t^2 + 5.368 \times t + 53.4821 \quad (r^2 = 0.97)$	$P_2(t) = -2.5457 \cdot 10^{-6} \times t^9 + 0.00025 \times t^8 - 0.0108 \times t^7 + 0.2415 \times t^6 - 3.0836 \times t^5 + 22.5684 \times t^4 - 89.6049 \times t^3 + 168.3375 \times t^2 - 96.4182 \times t + 148.61 \quad (r^2 = 1)$ $P_3(t) = 0.075 \times t^4 - 5.564 \times t^3 + 148.704 \times t^2 + 1,695.417 \times t + 7,168.233 \quad (r^2 = 1)$
Temperature	$\int_0^{24} P_1(t) \cdot dt = 1,884 \mu\text{mol NO}_3^- \text{ d}^{-1} \text{ g}^{-1} \text{ root dry wt}$ $0.0081 \times \theta^4 - 0.4552 \times \theta^3 + 8.6933 \times \theta^2 - 60.0666 \times \theta + 150.98 \quad (r^2 = 0.99)$	$\int_0^{12} P_2(t) \cdot dt + \int_{12}^{24} P_3(t) \cdot dt = 4,899 \mu\text{mol NO}_3^- \text{ d}^{-1} \text{ g}^{-1} \text{ root dry wt}$ $0.0055 \times \theta^4 - 0.2625 \times \theta^3 + 3.7343 \times \theta^2 - 10.1052 \times \theta + 41.2233 \quad (r^2 = 0.99)$
Ontogeny	Sowing $\leq$ d < E: $-0.049 \times d + 127.434 \quad (r^2 = 0.89)$ E $\leq$ d < F2: $1.940 \times d - 1,673.826 \quad (r^2 = 1)$ F2 $\leq$ d < G4: $-5.077400521223773 \cdot 10^{-7} \times t^3 + 2.911502508142226 \cdot 10^{-3} \times t^2 - 5.103545398986412 \times t + 2,852.657341762818 \quad (r^2 = 1)$	Sowing $\leq$ d < C2: $154.4295429470372 \times d^{0.0647} \quad (r^2 = 0.89)$ C2 $\leq$ d < D2: $6.233 \times d - 4,703.688 \quad (r^2 = 1)$ D2 $\leq$ d < E: $6.614485723735082 \cdot 10^{-4} \times d + 365.7319512732885 \quad (r^2 = 1)$ E $\leq$ d < G2: $-1.788658200706148 \times d + 1,978.497036513341 \quad (r^2 = 0.99)$ G2 $\leq$ d < G4: $-4.390933585646382 \cdot 10^{-4} \times d^2 + 1.226621449509313 \times d - 796.8996277255961 \quad (r^2 = 1)$
PAR	G4 $\leq$ d: N uptake is considered null for HATS and LATS From C2 to harvest: $1.091666666666667 \cdot 10^{-8} \times \text{PAR}^4 - 7.111851851851857 \cdot 10^{-5} \times \text{PAR}^3 - 8.090277777777776 \cdot 10^{-4} \times \text{PAR}^2 + 1.109485582010582 \times \text{PAR} + 5.01 \quad (r^2 = 0.96)$	From C2 to harvest: $7.38 \cdot 10^{-6} \times \text{PAR}^3 - 8.51 \cdot 10^{-3} \times \text{PAR}^2 + 2.96 \times \text{PAR} + 44.34 \quad (r^2 = 0.92)$

