Accurate simulation models for short-term (≈hours) changes in hydroponic crop growth and nitrate uptake are needed for rapid fault detection in hydroponic systems. Comparison between model-predicted and measured values for crop growth and nitrate uptake is proposed as the basis for such a fault detection system. To this end, the Nitrate Control in Lettuce (NiCoLet) model was used to evaluate both short- and long-term changes in growth and nitrate accumulation. Three replicated experiments were conducted with lettuce (Lactuca sativa L. cv. Flandria), including: (1) plants subjected to either low or high nitrate concentration treatments for model calibration; (2) collection of growth data every 2 days for model validation; and (3) frequent (every 4 mol m$^{-2}$ of accumulated light) collection of growth and shoot nitrate concentration data to validate short-term predictions. After a minor modification (maximum nitrogen uptake rate restricted) and calibration, the NiCoLet model accurately simulated lettuce crop growth and nitrate uptake on a long-term basis and provided evidence of short-term behaviour, including statistically significant predictions of diurnal patterns. This is a first step in realising fault detection systems based on mechanistic simulation models.

1. Introduction

Using crops to recycle carbon dioxide and generate oxygen is a promising strategy for maintaining sustainable regenerative life support systems in closed habitats for space exploration (Wheeler et al., 2001). This study was designed to address fault or problem detection in hydroponic crop production systems, which is one of the main bio-regenerative system components being considered by the National Aeronautics and Space Administration (NASA). A fast and reliable fault detection system is necessary to warn the crew before there is irreversible crop damage in the hydroponic production system, as well as its possible effects on the entire life support system. Comparison between model-predicted and measured values for crop growth and nitrate uptake is proposed as the basis for such a fault detection system. Frequent (e.g. 1–2 days) plant harvests meet the NASA objective of producing salad-type crops for supplementing the crew’s diet in a space station or long duration mission, as well as providing plant fresh mass growth data for model comparison. Although plant tissue nitrate concentration data is needed to calibrate the model, once calibrated, measurement of nitrate uptake from solution using, for example, nitrate electrodes could be implemented for real-time fault detection. Alternatively, addition of individual nutrients is possible using measurements of electrical conductivity, pH, nutrient ratios, and the quality of the source water (Savvas, 2002). Short-term (≈hours), accurate
models for hydroponic crop growth and nitrate uptake are the first step in achieving a fault detection system. Many lettuce growth simulation modelling efforts were reviewed by Pearson et al. (1997). As outlined, Sweeney et al. (1981) defined for the first time a structural and storage carbon pool in the plant following the approach of de Wit, which has been more recently described in Bouman et al. (1996). The two state variables were the structural and non-structural dry matter. Prior to this study, Soribe and Curry (1973) considered dry mass production as the difference between photosynthesis and respiration, and Wheeler et al. (1993) used the work to develop a simple model for lettuce growth at elevated carbon dioxide concentrations. The model presented by Wheeler et al. was later modified by Pearson et al. (1997) to incorporate the mechanistic features of Sweeney et al. (1981). More recently, Critten (1991) and van Henten (1994) adapted the Sweeney et al. (1981) model for greenhouse conditions. Spitters et al. (1989) developed the Simple and Universal Crop Growth Simulator (SUCROS87), which used total dry mass as the state variable. SUCROS87 was later used by Ascher (1993) and Both (1995) to develop greenhouse lettuce models, and is the starting point for the crop model HORTISIM—a greenhouse climate and crop growth simulator (Gijzen et al., 1998).

Based on the work of Sweeney et al. (1981), Seginer et al. (1991), and van Henten (1994), Seginer et al. (1998) developed the Nitrate Control in Lettuce (NiCoLet) model with follow-up versions in Seginer et al. (1999), Seginer (2003, 2004), and Seginer et al. (2004). The NiCoLet model was developed to determine climate control strategies that would prevent high nitrate
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concentration in lettuce (Ioslovich & Seginer, 2002a; de Graaf et al., 2004; Lopez Cruz et al., 2003). Accordingly, this model predicts crop growth (dry and fresh matter accumulation) and shoot nitrate concentration under normal and nitrogen-deficient conditions.

The NiCoLet model is described by continuous differential equations, and can therefore be used to calculate expected ‘instantaneous’ crop growth and nitrate uptake. However, the NiCoLet model was developed to predict mid- to long-term shoot growth and nitrate concentration, and its ability to predict correctly short-term changes has not been tested experimentally. A model predicting correctly the short-term effects of sudden changes in the root zone is one of the elements needed to develop a fault detection system for hydroponic production systems. The primary objective of this study is to investigate the ability of the NiCoLet model to simulate short-term shoot growth and nitrate uptake as well as identify model components that may require modification.

2. Model description

The NiCoLet model, as described in Seginer (2003), is a carbon balance model in which photosynthesis, growth, and a mechanism for maintaining turgor pressure are used to predict growth as well as the dynamic fluctuations of soluble carbon and nitrate. The concentration of primary assimilates (mostly carbohydrates and organic acids) are determined by the balance between source activity (carbon assimilation) and sink activity (processing of assimilates). Source activity is the supply of soluble carbon by photosynthesis (controlled in the model by light, carbon dioxide, and size of the structural compartment), and sink activity is the demand of soluble carbon by growth and maintenance respiration (controlled in the model by temperature and the size of the structural compartment).

