

LETTUCE GROWTH LIMITED BY NITRATE SUPPLY

Ido Seginer
Agricultural Engineering Department
Technion, Haifa 32000
Israel
E-mail: segineri@tx.technion.ac.il

Gerrit van Straten
Agricultural, Environmental and System
Technology Department
Wageningen Agricultural University
6703 HD Wageningen
The Netherlands

Fokke Buwalda
Research Station for Floriculture and
Glasshouse Vegetables
1431 JV Aalsmeer
The Netherlands

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Abstract

Limiting the supply of nitrate to winter-lettuce in greenhouses is one approach to assuring an acceptably low nitrate level in the marketed produce. To optimize growth under such conditions, a two-state-variable lettuce model, originally developed to predict the nitrate concentration when the supply of nutrients is unlimited (Seginer *et al.*, 1998), has been modified to take account of growth limited by nitrate supply.

Nitrate in the plant serves two functions: it is a vital raw material for protein synthesis and also an important osmoticum in the cell sap. The plant model responds to limited nitrate supply by placing a higher priority on its role as raw material. Nitrate in the cell sap is replaced, as much as needed, by assimilates (sugar), freeing the recovered nitrate for protein synthesis.

The modified model includes a balance equation for nitrate in the plant

$$dS_{Nv}/dt = F_{Nrv} - rF_{Cvs} ,$$

where S_{Nv} is nitrate-N content, F_{Nrv} is nitrate-N uptake by the plant, F_{Cvs} is the rate of structural growth and r is the nitrogen content of the structural material. dS_{Nv}/dt is determined by the rate of change of the assimilate content of the cell sap, as described by the original model. If nitrate supply is abundant, its rate of uptake, namely F_{Nrv} , is determined as a remainder. If nitrate supply is limiting, and therefore is all taken up by the plant, it is the nitrate used for growth, rF_{Cvs} , that is determined by the balance.

Comparison with limited experimental results produced a good agreement.

1. Introduction

Nitrate concentration in greenhouse lettuce varies considerably between seasons, being higher in winter than in summer (UK Ministry of Agriculture, 1997). In northern latitudes, with poor light conditions in winter, excessively high nitrate concentrations may render the produce unmarketable. To explore the possibilities for a preventive environmental control, a simple two-compartment, dynamic lettuce model has been recently developed (Seginer *et al.*, 1998), based on a source-sink (photosynthesis-growth) relationship, together with a turgor maintenance mechanism. It successfully predicted the seasonal variation of nitrate concentration in greenhouse lettuce grown with abundant supply of nutrients by Drews *et al.* (1995). Other dynamic models to predict nitrate uptake and concentration in plants are available (Scaife, 1989; Burns, 1994; Cárdenas-Navarro *et al.*, 1998), but none of them

utilizes the turgor requirement, which is a corner stone of the new model.

According to the model, nitrate concentration in the crop may be reduced by increasing source activity and/or by reducing sink activity. The latter can be achieved, among other means, by limiting the supply of a vital nutrient, such as nitrate itself, either throughout the growing season or towards its end, as demonstrated by Blom-Zandstra and Lampe (1983) and by Burns (1994).

The simulation of crop development, when growth is limited by nitrate supply, requires a modified version of the original model. The following sections will first briefly review the original model, then introduce the nitrate-limiting modification, develop a steady-concentration trajectory and finally compare model predictions with a set of experimental data provided by Veen (private communication).

2. Original model

In a nutshell, the original model (Seginer *et al.*, 1998) is as follows: Lettuce is represented by a single vegetative organ with two compartments, designated *vacuoles* (v) and *structure* (s) (Figure 1).