Because the model was moderately sensitive to variations in root biomass, potential errors due to our method of root biomass approximation are negligible. It also shows that an under- or overestimation ( $\pm 20\%$ ) of root biomass would have a limited impact on the simulated N uptake. As a consequence, it can be hypothesized that simulation of N uptake was mainly controlled by the studied factors and that they represent "meta mechanisms" of nitrate uptake regulations. Sensitivity analysis shows that the model was responsive to a variation of Im IHATS and environmental variables such as temperature. Nevertheless, it should be noted that each factor was studied individually and integrated in a cumulative way into the model. As a result, further experiments should be undertaken to simultaneously study interactions between endogenous and environmental factors on  $\text{NO}_3^-$  uptake to avoid pitfalls due to this mode of calculation. Furthermore,  $\text{NH}_4^+$  uptake

must be considered to improve the model.  $\text{NH}_4^+$  is the most abundant form of available N in waterlogged and acid soils or during cold periods, conditions under which nitrification is restricted. The uptake of both forms,  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , can be affected by internal plant factors such as N or carbohydrate status and by external factors such as temperature,  $\text{O}_2$  level, and rhizosphere pH. Developmental stage can also influence N uptake. Moreover, concentrations of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in soil do not reflect the uptake ratio. Many studies have shown that the presence of  $\text{NH}_4^+$  in the nutrient solution down-regulates  $\text{NO}_3^-$  uptake (Clarkson et al., 1986; Macduff et al., 1987; Macduff and Jackson, 1991). As a consequence, interaction between  $\text{NH}_4^+$  and  $\text{NO}_3^-$  uptake should also be taken into account in our model.

The model has been tested with a new set of data from another experiment conducted in field conditions under high N fertilization ( $225 \text{ Kg N ha}^{-1}$ ). The

results presented in Figure 6 show that these experimental data fitted well with the exported N simulated by our model.

In conclusion, the satisfactory predicted values for all N fertilization treatments in the model indicates that this mechanistic approach is a powerful tool for modeling  $\text{NO}_3^-$  over the whole growth cycle. The mechanistic concepts of this model allow prediction of the N requirements of crops and provide a context within which the different scales and approaches of plant physiologists and agronomists can be reconciled. The present model of N uptake will be extended in the future to other plant species and will allow the prediction of the best period when N fertilizer should be applied to improve yield production. On the other hand, the availability of transporter gene NRT mutants of HATS and LATS in Arabidopsis will allow the study and validation of hypotheses used to build this model. Furthermore, this model will be useful as a component of a mechanistic description of N allocation within plant tissues with emphasis on the origin of N used for pod filling (from current uptake or derived from N recycled from senescing leaves).

## MATERIALS AND METHODS

### Physiological Experiments

#### *Plant Culture for Experiments 1, 2, and 4*

Seeds of oilseed rape (*Brassica napus* L. cv Capitol) were germinated and grown in hydroponic solution (25 seedlings per plastic tank) in a greenhouse in 1999. The aerated nutrient solution contained 1 mM  $\text{KNO}_3$ , 0.40 mM  $\text{KH}_2\text{PO}_4$ , 1.0 mM  $\text{K}_2\text{SO}_4$ , 3.0 mM  $\text{CaCl}_2$ , 0.50 mM  $\text{MgSO}_4$ , 0.15 mM  $\text{K}_2\text{HPO}_4$ , 0.20 mM Fe-Na EDTA, 14  $\mu\text{M}$   $\text{H}_3\text{BO}_3$ , 5.0  $\mu\text{M}$   $\text{MnSO}_4$ , 3.0  $\mu\text{M}$   $\text{ZnSO}_4$ , 0.7  $\mu\text{M}$   $\text{CuSO}_4$ , 0.7  $\mu\text{M}$   $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$ , and 0.1  $\mu\text{M}$   $\text{CoCl}_2$  and was renewed every 2 d. pH was maintained at  $6.5 \pm 0.5$  by adding  $\text{CaCO}_3$  (200 mg  $\text{L}^{-1}$ ). The natural light was supplemented with phyto lamps (150  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) of PAR at the height of the canopy) for 16 h per day. The thermoperiod was  $24^\circ\text{C} \pm 1^\circ\text{C}$  (day) and  $18^\circ\text{C}$  (night).

