

NITRATE CONCENTRATION IN GREENHOUSE LETTUCE: A MODELING STUDY

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Abstract

A simple two-state-variable model was developed to describe the nitrate concentration in lettuce when nitrate supply is unlimited. A central element of the model is a negative correlation between the concentrations of soluble carbohydrates and nitrate in the cell sap, a correlation which reflects the equivalent roles of nitrate and organic solutes in the maintenance of cell turgor. A conventional carbon balance model was used to predict the fluctuations of soluble carbohydrates as a function of time. The carbohydrate-to-nitrate correlation was then used to compensate for these fluctuations by adjusting the nitrate concentration.

The concentration of carbohydrates in the non-structural buffer (vacuoles) is determined by the balance between source activity (supply of carbohydrates by photosynthesis, controlled by light and CO₂) and sink activity (demand for carbohydrates by growth and maintenance, controlled by temperature). As a result, winter conditions, characterized by source-limited growth, result in a depletion of carbon from the cell sap and, via the negative correlation, in a high nitrate concentration. Conversely, summer conditions result in low nitrate levels.

A fair agreement was obtained when the model was tested with an available experimental data set for all-year-round production of greenhouse lettuce in Northern Germany.

1. Introduction

High nitrate (NO₃) concentration in vegetables is undesirable, since it may constitute a health hazard (Maynard, *et al.*, 1976; Walters and Walker, 1979). From observations in northern countries it appears that the low light level in winter is the primary cause of high nitrate concentration in vegetables. Blom-Zandstra & Lampe (1985) and Veen & Kleinendorst (1985) postulated that plants may actively accumulate nitrate in order to compensate osmotically for the low concentrations of organic osmotica, resulting from decreased photosynthetic activity when light level is low.

The concentration of the primary assimilates (mostly carbohydrates, CH) is the result of two counteracting fluxes: a source term driven by photosynthesis, and a sink term due to growth and maintenance (e.g., Sweeney, *et al.*, 1981; Gary, 1988; Seginer, *et al.*, 1994; Van Henten, 1994). The rate of production of assimilates by photosynthesis is mainly determined by light and carbon-dioxide (CO₂) concentration, whereas the rates of

growth and maintenance are mainly controlled by temperature (Gent and Enoch, 1983). As a result, the concentration of CH in the vacuoles decreases when growth is source limited, and, in response, the concentration of nitrate (among other inorganic compounds), must increase. This implies a negative correlation between CH and nitrate in the vacuoles (cell sap).

Alberda (1965) already described the effects of temperature and light level on the balance between CH and nitrate in *Lolium perenne* L. A negative correlation has in fact been observed by Veen and Kleinendorst (1985), who described the roles of nitrate and CH in the adjustment of *Lolium multiflorum* Lam. to osmotic stress. Several studies with lettuce (Blom-Zandstra and Lampe, 1985; Blom-Zandstra, *et al.*, 1988; Behr and Wiebe, 1988; Drews, *et al.*, 1995) produced clear negative linear correlations between sugar and nitrate in the cell sap (Figure 1).

Based on the preceding description, the nitrate adjustment mechanism can be summarized as follows: The carbon sink-source relationship actively determines the CH concentration in the cells, while available nitrate is moved in and out of the cells to maintain the turgor pressure at the desired level.

With this view in mind, lettuce growers may attempt to control the nitrate level by manipulating the shoot and/or root environments and hence the sink-to-source ratio. Here we refer only to the shoot environment. Since supplementary lighting appears to be too expensive for lettuce, practical options to decrease the nitrate concentration by changing above-ground conditions are restricted to increasing the carbon-dioxide concentration and/or lowering the temperature.

The aim of the present study is to formulate a simple dynamic lettuce model, capable of predicting the response of plant nitrate levels to shoot environmental conditions. We attempt to achieve this by combining a simple photosynthesis-growth (carbon balance) model with a mechanism for turgor maintenance.

2. Basic model

Our current model makes no distinction between shoot and root. Furthermore, no provision is made for long-term storage of assimilates, such as in the form of starch, nor for buffers of compounds other than carbohydrates (CH) and nitrate. These additional elements may become necessary for more refined versions of the model, which in its present form should be viewed as a first approximation.

The two compartments of the model (Figure 2) are designated *vacuoles* (v), where soluble, non-structural material is stored, and *structure* (s), which is composed of structural material, including proteins. It is assumed that the volume occupied by the vacuoles is a fixed fraction of the total volume of the plant. The carbon-to-nitrogen ratio in the structure is fixed, while the ratio in the vacuoles is variable, but constrained by the need to maintain a constant turgor pressure (Figure 1). The plant grows by building new cells with exactly the same proportions as the already existing cells.