As shown in Fig. 1, the model has three compartments, namely the metabolically active ‘structure,’ the soluble raw-material buffer referred to as the ‘vacuole’, and the ‘excess-C’ compartment that stores excess carbon. The carbon in the ‘structure’ compartment is assumed to correlate to nitrogen and water within the compartment while the carbon in the ‘excess-C’ compartment is not correlated to nitrogen or water. The ‘excess-C’ compartment allows growth to continue when nitrogen supply is low and assimilated carbon cannot be matched by nitrogen.

The balance between the source and sink activity determines the soluble non-structural carbon and nitrate concentrations, which complement each other to maintain a constant osmotic pressure. Such a complementary role for soluble non-structural carbon and nitrate has been reported in numerous studies (Blom-Zandstra & Lampe, 1985; Blom-Zandstra et al., 1988; Behr & Wiebe, 1988, 1992; Drews et al., 1995; Buwalda & Warmenhoven, 1999). In these studies, nitrate concentration has been found to vary with concentrations of soluble non-structural carbon, thereby incorporating a regulating feedback for nitrate uptake related to the osmotic pressure in the plant. However, this relation ship has not been strongly supported by all data sets examined, and the regulation mechanism has not been characterised (Buwalda & Warmenhoven, 1999).

The balance equations of the model are shown in Eqns (1)–(5) and the fluxes of carbon and nitrogen are shown schematically in Fig. 1.

\[
\frac{dM_{Ce}}{dt} = F_{CP} - F_{Cex} - F_{Ca} - F_{Cm} + F_{Cve} \quad (1)
\]

\[
\frac{dM_{Cs}}{dt} = F_{Ccs} \quad (2)
\]

\[
\frac{dM_{Ce}}{dt} = F_{Cve} - F_{Cev} \quad (3)
\]

\[
\frac{dM_{Ne}}{dt} = F_{Nnu} - F_{Nve} \quad (4)
\]

\[
\frac{dM_{Ns}}{dt} = F_{Nvs} \quad (5)
\]

In these equations, \(M_C\) and \(M_N\) denote the content of carbon and nitrogen, respectively, per unit ground or growth area in mol m\(^{-2}\) and the subscripts \(v\), \(s\), and \(e\) refer to the ‘vacuole’, ‘structure’, and ‘excess-C’ compartments.

**Fig. 1.** Schematic (Seginer, 2003) of the three compartments of the model, ‘excess-C’ (\(e\)), ‘vacuole’ (\(v\)), and ‘structure’ (\(s\)), with corresponding mass of carbon (\(M_C\)) and nitrogen (\(M_N\)); flux of nitrogen from the environment to the ‘vacuole’ represents nitrate uptake (\(F_{Nu}\)); photosynthesis (\(F_{Cp}\)) provides the flux of carbon to the ‘vacuole’, and maintenance (\(F_{Cm}\)) and growth (\(F_{Cg}\)) respiration account for the flux of carbon from the ‘vacuole’; carbon (\(F_c\)) and nitrogen (\(F_N\)) flux terms have lower case subscripts denoting the origin and destination compartments.
compartments, respectively. For example, the carbon flux from the ‘vacuole’ to the ‘structure’ compartment is expressed as $F_{Cv}$ and the nitrogen uptake rate is expressed as $F_{Nu}$. The flux for photosynthesis, growth respiration, and maintenance respiration are $F_{Cp}$, $F_{CP}$, and $F_{CN}$ in mol m$^{-2}$s$^{-1}$, respectively. All carbon flux equations are described in detail in the Appendix.

A constant nitrogen-to-carbon (N:C) ratio and water content $V$ in m$^3$ m$^{-2}$ [ground] are assumed within the ‘structure’, and are described in Eqs (6) and (7), respectively, where $r_N$ in mol [N] mol$^{-1}$ [C] is the N:C ratio and $\lambda$ in m$^3$ [H$_2$O] mol$^{-1}$ [C] is the volume of water per mole of structural carbon.

$$M_{Ns} = r_N M_{Cs}$$ (6)
$$V = \lambda M_{Cs}$$ (7)

The osmotic balance is expressed in Eqn (8), where $\beta_C$ in m$^{-1}$ [H$_2$O] kPa mol$^{-1}$ [C] and $\beta_N$ in m$^{-1}$ [H$_2$O] kPa mol$^{-1}$ [N] are the osmotic pressures associated with one unit of vacuolar carbon and nitrogen, respectively and $\Pi$ in kPa is the total osmotic pressure, which is assumed to be constant.

$$\beta_C M_{Cs} + \beta_N M_{Ns} = \lambda \Pi M_{Cs}$$ (8)

3. Materials and methods

3.1. Seedling production

Lettuce (Lactuca sativa L. cv. Flandria) seedlings were grown for 14 days in a growth chamber using deep trough hydroponic cultivation. A single lettuce seed was sown in a Peatlite (15 l of sifted vermiculite, 15 l of sifted peat moss, and 70 m$^3$ of fine dolomitic limestone) filled hole (diameter 1 cm, depth 2 cm) in a Rockwool cube (16×4 cm$^3$), and finally covered with a light layer of Peatlite (thickness 2 mm). The seeds were sprayed with reverse-osmosis water and maintained in a high-humidity chamber for 2 days. Upon germination, the seedlings were transferred to the hydroponic system and floated using foam insulation board. The nutrient solution recipe was transferred to the hydroponic system and floated using a Rockwool cube. Upon germination, the seedlings were reverse-osmosis water and maintained in a high-humidity chamber with a relative humidity averaged 72±7% for the experiments. The greenhouse temperature (day and night set points 24 and 19°C, respectively) and light integral (16 mol m$^{-2}$ day$^{-1}$) were controlled using a custom computer control program in which supplemental lighting was used as needed to reach the daily light integral (Albright et al., 2000). The greenhouse relative humidity was not controlled; however, the daily relative humidity averaged 72±7% for the experiments. The root zone temperature was continuously maintained above 5 mol l$^{-1}$ using a mechanical aerator in the large tank and pure oxygen injection in tanks 1–3. The nutrient solution was cooled using a heat exchanger, which prevented the root zone temperature from exceeding 27°C.

