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# On the mechanism for winter stem pressure build-up in walnut trees

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## Abstract

Xylem embolism is a significant factor in tree mortality. Restoration of hydraulic conductivity after massive embolisation of the vascular system requires the application of positive pressure to the vessels and/or the creation of new conductive elements. Some species generate positive pressure from the root system to propagate pressure in distal, aboveground, organs in spring, whereas other species generate positive pressure locally at the stem level during winter. We provide a mechanistic explanation for winter stem pressure build-up in the walnut tree. We have developed a physical model that accounts for temperature fluctuations and phase transitions. This model is based on the exchange of water and sugars between living cells and vessels. Our computations demonstrate that vessel pressurization can be attributed to the transfer of water between vessels across the parenchyma rays, which is facilitated by a radial imbalance in sugar concentration. The ability to dispose of soluble sugars in living cells, and to transport them between living cells and up to the vessels, are identified as the main drivers of stem pressure build-up in the walnut tree.

## Keywords

Embolism recovery ; Modelling ; Sugar transport

## Introduction

Massive embolism in xylem causes a decline in trees' hydraulic conductivity and is among the factors causing their death (Sperry and Tyree, 1988; Brodribb and Cochard, 2009; Mantova et al., 2022). This embolism can occur as a result of either drought, leading to air seeding in xylem conduits (Hargrave et al., 1994; Choat et al., 2016), or due to freeze-thaw cycles (Sperry and Sullivan, 1992; Charra-Vaskou et al., 2016). Despite previous studies (Salleo et al., 1996; Nardini et al., 2011), hydraulic conductivity seemingly cannot be re-established while the xylem remains under tension (Charrier et al., 2016). Hydraulic conductivity recovery happens through creating new conducting vessels (Cochard and Tyree, 1990), pressurizing xylem conduits to remove air bubbles (Sperry et al., 1987; Hacke and Sauter, 1996), or a combination of both (Cochard et al., 2001; Améglio et al., 2002). In species that pressurize their xylem conduits, it is important to differentiate between species where the pressure comes only from the roots in spring (Fisher et al., 1997), and species where the pressure can also come from the stem in winter (Améglio et al., 2001). The walnut tree has the ability to use both strategies to generate positive pressure (Ewers et al., 2001).

Note that for many species during winter, there are pressure variations associated with freezing events (Robson and Petty, 1987; Milburn and O'Malley, 1984; Améglio et al., 2001). They are related to phase changes and freeze-induced water fluxes (Ceseri and Stockie, 2013; Graf et al., 2015; Bozonnet et al., 2023; Zarrinderakht et al., 2024). What we call here "stem pressure" is a pressure that stays positive in a stem conduit even after sap thawing and that could subsequently lead to embolism recovery by refilling, once capillary forces between an embolized and a pressurized conduit are overcome. The term "build-up" refers to the gradual increase of this pressure with time.

41 Winter stem pressure has been extensively studied for walnut tree (Améglio and Cruiziat, 1992; Améglio  
42 et al., 2001; Ewers et al., 2001; Améglio et al., 2002, 2004), and maple tree (Milburn and O’Malley, 1984; Tyree,  
43 1983; Cirelli et al., 2008; Ceseri and Stockie, 2013; Graf et al., 2015; Zarrinderakht et al., 2024). Note that the  
44 work of Graf et al. (2015) also includes a comparison with experimental results for walnut tree. In this work,  
45 we focus on walnut tree.

46 Walnut winter stem pressure has been studied using laboratory experiments in (Améglio et al., 2001) that  
47 demonstrated many features associated with this phenomenon: 1) the pressure can be generated for stems  
48 disconnected from the rest of the tree; 2) the pressure rise starts at positive temperature and stops at higher  
49 temperature (typically  $> 5^{\circ}\text{C}$ ); 3) the pressure rises much more during successive freeze-thaw cycles rather  
50 than during continuous exposure to low and non-freezing temperature; 4) at the end of the experiments, the  
51 magnitude of the pressure build-up is positively correlated with the osmolarity of the xylem sap; 5) earlier stem  
52 defoliation and exposure to high temperature (typically  $18^{\circ}\text{C}$ ) before successive freeze-thaw cycles both reduce  
53 the xylem sap osmolarity and xylem pressure.

54 During winter, when sap mineral content is low, xylem sap osmolarity depends on a balance of sugar fluxes  
55 between vessels and vessel-associated cells (VACs, Améglio et al. (2004)), mediated by temperature dependent  
56  $\text{H}^+$ /sugar co-transport (Alves et al., 2007). Particularly, sugar fluxes from VACs to vessels are thought to be  
57 diffusive (facilitated by a specific protein), whereas a  $\text{H}^+$ /sugar co-transport, related to the ATP-ase activity,  
58 drives the fluxes from vessels to VACs at sufficiently high temperature (Améglio et al., 2004; Decourteix et al.,  
59 2008). Pressure changes in the thawed state are thus believed to be due to the exchange of water between VACs  
60 and vessels, which are triggered by the corresponding sugar fluxes.

61 In this work, we built a mechanistic model for pressure build-up in walnut tree that is intended to reproduce  
62 the specific features listed above. We particularly explored the link between xylem pressure rise and xylem sap  
63 osmolarity.

64 To do so we have developed a comprehensive physical model that integrates water and heat fluxes, phase  
65 changes, pressure-volume relationships, and sugar fluxes within various tissues. The model incorporates changes  
66 in stem diameter that are related to water flows between living cells (in bark or xylem tissues) and apoplast.  
67 During freeze-thaw cycles, pressure and stem diameter changes are inter-related as we demonstrated in our  
68 previous work (Bozonnet et al., 2023). This feature allows future comparison of the model results with non-  
69 invasive measurements of stem diameter changes.

70 After a presentation of the effects of sugar fluxes on pressure changes, we explored the role of sugar perme-  
71 abilities and initial concentration on pressure build-up. We then compared the outputs of the model (pressure  
72 level, xylem sap osmolarity) to the experimental results of Améglio et al. (2001). We finally presented a thorough  
73 analysis of the model results, highlighting key findings and potential avenues for further research. The model  
74 is freely available along with the paper so that other scientists could benefit from its use and contribute to its  
75 development.

## 76 **Material and methods**

### 77 **General description of the numerical model**

78 The present model is a modified version of a previous one presented and validated in Bozonnet et al. (2023).  
79 This model was based on earlier modeling efforts about pressure changes in maple trees (Ceseri and Stockie,  
80 2013; Graf et al., 2015). We provide here a general description of the model and highlight its difference with its  
81 previous version.

82 The model relies on a wood anatomy description in the transversal plane of a wood section, as described by  
83 Alves (Alves et al., 2007). The structure of our model (figure 1) groups the essential anatomical elements to  
84 simulate the processes described above: xylem vessels, vessel-associated cells (VACs) and bark cells. It shares  
85 some similarities with the model of Hölttä et al. (2006): living cells are interconnected by a parenchyma ray,  
86 connected at its periphery to the bark cells, and connected to a radial alignment of vessels. We assume that  
87 the external temperature field is homogeneous around the stem, i.e. axi-symmetric, so that radial exchanges  
88 are only modelled along one ray and rescaled at the xylem/bark interface by  $N_{ray}$ , the number of parenchyma  
89 rays. The longitudinal dimension is not considered. The parenchyma ray itself is not described explicitly, i.e.,  
90 individual (isolated) ray cells are omitted, but rather represented by a hydraulic resistance between VACs, and

91 between VACs and bark cells. Water flows and volume changes are computed for one VAC per vessel and for  
 92 one bark cell in the bark tissue. These water flows and volume changes are then rescaled by  $N_{vac}$ , the number of  
 93 VACs connected to each vessel, and  $N_{bark\ cell}$ , the number of living cells in the bark, similarly to what is done  
 94 in Graf et al. (2015) for the fiber/vessel fluxes.

95 Elastic living cells, i.e., bark cells and VACs, are assumed to contain only water and soluble sugar. We  
 96 therefore assume that intracellular ice does not form in the temperature range we study. Rigid vessels contain  
 97 sugar, liquid water or ice depending on local temperature, and gas. This gas compresses or expands in response  
 98 to water flows entering or leaving the vessels, thus creating pressure variations according to the ideal gas law,  
 99 as done in Ceseri and Stockie (2013); Graf et al. (2015).

100 Heat transfer and phase changes are calculated at the tissue scale and driven by external temperature  
 101 variations. Vessel sugar content impacts tissue-scale phase change through freezing point depression (FPD).

102 Water fluxes occur between the different elements (blue arrows in figure 1). These water fluxes are driven  
 103 by the differences in water potential (hydrostatic/turgor, osmotic, cryostatic) across cell membranes. For each  
 104 elastic compartment (VACs, bark cells), the balance of water fluxes results in volume changes, which are then  
 105 used to calculate changes in tissue dimensions, as well as changes in turgor and osmotic potential.

106 The only difference with our previous work is that sugar quantities are now assumed to vary with time. Sugar  
 107 fluxes occur between the different elements (yellow arrows in figure 1). For simplicity, these fluxes are assumed  
 108 to come from passive diffusion, i.e., they are proportional to the concentration gradient between two successive  
 109 elements. These variations in sugar quantities are then used to calculate sugar concentrations in living cells and  
 110 vessels, which impacts osmotic potential, thus generating water fluxes, and vessel FPD.