Photosynthesis is assumed to depend on light and carbon-dioxide concentration, but not on temperature, while respiration and growth are assumed to depend on temperature, but not on the other environmental variables. Photosynthesis and growth also depend on the size of the crop. Nitrate is assumed to flow into the vacuoles *as needed* to support growth and to maintain turgor.

Previous dynamic models of lettuce (Sweeney, *et al.*, 1981; van Henten, 1994) already described the processing of carbon in essentially the same terms, but they did not refer to nitrogen, which is a central element of the present model. Scaife (1989), who focused on nitrate uptake, made the removal of nitrate from the vacuoles into structure proportional to the photosynthesis rate, rather than to growth rate, as we propose here. Furthermore, the substitution of nitrate for sugar, and vice versa, to maintain osmotic pressure, is not present in his model, which assumes constant influx and gradient-driven efflux of nitrate.

2.1. Carbon dynamics

We consider two state variables, namely the carbon content of the vacuoles and the carbon content of the structure (S_{Cv} and S_{Cs} , respectively). The dynamic carbon balances of the vacuoles and structure compartments (Figure 2) are, respectively

$$dS_{Cv}/dt = F_{Cav} - F_{Cm} - F_{Cg} - F_{Cvs} \quad [1]$$

and

$$dS_{Cs}/dt = F_{Cvs}, \quad [2]$$

where F_{Cav} is the photosynthetically generated carbon (subscript C) flux from the atmosphere (a) to the vacuoles (v); F_{Cm} and F_{Cg} are the maintenance (m) and growth (g) respiration fluxes; and F_{Cvs} is the flux of carbon into the structure (s). When growth is sink limited, $dS_{Cv}/dt > 0$ and when it is source limited, $dS_{Cv}/dt < 0$.

The photosynthesis flux, F_{Cav} , and the growth flux, F_{Cvs} , are each a product of three factors: (1) The uninhibited flux of a closed-canopy crop, (2) a measure of light interception (surface cover) by the canopy, and (3) an inhibition function. Thus,

$$F_{Cav} = p\{I, C_{Ca}\} f\{S_{Cs}\} h_p\{C_{Cv}\} \quad [3]$$

and

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

where $p\{I, C_{Ca}\}$ is the gross-photosynthesis rate, determined by light, I , and by atmospheric (or greenhouse) CO_2 concentration, C_{Ca} ; $g\{T\}$ is the potential growth rate, which is a function of temperature, T ; $f\{S_{Cs}\}$ is a measure of light interception (size of crop), which approaches one as the canopy closes; and $h_p\{C_{Cv}\}$ and $h_g\{C_{Cv}\}$ are dimensionless photosynthesis and growth inhibition functions, respectively, which depend on the carbon concentration in the vacuoles. Using the same size factor, f , in both the photosynthesis and growth terms, is not self-evident, but we chose to follow here the example of Seginer, *et al.* (1994).

The respiration fluxes (Equation [1]) are formulated conventionally as

$$F_{Cm} = S_{Cs}e\{T\} \quad [5]$$

and

$$F_{Cg} = \theta F_{Cvs}, \quad [6]$$

where maintenance respiration is made proportional to structural matter and to a function of temperature, and growth respiration is a constant fraction, θ , of the rate of growth. The chosen light interception (size) factor, $f\{S_{Cs}\}$, photosynthesis function, $p\{.\}$, respiration function, $e\{.\}$, and growth function, $g\{.\}$, are all similar to those of current models (details in the appendix).

2.2. Inhibition functions

The inhibition functions, h_p and h_g (Equations [3] and [4]), are both functions of C_{Cv} , the carbon concentration in the vacuoles. Expressing C_{Cv} in terms of the state variables, S_{Cv} and S_{Cs} , namely carbon quantities per unit ground area, requires certain assumptions: We assume that the vacuoles occupy a constant fraction of the total volume of the crop (which consists of identical cells):

$$V_v = \phi (V_v + V_s), \quad [7]$$

where V denotes volume per unit of ground area and ϕ (<1) is a constant. Furthermore, we assume that the concentration of carbon in the structure is constant

$$C_{Cs} \equiv S_{Cs}/V_s = \kappa_s. \quad [8]$$

Recalling that, by definition,

$$C_{Cv} \equiv S_{Cv}/V_v, \quad [9]$$

V_v and V_s can be eliminated between Equations [7], [8] and [9], to obtain

$$C_{Cv} = [\kappa_s(1-\phi)/\phi] [S_{Cv}/S_{Cs}]. \quad [10]$$

Defining

$$\kappa \equiv [\kappa_s(1-\phi)/\phi], \quad [11]$$

the required dependence of C_{Cv} on the state variables is obtained:

$$C_{Cv} = \kappa [S_{Cv}/S_{Cs}]. \quad [12]$$

The inhibition functions should prevent the carbon concentration in the vacuoles from attaining unreasonably extreme values. One hard constraint on this concentration is obviously $C_{Cv} > 0$. The other hard constraint can be obtained from the correlation between sugar and nitrate in the vacuoles (Figure 1):

$$\beta C_{Nv} + \gamma C_{Cv} = \pi_v, \quad [13]$$

where π_v is the osmotic pressure in the vacuoles and β and γ are constants. This places the upper hard limit of C_{Cv} , (where $C_{Nv}=0$), at π_v/γ , leading to

$$C_{Cv} < \pi_v/\gamma. \quad [14]$$

The inhibition functions which we selected (Appendix; Figure 3) ensure that h_p approaches 1 as the carbon buffer is emptied and approaches 0 as it is filled, while the opposite is true for h_g . The scale for nitrate concentration (in $\text{mg}[\text{NO}_3]\text{kg}^{-1}[\text{FM}]$), appearing in the upper part of Figure 3 is intended to associate the model with the marketing regulations. In particular, the summer upper-limit, $3500 \text{ mg}[\text{NO}_3]\text{kg}^{-1}[\text{FM}]$, is situated at the edge of the no-inhibition region, and the winter upper-limit, $4500 \text{ mg}[\text{NO}_3]\text{kg}^{-1}[\text{FM}]$, is well within the growth limiting range.

Given the initial conditions of the state variables S_{Cv} and S_{Cs} , as well as the environmental conditions, I , C_{Ca} and T as functions of time, the system Equations [1] and [2], with substitutions from Equations [3] to [6] as well as from the equations of the appendix, can be solved for the time trajectories of S_{Cv} and S_{Cs} .

3. Constant-concentration trajectory

In principle, it should be possible to control the growth in such a way that the nitrate concentration remains constant all the time. Normal growing practices are probably not too far from such a policy. Equation [12] indicates that if the state-variable ratio S_{Cv}/S_{Cs} is somehow kept constant, the carbon concentration in the vacuoles will also remain

constant. The state-ratio will stay constant if the incremental growth of the state variables maintains, at all times, a constant ratio, namely

$$dS_{Cv}/dS_{Cs} = S_{Cv}/S_{Cs} . \quad [15]$$

In view of Equation [12] this leads to

$$dS_{Cv}/dS_{Cs} = C_{Cv}/K . \quad [16]$$

From Equations [1], [2], [6] and [16] it now follows that

$$C_{Cv} = \kappa [F_{Cav} - F_{Cm} - (1 + \theta)F_{Cvs}] / F_{Cvs}, \quad [17]$$

and substitutions for F_{Cav} , F_{Cm} and F_{Cvs} from Equations [3], [4] and [5], lead to

$$C_{Cv} = \kappa \left[\frac{h_p\{C_{Cv}\}p\{I, C_{Ca}\} S_{Cs}e\{T\}}{g\{T\}h_g\{C_{Cv}\} g\{T\}f\{S_{Cs}\}h_g\{C_{Cv}\}} - (1 + \theta) \right] . \quad [18]$$

To maintain a constant concentration C_{Cv} , for which this is an implicit equation, the three terms in the square brackets must sum to a constant, independent of the size of the crop, S_{Cs} . In general, as the crop grows, so does the burden of maintenance ($S_{Cs}/f\{S_{Cs}\}$ increases), and this increases the relative magnitude of the second term. Therefore, if constant vacuole concentrations are desired, the first term must be increased over time by increasing light and carbon dioxide concentration and/or by lowering the temperature.

4. Experimental data for the constant-concentration case

Recently, Drews, *et al.* (1995) published the results of an experiment where ten crops of lettuce were grown to a harvestable stage under normal greenhouse conditions of northern Germany. The crops were harvested over a period of one year at about one month intervals. Although the experiments, which were conducted with natural light, did not strictly meet the requirements of the steady-concentration model, the differences between winter and summer were large enough to justify a fitting attempt.

Normalized carbon and nitrogen concentrations, C_v and C_n (Appendix), both as converted from the data of Drews, *et al.* (1995) and as predicted by Equation [18], are shown in Figure 4. Most model parameters were determined on the basis of available literature. The parameters m (Appendix) and K were adjusted to produce a fair agreement between data and model.