#### *Plant Culture for Experiment 3*

Seeds of oilseed rape cv Capitol plants were taken from a field plot located in Saint-Aubin d'Arquenay (Normandie, France) when they were vernalized at the three- to four-leaf stage. Plants with a well-developed taproot were harvested cautiously at the bolting stage (three–four leaves), taking care not to damage the root system. The roots were gently rinsed with de-ionized water before their transfer to a hydroponic system, and plants were then grown in the greenhouse as previously described.

### Experimental Treatments for $\text{NO}_3^-$ Influx Measurements

#### *Experiment 1, Light/Darkness Cycle*

Fifteen-day-old seedlings were transferred from the greenhouse to a culture room for 1 week. Light was provided by high-pressure sodium lamps (300  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of PAR at the height of the canopy), and the thermoperiod was  $20^\circ\text{C}$  (day) and  $15^\circ\text{C}$  (night). Before each measurement, plants were acclimated for 1.5 h in a nutrient solution containing either 100  $\mu\text{M}$  or 5 mM  $\text{KNO}_3$ .  $\text{NO}_3^-$  influx was then determined at  $t = 3, 6, 9$ , and 12 h after the beginning of the diurnal period (i.e. 9 AM, 12 AM, 3 PM, and 6 PM) and at  $t = 0, 2, 4, 6$ , and 8 h after the beginning of the dark period (i.e. 10 PM, 12 PM, 2 AM, 4 AM, and 6 AM).

#### *Experiment 2, Low Root Temperatures*

Two days before the experiments, 15-d-old seedlings were transferred from the greenhouse to a control room under the previously described conditions (Experiment 1). After acclimation for 1.5 h in a nutrient solution containing either 100  $\mu\text{M}$  or 5 mM  $\text{KNO}_3$  at different root temperatures ( $4^\circ\text{C}$ ,  $8^\circ\text{C}$ ,  $12^\circ\text{C}$ ,  $16^\circ\text{C}$ ,  $20^\circ\text{C}$ , and  $24^\circ\text{C}$ ) maintained with a cryostat,  $\text{NO}_3^-$  influx was measured. All influx measurements lasted about 45 min from 12 PM. Temperatures of the solution used for  $\text{NO}_3^-$  influx measurements were similar to those applied during the pretreatment.

#### *Experiment 3, Variation during the Developmental Stage*

For each studied developmental stage (C2, D2, E, F2, G2, G4, and G5; Table II), plants were acclimated for 1.5 h to  $\text{KNO}_3$  concentrations (100  $\mu\text{M}$  or 5 mM) as previously described, before influx measurements were made at 12 PM.

#### *Experiment 4, PAR*

Fifteen-day-old seedlings were transferred from the greenhouse to a controlled culture room 2 d before the start of treatment at the tested PAR varying from 0 to 500  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Different PARs were obtained by varying the height between the top of the canopy and the lamps. After acclimation for 1.5 h in a nutrient solution containing either 100  $\mu\text{M}$  or 5 mM  $\text{KNO}_3$ ,  $\text{NO}_3^-$  influx was measured at 12 PM.

When a factor was tested on  $\text{NO}_3^-$  uptake, the others remained constant during the experiment, except for the day/night cycle experiment. The thermoperiod was  $20^\circ\text{C}$  and  $15^\circ\text{C}$  during the light and the dark period, respectively. Given temperature had no effect on  $\text{NO}_3^-$  uptake between  $15^\circ\text{C}$  and  $20^\circ\text{C}$ , it can be assumed that its incidence on  $\text{NO}_3^-$  uptake was limited during the light/darkness cycle experiment.

#### *Measurement of $\text{NO}_3^-$ Influx and Harvest*

$\text{NO}_3^-$  influx rate was measured from three batches of 25 seedlings (Experiments 1, 2, and 4) or from six plants (Experiment 3). The root system was rinsed twice with 1 mM  $\text{CaSO}_4$  solution for 1 min and then placed in a complete nutrient solution for 5 min containing either 100  $\mu\text{M}$  or 5 mM  $\text{K}^{15}\text{NO}_3^-$  ( $^{15}\text{N}$  excess of 99%). The extent of  $\text{NO}_3^-$  depletion from these solutions during the influx assays was less than 4% in each case. At the end of feeding, roots were given two 1-min washes in 1 mM  $\text{CaSO}_4$  at  $4^\circ\text{C}$  before being harvested. At harvest, shoots and roots were sampled separately, weighed, dried, ground into a fine powder, and kept in a vacuum with  $\text{CaCl}_2$  until total N and isotopic analyses. For Experiment 3, the root system of harvested plants was separated into taproot and lateral roots.

