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# Modelling higher plants gas exchange in reduced gravity environment

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Long-duration human space missions and the establishment of permanent off-Earth bases (e.g. on the Moon or Mars) is one of the main focuses of today's space exploration. This poses many severe challenges at the life-support level, which needs to recycle atmosphere, water and waste for crew survival. The European Space Agency (ESA) project Micro-Ecological Life Support System Alternative (MELiSSA) can ensure these functions. It is a closed-loop bio-regenerative life-support system functioning with microorganisms and higher plants and providing a circular cycling of mass, including O<sub>2</sub> production, CO<sub>2</sub> capture, water recycling and food production. The growth and development of higher plants are strongly influenced by environmental conditions (e.g. gravity, pressure, temperature, relative humidity, partial pressure of O<sub>2</sub> or CO<sub>2</sub>) so bio-regenerative life support systems require a high level of control and management. The goal is to develop a mechanistic physical model of plant growth to predict the effects of microgravity or of a reduced gravity environment (like on Mars or on the Moon) on plant growth at its morphological, physicochemical and biochemical levels. Current existing plant growth models are developed for agronomy and are therefore not adapted for modeling plant growth for applications in life-support systems, which require being able to extrapolate plants behavior for a wide range of environmental conditions. The first mechanistic plant growth model developed in the framework of the MELiSSA project has attempted to address these limitations. Based on this work, a preliminary structure of the model was defined. In this presentation, the addition of gravity as a parameter is addressed, taking into account the altered gas exchanges due to the low or lack of free convection in reduced gravity environments. The influences of forced and free convection are studied according to the levels of gravity and the interdependence of low gravity and ventilation are addressed.

## I. Introduction

### A. Life-Support Systems and higher plants in space

**A**STRONAUTS going to Mars or further into the solar system will need food, water, and oxygen for the whole length of the mission - about 1000 days for a typical scenario of a mission to Mars<sup>1</sup>. Since sending all consumables from Earth or resupplying are not viable options<sup>2-4</sup>, sustainable solutions are key to succeed in future human space exploration. On the International Space Station (ISS), partial recycling is already a standard step for water and oxygen using physicochemical technologies<sup>5,6</sup>. However, these technologies cannot provide food to the astronauts; this can only be achieved with biological processes, i.e. by the use of microbial bioconversions and higher plants cultivation<sup>7,8</sup>. Future regenerative life-support systems (LSS) will likely be a combination of

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physicochemical technologies and biological processes to ensure matter and water recycling and oxygen and food production<sup>9</sup>.

## **B. Principles of mechanistic modelling and goal of our study**

In order to have efficient and reliable LSS and reduce the risk of system failure when humans embark on long trips into the solar system, it is crucial to be able to predict the behaviour of any recycling system whatever it is a combination of biological processes<sup>10</sup>. The European Space Agency (ESA) project Micro-Ecological Life-Support System Alternative (MELiSSA) is a closed-loop LSS inspired by a lake ecosystem and based on microorganisms and higher plants and aimed at sustaining humans on long-duration space travels<sup>11</sup>. The approach of this project is to develop a mechanistic model of the whole loop in order to achieve a deep understanding of underlying mechanisms and acquire a good knowledge about systems control. The three microbial compartments and the algae compartment of the MELiSSA loop are effectively modelled mechanistically<sup>12,13</sup>, while modelling efforts on the higher plants compartment are currently on-going<sup>14-16</sup>. The ground basis of such model was laid by Hézard in 2012 for Earth gravity conditions<sup>16</sup>. The aim of a mechanistic model of plant growth is to get a thorough multi-scale description of elementary mechanisms of plant growth accounting both for physical or biochemical phenomena, including transport phenomena and metabolic regulations. An integration synthesis then enables to go back to whole-system variables. Figure 1 gives an overview of the definition of mechanistic models in comparison to empirical models, when applied to a higher plant system. The ultimate goal of the plant growth model described hereafter is to remove empirical equations and parameters from the model to achieve a mechanistic description of each process. The first iterations of the mechanistic model will be less accurate on, for example, yield predictions than commonly used empirical models, but when all processes are described mechanistically, it will be well adapted for prediction and control of a plant chamber in a closed-loop LSS for a wide range of parameters, since each growth mechanism will be fully understood.

Typically in agronomy the two main types of models used are the process-based models (e.g. CERES, ORYZA, STICS), mainly used for predicting yield of a given crop in a given field with a given range of environmental conditions, and the functional-structural models (e.g. GREENLAB, L-PEACH), based on a geometrical description of plant morphology<sup>16</sup>. In both cases, some processes are described empirically, mainly because of accuracy of predictions, since these models are meant to be used for assisting agricultural production, and not for plant growth mechanism understanding. However plants in bioregenerative LSS will grow in confined environments and uncanny conditions. Thus it must be understood how slight variations in environmental parameters in space conditions can affect the whole plant system, since there are no buffer effects of the atmosphere, oceans or soil and genetic regulations that may regulate the effects of space environmental variations. Therefore it is critical to take into account the influence of non-terrestrial gravity conditions on elementary plant growth mechanisms, as well as for the variations of the physical environmental surrounding the plants.

Globally the gravity may have influence at three levels:

- Orientation of growth of the roots i.e. gravitropism;
- Fluid migration between root, stems and leaves;
- Gas exchange and influence on photosynthesis rate.

This work is concerned by the last item, knowing that various models of plant gas exchange in Earth gravity have been developed in recent years but to our knowledge no model accounts for changes in gravity levels. The goal of this study is to investigate and propose a preliminary model of plant gas exchanges with gravity as a parameter.

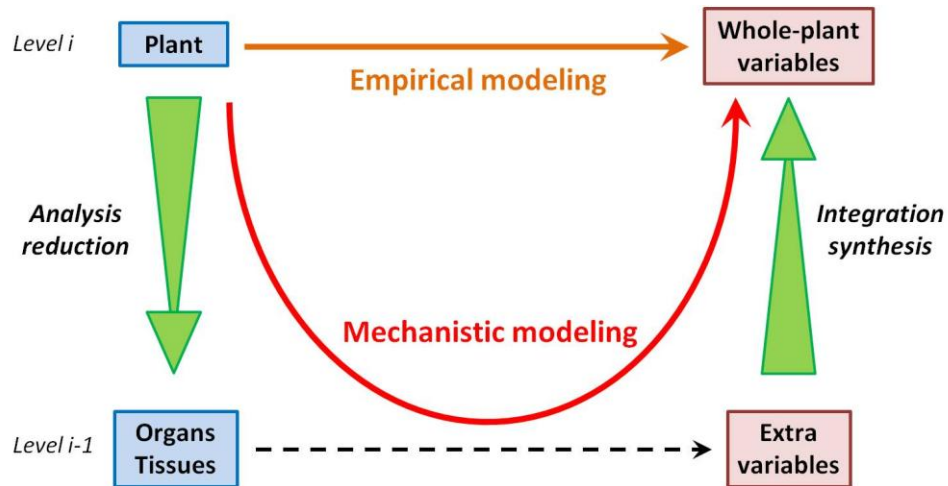


Figure 1. Mechanistic modeling vs. empirical modeling.<sup>17</sup>

### C. Plants gas exchanges and the influence of the environment

Gas exchanges in plants occur during photosynthesis, respiration and transpiration. When plants receive light, typically during the day, they photosynthesize: they absorb  $\text{CO}_2$  and release  $\text{O}_2$  and water vapour. Without light, typically during night time, they respire: they absorb  $\text{O}_2$  and release  $\text{CO}_2$ . In BLSS, gas exchanges in plants ensure air revitalization via  $\text{O}_2$  production and water recycling via transpiration. Indeed non-limiting irrigation methods such as hydroponics or nutrient-film technique are envisioned for plant irrigation in BLSS. Food production also relies on proper gas exchanges since higher plants growth depends on photosynthesis, which is a coupled phenomenon between photon capture, water evaporation and  $\text{CO}_2$  and  $\text{O}_2$  counter-diffusion.