## 111 Mathematical description of the numerical model

### 112 Anatomy

113 The anatomical description used in the model is shown in figure 1. Vessels are arranged regularly along the ray,  
 114 with the vessel number,  $N_{vessels}$ , computed using a linear vessel density,  $lvd$  and the size of the xylem tissue:

$$N_{vessels} = lvd \times (R_{xylem} - R_{pith}), \quad (1)$$

115 where  $R_{xylem}$  and  $R_{pith}$  are the xylem and the pith radius, respectively. Each vessel has a given number of  
 116 VACs associated with it,  $N_{vac}$ , calculated using a ratio of the vessel-VAC exchange area to the projected VAC  
 117 area:

$$N_{vac} = \frac{A_{vac-v}}{2R_{vac}l_{vac}}, \quad (2)$$

118 where  $A_{vac-v}$ ,  $R_{vac}$  and  $l_{vac}$  are the vessel-VAC exchange area, the VAC radius and VAC length, respectively.  
 119 The number of parenchyma rays is computed using a tangential ray density,  $trd$ , and the branch diameter,  
 120  $R_{branch}$ :

$$N_{ray} = trd \times 2\pi R_{branch}. \quad (3)$$

121 The number of bark cells connected to the parenchyma rays is

$$N_{bark\ cell} = \frac{V_{w,bark}^0}{V_{w,bark\ cell}^0} = \frac{BWF \times V_{bark}^0}{V_{w,bark\ cell}^0}, \quad (4)$$

122 with  $V_{w,bark}^0$  the initial volume of water in the bark accessible from the rays, equal to  $BWF \times V_{bark}^0$ , with  $BWF$   
 123 the bark water fraction accessible from the rays, and  $V_{bark}^0$  the initial bark volume.  $V_{w,Bark\ cell}^0$  is the initial  
 124 bark cell water volume.

### 125 Heat transfer and phase change

126 Heat transfer and phase change are calculated at the tissue scale through the heat equation in a 1D axi-symmetric  
 127 model in cylindrical coordinates:

$$\frac{dH}{dt} = \frac{1}{\rho} \left( \frac{\partial}{\partial r} \left( k_{th} \frac{\partial T}{\partial r} \right) + \frac{k_{th}}{r} \frac{\partial T}{\partial r} \right), \quad (5)$$

128 where  $H$  is the enthalpy,  $T$  the temperature,  $k_{th}$  the thermal conductivity and  $\rho$  the density. This equation is  
 129 used for  $r$ , the radial coordinate, in  $]R_{pith}; R_{xylem}[$  and completed with the following boundary conditions (Graf  
 130 et al., 2015):

$$T(R_{xylem}, t) = T_{ext}(t), \quad \text{and} \quad \left. \frac{\partial T}{\partial r} \right|_{R_{pith}} = 0, \quad (6)$$

131 where  $T_{ext}(t)$  is the external air temperature. Equation (5) must be completed by thermodynamic relationship  
 132 between  $H$  and  $T$  as well as between  $H$  and the physical properties (density and thermal conductivity) in order  
 133 to account for phase change. The procedure we used is explained in Bozonnet et al. (2023). The phase change  
 134 temperature at the tissue scale is computed locally based on  $C_s^v$ , the local vessel sugar content:

$$T_m^v = T_c - 0.001853 \times C_s^v, \quad (7)$$

135 with  $T_c = 273.15\text{K}$ . Note that Eq. (7) is only valid for water. More details on the implementation can be found  
 136 in Bozonnet et al. (2023).

### 137 Water fluxes

138 Water fluxes between elements are computed using Darcy's law. Along the parenchyma ray, the flow rate  $dV_{ray}/dt$   
 139 is:

$$\frac{dV_{ray}}{dt} = Q_{ray} = \frac{k_{ray}\pi R_{vac}^2}{\frac{\mu_{++}\mu_-}{2}\Delta l_v} \left( [p_t - C_s R_g T]_+ - [p_t - C_s R_g T]_- \right), \quad (8)$$

140 where  $k_{ray}$  is the ray water permeability,  $\Delta l_v$  is the distance along the ray between two vessels,  $\mu$  is the dynamic  
 141 viscosity of the water and sugar solution computed locally using the law given in Chenlo et al. (2002),  $p_t$ ,  $C_s$   
 142 and  $T$  are the living cell (VAC or bark cell) turgor pressure, sugar concentration and temperature, respectively.  
 143 The  $+$  and  $-$  signs represent a differentiation along the ray from the inside to the outside of the stem (up to  
 144 the bark cells).  $Q_{ray}$  is positive for water fluxes going towards the inside of the branch. Between one vessel and  
 145 one corresponding VAC, the flow rate  $dV_{vac-v}/dt$  is

$$\frac{dV_{vac-v}}{dt} = Q_{vac-v} = -\frac{k_{vac}A_{vac-v}}{\mu_{vac}WN_{vac}} (p_w^v - C_s^v R_g T + p_{ice}^v - [p_t - C_s R_g T]_{vac}), \quad (9)$$

146 where  $k_{vac}$  is the vessel-VAC membrane water permeability,  $W$  the vessel-VAC wall thickness, and  $p_w^v$  the vessel  
 147 water pressure.  $Q_{vac-v}$  is positive for water fluxes going towards the vessels. The cryo-suction pressure induced  
 148 by vessel freezing is computed at each vessel location as (Loch, 1978; Beck et al., 1984)

$$p_{ice}^v = \rho_w L \ln \left( \frac{T}{T_c} \right) \delta_{iv}, \quad (10)$$

149 where  $\rho_w$ ,  $L$ , and  $\delta_{iv}$  are the water density, water latent heat of fusion, and vessel ice volume fraction, respectively.  
 150 Eq. (9) implies that cryo-suction will draw water in a vessel from its VACs once this vessel is frozen.

### 151 Pressure-volume relationships in living cells

152 In living cells, the balance of water fluxes results in volume changes. For the VACs, the changes in water volume,  
 153  $dV_{vac-v}/dt$ , is

$$\frac{dV_{vac}}{dt} = -Q_{vac-v} + \sum_{\text{in-out}} Q_{ray}, \quad (11)$$

154 where the second term on the right hand side represents the balance of fluxes entering/leaving the VAC from/to  
 155 the ray. Between the xylem tissue and the bark, the total water flux is

$$Q_{xylem-bark} = -N_{vac} N_{ray} Q_{ray}^{R_{xylem}}, \quad (12)$$

156 where  $Q_{ray}^{R_{xylem}}$  is the water flux computed between one bark cell and the VAC closest to the bark. This water  
 157 flux is rescaled by the number of bark cells to obtain the volume change at the bark cell scale,  $V_{bark\ cell}$  :

$$\frac{dV_{bark\ cell}}{dt} = \frac{1}{N_{bark\ cell}} Q_{xylem-bark}. \quad (13)$$

158 These living cells' volume changes are related to turgor pressure variation through (Steudle et al., 1977)

$$\frac{dp_t^{vac}}{dt} = \frac{B_{vac}}{V_{vac}} \frac{dV_{vac}}{dt} \quad (14)$$

159 for the VACs, and

$$\frac{dp_t^{bark\ cell}}{dt} = \frac{B_{bark\ cell}}{V_{bark\ cell}} \frac{dV_{bark\ cell}}{dt} \quad (15)$$

160 for the bark cell. In the previous two equations,  $B_{vac}$  and  $B_{bark\ cell}$  are the VAC and bark cell elastic modulus,  
 161 respectively. We use the procedure introduced in Bozonnet et al. (2023) to account for turgor loss. Volume  
 162 changes also result in osmotic pressure changes through changes in sugar concentration, which are also related  
 163 to changes in sugar content:

$$C_s^{vac} = \frac{n_s^{vac}}{V_{vac} - K_{vac}} \quad \text{and} \quad C_s^{bark\ cell} = \frac{n_s^{bark\ cell}}{V_{bark\ cell} - K_{bark\ cell}}, \quad (16)$$

164 where  $n_s^{vac}$  and  $n_s^{bark\ cell}$  are the variable sugar quantities in the VAC and bark cell, and  $K_{vac}$ ,  $K_{bark\ cell}$ , are  
 165 the cell volume where no sugar can be contained (certain cell organelles), respectively. These changes in sugar  
 166 concentration also result in living cell FPD for the VACs and the bark cells, with an equation similar to eq. (7).