#### *Total N and Isotopic Analyses*

Total N and  $^{15}\text{N}$  in the plant samples were determined with a continuous flow isotope mass spectrometer (Twenty-twenty, PDZ Europa Scientific Ltd., Crewe, UK) linked to a C/N analyzer (Roboprep CN, PDZ Europa Scientific Ltd.). Influx of  $\text{NO}_3^-$  was calculated from  $^{15}\text{N}$  contents of roots and shoots.

#### *Statistical Analysis*

All experiments were performed with three or six replicates. The resulting variation in the measurements was expressed as the mean  $\pm$  SE for  $n = 3$  or 6.

### Model Description

A mechanistic single root model that explains nitrate uptake of plants mainly based on  $\text{NO}_3^-$  concentration around the root system was developed. The proposed thermal time step model simulates total  $\text{NO}_3^-$  uptake by rape crops from the root transport processes formalized by kinetic equations of the different  $\text{NO}_3^-$  transport systems involved in N uptake.

### Kinetic Equations of Nitrate Transport Systems

Faure-Rabasse et al. (2002) have determined the kinetics of the constitutive and inducible components of HATS and HATS + LATS in 15-d-old seedlings of rape using  $^{15}\text{N}$  labeling experiments. These authors demonstrated that influx rates approximated Michaelis-Menten kinetics below 200  $\mu\text{M}$  (CHATS and IHATS), whereas at higher  $\text{NO}_3^-$  concentrations ( $>1$  mM), influx rates of CHATS + CLATS and IHATS + ILATS exhibited non-saturable kinetics. Parameters for Michaelis-Menten ( $I = I_m \times [\text{NO}_3^-] / ([\text{NO}_3^-] + K_m)$ ) for HATS components (constitutive and inducible) and linear equations ( $I = a \times [\text{NO}_3^-] + b$ ) for HATS + LATS are described in Table II and constitute the basis of the model. Influx values of CLATS and ILATS were estimated in our model by subtracting the  $I_m$  value of CHATS from CHATS + CLATS influx and IHATS from IHATS + ILATS influx, respectively. It is noteworthy that LATS is considered to operate when soil  $\text{NO}_3^-$  concentration is above 900  $\mu\text{M}$  and 1 mM for the CLATS and ILATS, respectively. At lower  $\text{NO}_3^-$  concentrations, LATS activities were integrated into the functioning of HATS because it is not physiologically possible to distinguish the HATS and LATS activities. Only the use of LATS mutants would allow quantification of the real contribution of LATS at low nitrate concentrations (for example, below 0.5 mM in *Arabidopsis*) as previously reported (Wang et al., 1998; Liu et al., 1999).

### Response Curves of Endogenous and Environmental Effects on HATS and HATS + LATS

The response curves of the effects of different factors such as the light/darkness cycle (16/8 h), ontogeny, application of low temperatures (from 24°C to 4°C) to the root system, or variations of PAR (from 0–500  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) on HATS and HATS + LATS activities were obtained by measuring  $\text{NO}_3^-$  influx at 100  $\mu\text{M}$  and 5 mM  $\text{K}^{15}\text{NO}_3$ , respectively. This made it possible to calculate HATS (CHATS + IHATS) activities at the initial concentration (100  $\mu\text{M}$ ) and LATS (CLATS + ILATS) activities from the difference in uptake rates measured for the two substrate concentrations used. Variations of influx as a function of the studied factors were subsequently fitted with polynomial equations (Table III).

