

CALIBRATION AND SENSITIVITY ANALYSIS OF A DYNAMIC MODEL FOR CONTROL OF NITRATE IN LETTUCE

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Abstract

Soluble carbohydrates and nitrate in the cell sap of leafy vegetables act as complementary osmotic solutes to maintain the plant's turgor pressure. The implication is that nitrate at harvest time can be kept low by manipulating the source-sink balance. Pseudo-static calculations based on a model developed earlier fairly well reproduced the seasonal variability of nitrate levels in crops grown and harvested at near-monthly intervals. This paper concentrates on the dynamic behaviour of this model by calibrating it to available data of lettuce growth, and by performing a sensitivity analysis

The model contains inhibition functions which are activated when the internal assimilate carbon concentration is near an upper or lower limit, in order to restrict photosynthesis or growth, respectively. Sensitivity analysis and calibration contour plots reveal that estimates of the growth parameters have a large uncertainty when growth is limited by carbon availability. In regions that are of interest for nitrate control, i.e. in the sink-limited case, all parameters determining the source-sink balance are important. Consequently, experiments must be designed such that they cover sink-limited conditions.

1. Introduction

High nitrate levels in lettuce and other leafy vegetables are considered a health hazard. There is considerable evidence that carbohydrates and nitrate in the cell sap of leafy vegetables act as complementary osmotic components to maintain the plant's turgor pressure (e.g. Blom-Zandstra and Lampe, 1983). This observation forms the basis for the conjecture that the nitrate content can be controlled by manipulating the source-sink balance of the carbohydrates. Experimental evidence that supports this hypothesis is growing (Buwalda and Warmenhoven, 1999).

A model developed earlier (Seginer *et al.*, 1998), used in a pseudo-static fashion, fairly well reproduced the seasonal variability of nitrate levels in crops grown and harvested with near-monthly intervals, as reported by Drews *et al.* (1995). The current paper concentrates on the dynamics of the model by performing a calibration and a sensitivity analysis. The final purpose is to use the model as a tool for the design of optimal operation strategies, such that nitrate at harvest time can be kept below limits set by health regulations.

2. Model description

The model has been described in detail by Seginer *et al.* (1998). It has two state variables: non-structural carbon content (S_{Cv}) and structural carbon content (S_{Cs}) expressed in moles [C] per unit soil surface area. The equations are summarised in

Appendix 1. A list of parameters is given in Appendix 2.

The dynamic back bone of the model is the carbon balance. The non-structural carbon content (S_{Cv}), mainly thought to be contained in the vacuoles, originates from assimilation by photosynthesis (F_{Cav}), driven by light (I) and CO_2 (C_{Ca}). Maintenance (F_{Cm}) and growth (F_{Cvs}) draw upon these resources under influence of temperature (T). The production of one unit of structural carbon (S_{Cs}) by growth needs θ additional units for growth respiration (F_{Cg}). The model assumes that the function that saturates the production as the canopy closes (Eqn. [10]) acts in the same way on growth.

Photosynthesis and growth can proceed uninhibited as long as the non-structural carbon concentration (C_{Cv}) in the vacuoles remains within certain limits. The carbon concentration in the vacuoles can be computed from the states (Eqn. [7]) under the assumption that the vacuoles occupy a fixed proportion of the plant's volume. If, due to the environmental conditions, the non-structural carbon concentration approaches zero then growth is reduced. In the model, this transition is implemented by introducing a smooth switching function $h_g\{C_{Cv}\}$ which is one for non-inhibiting C_{Cv} levels, but falls off to zero rapidly when the assimilate stock becomes empty. When carbon assimilates in the vacuoles are high a similar switching function $h_p\{C_{Cv}\}$ brings photosynthesis to a halt. The nitrogen concentration follows algebraically from the negative correlation between carbon concentration and nitrogen concentration in the vacuoles (Eqn. [15]). In this paper it is assumed that the demanded supply is always available. In a separate paper (Seginer *et al.*, this volume) this restriction is relaxed.

