Is it possible to simulate rapeseed organ mass in relation to N nutrition? Calibration of the functional-structural model GREENLAB for the oilseed rape *Brassica napus* L. during the vegetative phase for two nitrogen nutrition levels

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Abstract

The dynamics of leaf and pod apparition and loss play a key role in rapeseed plant growth, as it controls the biomass production and repartition among organs. The source/sink ratio in turn influences appearance and disappearance of organs. Our objective is to investigate this interaction and its consequences on seed growth and development in order to better understand yield variability and seed quality (oil and protein content). In order to quantify the dynamics of C offer and demand, the functional-structural model GREENLAB is being used. It models the interactions between morphogenesis and growth. Biological processes are described according to needs. The mathematical form of GREENLAB allows estimating the ecophysiological parameters of the model (photosynthesis parameters, sink strengths, sink functions) from experimental data by using optimization procedures. The model is being calibrated for rapeseed plant and will be used to investigate the interactions between morphogenesis and biomass production by simulating plant development and growth in response to nitrogen (N) nutrition. In this study we present the calibration procedure for the vegetative phase over autumn and winter for two nitrogen nutrition levels.

Key words : Oilseed rape, *Brassica napus*, architectural model, fitting, environmental control.

Introduction

The negative environmental impact of the massive use of fossil fuel and the problem of limited petroleum resources are driving the conversion of vegetal material into new bioenergies. However, oilseed rape is a nitrogen-gruzzling crop (Dejoux et al. 2003) and its development for biofuel production depends on an improvement in its environmental impact, yield and/or seed quality; therefore, its nitrogen use efficiency and/or needs. At this time, the models being used to study rapeseed yield and seed quality elaboration in relation with nutrition level, describe crop development and growth at the crop scale (Habekotté 1997; Gabrielle et al. 1998a, b; Jeuffroy et al. 2003; Brisson et al. 2004). An approach on a refined scale would allow us to simulate the allocation of biomass in carbon (C) and nitrogen (N) into a variable number of ramifications, pods per ramification, seeds per pod, and seed biomass, and thus to better understand the elaboration of yield and seed quality. Therefore, we need to develop a functional-structural ecophysiological model that will help us explain the repartition of assimilate within the plant at the organ scale. Among the models dealing with the interactions between morphogenesis and growth, some are more dedicated to the fine study of the determinism of architecture and its regulation by the local environment (Perttunen et al. 1996; Sievänen et al. 2000; Drouet and Pagès 2003). These models have a high number of parameters and are difficult to calibrate because some parameters are not experimentally estimable. Compared to these models, GREENLAB is a mathematical model based on recurrence equations (de Reffye and Hu 2003; Yan et al. 2004). It calculates biomass production as a function of leaf surface and environmental potential and allocates produced biomass to organs according to their sink strength. Parameters of the equations are not accessible by measurements (photosynthesis resistance, sink strength, sink variation, see Table 1) but are easy to calculate through mathematical optimisation procedures allowed by the mathematical structure of the model (fitting and multifting, see Guo et al. 2006). It works like a source/sink solver that calculates a source/sink dynamics of the plant that explains the observed plant growth and architecture. Thus, the model will help us explain how nutrition level modifies plant architecture, source/sink dynamics and finally yield and quality. The GREENLAB model has already been calibrated for several species like sunflower (Guo et al. 2003), maize (Guo et al. 2006) or Chrysanthemum (Kang et al. 2006) but not for rapeseed. Thus, this paper has two objectives. The first aim is to calibrate the model for rapeseed during the vegetative period without limitation of N nutrition. The second aim is to use the model to calculate modifications of the source/sink relationships within the plant induced by a limitation of N nutrition.