### Introduction of Auxiliary Variables in the Model

Environmental and endogenous factors, introduced into the model as auxiliary variables, allowed integration of regulations by N demand on  $\text{NO}_3^-$  uptake. Light/darkness cycle and ontogeny were chosen and incorporated into the model to take short- (light/darkness cycle) and long- (ontogeny) term regulations acting on N transport systems into account. Environmental variables such as temperature and radiation were introduced because of their well-known impact on growth and N uptake. As a consequence, endogenous factors can be considered as "meta regulation mechanisms," influenced by climatic factors (PAR and temperature) permitting access to a higher level of nitrate uptake regulations by N demand.

To integrate the effects of these different factors on HATS and HATS + LATS activities, a standard influx (SI) value was determined for each studied factor:  $SI_{\text{light/darkness}}$ ,  $SI_{\text{ontogeny}}$ ,  $SI_{\text{temperature}}$ , and  $SI_{\text{PAR}}$ . These values were obtained in the following conditions: 12 AM for a light/darkness cycle of 16/8 h, 20°C for root temperature, 300  $\mu\text{mol s}^{-1} \text{m}^{-2}$  for PAR, and B4 stage for ontogeny (i.e. 4-leaf stage, source: CETIOM). These conditions were similar to those used by Faure-Rabasse et al. (2002) to determine nitrate influx kinetics. Because effects of these factors were measured in different experiments, SI values allowed us to adjust the fluctuations of measured influx between these experiments. Thus, a corrected influx was determined for each factor studied by application of a correction factor defined as the ratio between the value of nitrate influx obtained by the adjusted polynomial equations and the SI value. For example, the averaged influx values per day (1,884 and 4,889  $\mu\text{mol NO}_3^- \text{d}^{-1} \text{g}^{-1}$  root dry weight for HATS and HATS + LATS, Table III) obtained by integrating equations  $P_1(t)$  for HATS and  $P_2(t)$  and  $P_3(t)$  for LATS from 0 to 24 h were divided by respective  $SI_{\text{light/darkness}}$  values.

### Calculation of Unregulated and Regulated Uptake

An unregulated uptake (expressed in kilograms per N  $\text{NO}_3^-$  per hectare) was calculated from kinetic equations by taking into account soil nitrate

concentration and root biomass at different depths, plant densities during all the growth cycle, and by operating a change of time scale (from hours to days) by multiplying by 24.

The regulated uptake in the model was determined by multiplying kinetic equations by correction factors. Integration of light/darkness and ontogenetic cycle factors allowed a change of time scale from hours to days and from days to growth cycle, respectively. The last auxiliary variables, temperature and PAR, were then integrated to simulate  $\text{NO}_3^-$  uptake in environmental field conditions. Changes in day length (minutes) during the year were also taken into account in the model with a day length reference value equal to 960 min.

### Sources of Input Variables

Input variables (soil nitrate concentrations and root biomass at different soil depths, temperature, and PAR) needed to run the model were obtained from the INRA oilseed rape database of experiments carried out at Grignon/Châlons/Laon/Reims (<http://www-bioclim.grignon.inra.fr>). Details about experiments can be found in Gosse et al. (1999).

The main difficulty encountered when running the model was to estimate the lateral root biomass, which was assumed to be the only part of the root system involved in N uptake, this assumption being based on the fact that  $\text{NO}_3^-$  uptake by the taproot was found to be insignificant (about 1%, P. Lainé, unpublished data). Independently of the studied developmental stage, previous experiments carried out under controlled conditions have shown that lateral root biomass represents an approximately constant proportion of 43% of the total root biomass (taproot + lateral root). This value was introduced as a parameter (P) in the model (Table II). To estimate lateral root biomass, a pattern of lateral root biomass distribution among soil layers was estimated from the frequencies of lateral root impact as a function of soil depth available in the database. Using this distribution and the total calculated lateral root biomass, lateral root biomass in each soil layer was assessed. N uptake (kilograms per hectare) in each soil layer was obtained by multiplying the regulated uptake by the root biomass calculated in each soil layer.