3.2. Experimental design

Three replicated experiments were conducted, including: (1) the ‘nitrate experiment’; (2) the ‘2-day harvest experiment’; and (3) the ‘frequent harvest experiment’.

In the ‘nitrate experiment’, the plants were grown in a nutrient solution initially containing 0 (low nitrate treatment) or 9.5 mM nitrate (high nitrate treatment). Shoot growth and nitrate concentration data were obtained at plants age 14, 18, 20, 22, and 24 days after seeding (DAS). During the ‘2-day harvest experiment’, growth data were collected every 2 days, starting at plant age 14 DAS until age 32 DAS. During the ‘frequent harvest experiment’, shoot growth and nitrate concentration data were collected frequently (every 4 mol m$^{-2}$ of accumulated light or approximately 4 h) at plants age 18 and 22 DAS with a final data point at a plant age of 26 DAS. Table 1 shows the harvest schedule of the ‘frequent harvest experiment’, as well as the planting densities for all three experiments.

3.3. Cultivation method

As shown in Fig. 2, the experiments were conducted in a greenhouse in which one large deep flow trough (DFT) hydroponic tank (large tank) was used for the ‘2-day and frequent harvest experiments’, and three DFT hydroponic tanks (tanks 1–3) were used for the ‘nitrate experiment’. The greenhouse temperature (day and night set points 24 and 19°C, respectively) and light integral (16 mol m$^{-2}$ day$^{-1}$) were controlled using a custom computer control program in which supplemental lighting was used as needed to reach the daily light integral (Albright et al., 2000). The greenhouse relative humidity was not controlled; however, the daily relative humidity averaged 72±7% for the experiments (for example, of the ‘2-day harvest experiment’ was the only exception with an average of 49±13%). The pH (5.8) and electrical conductivity (120 mS m$^{-1}$) were manually adjusted daily, while the dissolved oxygen was continuously maintained above 5 mol l$^{-1}$ using a mechanical aerator in the large tank and pure oxygen injection in tanks 1–3. The nutrient solution was cooled using a heat exchanger, which prevented the root zone temperature from exceeding 27°C.

3.4. Experimental procedures

For each experiment, seedlings were transplanted into the greenhouse at plant age 14 DAS. A batch hydroponic production system was used, in which all plants in
Plant densities for experiments and harvest schedule for the ‘frequent harvest experiment’: 0 mol m\(^{-2}\) of light integral corresponds to a measurement taken at dawn and 16 mol m\(^{-2}\) of light integral corresponds to a measurement taken at the end of the light period; for the ‘frequent harvest experiment’, 20 min data for light integral and air temperature as well as more frequent data for plant density were used as compared to the daily values used in the ‘nitrate and 2-day harvest experiments’

<table>
<thead>
<tr>
<th>Plant age days after seed (DAS)</th>
<th>Plant density *1 Plants m(^{-2})</th>
<th>Light integral mol m(^{-2})</th>
<th>Light integral mol m(^{-2})</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>180</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>17</td>
<td>180</td>
<td>16</td>
<td>—</td>
</tr>
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<td>131</td>
<td>0, 4, 8, 12, 16</td>
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<td>96</td>
<td>4, 8, 12, 16</td>
<td>0, 8, 12, 16</td>
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<td>22</td>
<td>76</td>
<td>—</td>
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<tr>
<td>30</td>
<td>41</td>
<td>—</td>
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</tr>
</tbody>
</table>

*The low nitrate treatment was re-spaced as needed to achieve a closed canopy.

*1The plant density remained unchanged for 2 days starting at 17:00h.

**Table 1**

**Fig. 2.** Schematic (not to scale) of experimental greenhouse (8.5 x 10.7 m with ridge orientation east–west) in which deep flow trough (DFT) hydroponic production systems with circulation pumps were used; glass clad greenhouse had staged ventilation rates, 20 high-pressure sodium lamps (400 W each), temperature and relative humidity sensors in an aspirated box, and a quantum sensor measuring photosynthetically active radiation located at canopy height; sensors were logged by the environmental control computer system every 2 min

a given tank were the same age at a given time. Starting at age 18 days, some of the plants were harvested and the remaining plants were re-spaced, with re-spacing occurring every 2 days except in the low nitrate treatments, where growth was reduced and re-spacing was done only as needed. The plant density, as indicated in Table 1, was selected to have the plant canopy closed or nearly closed for the entire experiment.