Materials and methods

GREENLAB model principles: the GREENLAB model has been precisely described in Yan *et al.* (2004). Optimisation procedures were also explained in Guo *et al.* (2006). Only the main principles and equations will be given here. The GREENLAB model simulates morphogenesis and growth of plants using a set of recurrent equations and morphogenetic rules based on rapeseed botany. The time scale used is the thermal time that is necessary to produce one metamer, that is the architectural unit comprising a node, internode and leaf, or the metamorphic variation of these organs. At each time step, the automaton produces new metamers that are used to calculate the organ demand. Biomass production (offer) is calculated by the environment potential (E) multiplied by the fraction of exposed green leaf area and by an empirical and constant coefficient of transpiration efficiency. When there is no environmental limitation, E is set to 1, its maximal value. The biomass produced is considered as a common plant pool that is allocated to organs according to their demand. Organ sink strength is relative to other organs, blade sink strength being set to 1. Organ size is calculated using allometric relations.

GREENLAB main equations: Production function: for all green leaves and for each GC, the following equation is implemented:

$$Q_m(i) = \frac{E(i)Sp}{r\,k}\left(1-\exp\left(-\frac{k}{Sp}\sum_{j=1}^{n(i)} S_j\right)\right) \qquad \text{Equation (1)}$$

where Qm(i) is the biomass production during GC(i); E(i) is the average potential of biomass production during GC(i) which is fixed by the environment; n(i) is the number of green leaves during GC(i); Sj is the blade surface of the jth leaf; Sp is the ground projection area of the leaf surface, which takes into account its inclination; r1 and r2 are empirical resistance parameters that are parameterized by optimization as described in Yan *et al.* (2004), with r1 setting leaf size effects on transpiration per unit area and r2 setting the effect of mutual shading of leaves according to Beer–Lambert's law. Sink functions: sinks receive an incremental allocation of biomass that is proportional to their relative sink strength. The sink strength for each type of organ denoted by o is defined as a function of its age in terms of GCs:

$$P_o(j) = P_o f_o(j) \qquad \text{Equation (2)}$$

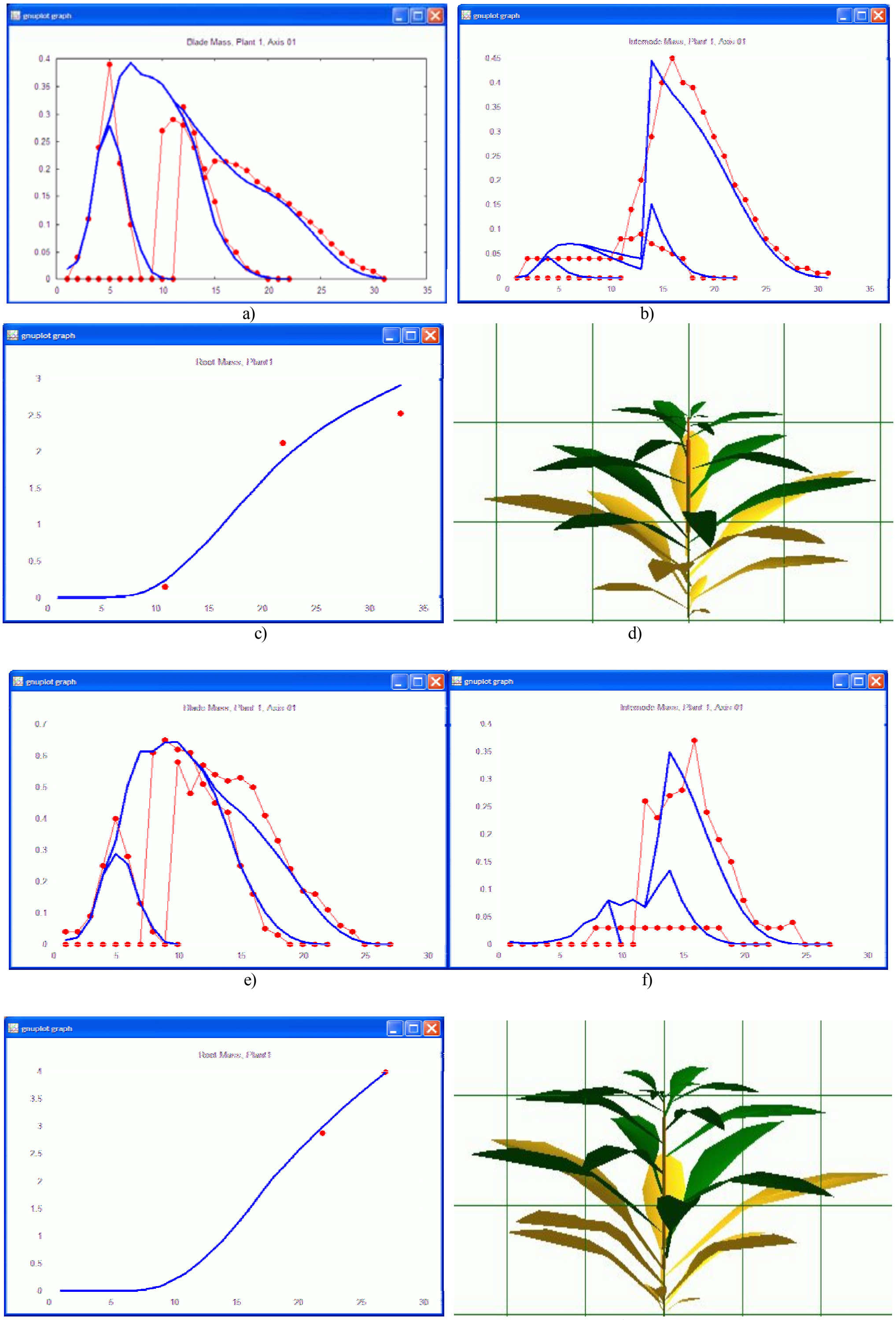
where o = indices for organ type (leaf blade, b; sheath, internode, e; cob, f; tassel, m); po is the coefficient of sink strength associated with organs of type o. For leaf blade Pb = 1 is set as normalized reference; fo(j) is an organ type specific function of sink variation in GC(j). A normalization constraint $Max(f_o(j)) = 1$ is set, with to the expansion duration of organ o for the rank k. f0 is a beta function with two parameters a0 and b0 that are specific to the organ type. The beta function shape is very flexible depending on a0 and b0 values that are calculated by fitting the value of B = a/(a+b) for each organ type (Guo et al. 2006). When B=0.5, the sink variation function is symmetric; when it is bigger to 0.5, organ demand is delayed; and when it is smaller than 0.5, organ demand is advanced. Organ age: GREENLAB uses a chronological age (CA) and a physiological (PA). The CA is the GC number. The PA corresponds to a degree of organ differentiation. Organs of a different PA will have a different sink function and their sink parameters will be calibrated separately.

Adaptations needed for rapeseed: the rapeseed vegetative development was described by 31 metamers (or GC) with one internode and one leaf each. Two PA were used. PA 1 one corresponds to the metamers 1 to 11 produced before the beginning of stem elongation. PA 2 corresponds to the metamers 12 to 31 produced during internode elongation in spring and corresponding to a different biomass repartition within the plant. 2 GC were added to simulate organ growth after the end of vegetative organ apparition on the main stem. E is set to one at each GC.

Field Experiments and Measurements: Field experiments were carried out in Thiverval-Grignon, France (48.9° N, 1.9° E) on a sandy-loamy soil on a parcel sown at a density of 60-70 plants/m2 the 30th August 2000. Two nitrogen treatments were applied: N-=140 kgN/ha and N+= 200 kgN/ha. N fertilisation was applied in three times: one of 60 kgN/ha at sowing (N+), a second of 70 kgN/ha the 21th February (N- and N+) and a third of 70 kgN/ha the 28th March (N- and N+). At three dates (N-: 5th October, 1st March and 29th March; N+: 5th October, 24th January and 27th February) 12 plants were harvested. For each plant, the length, surface and dry mass of each leaf were measured as well as the length, the diameter and the dry mass of each internode. The 9 parameters (Table 1) of the production and the sink functions were calculated by fitting procedures using observations collected on the plants at different times of their growth cycle (multi-fitting).