Nitrate concentrations in the different soil layers were determined every 15 d using the Skalar method (Gosse et al., 1999). Soil  $\text{NO}_3^-$  limitation was defined as the maximum soil nitrate stock available for N uptake. Thus, soil nitrate limitation was calculated every 15 d over the whole growth cycle from the database corresponding to soil  $\text{NO}_3^-$  concentration and soil water content in the different soil layers and N uptake by plants between two harvest dates. An interpolation was made between these two dates.

Finally, model output (i.e. predicted N uptake by the crop) is the sum of N uptake along the root profile. This model was tested to compare observed and predicted N uptake by rape with three levels of N fertilization (N0, 0 kg N ha $^{-1}$ ; N1, 135 kg N ha $^{-1}$ ; and N2, 273 kg N ha $^{-1}$ ). The highest soil  $\text{NO}_3^-$  concentrations were found in the first soil layer. The variation scale in this soil layer ranged from 0.23 to 4.1, from 0.15 to 4.1, and from 0.24 to 7.0 mM for N0, N1, and N2 fertilization, respectively. The model was built using Model Maker software (Cherwell Scientific, Reading, Berkshire, UK).

### Basic Assumptions for Model Construction

Both  $\text{NO}_3^-$  and  $\text{NH}_4^+$  can be used for N nutrition by many crop species. However, it has been reported that Brassicaceae are characterized as  $\text{NH}_4^+$ -sensitive plants (for review, see Britto and Kronzucker, 2002). Even if  $\text{NH}_4\text{NO}_3$  is used to fertilize plots, ammonium in the soil is readily oxidized to  $\text{NO}_3^-$  by nitrifying bacteria present in the soil.  $\text{NO}_3^-$  is the prominent form of N available to most cultivated plants grown under normal field conditions. Moreover, availability of  $\text{NO}_3^-$  in the soil is often considered as rate limiting for plant growth (Redinbaugh and Campbell, 1991). For these reasons,  $\text{NO}_3^-$  was assumed to be the sole N source used for N nutrition in our work. No  $\text{NO}_3^-$  efflux was considered under field conditions. In the kinetic equations used to build the model,  $K_m$  and  $I_m$  for HATS and  $a$  and  $b$  for HATS + LATS, have been fixed to values determined by Faure-Rabasse et al. (2002). However, variations of SI values determined for the endogenous and environmental studied factors take into account changes of  $K_m$ ,  $I_m$ ,  $a$ , and  $b$  during the growth cycle. The magnitude of variation of these parameters cannot be directly estimated in the present work.  $\text{NO}_3^-$  transporters were assumed to have a homogenous spatial distribution along lateral roots. A minimum temperature of 4°C was assumed to be the lowest



temperature at which growth may occur. As a consequence, nitrate uptake by transport systems when temperature was below 4°C was considered as negligible.

It was hypothesized that the taproot to lateral root ratio assessed under controlled conditions was similar under field conditions and remained constant throughout the growth cycle. No competition for water, light, or mineral nutrient acquisition was considered between rape plants.

Concerning auxiliary variables, plants at the vegetative stage (B4) were used to study the effect of root temperature, light/darkness cycle, and PAR on  $\text{NO}_3^-$  uptake. Effects of these factors were formalized by polynomial equations (Table III) and assumed to be the same for all developmental stages. Up- and down-regulations of nitrate transport systems that may occur at the plant level through the effects of different phloem or root compounds issued from nitrate assimilation or photosynthetic activity (amino acids, organic acids, and sugars) were implicitly included through the light/darkness cycle or through ontogeny. The two constitutive and inducible components of each transport system (high or low affinity) were assumed to be similarly regulated by these compounds. The effect of PAR was taken into account in the model from bolting to harvest according to Chapman et al. (1984) and Mendham et al. (1981), who have demonstrated a decrease of about 60% to 80% of PAR transmitted inside the rape canopy at the beginning of flowering. No interaction between climatic factors (temperature and PAR) was taken into account.

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