### 167 Pressure-volume relationships in vessels

168 Vessels contain gas that compresses or expands depending on water fluxes leaving or entering vessels. Following  
 169 Ceseri and Stockie (2013); Graf et al. (2015); Bozonnet et al. (2023), we assume that this gas is contained in one  
 170 cylindrical bubble located at the center of each vessel. Applying flow rate conservation between the gas/water  
 171 (or gas/ice) interface and the vessel/VAC membrane, the bubble radius,  $r_g^v$ , varies as

$$\frac{dr_g^v}{dt} = -\frac{N_{vac} Q_{vac-v}}{2\pi r_g^v L_z}, \quad (17)$$

172 where  $L_z$  is a vertical dimension that is introduced for unit consistency but that has no influence on model  
 173 results. Changes in  $r_g^v$  induce changes in gas pressure through the ideal gas law:

$$p_g^v = \frac{n_g^v R_g T(r, t)}{\pi r_g^{v2} L_z}. \quad (18)$$

174 In previous equation,  $n_g^v$  is the gas quantity inside the gas bubble and  $R_g$  the ideal gas constant. Finally, the  
 175 pressure in the liquid water/ice phase is obtained using Laplace equation:

$$p_w^v = p_g^v - \frac{\sigma_{gw}}{r_g^v}, \quad (19)$$

176 where  $\sigma_{gw}$  is the liquid water/gas interface surface tension. In the results section, we will also use the evolution  
 177 of the mean vessel pressure over all vessels, defined as

$$\overline{p_w^v} = \frac{1}{N_v} \sum_{N_v} p_w^v. \quad (20)$$

178 Similarly to living cells, water volume and sugar content changes in vessels also result in sugar concentration  
 179 changes:

$$C_s^v = \frac{n_s^v}{\pi(R_v^2 - r_g^{v2})L_z}, \quad (21)$$

180 where  $n_s^v$  and  $R_v$  are the variable vessel sugar quantity, and vessel radius, respectively. These changes in vessel  
 181 sugar concentration impact phase change at the tissue scale through Eq. (7). We will also use in the results  
 182 section the average vessel sugar concentration,  $\overline{C_s^v}$ , defined in a way similar to Eq. 20.

## 183 Sugar fluxes

184 Sugar fluxes occur due to passive diffusion between elements. Each vessel sugar content is computed as

$$\frac{dn_s^v}{dt} = \frac{D_s^{vac} A_{vac-v}}{W} (C_s^{vac} - C_s^v) \delta_a, \quad (22)$$

185 with  $D_s^{vac}$  the sugar diffusion coefficient between one vessel and one VAC, and  $\delta_a$  is an activation coefficient.  
 186  $\delta_a$  goes linearly from 0 at  $-0.5^\circ\text{C}$  to 1 at  $0^\circ\text{C}$ , and outside this interval it is equal to 0 for lower temperature  
 187 and 1 for higher temperature, hence progressively blocking sugar diffusion at negative temperature. It appeared  
 188 essential for numerical stability to block sugar diffusion at negative temperature, as the code had difficulties to  
 189 converge at negative temperature when ice was blocked in vessels whereas sugar fluxes would still induce water  
 190 flows in-between living cells. Each VAC sugar content is computed as

$$\frac{dn_s^{vac}}{dt} = -\frac{1}{N_{vac}} \frac{dn_s^v}{dt} + \sum_{\text{in-out}} F_s^{ray}, \quad (23)$$

191 where the first term on the right-hand side represents the sugar flux leaving the VAC towards its vessel, and the  
 192 second term represents the sum of sugar fluxes leaving or entering each VAC to/from the ray. These fluxes are  
 193 computed as

$$F_s^{ray} = \frac{D_s^{ray} \pi R_{vac}^2}{\Delta l_v} (C_s^{vac}(+) - C_s^{vac}(-)) \delta_a, \quad (24)$$

194 where  $D_s^{ray}$  is the sugar diffusion coefficient across the ray, and the  $+$  and  $-$  signs represent a differentiation  
 195 along the ray from the inside to the outside of the stem (up to the bark cells).  $F_s^{ray}$  is positive for sugar transport  
 196 towards the inside of the stem. The bark sugar content is computed as

$$\frac{dn_s^{bark}}{dt} = -\frac{N_{vac} N_{ray}}{N_{bark\ cell}} F_s^{ray} (R_{xylem}) \quad (25)$$

## 197 Diameter changes

198 Finally, diameter changes are obtained from living cell volume changes, Eqs. (11) and (13). The total volume  
 199 of water in VACs is computed at each instant:

$$V_{vac}^{tot}(t) = N_{vac} N_{ray} \sum_{N_{vessels}} V_{vac}(t), \quad (26)$$

200 with the volume variation equals to

$$\Delta V_{vac}^{tot}(t) = V_{vac}^{tot}(t) - V_{vac}^{tot}(0). \quad (27)$$

201 The xylem diameter, considered as a cylinder, is computed as

$$D_{xylem}(t) = \sqrt{D_{xylem}(0)^2 + \frac{4}{\pi L_z} \Delta V_{vac}^{tot}(t)}. \quad (28)$$

202 At each instant, the volume of the bark tissue is equal to the initial volume minus the volume lost by dehydration:

$$V_{bark} = V_{bark}^0 \left( 1 - BWF \left( 1 - \frac{V_{bark\ cell}}{V_{bark\ cell}^0} \right) \right), \quad (29)$$

203 from which the stem diameter, considered as a cylinder, can be computed:

$$D_{stem}(t) = \sqrt{D_{xylem}(t)^2 + \frac{4}{\pi L_z} V_{bark}}. \quad (30)$$

## 204 Numerical resolution and parameter choices

205 The model is implemented in the Matlab software version R2018a (MATLAB, 2018). Spatial discretisation of  
206 Eq. (5) is ensured using the finite difference method. The system of differential equations formed by equations  
207 (5), (8), (9), (11), (13), (14), (15), (17), (22), (23) and (25) is advanced in time using Matlab’s variable order  
208 *ode15s* solver based on numerical differentiation formulas, which is specifically designed for stiff equations, with  
209 a maximal time step of 1s, which is a sufficient value to resolve any stiffness in the problem under study. The  
210 other equations are state equations computed at each time step. Note that we verified the implementation of  
211 Eq. (5) using an analytical solution (Prapainop and Maneeratana, 2004) for a 1D freezing-front propagation  
212 (Stefan problem,  $R^2 > 0.9999$ ), and using a reference finite element solver (Comsol Multiphysics (COMSOL,  
213 2020)) for the 1D axi-symmetric implementation ( $R^2 = 0.9998$ ). The reference result presented in figure 2 takes  
214 a computational time of around 10 minutes on a Dell Latitude 7490 with 1.7 GHz quad-core Intel i5 processor.  
215 The source code can be downloaded at <https://github.com/cyrilbz/pressurebuildup>.

216 All model and state variables are regrouped in table 1. All parameters are in table 2. All values are either  
217 justified based on the literature, have been specifically measured, or calibrated and justified in our previous  
218 work (Bozonnet et al., 2023), except the initial sugar content in living cells, and the sugar diffusion coefficients.  
219 The initial sugar content in living cells has been estimated based on measurements on whole stems in Charrier  
220 et al. (2013). The starting values for the diffusion coefficients have been computed using a solute permeability  
221 coefficient  $P_s^{ray} = P_s^{vac} \approx 3 \times 10^{-9}$  m/s (Gunning, 1977; Tyree et al., 1994), which leads to  $D_s^{vac} = P_s^{vac}W \approx$   
222  $1 \times 10^{-14}$  m<sup>2</sup>/s and  $D_s^{ray} = P_s^{ray}\Delta l_v \approx 1 \times 10^{-12}$  m<sup>2</sup>/s. We note that the permeability coefficients in the  
223 reference we used correspond to solute flow across roots, hence the actual values might be underestimated.

224 For simplicity, we further assume that all living cells have the same mechanical properties and the same  
225 initial sugar concentrations. However, the model is already capable of handling different parameter values  
226 between VACs and bark cells.

## 227 Results

228 In this section we present the results obtained using the model described previously. Unless stated otherwise,  
229 all parameter values are presented in table 2. In the following, and unless stated otherwise, the term pressure  
230 always refers to the vessel pressure in the liquid water/ice phase,  $p_w^v$ .

### 231 Effect of sugar fluxes

232 In this section we describe the model results obtained with or without sugar fluxes. The stem undergoes a three-  
233 day period of continuous temperature fluctuations, ranging between +5°C and -10°C, occurring in 24-hours  
234 cycles, as shown in figure 1. The expression for  $T_{ext}(t)$  is given in table 2.

235 Without sugar fluxes ( $P_s^{ray} = P_s^{vac} = 0$ ; continuous red line), the mean pressure (figure 2a) shows an  
236 alternating sequence of increases at freezing and drops at thawing. As explained in Bozonnet et al. (2023), this  
237 is due to water going from living cells to xylem vessels under the influence of the low ice potential in vessels and  
238 reversing at thawing. The effect of these water flows can be seen in stem diameter changes (figure 2b): at freezing  
239 we observe a shrinkage in diameter, followed by a swelling at thawing. Pressure and stem diameter changes are  
240 fully reversible. In the end of the simulation the pressure distribution (figure 2c) is nearly homogeneous across  
241 the stem, i.e., pressure values are equal for all radial positions, and the mean pressure is equal to its initial value.

242 When sugar fluxes between VAC and vessels are included ( $P_s^{vac} = 3 \times 10^{-9}$  m/s,  $P_s^{ray} = 0$ ; green dashed  
243 line), we observe a slight increase of the final pressure compared to the case where these fluxes were missing  
244 ( $\overline{p_w^v}(72h) = 20.7$ kPa compared to  $\overline{p_w^v}(72h) = 20$ kPa, see figure 2a). The maximum pressure in the frozen state  
245 increases, but more significantly (+15kPa). Stem diameter changes are also affected: compared to the previous  
246 case, the maximum shrinkage at freezing increases, i.e., the minimal diameter decreases, and at thawing the  
247 diameter does not come back to its initial value (figure 2b). The radial pressure profile (figure 2c) does not  
248 show much difference with the previous case. Note that increasing the number of VACs per vessel ( $N_{vac} = 2260$   
249 compared to  $N_{vac} = 300$  in the present case) presents only a slight vessel pressure increase after 72 hours  
250 ( $\overline{p_w^v}(72h) = 26.2$ kPa, results not shown).