Outputs of the model are variables that are directly accessible for measurements, such as plant dry weight and nitrate content. They are computed from the states of the model according to the equations in the output section of Appendix 1.

3. Calibration

The original parameterisation of the model was largely based upon the available literature, and sufficed to establish good quasi-equilibrium results. In the dynamic situation studied here, independent data collected by Van Henten (1994) were used to perform a calibration of the carbon part of the model. The key parameters in the model are those that are related to the source-sink balance, in particular the growth parameter m , and the photosynthesis parameters ϵ and σ . They were incorporated in the calibration, while all others were kept at their original values. A simple non-weighted least squares procedure was used to fit the dry weight predicted as output of the model (Eqn.[19]) to values measured at weekly intervals. An excellent fit could be obtained, as shown in Figure 1. Figure 1a (experiment) refers to an autumn period where the light intensity is decreasing towards harvest time. In the second experiment (Fig. 1b) the light is increasing to levels higher than the final values of experiment 1. The calibration yields quite different parameter estimates for m in both cases, but hardly effects ϵ and σ (Table 1).

Table 1 - Characterisation of input data sets and calibration results for the two sets. The environmental data given are the averages over the last two weeks before harvest.

Experiment no.		1 autumn/winter	2 winter/spring
Global radiation	$W m^{-2}$	11.7	37.5
CO_2	ppm	589	415
Temperature	$^{\circ}C$	10.3	12.9
Calibrated parameters			
m	$mol[C] m^{-2}$	8	23
ϵ	$mol[C] mol[phot]^{-1}$	0.07	0.07
σ	$m s^{-1}$	0.0014	0.0012

A cross-validation, shown in Figure 1 as well, reveals that with either set the model runs stayed within the sample error of the data. It appears that the estimate for the growth

parameter m , which can be seen as the structural C content where maintenance respiration equals uninhibited growth, ranges from 8 to 23 $\text{mol}[\text{C}] \text{m}^{-2}$. Figure 2 shows the sum of squares contour plot for simultaneous changes in m and the apparent light use efficiency ϵ . It is clear that around the optimum choice for ϵ the value of m is not well determined. Sensitivity analysis is used to study this issue in more detail.

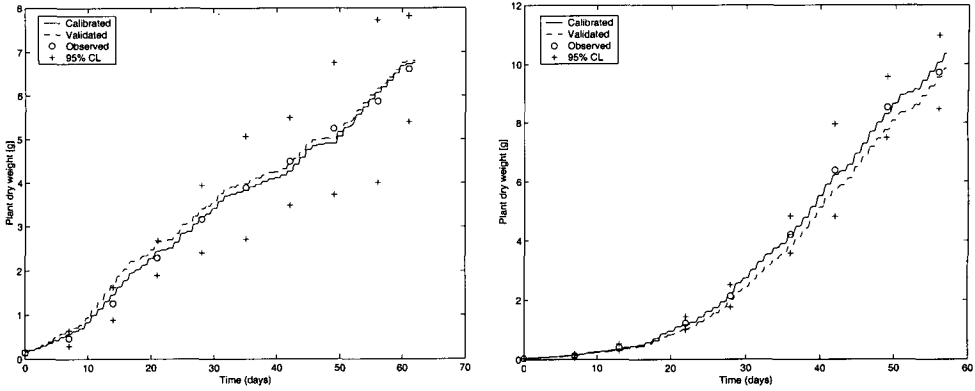


Figure 1. Calibration. Left (a): experiment 1, right (b): experiment 2 of Van Henten. Cross-validations use parameters obtained from the opposite calibration. Error bounds are 2 standard deviations from 6-fold samples).