$\text{CO}_2$  and water vapour transfer is made by diffusion between the leaf and the boundary layer through the stomata<sup>18</sup> (Figure 2). When plants are under high water stress, they reduce stomatal conductance to reduce risks of damaging the leaves and the plant<sup>19</sup>. Stomatal conductance corresponds to the rate of  $\text{CO}_2$  entering or water vapour exiting the leaf through the stomata, as opposed to the stomatal resistance. It depends on stomatal density, stomatal aperture, and stomatal size.

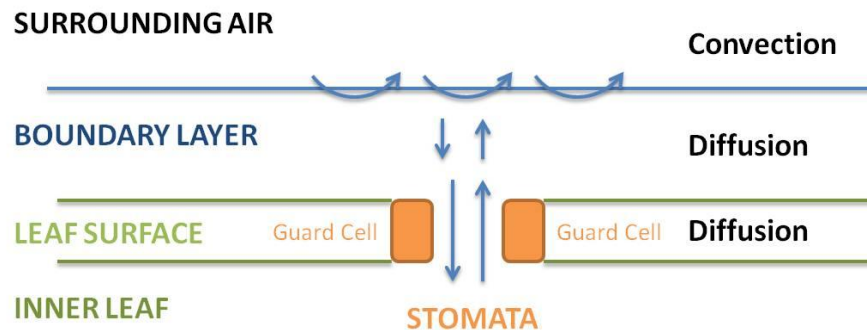


Figure 2. Mechanisms of gas exchange between the leaf and the surrounding air through diffusion and convection.

Furthermore, exchange between the boundary layer and the surrounding air is ensured by convection (Figure 2) and the gas transfer between the leaf surface and the gas are dependent on the convective system of the outside air. A mechanistic model consists in considering that the gas transfer resistance is represented by the diffusion of the species ( $\text{CO}_2$ ,  $\text{O}_2$  and vapour water) in a boundary layer at the leaf surface, the thickness of which is characteristic of bulk gas turbulence. Kitaya et al. showed that when ventilation is increased from 0.01 to 1 m/s in a chamber containing sweet potato plant, the leaf boundary layer resistance decreased (at 0.2 m/s it was one third of the leaf boundary layer resistance at 0.01 m/s), inducing an increase in transpiration and photosynthesis rate, though the

latter was almost constant between 0.5 and 1 m/s<sup>20</sup>. The environmental conditions at the leaf surface can indeed be considered like a micro-climate in the boundary layer of the leaf, with high gradients in temperature, CO<sub>2</sub> concentrations and water vapour<sup>21</sup>. In addition ventilation was shown to have a direct effect on the relative humidity in the surrounding of the leaf surface and an increase in relative humidity was observed 5 mm from the underside of leaves during day-time when crop transpiration reached its maximum<sup>21</sup>. Kitaya et al. also showed that the net photosynthesis rate of a plant canopy can be doubled, by increasing the air velocity from 0.1 m/s to 1 m/s above a tomato leaflet canopy<sup>20</sup>, and from 0.01 m/s to 0.8 m/s above a rice plant canopy<sup>22</sup>. The air current speed inside a tomato leaflet canopy indeed decreased to 30% of the air velocity above the canopy<sup>20</sup>. This led them to conclude that the boundary layer of a whole canopy was greater than the boundary layer on a single leaf. They also concluded that the minimum air current velocity to improve gas exchanges was 1 m/s above a plant canopy and 0.2 m/s around the leaves<sup>20</sup>. These results were confirmed under an elevated CO<sub>2</sub> level<sup>23</sup>.

As a confirmation of the diffusive character of CO<sub>2</sub> through the boundary layer between the gas and the leaf surface, it was been demonstrated that elevated CO<sub>2</sub> is known to promote photosynthesis of C3 and C4 plants<sup>24</sup> and is associated with an increase in biomass production, when it is coupled to high photosynthetic rates<sup>25,26</sup>, otherwise super-elevated CO<sub>2</sub> levels are known for triggering stomata closure, resulting in a decrease in photosynthetic rate<sup>24,27</sup>. However Wheeler et al. showed that super-elevated CO<sub>2</sub> levels (10000 ppm) can increase stomatal conductance during dark periods, with a consequence of increasing water use, i.e. transpiration, which could be used for throttling up or down water purification<sup>25</sup>.

#### **D. Plants gas exchanges in reduced gravity environments**

The effects of microgravity on plant growth have been extensively reviewed<sup>10,28-32</sup>. From the above, it is clearly demonstrated that the thickness of the gas boundary layer and of CO<sub>2</sub> partial pressure gradient between the bulk air and the leaf surface directly acts on photosynthetic activity. As the thickness of the boundary layer depends on bulk agitation, the question is therefore to understand how this could be altered by reduced gravity. One of the indirect effects of a weightless environment is the resulting altered gas exchanges. Early plant growth experiments on the ISS<sup>33</sup> and experiments in parabolic flights<sup>34-36</sup> have shown that poor ventilation or lack of ventilation in spaceflight induces stagnant air boundary layers with decreased conductance around the leaves due to the lack of free convection in microgravity, decreasing heat and gas transfer at the leaf surface<sup>37,38</sup>. In 2001 and 2003 Kitaya et al. studied leaf temperature in weightlessness during a parabolic flight campaign<sup>34,35</sup> and in 2009 Hirai et al. investigated leaf transpiration in weightlessness<sup>36</sup>. They found that leaf temperature increased by 1°C and that gas exchanges were reduced with decreased gravity levels without providing adequate ventilation to the plants.

This deficiency in optimal gas exchange is directly linked to photosynthetic activity (CO<sub>2</sub> uptake and O<sub>2</sub> release) and also to water evapotranspiration that in turns regulates leaf temperature. In turn, the entire cascade of metabolic events and finally, overall plant growth may be affected.

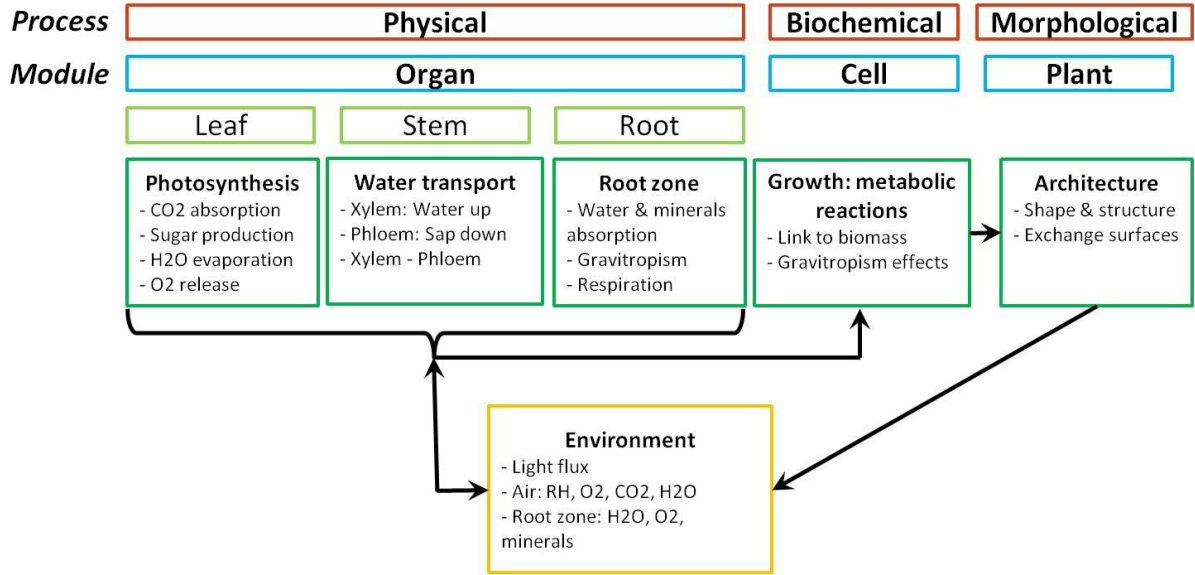
Later experiments on the MIR space station and on the ISS showed that plant gas exchange processes in microgravity, including transpiration and photosynthesis, are similar to that in Earth gravity, when adequate ventilation is provided<sup>39,40</sup> at moderate light levels<sup>41</sup>.