251 When radial sugar fluxes are added ( $P_s^{vac} = P_s^{ray} = 3 \times 10^{-9}$  m/s; continuous blue line), the mean vessel  
 252 pressure shows a much larger increase at the end of the simulation:  $\bar{p}_w^v(72h) = 63\text{kPa}$  (figure 2a). It progressively  
 253 increases in both states: in the frozen state, the maximum pressure increases all over the simulation, as well as  
 254 in the thawed state, where sugar fluxes are active. Stem diameter variations (figure 2b) also show differences  
 255 in both states: in the frozen state, the maximum shrinkage progressively increases over the three cycles, and  
 256 in the thawed state, the maximum diameter decreases with time. The radial vessel pressure profile shows  
 257 spectacular differences with the other two previously cases (figure 2c): vessel pressure is slightly lower ( $-25\%$ )  
 258 than previously near the pith (lowest  $r$  values), and significantly higher (up to  $+1530\%$ ) near the bark (highest  
 259  $r$  values).

## 260 Effect of sugar permeabilities

261 In figure 3a we show the effect of both sugar permeability coefficients ( $P_s^{vac}$  and  $P_s^{ray}$ ) on the pressure level after  
 262 72h. We consider three cases: one case where  $P_s^{ray}$  is kept equal to its original value ( $3 \times 10^{-9}$  m/s) and only  
 263  $P_s^{vac}$  varies (continuous line), and two cases where  $P_s^{vac}$  is kept constant and only  $P_s^{ray}$  varies, for 2 values of  
 264  $P_s^{vac}$  (dashed lines).

265 We observe that when  $P_s^{vac}$  only is increased, the pressure build-up increases too and reaches a plateau at  
 266 high  $P_s^{vac}$ . When  $P_s^{ray}$  only is varied the pressure build-up is low for extreme  $P_s^{ray}$  values and passes through  
 267 a maximum value. This is valid for both data series at varying  $P_s^{ray}$ , with the data serie for the highest  $P_s^{vac}$   
 268 showing the highest pressure levels.

269 In figure 3b we show the effect of the ray sugar permeability on the long term evolution (20 days) of the  
 270 mean pressure. The case with  $P_s^{ray} = 8 \times 10^{-8}$  m/s shows the fastest pressure increase followed by a decline after  
 271 only 2 days. After 10 days, the case with  $P_s^{ray} = 3 \times 10^{-9}$  m/s, the lowest  $P_s^{ray}$  value in this figure, generates  
 272 the highest pressure level. For a sufficiently long simulation, and for the parameter range considered here, the  
 273 maximal mean pressure level reached during a simulation decreases with an increase in ray sugar permeability.

274 In figure 3c we show the effect of the ray sugar permeability on the long term (20 days) variations of stem  
 275 diameter. One can see that increasing the permeability decreases the diameter, both in frozen and thawed states.

## 276 Spatio-temporal vessel pressure variations

277 In figure 4a we draw from figure 3b the changes in mean vessel pressure for the case with  $P_s^{ray} = P_s^{vac} = 2.1 \times 10^{-8}$   
 278 m/s, with a custom color code that represents the time course. We chose this case as it shows a pressure build-up  
 279 followed by a decline. In figure 4b we show for the same case the vessel pressure profile as a function of the  
 280 radial coordinate and for different instants of the simulation (one profile every 24 hours) with the same color  
 281 code. Similarly to figure 2c and compared to the initial value, the pressure decreases near the pith and increases  
 282 near the bark. One can see that in only 2 days the vessel pressure reaches its maximal value near the bark.  
 283 Then the pressure curve progressively spreads towards the interior of the stem. For a much longer simulation,  
 284 the vessel pressure will become homogeneous along the radius (results not shown), similarly to cases with no ray  
 285 sugar fluxes in figure 1a, but with a slightly higher pressure value.

## 286 Effect of initial sugar content and validation

287 Figure 5a illustrates the effect of the initial sugar concentration in living cells on the mean pressure for two sugar  
 288 permeability values. The initial sugar concentrations were chosen to reflect the potential changes in soluble  
 289 carbohydrates content across the winter season (Charrier et al., 2018), and with the treatments applied to the  
 290 trees (defoliation, exposure to low or high temperature). We remind the reader that all living cells initially  
 291 have the same sugar content. We observe an increase of the mean pressure with the initial sugar content.  
 292 This increase is even higher at higher sugar permeability. For low initial sugar concentration ( $200\text{mol/m}^3$ ) the  
 293 difference between both cases is only 8kPa, whereas it reaches 160kPa for the highest initial sugar concentration  
 294 ( $1600\text{mol/m}^3$ ).

295 In figure 5b we show the relation between the mean vessel sugar concentration and the mean vessel pressure,  
 296 both after 72h, for the cases presented in figure 5a. In the same figure we also show the experimental results from  
 297 Améglio et al. (2001), for stems that were defoliated during summer and the control group. The mean pressure

298 level rises with the increase of the mean vessel sugar concentration for both the model and the experimental  
299 data. For the greatest permeability coefficient, both the average sugar concentration and mean pressure reach  
300 significantly higher values. Model results and experiments have similar order of magnitude for both quantities.

## 301 Discussion

### 302 Mechanism behind pressure build-up

303 As we shown in figure 2a, in our model sugar fluxes across the parenchyma ray are absolutely essential for  
304 pressure build-up to occur. They also dramatically change the pressure radial distribution within the stem, with  
305 the pressure decreasing near the pith and increasing elsewhere (figure 2c). They are initially induced by the  
306 sugar fluxes from VACs to vessel, that decrease the VACs sugar concentration. These ray sugar fluxes act on the  
307 pressure through two mechanisms. First, the sugar flux from the bark cells to the vessels (through the VACs)  
308 induce a flow of water from the bark cells towards the vessels, thus increasing the pressure. Secondly, because of  
309 the spatial distribution of the vessels along the parenchyma rays, the vessels close to the bark are preferentially  
310 loaded with sugars. This creates a radial gradient in sugar concentration that induces a water flux from the near  
311 pith vessels to the near bark vessels, thus creating the distribution in pressure observed in figure 2c.

312 The first mechanism, the sugar and water fluxes from the living cells towards the vessels, can also be evidenced  
313 through stem diameter changes: a reduction in diameter occurs both in frozen and thawed states. Particularly,  
314 the increase in the freeze-induced stem shrinkage is due to the decrease in living cell sugar content, as already  
315 shown in Bozonnet et al. (2023). The second mechanism, although not directly observable on stem diameter has  
316 a much greater impact on vessel pressure: the case with the highest stem pressure does not have the smallest  
317 diameter in the frozen state (see figure 3b and c). We have thus demonstrated that pressure build-up can be  
318 due to a transfer of water between vessels, across the parenchyma rays, induced by a radial imbalance in vessel  
319 sugar concentration.

320 For low and high ray sugar permeabilities ( $P_s^{ray}$ ), the radial imbalance is greatly reduced, and no pressure  
321 rise occurs, except the one due to the water flows coming from living cells, as shown in figure 3a with the effect of  
322  $P_s^{ray}$ . Sugar transportation along the ray alone is not sufficient: if VAC to vessel transport is too low, no pressure  
323 accumulates, as observed in 3a, with the effect of  $P_s^{vac}$ . Note that, in the short term, there will necessarily be  
324 no sugar radial imbalance, thus no pressure rise. This is also true for sufficiently long simulations for which the  
325 sugar concentrations will become homogeneous across the stem (figure 3b). The duration required to achieve  
326 this homogenisation, as well as the duration required to reach the maximum pressure build-up, is contingent on  
327 the ray sugar permeability values.

328 The spatio-temporal variations of the vessel pressure give even more insights into the process. Figure 4b  
329 enlightens that at short times there is a pressure decrease near the pith and an increase near the bark, indicating  
330 a flow of water in-between vessels through the parenchyma rays. The decrease near the pith continues while  
331 the pressure profile in the vicinity of the bark progressively flattens. The mean pressure reaches its maximum  
332 during this part of the process (figure 4a). Eventually the pressure near the pith progressively starts to increase  
333 while the pressure profile tends towards homogeneity. For sufficiently long simulations, pressure values will be  
334 equal for all vessels, with a value slightly higher than for the case where no ray sugar fluxes were included in the  
335 processes (figure 2).

### 336 Comparison with experiments

337 Stems that underwent early defoliation showed low pressure build-up compared to controls (Améglio et al., 2001).  
338 Early defoliation can indeed reduce the amount of stored carbohydrates in living cells, thus preventing them to  
339 hydrolize starch during winter in order to increase their sugar concentration (Charrier et al., 2018). Similarly, a  
340 treatment at high temperature before the experiments was also likely to reduce the accumulation of soluble sugar  
341 in living cells. Both of these treatments lowered the measured pressure level in Améglio et al. (2001). The model  
342 predicts the same relation between the initial sugar concentration and the mean vessel pressure level (figure 5a):  
343 a decrease in the concentration leads to a decrease in pressure. The effect of the initial sugar concentration is  
344 even greater at high sugar permeability, which is fully expected as higher permeabilities lead to higher pressure  
345 (figure 3a).