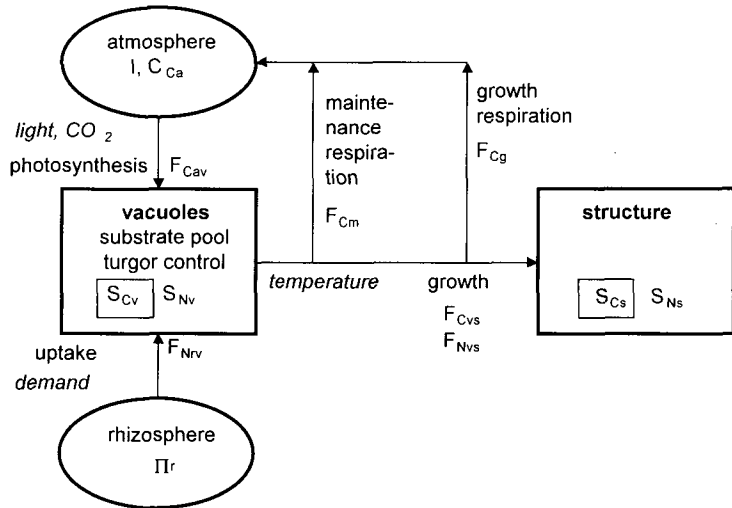


Figure 1: A schematic representation of the two-compartment lettuce model. F are fluxes and S are quantities per unit ground area. S_{Cv} and S_{Cs} were selected as the two state variables. S_{Nv} and S_{Ns} can be determined algebraically from the state variables. The words 'light', 'CO₂', 'temperature' and 'demand' indicate the dominant influencing factors for the transfer.

The volume occupied by the vacuoles is a fixed fraction of the total volume of the plant. The nitrogen-to-carbon ratio in the structure is also fixed, while the ratio in the vacuoles is variable, but constrained by the need to maintain a constant turgor pressure. Photosynthesis is assumed to depend on light and carbon-dioxide concentration, while respiration and growth are assumed to depend on temperature. In the original model, nitrate is assumed to flow into the vacuoles *as needed* to support growth and to maintain turgor.

The model has two state variables, 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 1) are, respectively

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

and

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

where F_{Cav} is the photosynthetically generated carbon flux (subscript C) 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 from the vacuoles into the structure (s), namely growth. Note that carbon consumption for nitrate reduction is included in growth respiration.

The photosynthesis flux, F_{Cav} , and the growth flux, F_{Cvs} , are modelled as products of three factors: (1) The uninhibited flux for 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, which approaches one as the canopy closes; and $h_p\{C_{Cv}\}$ and $h_g\{C_{Cv}\}$ are, respectively, dimensionless photosynthesis and growth inhibition functions, which in the uninhibited state are equal to one. The first approaches zero for high values of carbon (sugar) concentration in the vacuoles, C_{Cv} , while the second approaches zero for low values of C_{Cv} .

The respiration fluxes (Equation [1]) were formulated as

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

and

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

where θ is a constant fraction and $e\{T\}$ is proportional to $g\{T\}$.

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], can be solved for the time trajectories of S_{Cv} and S_{Cs} , provided that specific expressions for $p\{I, C_{Ca}\}$, $g\{T\}$, $f\{S_{Cs}\}$, $h_p\{C_{Cv}\}$ and $h_g\{C_{Cv}\}$ are available.

The nitrate concentration in the vacuoles, C_{Nv} , can be obtained from the carbon concentration, C_{Cv} , via an empirically observed negative correlation between sugar and nitrate in the cell sap (Blom-Zandstra and Lampe, 1985; Blom-Zandstra *et al.*, 1988; Behr and Wiebe, 1988; Drews *et al.*, 1995). It may be expressed as

$$\beta C_{Nv} + \gamma C_{Cv} = \Pi_v , \quad [7]$$

where β and γ are constants and Π_v is the osmotic pressure in the vacuoles. The reader is referred to Seginer *et al.* (1998) for further details and justification of the model.

3. Growth limited by nitrate supply

The model described in the previous section assumes unlimited availability of nitrate in the rhizosphere. The model can be modified, however, to accommodate a situation where nitrate is supplied at a lower rate than required for potential growth under the prevailing shoot environment. When nitrate supply is limiting, Equations [1] and [2], as well as Equation [3] still hold, but the growth inhibition function, h_g , in Equation [4] needs to be modified. Originally, h_g became limiting when the carbon concentration in the vacuoles was low. Now growth may be inhibited even when the carbon concentration is high.

To modify the model, a nitrogen balance of the vacuoles is introduced,

$$dS_{Nv}/dt = F_{Nrv} - rF_{Cvs} , \quad [8]$$

where dS_{Nv}/dt is the rate of change of the nitrate-N content of the vacuoles, F_{Nrv} is the uptake of nitrate from the rhizosphere, and r is the ratio of nitrogen to carbon in the structure, assumed here to be constant (but could also be made to depend on the state, say $r\{S_{Cs}\}$). If growth is limited by nitrate supply, then uptake is equal to supply and F_{Nrv} represents the rate of supply. Note that in this case the boundary condition at the rhizosphere is a nitrate flux, F_{Nrv} , rather than a nitrate concentration. This produces a simpler model which, nevertheless, is suitable for comparison with certain experiments.