All these observations show that there is at the boundary layer level a mixed effect between natural convection (mostly driven by density gradients and dependent on gravity) and forced convection in the bulk. The objective is here to investigate how these microscopic effects interact on the overall plant growth.

## **II. MELiSSA Plant growth model description**

### **A. Current MELiSSA plant model – mass balance**

The model as defined by Hézarad is divided into four modules; three are related to the plant itself: morphological, physical and biochemical, and the fourth one links the environment surrounding the plants to the plant modules. Figure 3 gives an overview of this model structure and Table 1 gathers the model parameters.



**Figure 3. Diagram illustrating the model structure.**

As a first approach, the approximation of a single leaf is made, represented by a total leaf area parameter ( $LA$ ), a total stem length ( $L_{stem}$ ), and an average number of vessels ( $N_{vessel}$ ). All of these parameters are proportional to the biomass fresh mass produced. The morphological module is still described with empirical equations and morphological laws are based on empirical coefficients for mature lettuce crops  $k_1$ ,  $k_2$ ,  $k_3$ :

$$\begin{aligned} LA &= k_1 \cdot Biomass \\ L_{stem} &= k_2 \cdot Biomass \\ N_{vessel} &= k_3 \cdot Biomass \end{aligned} \quad (1)$$

with  $k_1$  the ratio the canopy surface to the biomass, equal to  $0.0012 \text{ m}^2/\text{g}$ ,  $k_2$  the ratio of the stem length to the biomass, equal to  $4.9 \cdot 10^{-4} \text{ m/g}$ , and  $k_3$  the ratio of the number of sap vessels to the biomass, equal to  $0.0979 \text{ g}^{-1}$ . These values  $k_1$ ,  $k_2$ ,  $k_3$  are average for any lettuce crop and were estimated from experiment and available literature. The single-leaf approach is justified by the fact that upper leaves contribute to most of the gas exchanges in a plant, since they are directly under solar radiation and absorb more of it<sup>42</sup>.

Equations in the physical module give the maximum fluxes for light interception,  $\text{CO}_2$  uptake, water vapour release via transpiration, as well as water absorption through the roots. All parameters used in the parameters below are detailed in Table 1. The three equations describing the process of photosynthesis at the leaf level depend on the leaf area:

- The intercepted light flux is derived from the Beer-Lambert law and depends on the light extinction coefficient  $k$ , the incident light flux  $I_0$ , and the leaf area index  $LAI$ <sup>16</sup>:

$$I = I_0(1 - e^{(-k \cdot LAI)}) \quad (2)$$

with  $LAI = LA \cdot Dens$ .

-  $\text{CO}_2$  uptake is calculated with Fick's first law of diffusion, the driving force being the gradient of  $\text{CO}_2$  concentration between the leaf ( $C_i$ ) and the outside air ( $C_a$ ). It depends on the leaf area  $LA$ , the  $\text{CO}_2$  diffusion coefficient  $D_c$ , and the mass boundary layer thickness  $\delta$  which surrounds the leaf<sup>16</sup>:

$$U_{CO2} = \frac{D_c(C_a - C_i)}{\delta_{mass}} \cdot LA \quad (3)$$

- The driving force for H<sub>2</sub>O transpiration is the water gradient between the leaf and the outside air (1-RH), and depends on a conductance parameter G, the saturating vapour pressure  $P_{(T)}^0$ , the gas constant R, the temperature for water vapour transfer T, and the leaf area LA<sup>16</sup>:

$$R_{H2O} = G \frac{P_{(T)}^0}{RT} (1 - RH) LA \quad (4)$$

The water absorption equation is derived from the Hagen-Poiseuille formula. The driving force for H<sub>2</sub>O absorption is the water potential gradient between the roots ( $\Psi_i$ ) and the nutrient solution in the root zone ( $\Psi_s$ ). It depends on the resistance to water flow given by the radius of the sap vessel  $R_{vessel}$ , the xylem sap dynamic viscosity  $\mu_{xylem}$ , and the stem length  $L_{stem}$ , multiplied by the sap vessel number  $N_{vessel}$ . M is the water molar mass M and  $\rho$  the water density<sup>16</sup>:

$$U_{H2O} = N_{vessel} \frac{\rho \cdot (\psi_s - \psi_i) \cdot \pi \cdot R_{vessel}^4}{M \cdot 8 \cdot \mu_{xylem} \cdot L_{stem}} \quad (5)$$

These four fluxes are limited by the metabolic reactions, reflecting the biochemical processes. The following four equations are evaluated to determine the limiting factor and constitute a stoichiometric test.

The quantum yield QY links the available incident light flux I to the CO<sub>2</sub> uptake by photosynthesis  $U_{CO2}$  with the following equation<sup>16</sup>:

$$\frac{I}{Dens} = \frac{U_{CO2}}{QY} \quad (6)$$

The oxygen availability is not considered to be a limiting factor, thus the respiration rate  $R_{CO2}$  is not included in the physical module. Instead, a fixed parameter Resp links the respiration rate to the total carbon uptake  $U_{CO2}$ , which is valid for both day and night behaviours<sup>16</sup>:

$$R_{CO2} = Resp \cdot U_{CO2} \quad (7)$$

Water lost by transpiration  $R_{H2O}$  is a fixed percentage of total water uptake  $U_{H2O}$ , which is illustrated with the fixed parameter Tr. The remaining water is the one accumulated in the plant, constituting the biomass<sup>16</sup>:

$$R_{H2O} = Tr \cdot U_{H2O} \quad (8)$$

This accumulated water (difference between water uptake  $U_{H2O}$  and transpiration rate  $R_{H2O}$ ) is relatively evaluated to the carbon content in biomass (difference between photosynthesis rate  $U_{CO2}$  and respiration rate  $R_{CO2}$ ) using the following equation<sup>16</sup>:

$$\frac{(U_{CO2} - R_{CO2}) \cdot BC_{mol}}{DM} = \frac{(U_{H2O} - R_{H2O}) \cdot 18}{1 - DM} \quad (9)$$

The ratio between carbon content and water content in biomass depends on the dry matter content DM and the biomass C-molar mass  $BC_{mol}$ .