The figure shows that the model was able to reproduce the general trends of carbon and nitrogen concentrations in the cell sap, which covered about 60% of the maximum possible range. The predicted C and N concentrations are exact mirror images of each other (Figure 4), while the measured concentrations deviate from the assumed correlation between sugar and nitrate (Equation [13]). These deviations (e.g., Harvest 4), may be due to measurement errors and/or they may reflect the over-simplification of the model.

The climatic conditions during the experiment of Drews, *et al.* (1995) were superimposed in Figure 5 over the predicted value of C_{Nv} (from Equations [18] and [13]), for a range of environmental conditions (PAR and temperature). For this purpose, and justified by the data, the second term in the brackets of Equation [18] was assumed to be the same for all harvests.

Figure 5 shows that crops 1, 2, 3, 9 and 10 (winter) grew under environmental conditions conducive of nitrate accumulation ($>3500 \text{ mg}[\text{NO}_3]\text{kg}^{-1}[\text{FM}]$), while the opposite is true for crops 4 to 8.

5. Discussion

Our simple model combines, we believe for the first time, a photosynthesis-growth model with a turgor-maintenance model. At present, the model is limited to two compartments, which represent an organ-less crop (no differentiation between shoot and root). The model emphasizes the dependence of the nitrate concentration on the carbohydrate source-sink relationships. When growth is source-limited and the carbon content of the vacuoles decreases, the model predicts an increase of the nitrate content. The opposite is true for sink-limited growth. It would seem, therefore, that when growth is source limited, any method of reducing sink activity or stimulating source activity will lead to a lower nitrate level.

Investigators discovered significant differences between lettuce cultivars (Blom-Zandstra and Eenik, 1986; Blom-Zandstra, *et al.*, 1988; Behr and Wiebe, 1988 and 1992). In our model these differences may be expressed in terms of different values for β and γ .

Future refinements of the model could include: (1) A distinction between root and shoot. (2) A starch compartment to store carbohydrates that in the present model are prevented from being created due to photosynthesis-inhibition. (3) An amino-acid buffer, which requires the introduction of a nitrate reduction module. We already extended the model to situations where growth is nitrate-limited, and it should also be possible to treat situations with limiting supply of other essential nutrients.

Figure 5 may be used as a guideline to growers concerning the expected nitrate concentration in their greenhouse. Even if the model is inaccurate for a particular cultivar and growing conditions, a grower may still expect a regular response of nitrate concentration to mean light and temperature conditions, which is not too different from Figure 5. Furthermore, a specific relationship for a particular greenhouse may be developed, over a few harvests, by plotting actual data as they become available.

Figure 5 indicates that low temperature and CO₂ enrichment are not likely to solve the nitrate problem in lettuce under dull winter conditions. Harvests 1, 2 and 10 will not be saved by going down to 5°C, and light, not CO₂ is limiting photosynthesis for these harvests.

Therefore, control via the rhizosphere might be the only viable solution.

Appendix: Details of certain functions

The following are specific formulations which we used in our simulations. The replacement of these by other suitable expressions should not materially change the results of this study.

The light interception, photosynthesis, and respiration terms are:

$$f\{S_{Cs}\} = 1 - \exp\{-aS_{Cs}\} . \quad [A1]$$

$$p\{I, C_{Ca}\} = \varepsilon I \alpha (C_{Ca} - C_{C*}) / \{ \varepsilon I + \alpha (C_{Ca} - C_{C*}) \} \quad C_{Ca} > C_{C*} \quad [A2]$$

$$e\{T\} = k \exp\{c(T - T_*)\} \quad [A3]$$

$$g\{T\} = m e\{T\} . \quad [A4]$$

In the light interception factor, a , the extinction coefficient, is constant. In the photosynthesis function, ε is the photosynthetic efficiency, α is the leaf conductance to CO₂, and C_{C*} is the CO₂ concentration at the compensation point. In the respiration function, k , c and T_* are constants. The temperature dependencies of growth and maintenance respiration were taken to be the same, following the example of Seginer, *et al.* (1994).