It is now necessary to express the rate of change of S_{Nv} in terms of the state variables S_{Cs} and S_{Cv} . By definition,

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

and

$$C_{Nv} \equiv S_{Nv}/V_v, \quad [10]$$

where V_v is the volume of the vacuoles per unit ground area. Substituting from Equations [9] and [10] into Equation [7], produces

$$\beta S_{Nv} + \gamma S_{Cv} = \Pi_v V_v. \quad [11]$$

Assuming that the osmotic pressure, Π_v , is constant, and differentiating Equation [11] with respect to time, leads to

$$\beta dS_{Nv}/dt + \gamma dS_{Cv}/dt = \Pi_v dV_v/dt. \quad [12]$$

As in the original model, the volume occupied by the vacuoles is assumed to be a fixed fraction of the total volume of the plant,

$$V_v/(V_v+V_s) = \phi, \quad [13]$$

and the structural carbon content is assumed to be proportional to the volume of the structural compartment

$$S_{Cs} = \kappa_s V_s. \quad [14]$$

Eliminating V_s between Equations [13] and [14], results in

$$S_{Cs} = [(1-\phi)/\phi]\kappa_s V_v \equiv \kappa V_v. \quad [15]$$

Finally, substituting Equation [15] into Equation [12], yields

$$dS_{Nv}/dt = (\Pi_v/\beta\kappa) dS_{Cs}/dt - (\gamma/\beta) dS_{Cv}/dt. \quad [16]$$

This equation, which is based entirely on the original model, specifies a relationship between the rates of change of the variables S_{Nv} , S_{Cv} and S_{Cs} that must be obeyed in order for a constant osmotic pressure, Π_v , to be maintained.

It is now possible to derive an expression for the rate of growth, F_{Cvs} , in terms of the limiting supply rate of nitrate, F_{Nrv} (and other quantities). Using Equation [16] to substitute for dS_{Nv}/dt in Equation [8] and then substituting for dS_{Cv}/dt from Equation [1], with F_{Cg} from Equation [6], the result is

$$(\Pi_v/\beta\kappa) dS_{Cs}/dt = F_{Nrv} - [(\gamma/\beta)(1+\theta) + r]F_{Cvs} + (\gamma/\beta)(F_{Cav}-F_{Cm}). \quad [17]$$

Substituting from Equation [2] for dS_{Cs}/dt , an expression for F_{Cvs} in terms of the other fluxes is obtained:

$$F_{Cvs} = \frac{\beta F_{Nrv} + \gamma(F_{Cav} - F_{Cm})}{\Pi_v / \kappa + \gamma(1 + \theta) + \beta r} \quad [18]$$

The denominator is a positive constant, F_{Nrv} is provided (when nitrate supply is limiting), and F_{Cav} and F_{Cm} can be calculated from the state and the environment. Using Equation [18] to substitute for F_{Cvs} in Equations [1] and [2], completes the modification required for the nitrate-limited growth. The new state-equations no longer contain the unknown h_g , and use instead the known nitrate flux, F_{Nrv} .

The behavior of Equation [18] for an abrupt interruption of the nitrate supply can be described, on a time scale of days, as follows: Following the interruption, namely the setting of F_{Nrv} to zero, growth is maintained for a while by the nitrate reserve in the vacuoles. In view of Equation [7], the depletion of the vacuolar nitrate results in an increase of vacuolar carbon concentration, eventually leading to photosynthesis inhibition. In the limit, an inhibition level is reached where the remaining photosynthesis, F_{Cav} , is just sufficient to support maintenance respiration, F_{Cm} . At this point the two terms of the numerator of Equation [18], as well as the growth rate, F_{Cvs} , all become zero.

Normally it is not required to deplete all vacuolar nitrate and to completely stop all growth. If nitrate is withheld as a corrective measure before harvest, the crop may be harvested as soon as the desired nitrate level is reached. If this level is not too low, so that photosynthesis inhibition does not in fact occur, there should be no loss of primary assimilate production. On the other hand, dry mass accumulation will be impeded, since some carbohydrates are diverted to replace nitrate in the vacuoles rather than to further grow the structure. As a result, the accumulation of dry and fresh matter also slows down, as the results of Blom-Zandstra and Lampe (1983) indicate.

Note that on a time scale of hours or shorter, Equation [18] may produce a negative growth rate. At night, $F_{Cav} - F_{Cm}$ is negative and if nitrate supply, F_{Nrv} , is zero, the growth rate, F_{Cvs} , must become negative. This would imply that structural material is consumed for nighttime maintenance.