Then the following metabolic equation enables the evaluation of instantaneous biomass production<sup>16</sup>:

$$J_{Biomass} = BC_{mol} \cdot U_{CO2} + 18 \cdot (U_{H2O} - R_{H2O}) \quad (10)$$

The integration of this equation on a time period gives the total biomass produced over this period<sup>16</sup>:

$$Biomass = \int_{t=0}^t J_{Biomass} \cdot dt \quad (11)$$

**Table 1. Model parameters for the MELiSSA plant growth model** <sup>16</sup>

Parameter	Description
I	Intercepted light flux
I <sub>0</sub>	Incident light flux
k	Light extinction coefficient
LAI	Leaf area index
U <sub>CO2</sub>	CO <sub>2</sub> uptake rate
Dc	CO <sub>2</sub> Diffusion coefficient
Ca	CO <sub>2</sub> concentration in the outside air
Ci	CO <sub>2</sub> concentration in the leaf
δ <sub>mass</sub>	Mass boundary layer thickness
LA	Leaf area
R <sub>H2O</sub>	H <sub>2</sub> O transpiration rate
G	Leaf conductance
T	Temperature for water vapour transfer
P <sup>0</sup> (T)	Saturating vapour pressure at T
R	Gas constant
T	Temperature for water vapour transfer T
RH	Relative humidity
U <sub>H2O</sub>	H <sub>2</sub> O uptake rate
N <sub>vessel</sub>	Sap vessel number
ρ	Water density
ψ <sub>s</sub>	Water potential gradient in the nutrient solution
ψ <sub>i</sub>	Water potential gradient in the roots
R <sub>vessel</sub>	Radius of the sap vessel
M	Water molar mass
L <sub>stem</sub>	Stem length
μ <sub>xylem</sub>	Xylem sap dynamic viscosity
Dens	Planting density
QY	Quantum yield
R <sub>CO2</sub>	Respiration rate
Resp	Respiration/Photosynthesis ratio
Tr	Transpired/Absorbed water ratio
DM	Dry Matter content per water content in biomass
BC <sub>mol</sub>	Biomass C-molar mass
J <sub>Biomass</sub>	Biomass production rate
Biomass	Biomass accumulation

## B. Gas exchanges modelling

The most common models used for gas exchanges are stomatal optimality, detailed mechanistic or empirical model. The first one expresses the stomatal conductance to CO<sub>2</sub> regulated by the plant to have maximum C assimilation over a defined time step, according to water availability<sup>19</sup>. Empirical models are models that are adapted for agronomy or ecology applications or to predict yields for given environmental parameters and plant species.

In the MELiSSA plant growth model presented above, gas exchanges are expressed via the CO<sub>2</sub> uptake and water vapour release:

$$U_{CO2} = \frac{D_c(C_a - C_i)}{\delta_{mass}} \cdot LA \quad (3)$$

$$R_{H2O} = G \frac{P(T)}{RT} (1 - RH) LA \quad (4)$$



Like other current mechanistic models of plant gas exchanges, it is adapted for plant growth on Earth and do not include gravity as a parameter. In order to study gas exchanges and plant growth in reduced gravity environment, we modified this model and expressed the transpiration and CO<sub>2</sub> uptake rate as functions of gravity.

### 1. Conductance

The water vapour conductance  $G$  is a combination of the stomatal and the boundary layer conductance (Figure 4). Since 95% of gas exchanges occur through stomatal pores<sup>43</sup>, we neglect cuticle conductance in front of stomatal conductance. We also neglect mesophyll conductance for CO<sub>2</sub> diffusion. Thus the conductance is a combination of stomatal and boundary layer conductance for water and CO<sub>2</sub>. Given the expression we have for the CO<sub>2</sub> uptake rate ( $U_{CO_2}$ ), the conductance is implicitly expressed with the product of the diffusion coefficient  $D_c$  with the difference between the outside and inside CO<sub>2</sub> concentration ( $C_a - C_i$ ) divided by the thickness of the boundary layer  $\delta_{mass}$ .

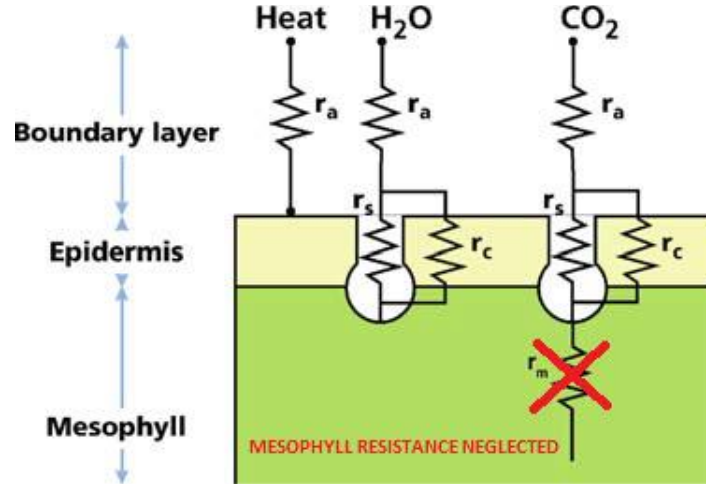


Figure 4. Diagram representing the different resistances to gas diffusion from the atmosphere to the leaf.<sup>44</sup>

New parameters used in this section are gathered in Table 2.

Table 2. Parameters for gas modelling.

Parameter	Description
$g_w^s$	Stomatal conductance
$g_w^{BL}$	Boundary layer conductance
$D_w$	diffusion coefficient of water vapour
$\rho_{mol,w}$	molar water vapour density
$l$	length of the diffusive pathway
$l_s$	depth of the stomatal pore
$a_s$	stomatal cross-sectional area
$d_s$	stomatal density
$v$	Total air velocity
$\eta$	air kinematic viscosity
$L$	characteristic length of a leaf
$g$	Acceleration of gravity
$\beta$	Thermal expansion coefficient
$h$	Characteristic length of the plant chamber
$T_{air}$	Temperature of the surrounding air
$T_{leaf}$	Temperature of the leaf

For the transpiration rate  $RH_2O$ , the total conductance  $G$  is found with the expression:

$$\frac{1}{G} = \frac{1}{g_w^s} + \frac{1}{g_w^{BL}}$$

Leading to:

$$G = \frac{g_w^{BL} g_w^s}{g_w^s + g_w^{BL}} \quad (12)$$

with  $g_w^s$  the stomatal conductance for water vapour and  $g_w^{BL}$  the boundary layer conductance for water vapour.

The stomatal conductance is species-dependent and is expressed as:

$$g_w^s = \frac{D_w \rho_{mol,w}}{l} \quad (13)$$

where  $D_w$  is the diffusion coefficient of the water vapour,  $\rho_{mol,w}$  is the molar water vapour density, and  $l$  is the length of the diffusive pathway. It can be expressed as a function of the stomata geometry, the depth of the stomatal pore  $l_s$ , the stomatal cross-sectional area  $a_s$ , and the stomatal density  $d_s$ :

$$l = \frac{l_s}{d_s a_s} \quad (14)$$

The stomatal density depends on the plant species but also on environmental factors such as light, relative humidity, water availability, and atmospheric  $CO_2$  concentration, and thus it can vary between individuals of a same species<sup>45</sup>. Stomatal density in *Sorghum* ranges between 107 and 177  $mm^{-2}$ <sup>46</sup>, whereas in most tree species in the Amazonian forest it is between 271 and 543  $mm^{-2}$ <sup>45</sup>, and in *Proteaceae* it ranges between 44 and 521  $mm^{-2}$ <sup>47</sup>. There is an inverse relationship between size and density of stomata on a given plant and in sun leaves stomatal density is higher than on shaded leaves<sup>45</sup>. It was also shown to be positively correlated with minor veins density on *Proteaceae*<sup>47</sup>. A typical stomatal size for *Sorghum* is 7.69 – 19  $\mu m$ , leading to a cross-sectional area of 46.4 to 283.5  $\mu m^2$ , similar to that found on Amazonian trees (7.5 – 22.5  $\mu m$ )<sup>45</sup>, but smaller than those of *Proteaceae* (20.5 – 67.7  $\mu m$ )<sup>47</sup>. Stomatal depth in *Sorghum* ranges between 10.6 and 11.7  $\mu m$ <sup>46</sup>. The values taken for our simulations are given in Table 3.