346 Finally, we have validated the model by comparing two of its outputs, the mean vessel sugar concentration  
347 and the mean vessel pressure after 72h, against the measured sap osmolarity and xylem pressure after 72h of  
348 experiments. We emphasize that both of these quantities are results, either from the model or the experiments,  
349 and not inputs or controlled parameters. The comparison is thus extremely favorable, as similar orders of  
350 magnitude are reached between the model and the experiments for both of these quantities, especially for the  
351 model results at high sugar permeability coefficients. This shows that the initial permeability coefficients we  
352 used were probably underestimated.

353 We note that the external temperature between the simulations and the experiments were not exactly the  
354 same: in Améglio et al. (2001), stems that underwent a high temperature treatment before the experiments  
355 were exposed during the freeze-thaw cycles to a maximal temperature up to 18°C, while the other stems were  
356 exposed to a maximal temperature of 1.5°C. In the simulations, we chose a maximal temperature of 5°C, that  
357 we estimate as being a threshold above which H<sup>+</sup>/sugar co-transport will bring sugar from the vessels back to  
358 the living cells and in-between living cells (at counter gradient). This mode of transport is not included in the  
359 model, but including it would be a way to go further in the exploration of temperature effects on stem pressure  
360 build-up. This would also be a way to verify if the current understanding of these temperature effects, as being  
361 the result of a balance between the H<sup>+</sup>/sugar co-transport and diffusion, is correct.

362 Similarly, starch-soluble sugar inter-conversion is not included in the model as we expect it to occur on a  
363 timescale longer than 3 days (Charrier et al., 2018). It can however have an impact on longer time scales.  
364 Including it in the model would be a way to dynamically compute the initial living cell sugar concentration,  
365 as a function of the environmental conditions and the tree’s carbohydrate reserves. This way, the effect of  
366 the different experimental treatments (defoliation, low/high temperature exposure) could be simulated directly  
367 within the model and not modelled with a varying initial sugar concentration.

368 In the model from Graf et al. (2015), pressure build-up, as we defined it in the introduction, occurs due to  
369 irreversible root absorption during freezing or thawing events in the stem. This is different from our model results  
370 and the experiments of Améglio et al. (2001), where it occurs during the day (at slightly positive temperature,  
371 after thawing and before freezing), and, particularly, in the absence of any connection with the root system. In  
372 Graf et al. (2015), freeze-thaw cycles are essentials for this build-up to occur, as in the experiments of Améglio  
373 et al. (2001). Our model does not reproduce such synergetic effect of freeze-thaw cycles: a case without freezing  
374 temperature shows the same pressure build-up as a complete case. Both models predict an increase in vessel  
375 pressure with sugar content, although in Graf et al. (2015) the sugar content does not change with time. Only  
376 our model reaches orders of magnitude that are consistent with the experimental results for both the final vessel  
377 pressure and vessel sugar concentration.

378 Although water fluxes between crown and roots are occurring in field experiments (Charrier et al., 2017),  
379 and are essentials for maple sap harvest (Tyree, 1984), we do not think that adding them in our model is the  
380 path to follow to obtain this synergetic effect of freeze-thaw cycle. This would indeed requires a connection  
381 with the rest of the tree, whereas following Améglio et al. (2001) it is not needed for pressure build-up to occur.  
382 It is possible that our omission of sugar transport at negative temperatures and our assumption of a constant  
383 quantity of gas in vessels,  $n_g^v$ , contributed to this lack of synergetic effect. It is well-established that freeze-thaw  
384 cycles create gas bubbles that may fill an entire section of the vessel, see references in the introduction. One  
385 could hypothesize that repeated freeze-thaw cycles could raise the value of  $n_g^v$ , thereby affecting the dynamics  
386 of pressure build-up. One other missing ingredient could be the starch to sugar conversion in living cells, which  
387 could still occur at slightly negative temperature, but as said previously we do not expect it to have an impact  
388 on such a short timescale. It would be worth investigating further this point to understand better the effect of  
389 freeze-thaw cycles on pressure build-up and how it could be impacted by a changing climate.

390 The pressure build-up mechanism we have highlighted leads to a pressure drop near the pith and a pressure  
391 rise elsewhere. In the context of embolism recovery, this means that this mechanism cannot repair embolism in  
392 the vicinity of the pith. We currently have no experimental data to validate such consequence. Such data could  
393 be obtained by scanning (X-ray microtomography) walnut branches before and after stem pressure generation  
394 to precisely locate the places where embolism recovery occurs, such as done for example on birch and maple tree  
395 in Robinson et al. (2023). This heterogeneous repair might however be related to the transition from sapwood to  
396 heartwood due to tyloses formation in embolized vessels (Kozłowski and Pallardy, 1997; Barnett, 2004). Another  
397 way to validate the mechanism would be to repeat the experiments of Améglio et al. (2001) with stem samples  
398 that have their bark removed and intact ones. The absence of pressure build-up for stems with their bark

399 removed would be a strong argument to validate the mechanism.

400 Compared to our previous model that did not generate any pressure build-up (Bozonnet et al., 2023), the  
401 only additional mechanism in the present one is the transport of sugar by diffusion in-between living cells and  
402 between living cells and vessels. The reason why some species are (or not) able to increase the pressure in  
403 their branches might therefore lie in this ability to transport sugar during winter. In the walnut tree, this is  
404 regulated by specific proteins in cell membranes, that could be less abundant during winter in other species.  
405 This might also depend on anatomical features such as the number of vessel associated cells, or the parenchyma  
406 rays anatomy.

407 In addition, it is worth investigating in the future whether this mechanism is relevant to the development of  
408 winter stem pressure in maple trees and the harvesting of maple sap. Particularly, one could start by coupling  
409 our work with recent modelling efforts on maple tree (Graf et al., 2015; Zarrinderakht et al., 2024). One major  
410 difference between maple and walnut trees lies in the need, when modelling pressure changes in maple tree, to  
411 include a hydraulic connection as well as an osmotic barrier between vessels and fibers. This is indeed required to  
412 reproduce the pressure drop observed at freezing inception in maple trees (Milburn and O'Malley, 1984; Cirelli  
413 et al., 2008; Ceseri and Stockie, 2013; Graf et al., 2015; Zarrinderakht et al., 2024), whereas the opposite is  
414 observed in walnut trees and all other species (Robson and Petty, 1987; Améglio et al., 2001). This pressure  
415 drop at freezing favours water entry from the roots while vessels are still in the liquid state. Water exchange  
416 with the roots, even though, in our opinion, not a key ingredient in the pressure build-up as discussed previously,  
417 can therefore have a much greater impact in maple tree compared to walnut tree. In both species, however,  
418 water fluxes between the crown and the roots could be directly driven by cryostatic suction, which is not taken  
419 into account in any of the existing models.

## 420 Conclusion

421 The initial aim of this work was to develop a mechanistic model capable of simulating the winter pressure build-  
422 up in walnut stems. We have shown that the pressure build-up can be explained by a transfer of water between  
423 vessels via the parenchyma rays, induced by a radial imbalance in sugar concentration.

424 Among the various features listed in the introduction, this mechanism succeeds in: generating a pressure  
425 build-up for stems disconnected from the rest of the tree, quantifying the relationship between pressure build-up  
426 and xylem sap osmolarity, and showing the effect of experimental treatments (defoliation, low/high temperature  
427 exposure) through the influence of the initial living cell sugar concentration. Temperature effects on vessel  
428 pressure are partially captured due to the lack of  $H^+$ /sugar co-transport in the model. The model does not yet  
429 capture the synergetic effect of freeze-thaw cycles on pressure build-up, which needs to be investigated in the  
430 future.

431 Finally, we have outlined two experiments to validate the pressure build-up mechanism we have identified.  
432 Both of these experiments could validate two crucial aspects of the mechanism: it leads to a heterogeneous  
433 embolism repair, and it requires sugar fluxes coming from the bark.

## 434 Data availability statement

435 The source code used to generate the data of the present paper can be downloaded at  
436 <https://github.com/cyrilbz/pressurebuildup>. Any result of the present paper can be reproduced using this code.