Simulation with the modified model requires two evaluations of F_{Cvs} at each time step, once with Equation [4] (abundant nitrate supply) and again with Equation [18] (limiting nitrate supply). The lower of the two values is the appropriate one to use.

4. Constant nitrate concentration

The harvestable state of a crop, commercially specified in terms of total fresh mass and nitrate concentration, may be reached via different growth trajectories. One possibility is to control the growth in such a way that the nitrate concentration remains constant with time. This happens when plants are continuously supplied with only a fraction of their nitrogen demand. The results of such an experiment will be used to test the predictions for a constant concentration trajectory.

Combining Equations [9] and [15] yields

$$C_{Cv} = \kappa[S_{Cv}/S_{Cs}] , \quad [19]$$

which indicates that if the state-variable ratio S_{Cv}/S_{Cs} is somehow kept constant throughout the growing period, the carbon concentration, and hence the nitrogen concentration in the vacuoles, will also remain constant. The state-ratio stays constant if the incremental growth of the state variables maintains, at all times, the same constant ratio, namely if

$$dS_{Cv}/dS_{Cs} = S_{Cv}/S_{Cs} . \quad [20]$$

Combining Equations [19] and [20], the result is

$$dS_{Cv}/dS_{Cs} = C_{Cv}/\kappa. \quad [21]$$

From Equations [1], [2], [6] and [21] it now follows that

$$C_{Cv} = \kappa [F_{Cav} - F_{Cm} - (1+\theta)F_{Cvs}] / F_{Cvs}. \quad [22]$$

Substituting for F_{Cvs} from Equation [18], the result is

$$C_{Cv} = \frac{\Pi_v + \gamma\kappa(1+\theta) + \kappa\beta r}{\beta F_{Nrv} / (F_{Cav} - F_{Cm}) + \gamma} - \kappa(1+\theta) \quad [23]$$

The carbon concentration, C_{Cv} , and hence the nitrate concentration, remain constant as long as the supply of nitrogen is proportional (by a factor ζ) to the surplus of photosynthesis over maintenance respiration, namely

$$F_{Nrv} = \zeta(F_{Cav} - F_{Cm}). \quad [24]$$

Substituting From Equation [24] into Equation [23] yields

$$C_{Cv} = \frac{\Pi_v + \kappa\beta r - (1+\theta)\kappa\beta\zeta}{\gamma + \beta\zeta} \quad [25]$$

The corresponding expression for the concentration of nitrate-N is obtained by using Equation [7] to substitute for C_{Cv} in Equation [25]

$$C_{Nv} = \frac{-\kappa\gamma r + [\Pi_v + (1+\theta)\kappa\gamma]\zeta}{\gamma + \beta\zeta} \quad [26]$$

Note that the carbon and nitrogen concentrations in the vacuoles (Equations [25] and [26]) are only indirectly, via ζ , functions of the shoot environment.

5. Data for constant nitrate limitation

Veen (private communication) conducted growth chamber experiments, in which lettuce was provided with nitrate at a constant fraction of the dry matter increment (Ingestad's method; Ingestad and Lund, 1979). Four treatments with limited supply ($\zeta=3.5\%$, 4.0% , 4.5% and 5.0% of potential dry matter production), were compared with a reference crop, to which nitrate was supplied in abundance.

Figure 2 shows the shoot nitrate concentration at harvest for each treatment, as a function of the nitrogen supply-fraction, ζ . The result for the treatment with unlimited nitrate supply was placed arbitrarily along the abscissa, indicating the level of the asymptotic concentration. The level of this asymptote depends on the shoot-environment in the growth chamber (light, CO_2 and temperature). Presumably, a higher asymptote is associated with a lower light level and/or a lower CO_2 concentration and/or a higher temperature.

The prediction with Equation [26] is also shown in Figure 2. The model parameter values are the same as previously obtained by Seginer *et al.* (1998), with the addition of a single new fitted value, namely the nitrogen-to-carbon ratio, $r=0.08 \text{ mol}[N]\text{mol}^{-1}[C]$. The qualitative agreement between the model and the measured data is fair.