The nutrient solution for the experimental period was the same as described for seedling production, except for the low nitrate treatment. In order to achieve low nitrate solution, it was necessary to use calcium sulphate
A nitrate stock solution (71.4 mM) was prepared for the low nitrate treatment using calcium nitrate, which was added daily following the addition rate technique (Hellgren & Ingestad, 1996). The nitrate addition at day zero \(D_0\) in mol [N] plant\(^{-1}\) or 14 DAS was calculated, as shown in Eqn (9), using the fresh weight at plant age 14 days \(\Theta_{FM,0}\) in g [FM] plant\(^{-1}\), the critical nitrogen concentration in the plant tissue \(\omega\) in mol [N] g\(^{-1}\) [FM], and a pre-determined nitrogen addition rate \(R\) in % of nitrate demand for normal growth.

\[
D_0 = \Theta_{FM,0} \frac{\omega}{R} \quad (9)
\]

The critical nitrogen concentration in the plant tissue has been reported to vary between 0.06–0.15 mol [N] g\(^{-1}\) FM (Linker et al., 2004); 0.15 mol [N] g\(^{-1}\) [FM] was used in this study. The nitrogen addition rate \(R\) was set at 12% of the nitrate demand for normal growth. The daily addition \(D_{d+1}\) in mol [N] plant\(^{-1}\) was calculated using the total number of plants tank\(^{-1}\) \(N\) on subsequent days as shown in Eqn (10).

\[
D_{d+1} = D_0 e^{R \cdot N} \quad (10)
\]

Complete nutrient analysis of the hydroponic nutrient solution of each treatment was performed at the end of the experiment, and all concentrations were found to be within acceptable ranges (Resh, 1993). However, it was found that there was residual nitrate (0.37 mM) at the beginning of the first replicate of the low nitrate treatment. This was most likely due to impurities in the agricultural grade fertilisers used to make the stock solutions. In addition, an incorrect addition rate (based on 100 plants instead of 98) was used for the first 6 days of the first replicate. Both sources of excess nitrate were accounted for in the simulation model.

### 3.5. Nitrate and carbon determination

Plants were harvested in each experiment according to the frequency described in the experimental design section. Shoot samples were either freeze-dried or oven-dried (70 °C). Shoot nitrate concentration was determined in both freeze-dried and oven-dried samples based on an Environmental Protection Agency method (EPA Method 300.1, 1993; Dionex Application Note 135, 2001). Dried tissue was ground using a Wiley Mill to pass through a 40 mesh screen and stored in a vacuum chamber with desiccant. Homogenised dried lettuce tissue powder was weighed (50 or 100 mg) and placed in an extraction cup. The samples were extracted with de-ionised water (5 or 10 ml) for at least 4 h on a rotary platform shaker. The samples were filtered through a 0.45-μm syringe filter and diluted 10 times with de-ionised water. Nitrate concentration in the extracts was determined using a Dionex DX-500 chromatograph system equipped with an analytical column (AS14-HC, 4 mm diameter and 250 mm length), a conductivity detector, and a temperature-controlled (8 °C) auto-sampler. The injection volume was 25 μl and the eluent was a solution of 3.5 mM sodium carbonate (Na\(_2\)CO\(_3\)) and 1.0 mM sodium bicarbonate (NaHCO\(_3\)) at a flow rate of 1.2 ml/min\(^{-1}\).

Soluble carbohydrate and malic acid were quantified in the freeze-dried tissue of the nitrate experiment. Homogenised freeze-dried lettuce tissue powder was weighed (20–25 mg) in a centrifuge tube. Next, 3 ml of 80% ethanol was added, which was followed by mixing (vortex), incubating for 10 min at 80 °C, and centrifuging at 3000 min\(^{-1}\) for 5 min. The supernatant was then transferred to a 15 ml tube, and this procedure was repeated two more times. The supernatant was combined in the tube and adjusted to a final volume of 10 ml with 80% ethanol and stored in a freezer (−80 °C) until analysis.

Before analysis, the samples were allowed to warm to room temperature, mixed, and centrifuged at 3000 rpm for 5 min. One millilitre of extract was transferred to a vortex-evaporation tube, in which the solvent was evaporated to dryness under vacuum at 40 °C. The dried extract was reconstituted in 5 ml or 2.5 ml de-ionised water for the determination of soluble carbohydrate or malic acid, respectively. After thorough mixing, the solution was filtered through a 0.45-μm filter into a high pressure liquid chromatography (HPLC) auto-sampler vial. Soluble carbohydrate and malic acid were also analysed using the Dionex DX-500 chromatography system. The separation of soluble carbohydrate was carried out on a CarboPac (PA-10) column using 52 mM carbonate-free sodium hydroxide at 1.3 ml/min\(^{-1}\) (Dionex Technical Note 20, 1989). A Dionex ion exclusion column (IonPac, ICE-AS6, 9 mm diameter and 250 mm length, 46023) with a guard column of the same packing material and an anion micro-membrane suppressor (AMMS-ICEII) were used in order to quantify malic acid in the extracts (Dionex Technical Note 135, 2001). The suppressor was operated in an external mode using 5 mM tetrabutyl ammonium hydroxide as the external regenerant. The eluent was 0.4 mM heptfluorobutyric acid, and set at a flow rate of 1.0 ml/min\(^{-1}\). Sample size for both carbohydrate and malic acid analyses was 25 μl.
All statistical evaluations were subjected to analysis of variance followed by Tukey’s multiple range tests with probability $P$ of 0.05 or 5% level of significance.