Replacing equation 14 into equation 13, the new expression for the stomatal conductance is:

$$g_w^s = \frac{d_s a_s D_w \rho_{mol,w}}{l_s} \quad (15)$$

The boundary layer conductance depends on the convective regime of the air surrounding the leaf and is expressed with the following expression:

$$g_w^{BL} = \frac{D_w \rho_{mol,w}}{\delta_{mass}} \quad (16)$$

where  $\delta_{mass}$  is the mass boundary layer thickness.

Combining equations 12, 15 and 16, we get the following expression for the total conductance for water vapour through the leaf:

$$G = \frac{D_w \rho_{mol,w} d_s a_s}{\delta_{mass} d_s a_s + l_s} \quad (17)$$

With this expression, the leaf conductance to water vapour, which was a constant of the previous model, is now dependent on the boundary layer thickness. It also depends on the stomatal geometry and thus on the plant species that is being studied.

Using the values of the parameters given in Table 3 and a typical boundary layer thickness of 8  $mm$ <sup>16,21</sup>, we find the length of the diffusive pathway for a lettuce crop equal to 4.62.10<sup>-4</sup> m.pores<sup>-1</sup>, a stomatal conductance for water vapour of  $g_w^s = 2.54 \text{ mol.m}^{-2}.\text{s}^{-1}$ , a boundary layer conductance for water vapour equal to  $g_w^{BL} = 0.15 \text{ mol.m}^{-2}.\text{s}^{-1}$ , and

thus we find  $G = 139 \text{ mmol.m}^{-2}.\text{s}^{-1}$ . The values for stomatal conductance are higher than values from the literature, since Holmberg et al. (2013) estimated a stomatal conductance ranging from 200 to 800  $\text{mmol.m}^{-2}.\text{s}^{-1}$  for MELiSSA crops using values from Nobel et al. (2009)<sup>18,48</sup>, and Smith et al. (2004) observed stomatal conductances in the range 460-660  $\text{mmol.m}^{-2}.\text{s}^{-1}$  for *Tradescantia virginiana* crops<sup>49</sup>. Holmberg et al. (2013) calculated a total conductance ranging from 63 to 1121  $\text{mmol.m}^{-2}.\text{s}^{-1}$ , so our total calculated conductance  $G$  is in agreement with values from the literature.

## 2. Boundary layer

We consider a laminar flow with a speed  $v$  circulating above our single leaf. The boundary layer is defined as the distance from the leaf surface where the concentration of a given compound is less than 99% of the ambient air value. It is expressed with the following expression<sup>50</sup>:

$$\delta_{mass} = 4.6 \sqrt{\frac{L\eta}{v}} \quad (18)$$

where  $\eta$  the air kinematic viscosity and  $L$  is the characteristic length of a leaf. Here  $L$  is expressed as the diameter of our single leaf:

$$L = 2 \sqrt{\frac{LA}{\pi}} \quad (19)$$

## 3. Convection

The air velocity results from free and forced convection. Air velocity from forced convection is a constant in our model and depends on the environment in which the plants are grown:

$$v = v_{free} + v_{forced} \quad (20)$$

Free convection depends on the temperature gradient between the surrounding air ( $T_{air}$ ) and the leaf ( $T_{leaf}$ ), and on the gravity levels and can be calculated using the following equation:

$$v_{free} = \sqrt{g\beta h(T_{air} - T_{leaf})} \quad (21)$$

where  $g$  is the acceleration of gravity,  $\beta$  the thermal expansion coefficient, and  $h$  the characteristic length of the plant chamber.

## 4. Transpiration rate as a function of gravity

Equation 21 enables a link between the gravity levels and the transpiration rate. Combining equations 4, 17, 18 and 21, we obtain the following expression for the transpiration rate:

$$R_{H_2O} = \frac{D_w \rho_{mol,w} d_s a_s}{4.6 \sqrt{\frac{L\mu}{\sqrt{g\beta h(T_{air} - T_{leaf})} + v_{forced}}}} \frac{P_{(T)}^0}{RT} (1 - RH) LA \quad (22)$$

This expression is a function of the gravity levels and can be used to study gas exchanges and plant growth in reduced gravity environments in a mechanistic way.  $L$  is no longer a constant deduced empirically from the literature but is a variable of the model, depending on the leaf area, which itself is a function of the produced biomass. Moreover with the species-specific values of  $l_s$ ,  $a_s$ , and  $d_s$ , this expression of the transpiration rate can be used for species-specific studies.

### 5. $CO_2$ uptake as a function of gravity

Combining equations 3, 18, and 21, we obtain the following expression for the  $CO_2$  uptake rate:

$$U_{CO_2} = \frac{D_c(C_a - C_i)}{4.6} \sqrt{\frac{g\beta h(T_{air} - T_{leaf}) + v_{forced}}{L\mu}} \cdot LA \quad (23)$$

Gas exchanges in the model are now expressed according to the gravity levels in the environment surrounding the plants.

## III. Simulations results and discussion

Simulations using this model were made using Matlab R2016b (9.1) version 13.5. Plants were grown for 50 days with a 14-hour photoperiod. The values of the different parameters used for the simulations are given on Table 3. Species-specific parameters are taken for a lettuce crop and taken from the literature.

**Table 3. Parameters values used for simulations. All values are taken from Hézard et al. (2012) unless otherwise indicated.**

Parameter	Value	Unit
$I_0$	1.44	$\text{mol.m}^{-2}.\text{h}^{-1}$
k	0.66	dimensionless
$D_c$	$6.12.10^{-2}$	$\text{m}^2.\text{h}^{-1}$
$C_a$	$4.087.10^{-3}$ (1000 ppm)	$\text{mol.m}^{-3}$
$C_i$	$1.23.10^{-3}$ (300 ppm)	$\text{mol.m}^{-3}$
$\psi_s$	-120	Pa
$\psi_i$	-1800	Pa
$R_{vessel}$	$4.8.10^{-5}$	m
$RH_{air}$	70	%
$RH_{leaf}$	100	%
M	18.01	$\text{g.mol}^{-1}$
$\mu_{xylem}$	$2.778.10^{-7}$	Pa.h
Dens	24	number of plants. $\text{m}^{-2}$
QY	0.054	$\text{mol}_C.\text{mol}_{photon}^{-1}$
Resp	0.27	$\text{mol}_{respired}.\text{mol}_{absorbed}^{-1}$
Tr	0.41	dimensionless
DM	0.07	$\text{g}_{dry}.\text{g}_{fresh}^{-1}$
$BC_{mol}$	30.5	$\text{g.mol}_C^{-1}$
$D_w$	0.0000282	$\text{m}^2.\text{s}^{-1}$
$\rho_{mol,w}$	41.58	$\text{mol.m}^{-3}$
$l_s$	$11.10^{-6}$ 18,46	m
$a_s$	$170.10^{-12}$ 18,46	$\text{m}^2$
$d_s$	$1.4.10^8$ 18,46	pores. $\text{m}^{-2}$
$\eta$	0.000018	$\text{m}^2.\text{s}^{-1}$
$\beta$	0.003412969	$\text{K}^{-1}$
h	1	M
$T_{air}$	293	K
$T_{leaf}$	296	K
Photoperiod	14	h
Initial Biomass	28	g

We looked at the dependence on the gravity parameter, for a given forced convection value for CO<sub>2</sub> uptake and transpiration, as well as biomass production; and the dependence on the forced convection, for a given gravity value.