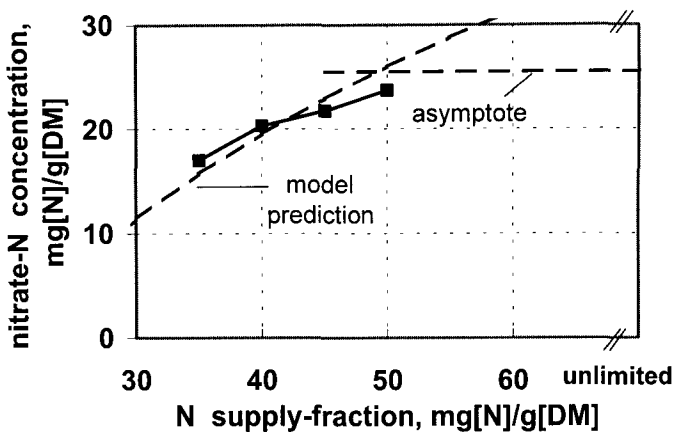


Figure 2: Measured nitrate concentrations in lettuce shoots (Veen, private communication), compared with the prediction of the nitrate-limiting model. The experimental data for unlimited nitrate supply are used to define the asymptotic level (horizontal line).

6. Discussion

Nitrate in lettuce serves two main functions: it is an important osmoticum in the cell sap and it is a vital raw material for protein synthesis. The plant model responds to a limited nitrate supply by placing a higher priority on its role as a raw material. Nitrate in the cell sap is therefore replaced, as much as needed, by assimilates (sugar), freeing the recovered nitrate for protein synthesis.

While the constant concentration trajectory is simple to analyze, it is not necessarily the optimal control solution for the low-light problem. Intuitively, a better approach would be to start with a high nitrate concentration in the nutrient solution. This is likely to promote growth, and specifically leaf-area expansion (Gary and Bertin, 1992), although accompanied by high nitrate concentration in the plant. Since the nitrate concentration only matters at harvest time, it should be possible to reduce the nitrate content over the final period of growth, by restricting the nitrate supply once a full cover has been established and further increase of light interception is no longer possible. Such a bang-bang like strategy is evident in nature, where many species (including lettuce) switch abruptly from the vegetative to the reproductive stage (e.g., Iwasa and Roughgarden, 1984). An example of a similar greenhouse management strategy is the switching from a higher to a lower temperature when a lettuce crop fully covers the ground (Seginer *et al.*, 1991).

The modified dynamic model makes it possible to simulate experimental procedures where availability of nitrate changes from abundant to scarce (relative to potential growth) and back again. The current version of the model does not, however, cover cases where the boundary condition at the rhizosphere is expressed in terms of nitrate concentration, the way many experiments are being carried out. This capability requires an additional modification.

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References

- Blom-Zandstra M, Lampe JEM. 1983. The effect of chloride and sulphate salts on the nitrate content in lettuce plants (*Lactuca sativa L.*). *J. Plant Nutrition* 6(7): 611–628.
- Burns IG. 1994. A mechanistic theory for the relationship between growth rate and the

- concentration of nitrate-N or organic-N in young plants derived from nutrient interruption experiments. *Annals of Botany* 74: 159–172.
- Cárdenas-Navarro R, Adamowicz S, Robin P. 1998. Diurnal nitrate uptake in young tomato (*Lycopersicon esculentum* Mill.) plants: test of a feedback-based model. *J. Experimental Botany* 49(321): 721–730.
- Drews M, Schonhof I, Krumbein A. 1995. Influence of growth season on the content of nitrate, vitamin C, β -Carotin and sugar of head lettuce under greenhouse conditions. *Gartenbauwissenschaft* 60(4): 180–187.
- Gary C, bertin N. 1992. La surface spécifique foliaire comme indicateur de l'équilibre source-puits chez la tomate (*Lycopersicon esculentum* Mill.). *Bull. Soc. Ecophysiol.* 17(2): 121–127.
- Ingestad T, Lund AB. 1979. N stress in birch seedlings. I. Growth technique and growth. *Physiol. Plant.* 45: 137–148.
- Iwasa Y, Roughgarden J. 1984. Shoot/root balance of plants: Optimal growth of a system with many vegetative organs. *Theoretical population biology* 25: 78–105.
- Scaife A. 1989. A pump/leak/buffer model for plant nitrate uptake. *Plant and Soil* 114: 139–141.
- Seginer I, Shina G, Albright LD, Marsh LS. 1991. Optimal temperature setpoints for greenhouse lettuce. *Journal Agricultural Engineering Research* 49: 209–226.
- Seginer I, Buwalda F, van Straten G. 1998. Nitrate concentration in greenhouse lettuce: A modelling study. *Acta Horticulturae* 456: 189–197.
- UK Ministry of Agriculture, Fisheries and Food. 1997. 1996/97 UK monitoring programme for nitrate in lettuce and spinach. *Food Surveillance Information Sheet.* 1997, No 121, 1–10. HMSO Publications Center, London, UK.