4. Results and discussion

4.1. Preliminary analysis

Although experimental data has been used in previous studies for calibrating the NiCoLet model (van Straten et al., 1999; Linker et al., 2004; Linker & Johnson-Rutzke, 2005), re-calibration of the model using the data collected in this study was necessary for two reasons: (1) the plant density in the current study was such that crowding was experienced throughout the growth cycle (canopy closure) rather than just before re-spacing or harvesting (Ioslovich et al., 2002b); and (2) the maximum air temperature in the current study was frequently greater than 24 °C (Broady et al., 2003).

To establish whether the continuously closed canopy had a detrimental effect on crop growth, growth data were compared with data collected using two plant densities used in Mathieu (2004) were 37 and 97 plants m$^{-2}$, respectively. The plant densities for the ‘2-day harvest experiment’ are shown in Table 1. Figure 3 shows the growth curves and second-order exponential polynomial regression equations (Both, 1995; Albright et al., 2000) for both sets of data. Plants from the current study were slightly smaller; specifically, the yield at age 32 DAS was 3-2% lower compared to that in Mathieu (2004). Such a reduction was minimal and indicates that the re-spacing procedures of the current study did not greatly affect growth. Therefore, using a production system that has a closed or nearly closed canopy (efficient use of growth space) throughout the growth cycle does not significantly affect growth.

Preliminary simulations using the model described in Linker et al. (2004) without re-calibration showed that the low nitrate treatments were simulated accurately by the first sample day (18 DAS); however, the high nitrate treatments were not simulated accurately. A rapid increase of nitrate concentration was simulated, reaching approximately 1.5 mmol [N] g$^{-1}$ [dry matter, DM] (Fig. 4), while the experimental data demonstrated a much more gradual increase. A detailed analysis of the simulation results showed that the rapid nitrate increase predicted by the model was due to unrestricted nitrogen uptake in the model. Consequently, the model was modified to restrict the maximum nitrogen uptake rate into the ‘structure’ $S$. The rate $F_{Nu}^S$ in mol [N] m$^{-2}$ s$^{-1}$ was made a function of the size of the carbon content in the structural compartment $MC_s$ in mol [C] m$^{-2}$, as shown in Eqn (11).

$$F_{Nu}^S = \phi \left[1 - e^{-\Psi}MC_s\right]$$

Equation (11) contains the potential uptake rate for a fully developed root system $\phi$ in mol [N] m$^{-2}$ s$^{-1}$ and a measure of effective root size $\Psi$ in m$^2$ mol$^{-1}$ [C]. This formulation is a simplification of the model used by Linker and Johnson-Rutzke (2005), and implicitly assumes a constant root-to-shoot ratio. Prior to Linker and Johnson-Rutzke (2005), there was no relationship between root size and nitrogen uptake capacity in the NiCoLet model. Nitrate uptake had been assumed to be unlimited.

![Fig. 3. Data and second-order exponential polynomial regression curves for cultivar Flandria from Mathieu (2004, ▲) and the current study (square), regression equations were used to estimate the shoot dry mass—the yield in the current study was only 3.2% lower than Mathieu (2004) at 32 DAS.](image)

![Fig. 4. Shoot nitrate concentration data and preliminary simulation results using the model of Linker et al. (2004) for the ‘nitrate experiment’ (not re-calibrated); 95% confidence intervals are shown using error bars; low nitrate treatment data (■) and simulation results (••••) and the high nitrate treatment data (▲) and simulation results (••••) are shown for both replicates, with replicate 1 in light grey.](image)
in NiCoLet modelling studies, which allowed unrealistically high nitrate uptake for plants with small root systems (Fig. 4). The results of this study supports limiting nitrate uptake based on the size of the structural compartment.

4.2. Model calibration using the nitrate experiment

Figure 5 shows the relationship between nitrate and carbon (glucose, sucrose, and malic acid) in the ‘vacuole’ for the present experiment, as well as for data from the literature (Blom-Zandstra et al., 1988; Behr & Wiebe, 1988; Linker et al., 2004). Other studies could not be used in Fig. 5 due to insufficient data reported (Blom-Zandstra & Lampe, 1985; Behr & Wiebe, 1992; Drews et al., 1995; Buwalda & Warmenhoven, 1999; Linker & Johnson-Rutzke, 2005). In Fig. 5, nitrate is graphed with the sum of glucose, sucrose, and malic acid concentrations. The results of the current study are within range of the previous studies, except for the high carbon concentrations (~65–110 mol [C] m\(^{-3}\)) observed in the current study. There were procedural differences in measuring nitrate and carbon concentrations among the studies included in Fig. 5. Differences in the relationship between carbon and nitrate could, therefore, be due to different measurement techniques or differences in the total osmotic pressure in the plant at the time of sampling. In the NiCoLet model, the osmotic pressure is assumed constant, but future work should include measurements of total osmotic pressure at the time of sampling to validate this assumption.

The results of the ‘nitrate experiment’ are shown in Fig. 6. The dry mass from the two replicates of the high nitrate treatment are highly reproducible as indicated by the 95% confidence interval error bars [Fig. 6(b)]. However, the low nitrate treatment ‘replicates’ are not true replicates due to excess nitrate in the first replicate. The measured ‘impurity’ nitrate in the initial nutrient solution and the incorrect addition rate used (see Section 3.4.) were both accounted for in the simulation. This does, however, account for the increased growth observed in the first replicate of the low nitrate treatment as compared to the second replicate [Fig. 6(a)].