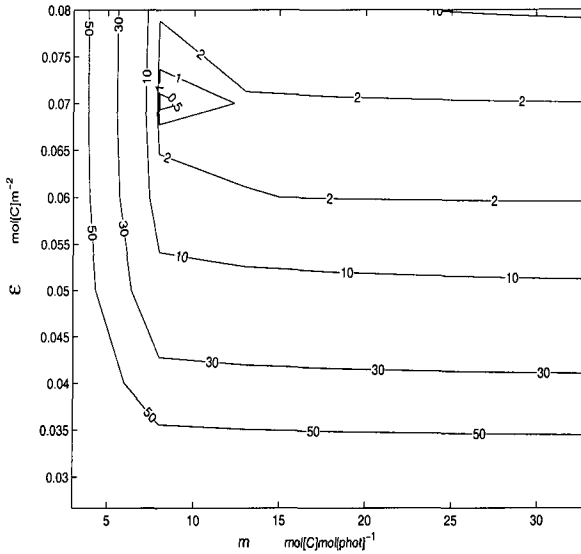


Figure 2. Uncertainty assessment of some key parameters. Lines of equal sum of squared error as function of light use efficiency ϵ and growth parameter m (parameterset 1, input data from exp. 1)

4. Sensitivity and parameter uncertainty

The sensitivities of the model were evaluated by solving the local sensitivity equations. (Van Henten and Van Straten, 1994). The local sensitivity $s_{ij}(t)$ of state x_i to parameter p_j is defined as $s_{ij} \equiv \partial x_i / \partial p_j$ and can be computed from

$$\dot{s}_{ij}(t) = \sum_{k=1}^n \frac{\partial f_i(t)}{\partial x_k} s_{kj}(t) + \frac{\partial f_i(t)}{\partial p_j} \quad s_{ij}(0) = 0$$

where f_i , $i=1, \dots, n$ represents the right hand side of the state equations (Eqns. [1] and [2]), and n the number of states. The state vector here is $x = [S_{C_s} \ S_{C_v}]^T$. Similar equations can be derived for the model outputs. The sensitivity system forms a set of simultaneous time-variant linear equations that can be solved in parallel with the model in one run. This is an advantage over one-by-one permutations of the parameters. The result in this case is a set of 2 (states) x 13 (parameters) = 26 plots for the states, and similarly for the outputs. The sensitivities evaluated this way are functions of time that depend upon the external conditions, as well as on the nominal parameters used.

Although the time evolution of the sensitivities of states and outputs is interesting in itself, for brevity Table 2 only presents the sensitivities of the outputs dry weight and nitrate content at final harvest time. This is done for all four combinations of calibration parameter sets and input data.

In the calibrations belonging to each input set (cal. 1/input 1 and cal. 2/input 2) the model appears to be sensitive to the apparent light use efficiency parameter ϵ . In contrast, the growth parameter m and the maintenance respiration k have much less influence. Inspection of the plots of h_g (not shown) reveals that growth inhibition due to depletion of assimilates frequently occurs. Despite this, the sensitivity of the dry weight to the switching function parameters is low. This can be explained by considering that with growth inhibition the cultivation is source limited. Changing the switching function parameters for the growth inhibition will cause growth to become limited a little bit earlier or later, but it is the photosynthesis that determines the biomass increase. Because the assimilate buffer will be near depletion most of the time, the nitrate content should be high. The threshold level for the growth inhibition function b_g still has some effect on the nitrate in this case.

The inhibition functions in the model merely serve to switch between unlimited growth, and either source or sink limited growth. The thresholds are governed by the parameters b_p and b_g . Maximum growth can be obtained if the environmental variables are manipulated such that neither the upper nor the lower threshold of the assimilate C concentration is ever approached (Tchamitchian and Ioslovich, 1998). In that case, the sensitivity to the parameters of the switching functions should be zero. However, using different light and temperatures can move the limiting regime from one end to the opposite. This is clearly illustrated in the case where the parameters of experiment 1, with growth parameter m low, are used with environmental data from experiment 2. It seems that the much higher radiation in data set 2' (Table 1) is enough to move the balance towards more assimilate production relative to consumption. Apparently, the light effect outweighs the opposing effect of the somewhat higher temperature. In this case, the biomass production is sink limited. Consequently, the model becomes sensitive to the growth parameters, such as m and a , and less to the photosynthesis parameters.