## A. Gravity dependence

### 1. Transpiration rate and CO<sub>2</sub> uptake

The forced convection air velocity was fixed to 1m/s. The transpiration rate (mol/h) and CO<sub>2</sub> uptake rate (mol/h) were computed for microgravity ( $10^{-5}$  m/s<sup>2</sup>), Martian gravity (3.711 m/s<sup>2</sup>) and Earth gravity (9.807 m/s<sup>2</sup>) (Figure 5 and Figure 6). The overall trend and orders of magnitude were similar for all three conditions, for both the transpiration rate and the CO<sub>2</sub> uptake rate. However the final transpiration rate on Earth gravity was higher than on Mars or in microgravity, 0.0146 mol/h, against 0.0132 mol/h and 0.0110 mol/h respectively. The final CO<sub>2</sub> uptake was also higher in Earth gravity conditions ( $1.28 \cdot 10^{-3}$  mol/h) than on Mars ( $1.16 \cdot 10^{-3}$  mol/h) and in microgravity ( $0.967 \cdot 10^{-3}$  mol/h).

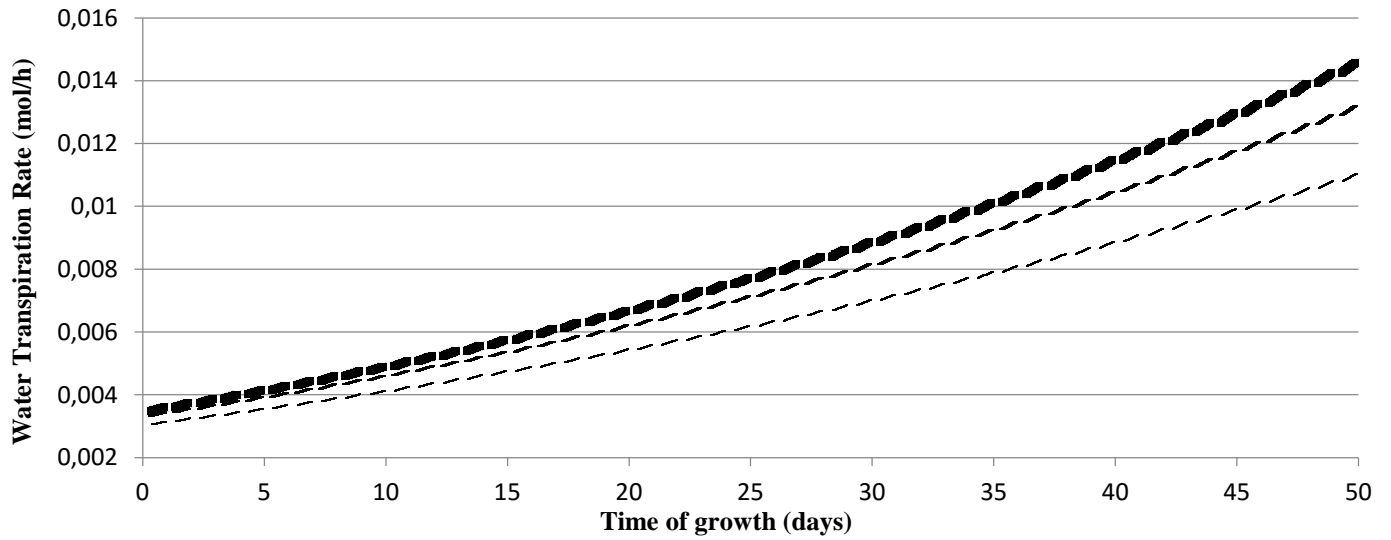


Figure 5. Water transpiration rate for microgravity (thin hyphenated line), Mars gravity (medium thickness hyphenated line), and Earth gravity (thick hyphenated line) over 50 days of growth on a lettuce crop.

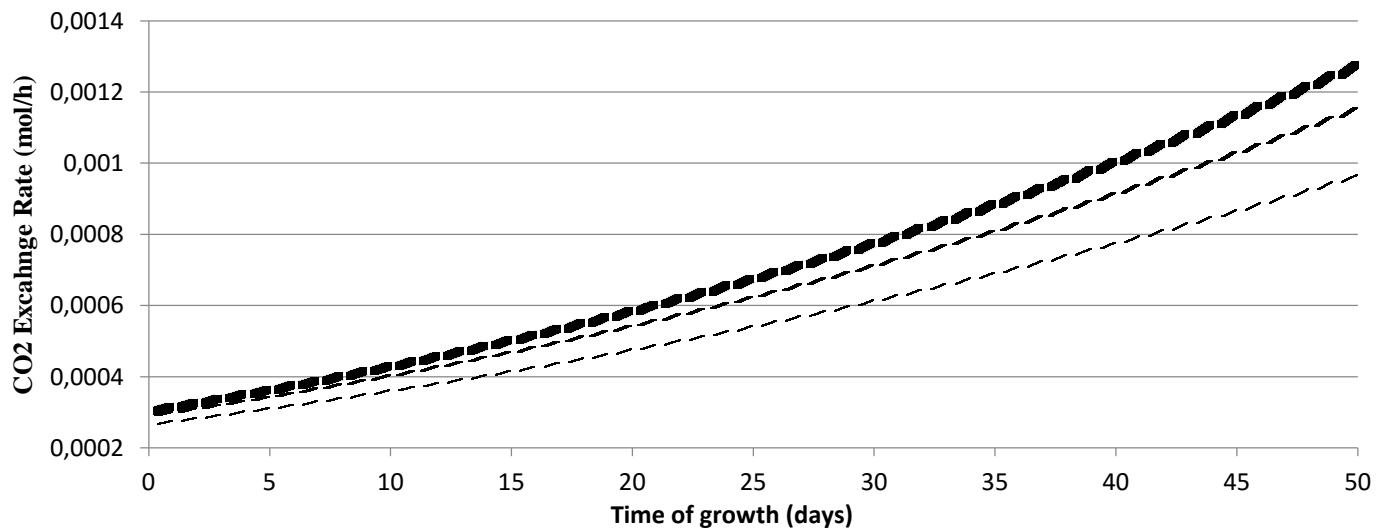
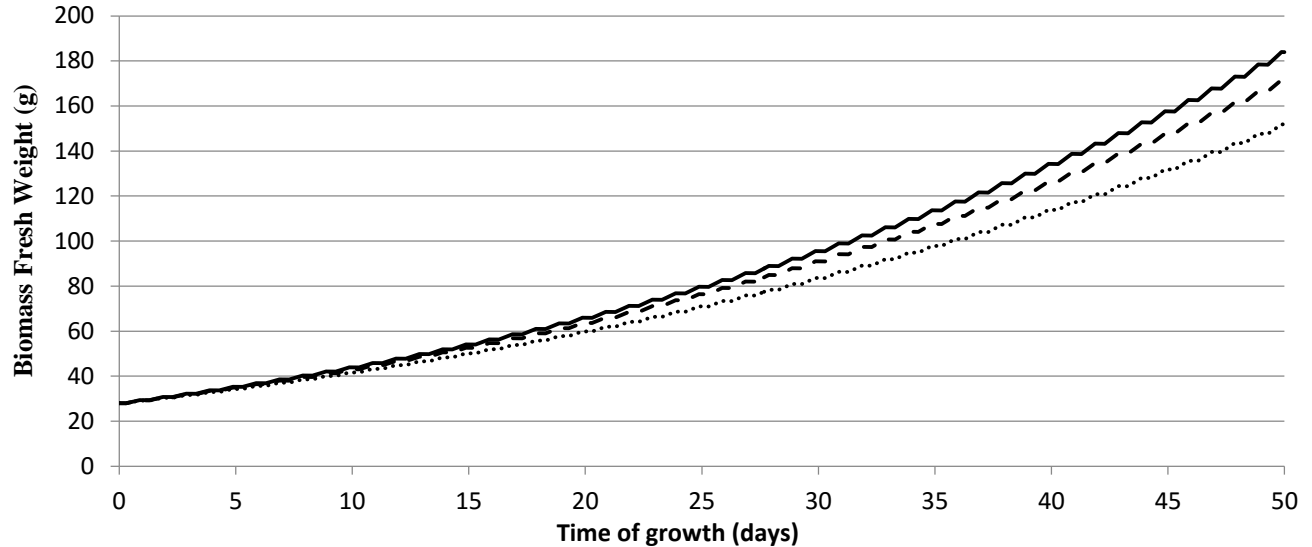