The inhibition functions are:

$$h_p\{C_{Cv}\} = 1 - 1/[1 + \exp\{-s_p(C_{Cv}/\Pi_v - b_p)\}] \quad [A5]$$

$$h_g\{C_{Cv}\} = 1/[1 + \exp\{-s_g(C_{Cv}/\Pi_v - b_g)\}], \quad [A6]$$

where a normalized carbon concentration may be defined as

$$C_v \equiv C_{Cv}/\Pi_v . \quad [A7]$$

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Figure 1: Linear correlations between sugar and nitrate in the cell sap of lettuce. Data of Blom-Zandstra (1985), Blom-Zandstra et al. (1988), Behr and Wiebe (1988 and 1992) and Drews et al. (1995). The dotted line is an extension of Drews' line. F and T denote Figures and Tables where data were found. Where the authors determined a regression line, it was copied into the figure. Some of the differences are due to difference in cultivars.

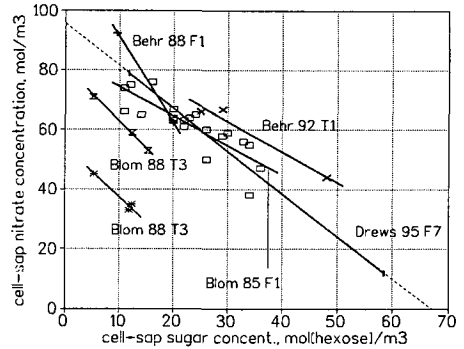


Figure 2: A schematic representation of the two-compartment lettuce model. F are fluxes and S are quantities. S_{Cv} and S_{Cs} were selected as the two state variables. S_{Nv} and S_{Ns} can be determined algebraically from the state variables. The words 'light', 'CO₂', 'temperature' and 'demand' indicate the dominant influencing factor for the transfer. h_p and h_g are the photosynthesis and growth inhibition functions.

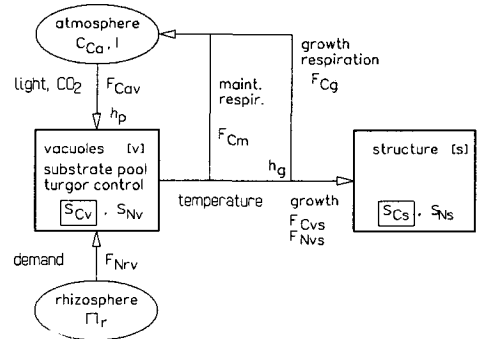


Figure 3: The inhibition functions h_p and h_g for $b_p=0.8$, $b_g=0.2$ and $s_p=s_g=30$ (Equations A5 and A6). The normalized carbon scale (under the figure) is defined by the model and is, therefore, exact. The absolute nitrate scale (above the figure) is an attempt to represent the real world and depends on the conversion factors and procedure used. The summer and winter upper limits on nitrate concentration are, according to this choice of parameters, within the range of moderate growth inhibition.

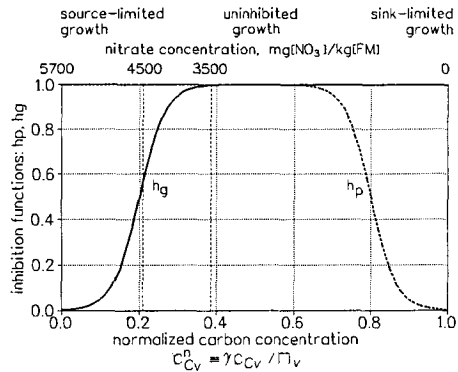


Figure 4: Comparison between measured and predicted carbon and nitrate concentrations. Sugar and nitrate concentrations, measured by Drews et al. (1995), were converted to model variables as described in the text. The adjusted parameters are $m=13 \text{ mol[C]m}^{-2}[\text{ground}]$ and $\kappa=1200 \text{ mol[C]m}^{-3}$.

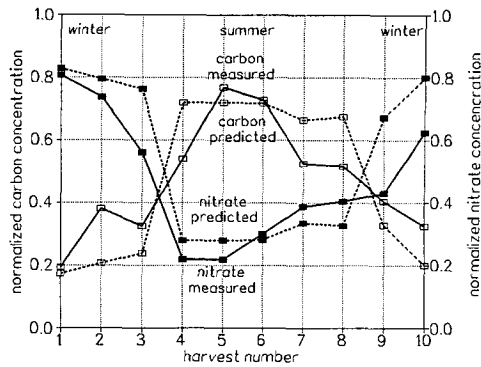


Figure 5: Environmental data of Drews et al. (1995) plotted over a surface approximately representing the cell-sap nitrate-concentration with abundant nitrate supply. The region where growth is completely uninhibited lies approximately between the 2500 and 3500 $\text{mg}[\text{NO}_3]\text{kg}^{-1}[\text{FM}]$ curves. The problematic combinations are low-light with high-temperature at the right-bottom corner. Numbers near crosses are harvest numbers.