Table 2 - Relative-to-relative sensitivities of dry weight (dw) and nitrate content at final harvest to parameter variations in % / % for calibration parameter sets 1 and 2 and model input sets 1 and 2. (Values larger than 0.7 %/% are bold-faced to facilitate interpretation). The last row shows simulated final nitrate content.

parameter set	1 (<i>m</i> = 8)		1 (<i>m</i> = 8)		2 (<i>m</i> = 23)		2 (<i>m</i> = 23)	
	1 (autumn/winter)		2 (winter/spring)		1 (autumn/winter)		2 (winter/spring)	
parameter	dw	nitrate	dw	nitrate	dw	nitrate	dw	nitrate
ϵ	0.90	-0.15	0.44	-1.20	0.99	-0.05	0.87	-0.05
σ	0.22	-0.03	0.14	-0.73	0.31	-0.01	0.46	-0.04
<i>a</i>	0.50	0.00	0.67	0.24	0.46	-0.00	0.45	0.01
<i>k</i>	-0.00	0.24	0.88	2.50	-0.22	0.10	-0.16	0.12
<i>c</i>	0.05	-0.16	-0.39	-1.13	0.14	-0.07	0.07	-0.05
<i>m</i>	0.21	0.18	0.94	2.11	0.02	0.06	0.02	0.10
θ	-0.19	0.04	-0.10	0.26	-0.23	0.01	-0.24	0.01
γ	-0.03	0.14	-0.22	-0.07	0.02	0.10	0.01	0.10
κ	-0.18	-0.71	-0.27	-1.02	-0.14	-0.73	-0.13	-0.76
s_p	0.01	0.00	0.02	-0.23	0.00	-0.00	0.00	-0.00
s_g	0.00	0.03	0.01	0.01	-0.01	-0.07	-0.01	-0.01
b_p	0.04	0.00	0.20	-2.26	0.00	-0.00	0.00	-0.00
b_g	-0.03	-0.30	-0.05	-0.02	-0.05	-0.29	-0.05	-0.31
Final nitrate mg NO ₃ /g FM	4554		1467		5013		4493	

Uninhibited saturated growth, i.e. growth with h_g and the canopy closure saturation function f equal to one, is mk times a temperature function (Eqn. [11]). The maintenance respiration rate is given by kS_{C_s} times the same temperature dependency as used for growth (Eqn.[13]). Hence, changing the specific respiration rate k also changes growth in the present implementation of the model. So, part of the sensitivity to k is already contained in the sensitivity to m . Since both functions depend upon temperature, the temperature coefficient c has a marked effect.

In the case of sink limitation, the assimilates in the vacuoles build up, and hence the nitrate will be low, as certified in Table 2. It is interesting to note that in this case the sensitivity of the nitrate content is high to both parameters related to growth (m , k , c), as well as the parameters related to photosynthesis and production inhibition (ϵ , σ , b_p).

The effect of γ and κ on biomass is limited. In fact, the sensitivities of the states to γ and κ are identical (not shown). This can be understood by inspecting the equations [12], [9] and [15]. There is always the product of γ and C_{Cv} , which itself is coupled to the state parameters by κ , the inverse of the vacuole volume per mole structural C . Hence, looking at the C -balance only, the number of free parameters could be reduced by considering the product $\gamma\kappa$ as new parameter. Its value can hardly be estimated from growth experiments alone. The coupling gets lost if the nitrate mass concentration in mg per g fresh weight is computed (Eqn. [20]), because this depends on the vacuole volume, and hence on κ (Eqn. [17]). As can be seen from Table 2, the nitrate concentration is therefore sensitive to this parameter. This can be turned to advantage: there is a fair chance that κ can be estimated from nitrate data.

5. Discussion

As already suggested during the original development of the model by Seginer *et al.* (1998) the long term dynamics determining total biomass appear to be less important in determining the nitrate and sugar contents at harvest time. In the case of source limitation the actual value of the growth parameter within a certain range hardly affects the final biomass. Therefore, in addition to growth experiments, direct measurement of the nitrate content will help to tie down the uncertainty range of the parameters. In view of the identifiability of this model by experiments it is interesting to look at the nitrate flux between assimilate pool and rhizosphere, that would be needed in order to obey the turgor pressure relationship at all times. While there is, on average, influx of N over the whole cultivation period, as expected, the simulated N-flux varies between an influx in the order of $0.02\text{-}0.03 \text{ mol[N] m}^{-2}\text{s}^{-1}$ during the night to an efflux from the plant of the same order of magnitude in periods with high photosynthesis. Experiments could be designed to verify this behaviour, in order to assess the validity of the algebraic turgor maintenance relationship under dynamic conditions.