In the ‘low nitrate treatment’, nitrate was added to the nutrient solution at a rate of 12% of the estimated nitrogen demand by adding the stock nitrate solution. Therefore, the plants had adequate nitrate supply until 14 DAS and then the treatment started in which the nitrate stock solution was added daily. The nitrate treatment did not affect dry mass accumulation until at least 6 days after applying the treatment [after 20 DAS as shown in Figs 6(a) and (b)]. Nitrogen in the form of amino acids, not nitrate, has been found to be transported from mature leaves to the apex in the phloem (van Helden et al., 1994), and Peuke et al. (1994) found an increase in phloem amino acid concentration under low nitrate conditions. However, the dry matter content, as shown in Figs 6(c) and 6(d), differed from the first sample day (plant age 18 DAS). In terms of the model, such an increase in dry matter content is due to the accumulation of excess carbon (e.g. starch) that is not matched by water. In the ‘low nitrate treatments’, the dry matter content increased with plant age. The shoot nitrate concentration also differed from the first treatment sample day [Figs 6(e) and (f)], and, as expected, the low nitrate conditions enhanced root growth (data not shown).

Model calibration was performed using the data from both replicates of the ‘nitrate experiment’ (Fig. 6). For the simulation, the osmotic pressure associated with one unit of carbon \(\beta_c\) and the osmotic pressure in the ‘vacuole’ compartment \(\Pi\) were estimated from the nitrate-carbon correlation observed in the experimental data using Eqn (8). Although the equation appears to have three parameters, one of the parameters can be set arbitrarily; thus, \(\beta_w\) was set to 6.0 m\(^3\) [H\(_2\)O] kPa mol\(^{-1}\) [N] as was proposed by Linker et al. (2004). In determining this relationship, the results were presented in terms of mol [C] m\(^{-3}\), where the m\(^{-3}\) was interpreted as the non-structural buffer cell water or cell sap calculated as the difference between fresh and dry mass. The soluble carbon concentration was calculated using
molar equivalence, in which fructose and glucose contain 6 mol [C] mol⁻¹, sucrose contains 12 mol [C] mol⁻¹, and malic acid contains 4 mol [C] mol⁻¹.

Calibration of the model was done following the procedure described by Linker et al. (2004), which relies on sensitivity analysis to determine the parameters that need to be adjusted. A simplified sensitivity analysis is available at www.technion.ac.il/~linker/nicolet/, with the website description given in Linker et al. (2005). The values of the parameters used are listed in Table 2. With few exceptions, the values are the same as reported in Linker et al. (2004). The parameters that were adjusted were the maintenance respiration rate, potential nitrogen uptake rate, and photosynthesis inhibition function border. In addition, the photosynthetic efficiency was adjusted after preliminary runs. Ioslovich and Seginer (2002) showed that the relative magnitude of the maintenance and growth respiration needed to be adjusted when fitting the NiCoLet model to data obtained under different spacing regimes, and this may explain the need to calibrate the maintenance respiration in the current study. Lopez-Cruz et al. (2004) reported that the maintenance respiration coefficient, the radiation extinction coefficient, and the uninhibited growth rate coefficient affected the NiCoLet model performance; the data in the current study supports this finding. In addition to these parameters the potential nitrogen uptake rate, which was previously estimated from the literature, was adjusted using the sensitivity analysis in the current study.

The predictions of the calibrated model are shown in Fig. 6. The calibrated model accurately predicted dry matter accumulation [Fig. 6(b)], dry matter content or equivalently fresh matter accumulation, [Figs 6(c) and (d)],
and shoot nitrate concentration [Figs 6(e) and (f)]. An exception was the dry matter accumulation in the ‘low nitrate treatment’ [Fig. 6(a)], which was greater than that predicted by the model. This may have been due to excess nitrate that was not accounted for in the simulation. In the ‘low nitrate treatment’ [Fig. 6(e)], there was a fast drawdown of simulated shoot nitrate concentration immediately after applying the treatment. The first replicate shows sustained levels of simulated shoot nitrate concentration for one day while the ‘impurity’ nitrate, mentioned above, was available. The simulated shoot nitrate concentration in the plants of the ‘high nitrate treatment’ [Fig. 6(f)] had both a diurnal oscillating pattern and continued to increase throughout the growth cycle.

4.3. Model validation using the ‘2-day and frequent harvest experiments’

Figure 7 shows the measured and predicted shoot dry mass [Fig. 7(a)] and dry matter content [Fig. 7(b)] using the calibrated model for the ‘2-day harvest experiment’. There was good agreement, not only between the measurements between the two replicates, but also between the measured and predicted values. The lower average relative humidity (49% versus 72%) in the first replicate of the ‘2-day harvest experiment’ (Section 3.3.) did not appear to affect crop growth.