Figure 6. CO<sub>2</sub> uptake rate for microgravity (thin hyphenated line), Mars gravity (medium thickness hyphenated line), and Earth gravity (thick hyphenated line) over 50 days of growth on a lettuce crop.

## 2. Biomass

The biomass production (in g) over the 50 days of growth was computed with the same growth conditions (forced convection air velocity fixed to 1m/s) and depends on the gas exchanges, namely CO<sub>2</sub> uptake rate and transpiration. As a result, the biomass produced in Earth gravity conditions is 21% higher than in microgravity and 7% only in Martian conditions (Figure 7). And biomass produced in Martian gravity conditions was 13% higher than in microgravity.



**Figure 7. Biomass accumulation for microgravity (dotted line), Mars gravity (hyphenated line), and Earth gravity (continuous line) over 50 days of growth on a lettuce crop.**

These simulations show a non negligible effect of the gravity parameter on gas exchanges, which have a direct incidence on biomass production. It is in accordance to experiments performed in the past on parabolic flights and on the MIR space station and on the ISS<sup>35,36,40</sup>.

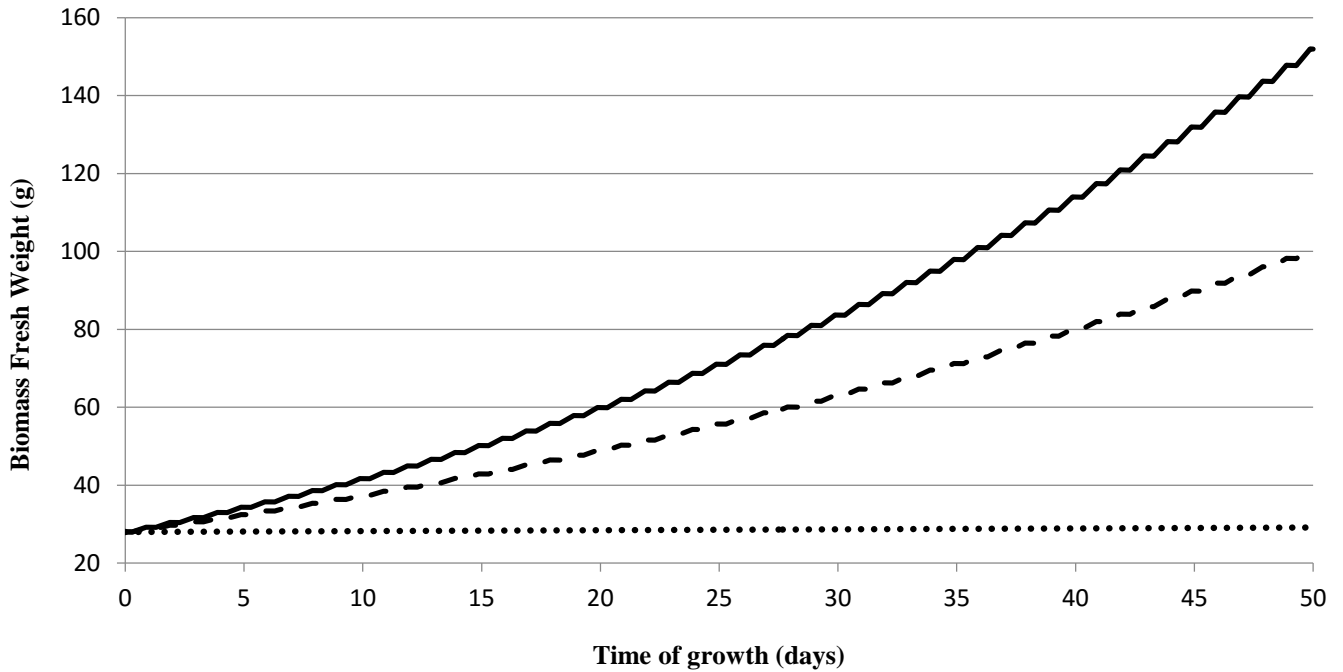
The importance of forced convection is highlighted here. Although there was an airspeed of 1m/s in each case, it was not enough to counter the indirect effects of low gravity resulting in lower free convection speeds. Consequently for future plant growth in space, forced convection will need to be adapted carefully in order to obtain optimal gas exchanges and biomass production.

## B. Ventilation dependence

In this section, only computations of biomass production are shown. For each gravity level: microgravity (Figure 8), Mars (Figure 9), Earth (Figure 10), three forced convection speeds are applied: 0 m/s, 0.5 m/s, and 1 m/s.

### 1. Microgravity

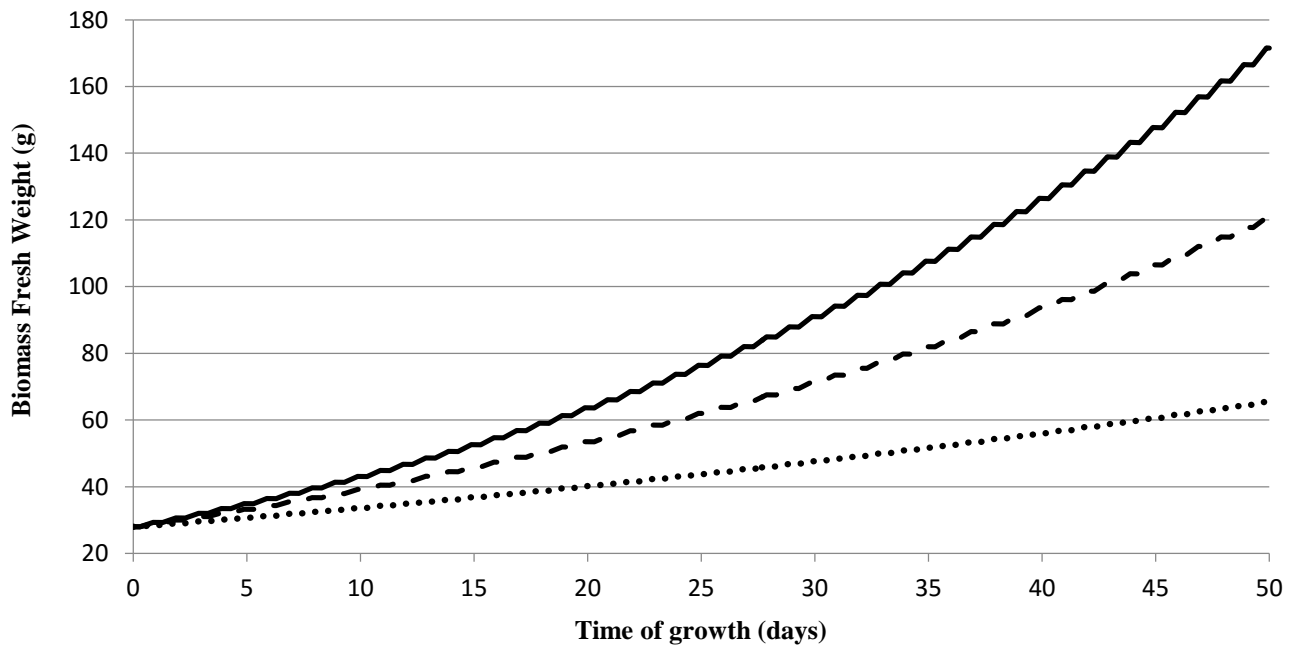
The biomass produced in microgravity with 0.5 m/s is 34% lower than with a forced convection of 1 m/s. When forced convection is removed, plants do not grow: 1.1 g only produced over 50 days. This is in accordance to what was observed in the past on plant growth in low Earth orbit<sup>33</sup>.