Results

Figure 1 presents the results of the model fitting for the blade, internode and the root masses as well as the 3D plant simulation for N- and N+ treatments. The simulated dynamics and values are in accordance with the observations (curve shapes and data values). To evaluate the effect of the N limitation, we can compare datafrom date 2 for N- (1st March) and from date 3 for N+ (27th February). At these dates, N reduction decreased the leaf number from 25 to 20 as well as the blade, internode and petiole (data not shown) masses (respectively divided by 3, 4 and 4) as it decreased root mass in a smaller extend (divided by 2). Thus, shoot/root ratio was decreased from 3.25 to 1.75. According to the model calculations, the effects of N limitation are due to an increase of the photosynthesis resistance (R1) as well as a delayed blade demand (Bb of the sink variation function) and an advanced petiole demand (Bp). Concerning the organ sink strengths that are relative, with blade sink strength being set to 1, internode sink strength was highly increased after stem elongation (Pi-2), petiole sink strength (Pp) was highly decreased and root sink strength was slightly decreased (Pr).



a)

b)

c)

d)

e)

f)

g)

h)

Figure 1: Blade mass (a, e), internode mass (b, f), root mass (c, g) and 3D visualisaton of the plants simulated by the model (d, h). Simulated lines and observed points were presented for both nitrogen levels (a, b, c, d : N^-; e, f, g, h: N^+) at three dates: the 05th October, the 1st March and the 29th March for N^-; and the 05th October, the 24th January and the 27th February for N^+. The 3D representation was possible thanks to allometric relations calibtrated between organ mass and organ size measured. Organ shapes (leaf, internode, petiol) were designed using SMB Software and is given for 27 GC (N^-: date 2; N^+: date 3). The yellow leaves represent dead leaves. The square size is 10cm.

Discussion

It was possible to calibrate the GL model for rapeseed. The use of the phyllochrone as time scale seems to be suitable to describe crop growth during winter. The effect of temperature on plant development is strong enough to take into account the ceasing of plant growth during winter without decreasing E (environment potential) in the model. A further improvement of the model simulations would be to take into account radiation variations as has been done for maize (Guo *et al.*, 2006). The

GREENLAB model calculates the impact of nitrogen nutrition on source/sink relationships within the plant in a satisfactory way. The parameter values show that N reduction induces a reduction in the total biomass produced, a delayed leaf growth, a favoured allocation to internodes and a quite stable allocation to root. This leads to a decreased shoot/root ratio. These results are in accordance with what is known about interactions between plant biomass production and N nutrition during vegetative growth of crops (Andrieu *et al.* 2006; Lemaire 2006).

Table1. Crop parameters optimised using a target file of field observations

Parameters	Description	N^-	N^+
P_b	Blade sink strength	1	1
P_i	Internode sink strength	0.149	0.582
P_p	Petiol sink strength	0.474	2.152
P_r	Root sink strength	3.947	4.264
$P_{i\_}2$	Internode sink strength of PA2	1.788	1.131
B_i	Internode sink variation (parameter of the beta function of blade expansion)	0.622	0.668
B_b	Blade sink variation (parameter of the beta function of internode expansion)	0.603	0.541
B_p	Petiole sink variation (parameter of the beta function of petiole expansion)	0.560	0.763
R1	Coefficient for leaf size effect on leaf resistance	75.33	67.16

Conclusion

This first step in modelling oilseed rape using the structure-function GREENLAB model has been achieved. The second step will be to model the reproductive phase. The model will then be calibrated for rapeseed varieties with contrasted architecture.

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