Figure 8 shows the simulated and measured shoot dry mass [Fig. 8(b)], dry matter content [Fig. 8(b)], and nitrate concentration [Fig. 8(b)] for the ‘frequent harvest experiment’. As shown in Fig. 8, the measured and predicted values were in good agreement. For the harvests centered around 19 DAS [Fig. 9(a)], using the combined data from both replicates, growth differences were statistically significant (Tukey’s multiple range test, probability $P = 0.05$) twice during the day. The harvests done at 0 and 4 mol m$^{-2}$ were statistically different from the harvests done at 12 and 16 mol m$^{-2}$. For the harvests between 22 and 23 DAS [Fig. 10(a)], growth differences were also statistically significant (Tukey’s multiple range test, probability $P = 0.05$) twice during the day. The harvests done at 0, 4, and 8 mol m$^{-2}$ of accumulated light were statistically different from the harvests done at 12 and 16 mol m$^{-2}$. This shows that it is possible to

### Table 2

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>For photosynthesis</strong></td>
<td></td>
</tr>
<tr>
<td>Light extinction coefficient $a$, m$^2$ [ground] mol$^{-1}$ [C]</td>
<td>1.7</td>
</tr>
<tr>
<td>Photosynthetic efficiency $e$, mol [C] mol$^{-1}$ [PAR]</td>
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</tr>
<tr>
<td>Leaf conductance to CO$_2$ $\sigma$, m s$^{-1}$</td>
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<tr>
<td>Photosynthesis inhibition function border $b_p$</td>
<td>0.9826</td>
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<tr>
<td>Photosynthesis inhibition function slope $s_p$</td>
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</tr>
<tr>
<td>Photosynthetic excess interpolation coefficient $\xi$</td>
<td>0.3</td>
</tr>
<tr>
<td><strong>For growth</strong></td>
<td></td>
</tr>
<tr>
<td>Structural growth coefficient $v$</td>
<td>20</td>
</tr>
<tr>
<td>Maintenance respiration rate at 20 °C $k$, mol [C] m$^{-2}$ [ground] s$^{-1}$</td>
<td>$3 \times 10^{-7}$</td>
</tr>
<tr>
<td>Respiration coefficient $c$, K$^{-1}$</td>
<td>0.0693</td>
</tr>
<tr>
<td>Growth respiration as fraction of growth $\theta$</td>
<td>0.3</td>
</tr>
<tr>
<td>Structural inhibition function border $b_s$</td>
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<tr>
<td>Structural inhibition function slope $s_s$</td>
<td>10</td>
</tr>
<tr>
<td><strong>Osmotic balance</strong></td>
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<tr>
<td>Osmotic pressure per mole of carbon $\beta_C$, m$^3$ [H$_2$O] kPa mol$^{-1}$ [C]</td>
<td>0.6</td>
</tr>
<tr>
<td>Osmotic pressure per mole of nitrogen $\beta_N$, m$_1$ [H$_2$O] kPa mol$^{-1}$ [N]</td>
<td>6.0</td>
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<tr>
<td>Osmotic pressure in ‘vacuole’ compartment $\Pi$, kPa</td>
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<tr>
<td><strong>Nitrogen uptake</strong></td>
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<tr>
<td>Potential nitrogen uptake rate $\phi$, mol [N] m$^{-2}$ [ground] s$^{-1}$</td>
<td>$1.54 \times 10^{-6}$</td>
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<tr>
<td>Exponent of effective root size $\Psi$, m$^2$ [ground] mol$^{-1}$ [C]</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>Miscellaneous</strong></td>
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<tr>
<td>Volume of water per mole of structural C $\lambda$, m$^3$ [H$_2$O] mol$^{-1}$ [C]</td>
<td>0.75 $\times 10^{-3}$</td>
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<tr>
<td>Nitrogen to carbon ratio in ‘structure’ $r_N$, mol [N] mol$^{-1}$ [C]</td>
<td>0.11</td>
</tr>
<tr>
<td>Parameter for carbon unit conversion $\varepsilon_C$, kg [DM] mol$^{-1}$ [C]</td>
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</tr>
<tr>
<td>Parameter for nitrogen unit conversion $\varepsilon_N$, kg [DM] mol$^{-1}$ [N]</td>
<td>0.148</td>
</tr>
</tbody>
</table>
get a statistically significant increase in growth two times a day through plant age 22 DAS using a sample size of 30 plants. This demonstrates that sufficient biomass accumulation occurred within a single day and could be used as an indicator in a fault detection system.

There were three periods in which harvests were done at the lights-out time and dawn the following morning as indicated by the dark period labels in Fig. 9. There was no significant difference in dry mass between these samples, showing that dry mass did not increase measurably during the dark period. The growth simulation results, also shown in Fig. 9(a), indicate a greatly reduced rate of growth during the dark period. The results for plants age 22 DAS are shown in Fig. 10(a); the dry mass increases throughout the day (dawn to lights-out time) for plants age 22 DAS. The NiCoLet growth model simulated the trend of diurnal growth accurately as illustrated best in Fig. 9(a).

The change in shoot nitrate concentration centred on 19 DAS is shown in Fig. 9(b), and demonstrates a diurnal pattern of nitrate concentration. Between the first two dark periods there is a decrease in nitrate concentration throughout the light period, which is due to the increase of nitrate assimilation during the light period. The first sample (0 mol m⁻²) and the last sample (16 mol m⁻²) are significantly (Tukey’s multiple range test, probability \( P = 0.05 \)) different for replicate 1, showing decreasing nitrate concentration during the light period. In the middle dark period of Fig. 9(b), there is a significant (Tukey’s multiple range test, probability \( P = 0.05 \)) increase in nitrate concentration during the dark period for replicate 1. These data suggest that there is a diurnal pattern of high shoot nitrate concentrations in the morning, decreasing nitrate concentration throughout the day, and increasing nitrate concentration during the night. This is due to the decrease of nitrate reductase activity during the dark in which uptake exceeds assimilation and results in nitrate accumulation. In terms of the source–sink relationship, during the light period the carbon production from photosynthesis fills the ‘vacuole’, reducing the need for nitrate. However, during the dark period the carbon stored in the ‘vacuole’ is mobilised to produce ‘structure’, and, consequently, more nitrate is needed to maintain turgor pressure in the ‘vacuole’. The results for plants age 22 days are shown in Fig. 10(b); the shoot nitrate concentration was simulated more accurately for replicate 1 than replicate 2.