**Figure 8. Biomass accumulation in microgravity with a forced convection air velocity of 0 m/s (dotted line), 0.5 m/s (hyphenated line), and 1 m/s (continuous line) over 50 days of growth on a lettuce crop.**

## 2. Mars gravity

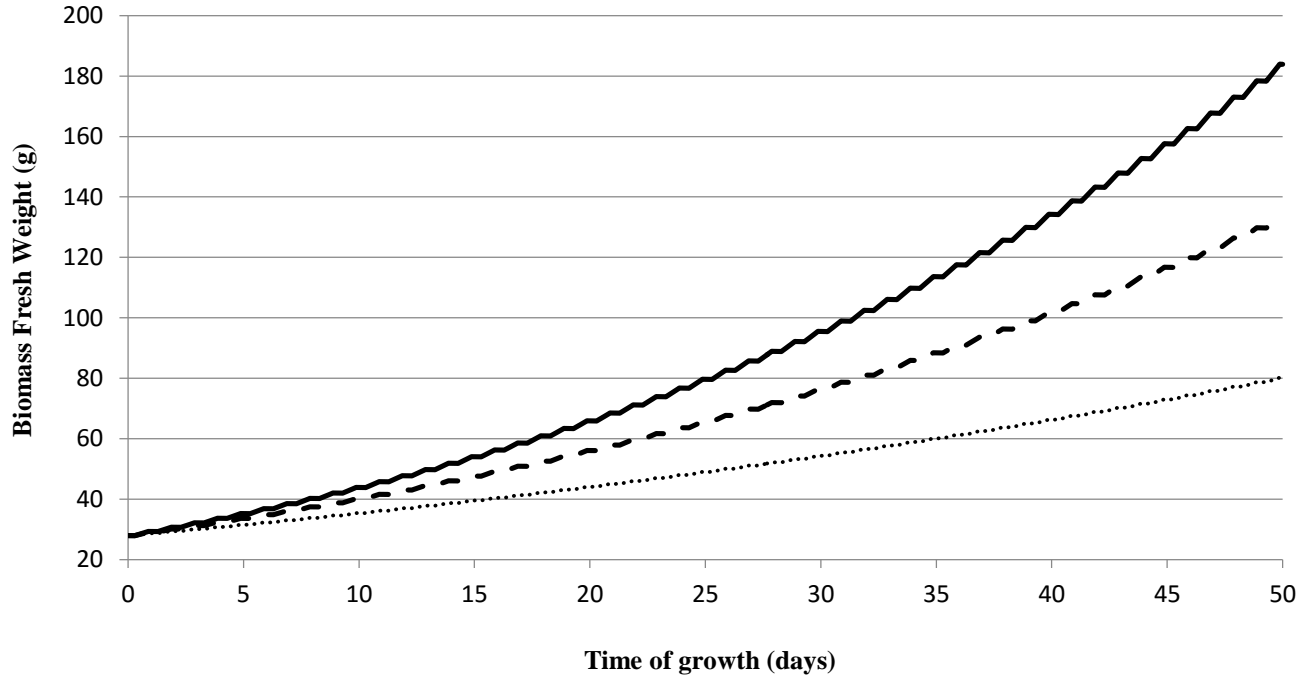
In Martian gravity, the effects of no forced gravity are less inhibitor than for microgravity, but biomass produced with no forced convection is 46% lower than with 0.5 m/s and 62% lower than with 1 m/s.



**Figure 9. Biomass accumulation in Martian gravity level with a forced convection air velocity of 0 m/s (dotted line), 0.5 m/s (hyphenated line), and 1 m/s (continuous line) over 50 days of growth on a lettuce crop.**

### 3. Earth gravity

These computations show the importance of a well ventilated growth chamber even on Earth. Indeed biomass produced with no forced convection is 40% lower than with 0.5 m/s and 56% lower than with 1 m/s.



**Figure 10. Biomass accumulation in Earth gravity level with a forced convection air velocity of 0 m/s (dotted line), 0.5 m/s (hyphenated line), and 1 m/s (continuous line) over 50 days of growth on a lettuce crop.**

In our example in part II.B.1, with the values used for the simulations, the stomatal conductance was  $2.54 \text{ mol.m}^{-2}.\text{s}^{-1}$ , while the boundary layer conductance was  $0.15 \text{ mol.m}^{-2}.\text{s}^{-1}$ , suggesting that plant gas exchanges are driven mostly by the stomata. Indeed the control of transpiration in plants is usually attributed to stomata<sup>18</sup> but our simulations show that the boundary layer and control of the convective regime surrounding the leaves also play a significant role in plant gas exchanges. In microgravity (no free convection) without forced convection, the boundary layer becomes so thick that gas cannot diffuse through it and plants did not grow; this proves that stomatal conductance solely is not enough. Gas exchanges at the leaf surface are the results of intricate coupled diffusion and convection mechanisms that need to be investigated further to fully understand plant growth in reduced gravity environments.

## IV. Conclusion and future work

Gas exchanges play a crucial role in plant growth and their dependency on gravity levels and forced ventilation have been studied experimentally in the past in parabolic flights and in Earth orbit. Building on the bases of the already existing MELiSSA plant growth model, we have added a more detailed description of the gas exchanges at the leaf level, with gravity as a varying parameter of the model. This has enabled us to study more in details the underlying mechanisms of gas exchanges and look at the influences of gravity and ventilation on the transpiration and  $\text{CO}_2$  uptake, and ultimately biomass production. The results shown and discussed above are mostly intended for studying the dependency on forced convection and gravity levels, not for accurate predictions of plant growth in reduced gravity environment. They show that an adequate ventilation of the plant chamber is more important than the indirect effect of reduced gravity on free convection. Indeed our results indicate that without forced convection plants do not grow very well, whatever the level of gravity. When adequate convection is provided, as expected from past experiments, plants in reduced gravity environment grow as well as plants on Earth in 1g.

An experiment in a parabolic flight planned for late 2017 will enable to gather enough data on plant gas exchanges to validate the model in microgravity. This will in return enable us to fine-tune the model and acquire more accurate simulations for better quantification of gas exchanges and biomass production. This will have implications on the



planning of future crewed space missions where plants will be used as food source and enable oxygen and water recycling for supporting humans.

Future versions of the model will include an accurate growth limitation in time and a coupling with the energy balance. Ultimately the morphological module presented above will also be described using a mechanistic approach and not only relying on empirical parameters.

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