6. Conclusion

The results reveal that estimates of the growth parameters have a large uncertainty when growth is inhibited by assimilate availability, i.e. when the system is source limited. However, in regions that are of interest for nitrate control, i.e. in the sink-limited case, all parameters determining the source-sink balance are important. Consequently, experiments for parameter estimation must be designed such that they cover sink-limited conditions.

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Appendix 1. Model equations.

State equations

$$\text{Carbon in vacuoles} \quad \frac{dS_{Cv}}{dt} = F_{C_{iv}} - F_{C_{m}} - (1+\theta)F_{C_{vs}} \equiv f_1\{S_{Cv}, S_{Cs}\} \quad [1]$$

$$\text{Carbon in structure} \quad \frac{dS_{Cs}}{dt} = F_{C_{vs}} \equiv f_2\{S_{Cv}, S_{Cs}\} \quad [2]$$

C-fluxes

$$\text{photosynthetic assimilation} \quad F_{C_{iv}} = p\{I, C_a\} h_p\{S_{Cv}, S_{Cs}\} f\{S_{Cs}\} \quad [3]$$

$$\text{growth} \quad F_{C_{vs}} = g\{T\} h_g\{S_{Cv}, S_{Cs}\} f\{S_{Cs}\} \quad [4]$$

$$\text{maintenance respiration} \quad F_{C_m} = e\{T\} S_{Cs} \quad [5]$$

$$\text{growth respiration} \quad F_{C_g} = \theta F_{C_{vs}} \text{ (implicit in [1])} \quad [6]$$

Additional relations

$$\text{carbon concentration in the vacuoles} \quad C_{Cv} = \kappa \frac{S_{Cv}}{S_{Cs}} \quad [7]$$

$$\text{uninhibited photosynthesis rate} \quad p\{I, C_a\} = \frac{eI\sigma(C_{Ca} - C_{Cs})}{eI + \sigma(C_{Ca} - C_{Cs})} \quad C_{Ca} > C_{Cs} \quad [8]$$

$$\text{photosynthesis inhibition function} \quad h_p\{C_{Cv}\} = 1 - \left(1 + \exp\left\{ -s_p \left(\frac{C_{Cv}\gamma}{\Pi_v} - b_p \right) \right\} \right)^{-1} \quad [9]$$

$$\text{canopy closure reduction function} \quad f\{S_{Cs}\} = 1 - \exp\{-aS_{Cs}\} \quad [10]$$

$$\text{maximum growth rate} \quad g\{T\} = mk \exp\{c(T - T^*)\} \quad [11]$$

$$\text{source depletion switching (inhibition) function} \quad h_g\{C_{Cv}\} = \left(1 + \exp\left\{ -s_g \left(\frac{C_{Cv}\gamma}{\Pi_v} - b_g \right) \right\} \right)^{-1} \quad [12]$$

$$\text{specific maintenance respiration} \quad e\{T\} = k \exp\{c(T - T^*)\} \quad [13]$$

$$\text{osmotic pressure in vacuoles (Pa)} \quad \Pi_v = P_v + \Pi_r \quad [14]$$

$$\text{nitrate concentration in the vacuoles} \quad C_{Nv} = \frac{\Pi_v - \gamma C_{Cv}}{\beta} \quad [15]$$