5. Conclusions

The objective of this study was to investigate the ability of the NiCoLet model to simulate short-term...
shoot growth and nitrate uptake in a spatially confined area, as well as identify model components that required modification. Using a hydroponic production system that has a closed or nearly closed canopy (e.g. efficient use of growth space) throughout the growth cycle did not affect growth significantly (3-2% reduction). However, it contributed to the need for model calibration, and in particular, the maintenance respiration rate. The results of this study supported limiting maximum nitrate uptake based on the size of the structural compartment, which prevented unrealistically rapid accumulation of nitrate in small plants.

The NiCoLet model was calibrated using the measured relationship between nitrate and carbon (glucose, sucrose, and malic acid) in the shoot. The nitrate and carbon data was mostly within the range of previously published work (carbon less than 65 mol [C] m$^{-3}$); however, carbon concentrations greater than 65 mol [C] m$^{-3}$ might be informed by measurements of total osmotic pressure during plant sampling in future studies. This would allow verification of the assumption that total osmotic pressure in the plant is constant. Calibration or adjustment of the following parameters was necessary: maintenance respiration rate, potential nitrogen uptake rate, photosynthesis inhibition function border, and photosynthetic efficiency. Besides the potential nitrogen uptake rate, this is a common list of parameters that must be fitted to specific environmental conditions prior to using the NiCoLet simulation model. A simplified sensitivity analysis is available at www.technion.ac.il/~linkerr/nicolet/.

There were three periods in which harvests were done at the lights-out time and dawn the following morning. There were no significant differences in dry mass between these samples, showing that dry mass did not increase measurably during the dark period (e.g. no growth). A decreasing nitrate concentration during the light period and an increase nitrate concentration during

Fig. 8. Shoot dry mass (a), dry matter content (b), and nitrate concentration (c) for the 'frequent harvest experiment', where ■ and Δ indicate measurements for replicates 1 and 2, respectively; 95% confidence intervals are shown using error bars; fresh and dry mass data consisted of at least 15 and 30 plants, respectively; 30 dried shoot samples from the frequent harvest experiment (each replicate) were divided into six samples (five shoots per sample) for nitrate determination; simulation results are shown as dashed and solid lines for replicates 1 and 2, respectively.
the dark period were shown to be statistically significant (Tukey’s multiple range test, probability $P = 0.05$). The NiCoLet simulation model captured this diurnal pattern of high shoot nitrate concentrations in the morning, increasing growth rate and decreasing nitrate concentration throughout the day, and decreasing growth rate and increasing nitrate concentration during the night. In terms of the source–sink relationship, carbon produc-
tion from photosynthesis fills the ‘vacuole’ during the light period, reducing the need for nitrate. During the dark period, the stored carbon is mobilised to produce structural material, and therefore nitrate uptake increases. These results are encouraging and constitute a first step towards the development of a model-based fault detection system.

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Sweeney D J; Hand D W; Slack G; Thornley J H M (1989). The flux equations defined in Eqns (A1)–(A5) are functions of the shoot environment, including light, temperature, and other environmental variables. The flux equations for the potential gross photosynthesis rate are defined by the mass of carbon in the ‘structure’ $M_{CV}$, and the normalised carbon concentration in the ‘vacuole’ $C_V$. The parameter $\xi$, which appears in Eqns (A1)–(A3), is an interpolation parameter (between 0 and 1) and controls the degree to which photosynthesis is effected by the inhibition function $h_p$. The flux equations for the potential gross photosynthesis and growth are formulated in Eqns (A6)–(A8), where $\varepsilon$ is the photosynthetic efficiency, $\sigma$ is the conductance to carbon dioxide, $k$ is the maintenance respiration rate at $T^*$, and $r$ and $c$ are constant coefficients.

\[ p[I, C_{CV}] = \frac{\varepsilon I \sigma C_{CV}}{\varepsilon I + \sigma C_{CV}} \]  
\[ g[T_a] = \varepsilon e^{\varepsilon e^{T_a - T^*}} \]  
\[ e[T_a] = k e^{\varepsilon e^{T_a - T^*}} \]  

The effect of crop size is modelled according to Eqn (A9), where $\alpha$ is the light extinction coefficient.

\[ f(M_{CV}) = 1 - e^{\varepsilon - a M_{CV}} \]  

The inhibition functions $h_p$ and $h_g$ are formulated in Eqns (A10) and (A11), where $h_p$, $b_p$, $s_p$, and $s_p$ control the shape of the inhibition functions.

\[ h_p[I_{CV}] = \frac{1}{1 + \left(1 - \frac{b_p}{1 - I_{CV}}\right)^s} \]  
\[ h_g[I_{CV}] = \frac{1}{1 + \left(\frac{b_g}{I_{CV}}\right)^s} \]