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## References

- 441 G. Alves, M. Decourteix, P. Fleurat-Lessard, S. Sakr, M. Bonhomme, T. Améglio, A. Lacoïnte, J.-L. Julien,  
442 G. Petel, and A. Guillot. Spatial activity and expression of plasma membrane H<sup>+</sup>-ATPase in stem xylem  
443 of walnut during dormancy and growth resumption. *Tree Physiology*, 27(10):1471–1480, 2007.
- 444 T. Améglio and P. Cruiziat. Tension-pressure alternation in walnut xylem sap during winter: The role of winter  
445 temperature. *Comptes Rendus de l'Académie des Sciences Serie 3 Sciences de la Vie (France)*, 1992.
- 446 T. Améglio, F. W. Ewers, H. Cochard, M. Martignac, M. Vandame, C. Bodet, and P. Cruiziat. Winter stem  
447 xylem pressure in walnut trees: effects of carbohydrates, cooling and freezing. *Tree Physiology*, 21(6):  
448 387–394, 2001.
- 449 T. Améglio, C. Bodet, A. Lacoïnte, and H. Cochard. Winter embolism, mechanisms of xylem hydraulic con-  
450 ductivity recovery and springtime growth patterns in walnut and peach trees. *Tree Physiology*, 22(17):  
451 1211–1220, 2002.
- 452 T. Améglio, M. Decourteix, G. Alves, V. Valentin, S. Sakr, J.-L. Julien, G. Petel, A. Guillot, and A. Lacoïnte.  
453 Temperature effects on xylem sap osmolarity in walnut trees: evidence for a vitalistic model of winter  
454 embolism repair. *Tree Physiology*, 24(7):785–793, 2004.
- 455 J. Barnett. Tree physiology | xylem physiology. In J. Burley, editor, *Encyclopedia of Forest Sciences*, pages  
456 1583–1590. Elsevier, Oxford, 2004. ISBN 978-0-12-145160-8. doi: [https://doi.org/10.1016/B0-12-145160-7/](https://doi.org/10.1016/B0-12-145160-7/00099-5)  
457 [00099-5](https://www.sciencedirect.com/science/article/pii/B0121451607000995). URL <https://www.sciencedirect.com/science/article/pii/B0121451607000995>.
- 458 E. Beck, E.-D. Schulze, M. Senser, and R. Scheibe. Equilibrium freezing of leaf water and extracellular ice  
459 formation in afroalpine ‘giant rosette’ plants. *Planta*, 162(3):276–282, 1984.
- 460 C. Bozonnet, M. Saudreau, E. Badel, T. Améglio, and G. Charrier. Freeze dehydration vs supercooling in tree  
461 stems: physical and physiological modelling. *Tree Physiology*, 2023. doi: 10.1093/treephys/tpad117. URL  
462 <https://doi.org/10.1093/treephys/tpad117>.
- 463 T. J. Brodribb and H. Cochard. Hydraulic failure defines the recovery and point of death in water-stressed  
464 conifers. *Plant physiology*, 149(1):575–584, 2009.
- 465 M. Ceseri and J. M. Stockie. A mathematical model of sap exudation in maple trees governed by ice melting,  
466 gas dissolution, and osmosis. *SIAM Journal on Applied Mathematics*, 73(2):649–676, 2013.
- 467 K. Charra-Vaskou, E. Badel, G. Charrier, A. Ponomarenko, M. Bonhomme, L. Foucat, S. Mayr, and T. Améglio.  
468 Cavitation and water fluxes driven by ice water potential in *Juglans regia* during freeze–thaw cycles. *Journal*  
469 *of Experimental Botany*, 67(3):739–750, 2016.
- 470 G. Charrier, M. Poirier, M. Bonhomme, A. Lacoïnte, and T. Améglio. Frost hardiness in walnut trees (*Juglans*  
471 *regia* L.): how to link physiology and modelling? *Tree Physiology*, 33(11):1229–1241, 2013.
- 472 G. Charrier, J. M. Torres-Ruiz, E. Badel, R. Burlett, B. Choat, H. Cochard, C. E. Delmas, J.-C. Domec,  
473 S. Jansen, A. King, et al. Evidence for hydraulic vulnerability segmentation and lack of xylem refilling  
474 under tension. *Plant Physiology*, 172(3):1657–1668, 2016.
- 475 G. Charrier, M. Nolf, G. Leitinger, K. Charra-Vaskou, A. Losso, U. Tappeiner, T. Améglio, and S. Mayr.  
476 Monitoring of freezing dynamics in trees: a simple phase shift causes complexity. *Plant Physiology*, 173(4):  
477 2196–2207, 2017.
- 478 G. Charrier, A. Lacoïnte, and T. Améglio. Dynamic modeling of carbon metabolism during the dormant period  
479 accurately predicts the changes in frost hardiness in walnut trees *Juglans regia* L. *Frontiers in Plant Science*,  
480 9:1746, 2018.
- 481 F. Chenlo, R. Moreira, G. Pereira, and A. Ampudia. Viscosities of aqueous solutions of sucrose and sodium  
482 chloride of interest in osmotic dehydration processes. *Journal of Food Engineering*, 54(4):347–352, 2002.

- 483 B. Choat, E. Badel, R. Burlett, S. Delzon, H. Cochard, and S. Jansen. Non-invasive measurement of vulnerability  
484 to drought-induced embolism by x-ray microtomography. *Plant Physiology*, 170(1):273–282, 2016.
- 485 D. Cirelli, R. Jagels, and M. T. Tyree. Toward an improved model of maple sap exudation: the location and  
486 role of osmotic barriers in sugar maple, butternut and white birch. *Tree Physiology*, 28(8):1145–1155, 2008.
- 487 H. Cochard and M. T. Tyree. Xylem dysfunction in *Quercus*: vessel sizes, tyloses, cavitation and seasonal  
488 changes in embolism. *Tree Physiology*, 6(4):393–407, 1990.
- 489 H. Cochard, D. Lemoine, T. Améglio, and A. Granier. Mechanisms of xylem recovery from winter embolism in  
490 *Fagus sylvatica*. *Tree Physiology*, 21(1):27–33, 2001.
- 491 COMSOL. version 5.6, 2020. URL <http://www.comsol.com/products/multiphysics/>. Accessed: 2023-09-10.
- 492 M. Decourteix, G. Alves, M. Bonhomme, M. Peuch, K. B. Baaziz, N. Brunel, A. Guilliot, R. Rageau, T. Améglio,  
493 G. Pétel, et al. Sucrose (jrsut1) and hexose (jrht1 and jrht2) transporters in walnut xylem parenchyma  
494 cells: their potential role in early events of growth resumption. *Tree physiology*, 28(2):215–224, 2008.
- 495 J. Dumais and Y. Forterre. “vegetable dynamicks”: the role of water in plant movements. *Annual Review of*  
496 *Fluid Mechanics*, 44:453–478, 2012.
- 497 F. W. Ewers, T. Améglio, H. Cochard, F. Beaujard, M. Martignac, M. Vandame, C. Bodet, and P. Cruiziat.  
498 Seasonal variation in xylem pressure of walnut trees: root and stem pressures. *Tree Physiology*, 21(15):  
499 1123–1132, 2001.
- 500 J. B. Fisher, A. Guillermo Angeles, F. W. Ewers, and J. Lopez-Portillo. Survey of root pressure in tropical vines  
501 and woody species. *International Journal of Plant Sciences*, 158(1):44–50, 1997.
- 502 I. Graf, M. Ceseri, and J. M. Stockie. Multiscale model of a freeze–thaw process for tree sap exudation. *Journal*  
503 *of the Royal Society Interface*, 12(111):20150665, 2015.
- 504 B. E. Gunning. Transfer cells and their roles in transport of solutes in plants. *Science Progress (1933-)*, pages  
505 539–568, 1977.
- 506 U. Hacke and J. Sauter. Xylem dysfunction during winter and recovery of hydraulic conductivity in diffuse-porous  
507 and ring-porous trees. *Oecologia*, 105:435–439, 1996.
- 508 K. Hargrave, K. Kolb, F. Ewers, and S. Davis. Conduit diameter and drought-induced embolism in *Salvia*  
509 *mellifera* Greene (Labiatae). *New Phytologist*, 126(4):695–705, 1994.
- 510 T. Hölttä, T. Vesala, M. Perämäki, and E. Nikinmaa. Refilling of embolised conduits as a consequence of ‘Münch  
511 water’circulation. *Functional Plant Biology*, 33(10):949–959, 2006.
- 512 T. T. Kozłowski and S. G. Pallardy. Chapter 3 - vegetative growth. In T. T. Kozłowski and S. G. Pallardy,  
513 editors, *Physiology of Woody Plants (Second Edition)*, pages 34–67. Academic Press, San Diego, second  
514 edition edition, 1997. ISBN 978-0-12-424162-6. doi: <https://doi.org/10.1016/B978-012424162-6/50020-X>.
- 515 J. Loch. Thermodynamic equilibrium between ice and water in porous media. *Soil Science*, 126(2):77–80, 1978.
- 516 M. Mantova, S. Herbette, H. Cochard, and J. M. Torres-Ruiz. Hydraulic failure and tree mortality: from  
517 correlation to causation. *Trends in Plant Science*, 27(4):335–345, 2022.
- 518 MATLAB. version 9.4.0 (R2018b). The MathWorks Inc., Natick, Massachusetts, 2018.
- 519 J. Milburn and P. O’Malley. Freeze-induced sap absorption in *Acer pseudoplatanus*: a possible mechanism.  
520 *Canadian Journal of Botany*, 62(10):2101–2106, 1984.
- 521 A. Nardini, M. A. L. Gullo, and S. Salleo. Refilling embolized xylem conduits: is it a matter of phloem unloading?  
522 *Plant Science*, 180(4):604–611, 2011.