Outputs

$$\text{dry matter (g m-2)} \quad DM = \eta_{DM/C}(S_{Cs} + S_{Cv}) + \eta_{DM/N}S_{Nv} \quad [16]$$

$$\text{with } S_{Nv} = C_{Nv} \frac{S_{Cs}}{\kappa} \quad [17]$$

$$\text{fresh matter (g m-2)} \quad FM = \frac{DM}{\eta_{DM/FM}} \quad [18]$$

$$\text{dry weight per head (g)} \quad y_{dw} = .DM/N_p \quad [19]$$

$$\text{nitrate content (mg[NO3] g[FM])} \quad y_{Nc} = \frac{\eta_{NO3/N}S_{Nv}}{FM} \quad [20]$$

$$\text{fresh weight per head (g)} \quad y_{fw} = .FM/N_p \quad [21]$$

$$\text{required N-flux from rhizosphere (mol [N] m-2)} \quad F_{Nrv} = \frac{dS_{Nv}}{dt} + r_s F_{C_{vs}} = \quad [22]$$

$$\frac{1}{\beta} \left[\frac{\Pi_v}{\kappa} \frac{dS_{Cs}}{dt} + \frac{S_{Cs}}{\kappa} \frac{d\Pi_v}{dt} - \gamma \frac{dS_{Cv}}{dt} \right] + r_s F_{C_{vs}} \quad [23]$$

Appendix 2. Parameter list (nominal calibration parameters).

Initial conditions

Non-structural carbon content	$S_{Cv}(0)$	0.006	$\text{mol}[C]\text{m}^{-2}$
Structural carbon content	$S_{Cs}(0)$	0.018	$\text{mol}[C]\text{m}^{-2}$

Parameter values of the state equations

Leaf area closure parameter	a	1.7	$\text{m}^2\text{mol}[C]^{-1}$
Apparent light use efficiency	ϵ	Table 1	$\text{mol}[C]\text{mol}[\text{phot}]^{-1}$
CO ₂ transport coefficient	σ	Table 1	ms^{-1}
Specific maintenance rate coefficient	k	0.25e-6	s^{-1}
Temperature effect parameter	c	0.0693	$^{\circ}\text{C}^{-1}$
Growth parameter	m	Table 1	$\text{mol}[C]\text{m}^{-2}$
Growth respiration loss factor	θ	0.3	
Regression par. of C/N ratio in vacuoles	γ	0.61	$\text{kPa}(\text{mol}[C]\text{m}^{-3})^{-1}$
Structural C per unit vacuole volume	κ	1350	$\text{mol}[C]\text{m}^{-3}$
Slope parameter of photosynthesis inhibition function h_p			s_p 30
Slope parameter of growth inhibition function h_g			s_g 30
Threshold parameter of photosynthesis inhibition function h_p			b_p 0.8
Threshold parameter of growth inhibition function h_g			b_g 0.2

Values of constants of the model

CO ₂ compensation point	C_c^*	0.0011	$\text{mol}[C]\text{m}^{-3}$
Reference temperature	T^*	20	$^{\circ}\text{C}$
Constant turgor pressure	P_v	530	kPa
Osmotic pressure in the rhizosphere	Π_r	50	kPa

Additional parameter values of output equations

N to C ratio of the structure	r_s	0.08	$\text{mol}[N] \text{mol}[C]^{-1}$
Regression parameter of C/N ratio in vacuoles	β	6.0	$\text{kPa} (\text{mol}[N]\text{m}^{-3})^{-1}$

Values of constants of the output equations

Organic matter in kg per mol C	$\eta_{OM/C}$	30e-3	kg mol^{-1}
Minerals in kg per mol N in vacuoles	$\eta_{MM/N}$	101e-3	kg mol^{-1}
Dry matter to fresh matter ratio	$\eta_{DM/FM}$	0.05	kg kg^{-1}
kg nitrate per mol N	$\eta_{NO3/N}$	62e-3	$\text{kg}[\text{NO}_3]\text{mol}[N]^{-1}$
kg sugars(hexose) per mol hexose	$\eta_{\text{sug/hex}}$	0.180	kg mol^{-1}
Moles of C in the vacuoles per mol hexose	$\eta_{C/\text{hex}}$	14	mol mol^{-1}
Plant density	N_p	18	plants m^{-2}
Density of water	ρ_w	1000	kg m^{-3}