- 523 J. Petty and M. A. Palin. Permeability to water of the fibre cell wall material of two hardwoods. *Journal of*  
524 *Experimental Botany*, 34(6):688–693, 1983.
- 525 R. Prapainop and K. Maneeratana. Simulation of ice formation by the finite volume method. *Simulation*, 26(1):  
526 56, 2004.
- 527 C. Rajashekar and M. J. Burke. Freezing characteristics of rigid plant tissues (development of cell tension during  
528 extracellular freezing). *Plant Physiology*, 111(2):597–603, 1996.
- 529 J. A. Robinson, M. Rennie, M. Clearwater, D. J. Holland, A. K. van den Berg, and M. Watson. Examination of  
530 embolisms in maple and birch saplings utilising microct. *Micron*, 168:103438, 2023.
- 531 D. Robson and J. Petty. Freezing in conifer xylem: I. pressure changes and growth velocity of ice. *Journal of*  
532 *Experimental Botany*, 38(11):1901–1908, 1987.
- 533 S. Salleo, M. A. L. Gullo, D. de Paoli, and M. Zippo. Xylem recovery from cavitation-induced embolism in  
534 young plants of *Laurus nobilis*: a possible mechanism. *New Phytologist*, 132(1):47–56, 1996.
- 535 J. S. Sperry and J. E. Sullivan. Xylem embolism in response to freeze-thaw cycles and water stress in ring-porous,  
536 diffuse-porous, and conifer species. *Plant Physiology*, 100(2):605–613, 1992.
- 537 J. S. Sperry and M. T. Tyree. Mechanism of water stress-induced xylem embolism. *Plant Physiology*, 88(3):  
538 581–587, 1988.
- 539 J. S. Sperry, N. M. Holbrook, M. H. Zimmermann, and M. T. Tyree. Spring filling of xylem vessels in wild  
540 grapevine. *Plant Physiology*, 83(2):414–417, 1987.
- 541 E. Steudle, U. Zimmermann, and U. Lüttge. Effect of turgor pressure and cell size on the wall elasticity of plant  
542 cells. *Plant Physiology*, 59(2):285–289, 1977.
- 543 M. Tyree. Maple sap exudation: How it happens. *Maple Syrup Journal*, 4(1):10–11, 1984.
- 544 M. T. Tyree. Maple sap uptake, exudation, and pressure changes correlated with freezing exotherms and thawing  
545 endotherms. *Plant Physiology*, 73(2):277–285, 1983.
- 546 M. T. Tyree, S. Yang, P. Cruiziat, and B. Sinclair. Novel methods of measuring hydraulic conductivity of  
547 tree root systems and interpretation using AMAIZED (a maize-root dynamic model for water and solute  
548 transport). *Plant Physiology*, 104(1):189–199, 1994.
- 549 M. Zarrinderakht, I. Konrad, T. R. Wilmot, T. D. Perkins, A. K. van den Berg, and J. M. Stockie. Experimental  
550 and computational comparison of freeze-thaw induced pressure generation in red and sugar maple. *Tree*  
551 *Physiology*, 01 2024. doi: 10.1093/treephys/tpae006. URL <https://doi.org/10.1093/treephys/tpae006>.

- 552 Figure legend list :
- 553 • Figure 1: Structure of the model. Depending on the type of element and its enthalpy level, water is  
554 assumed to be in different phases (solid, liquid, gas). Water fluxes (blue arrows) and sugar fluxes (yellow  
555 arrows) occur between different anatomical elements across cell membranes. Scaling coefficients ( $N_{vac}$ ,  
556  $N_{ray}$ ,  $N_{bark\ cell}$ ,  $N_{vessels}$ ) are used to obtain a more accurate anatomical description.
  - 557 • Figure 2: Effect of sugar fluxes on mean pressure (a), stem diameter changes (b), and pressure field at the  
558 end of the simulation (c). In (c),  $r$  is the radial coordinate. Continuous red line:  $P_s^{ray} = P_s^{vac} = 0$ ; green  
559 dashed line:  $P_s^{vac} = 3 \times 10^{-9}$  m/s,  $P_s^{ray} = 0$ ; continuous blue line:  $P_s^{vac} = P_s^{ray} = 3 \times 10^{-9}$  m/s.
  - 560 • Figure 3: Effect of sugar permeabilities ( $P_s^{vac}$  and  $P_s^{ray}$ ) on the mean vessel pressure after 72 hours (a).  
561 Effect of the ray sugar permeability  $P_s^{ray}$  on the mean pressure long term evolution (b), and on stem  
562 diameter changes (c). Note that in b only one data point every 24 hours (in thawed state) is shown to  
563 enhance readability. In b and c:  $P_s^{vac} = 2.1 \times 10^{-8}$  m/s. For the legend in figure c, please refer to figure b.
  - 564 • Figure 4: Spatio-temporal variations of vessel pressure for case  $P_s^{ray} = P_s^{vac} = 2.1 \times 10^{-8}$ . a) Mean pressure  
565 signal (reproduced from figure 3 with a custom color code), for color interpretation see the scale in figure  
566 b. In a, only one data point every 24h is shown to enhance readability. b) Radial pressure profile every  
567 48h.
  - 568 • Figure 5: a) Effect of initial living cell sugar concentration on the mean vessel pressure after 72h. b) Link  
569 between mean vessel pressure and mean vessel sugar content after 72h + comparison with experiments  
570 from Améglio et al 2001 (after 72h too).



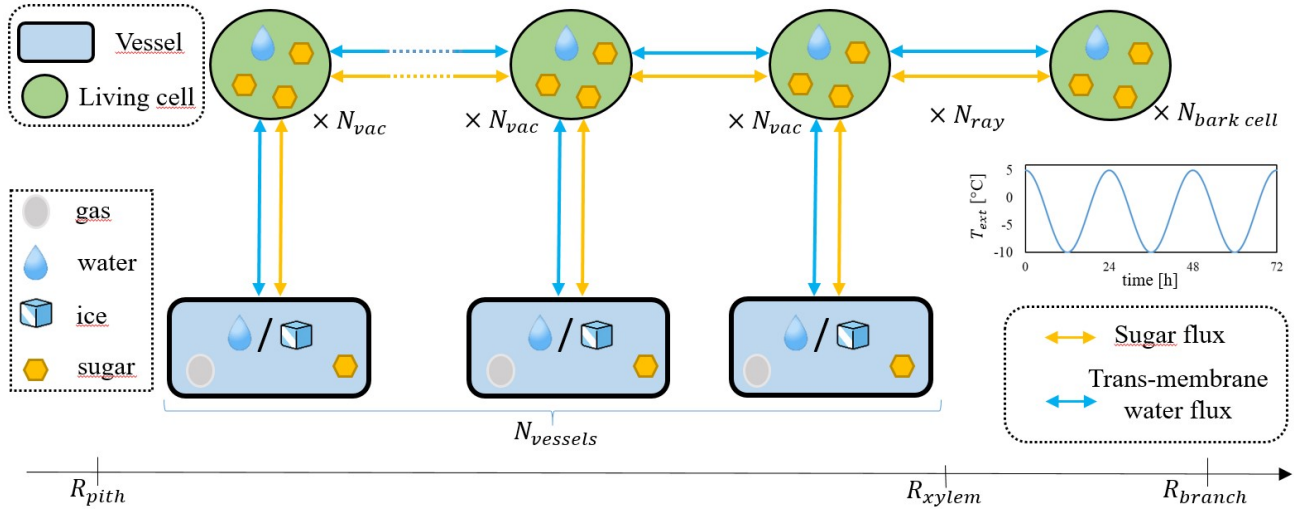


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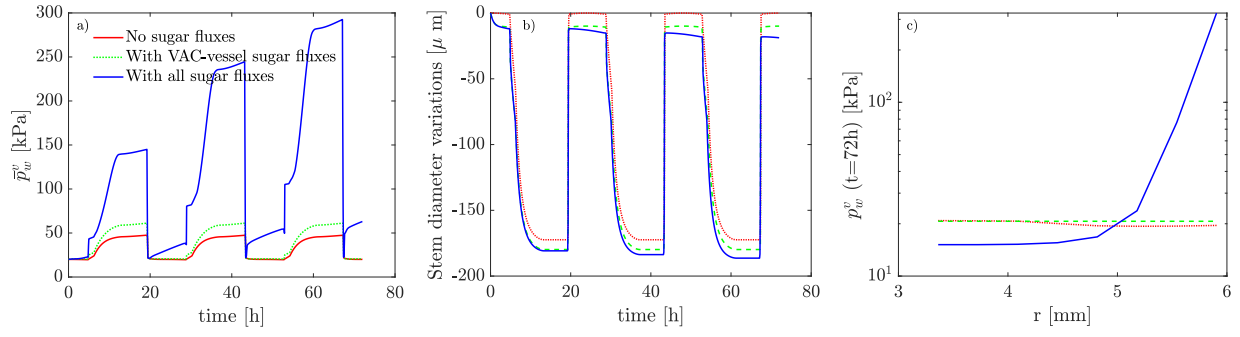


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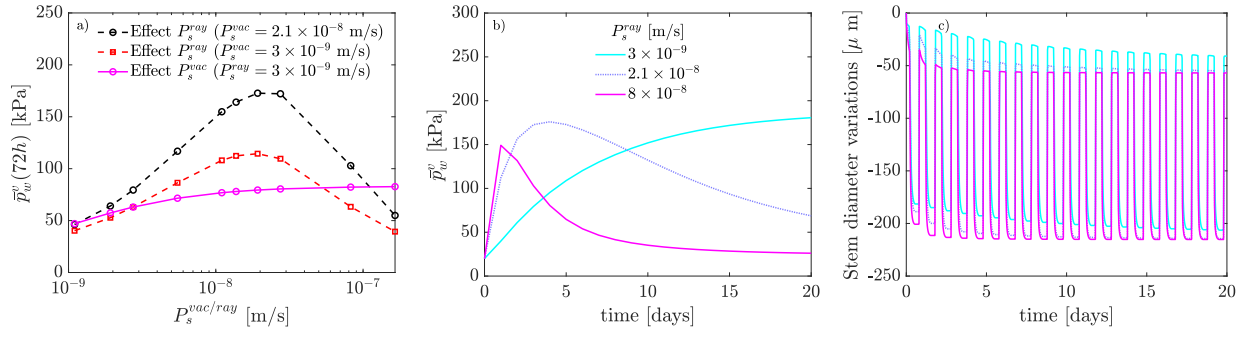


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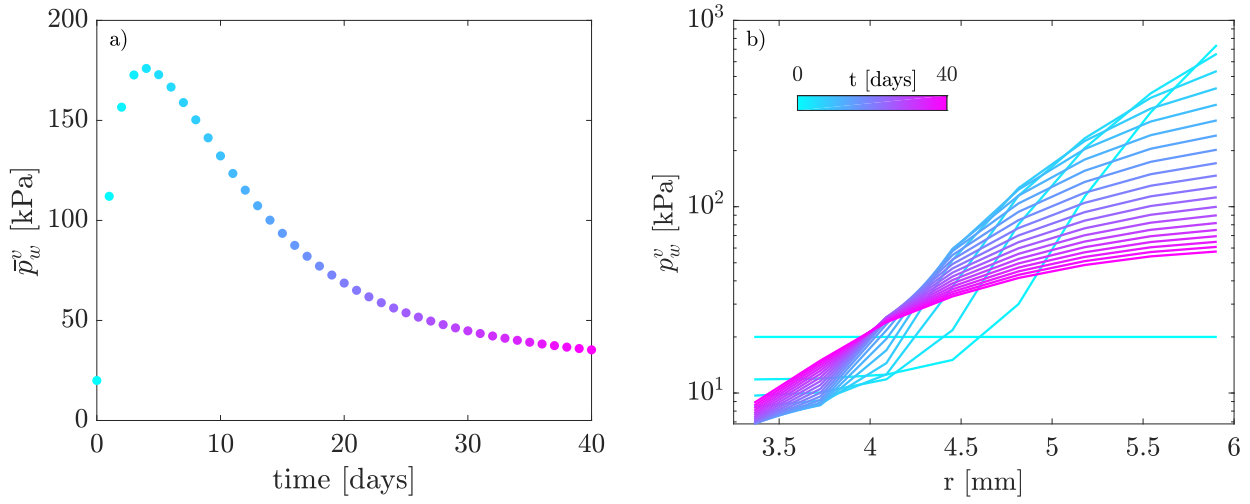


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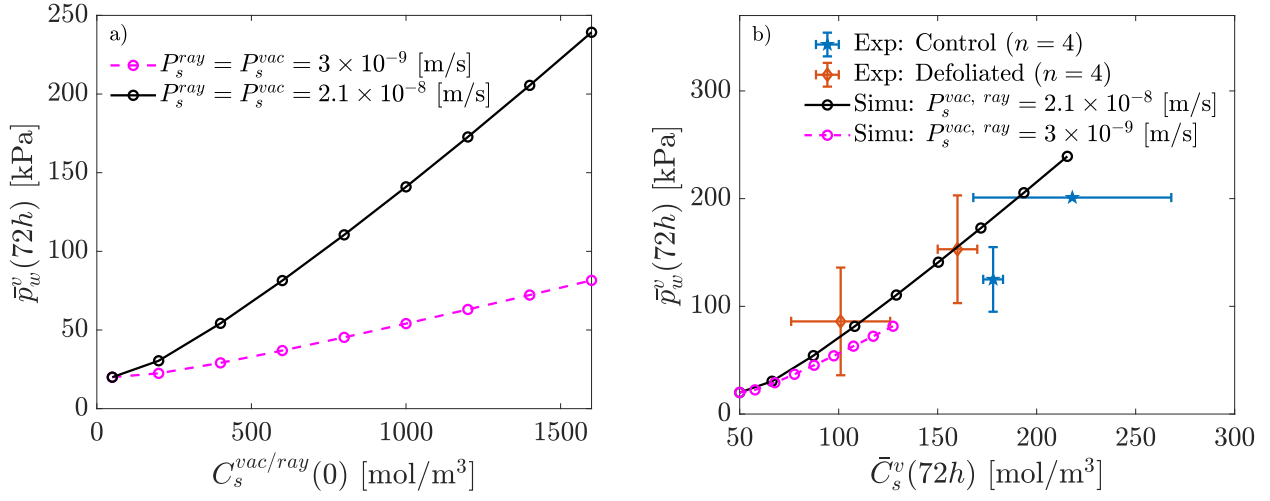


Figure 5: a) Effect of initial living cell sugar concentration on the mean vessel pressure after 72h. b) Link between mean vessel pressure and mean vessel sugar content after 72h + comparison with experiments from Améglio et al 2001 (after 72h too).

Variable name	Description	Unit	Expression
Model variables			
$t$	Physical time	s	-
$r$	Radial coordinate	m	-
$H$	Enthalpy	J/Kg	Eq. (5)
$V_{ray}$	Volume exchanged across the ray	$m^3$	Eq. (8)
$V_{vac-v}$	Volume exchanged VAC-vessel	$m^3$	Eq. (9)
$V_{vac}$	VAC water volume	$m^3$	Eq. (11)
$V_{bark\ cell}$	Bark cell water volume	$m^3$	Eq. (13)
$p_t^{vac}$	VAC turgor pressure	Pa	Eq. (14)
$p_t^{bark\ cell}$	Bark cell turgor pressure	Pa	Eq. (15)
$r_g^v$	Vessel gas bubble radius	m	Eq. (17)
$n_s^v$	Vessel sugar content	mol	Eq. (22)
$n_s^{vac}$	VAC sugar content	mol	Eq. (23)
$n_s^{bark}$	Bark cell sugar content	mol	Eq. (25)
State variables			
$T$	Temperature	$^{\circ}C$	Bozonnet et al. (2023)
$T_m^v$	Vessel melting point	$^{\circ}C$	Eq. (7)
$\delta_{iv}$	Vessel ice volume fraction	-	Bozonnet et al. (2023) (figure 2b)
$\delta_a$	Activation coefficient for sugar diffusion	-	= 1 for $T > T_c$ ; = 0 otherwise
$C_s^v$	Vessel sugar concentration	$mol/m^3$	Eq. (21)
$C_s^{vac}$	VAC sugar concentration	$mol/m^3$	Eq. (16)
$C_s^{bark\ cell}$	Bark cell sugar concentration	$mol/m^3$	Eq. (16)
$p_{ice}^v$	Vessel cryo-suction	Pa	Eq. (10)
$p_g^v$	Vessel gas pressure	Pa	Eq. (18)
$p_w^v$	Water/ice vessel pressure	Pa	Eq. (19)
$V_{vac}^{tot}$	Total VAC water volume	$m^3$	Eq. (26)
$V_{bark}$	Bark volume	$m^3$	Eq. (29)
$D_{xylem}$	Xylem diameter	m	Eq. (28)
$D_{stem}$	Stem diameter	m	Eq. (30)
$\mu(C_s, T)$	Sugar-water solution visco.	Pa.s	See Chenlo et al. (2002)

Table 1: Model and state variables

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Name	Description	Units	Value/expression	Comments
Anatomical description				
$R_{branch}$	Branch radius	m	0.0075	
$R_{pith}$	Pith radius	m	0.003	Measurement
$lvd$	Linear vessel density	$m^{-1}$	2500	Measurement
$W$	Vessel-VAC wall thickness	m	$3.64 \times 10^{-6}$	Petty and Palin (1983) on Maple tree
$R_v$	Vessel radius	m	$80 \times 10^{-6}$	Measurement
$L_z$	Vertical dimension	m	0.001	Only used for unit consistency
$\delta_{bark}$	Bark thickness	m	function of $R_{branch}$	Fit of experimental measurements
$R_{xylem}$	Xylem radius	m	$R_{branch} - \delta_{bark}$	
$trd$	Tangential ray density	$m^{-1}$	5000	Measurement
$A_{vac-v}$	Vessel-VAC exchange area	$m^2$	$f_{vac} 2\pi R_v L_z$	
$f_{vac}$	Vessel-VAC wall fraction	-	0.12	Bozonnet et al. (2023)
$R_{vac}/R_{bark\ cell}$	VAC/Bark cell radius	m	$5 \times 10^{-6}/10 \times 10^{-6}$	Measurement
$l_{vac}/l_{bark\ cell}$	VAC/Bark cell length	m	$20 \times 10^{-6}/15 \times 10^{-6}$	Measurement
$\Delta_r$	Thermal grid cell size	m	$132 \times 10^{-6}$	Obtained through mesh convergence study
$\Delta l_v$	Distance between two vessels	m	$(R_{xylem} - R_{pith}) / (N_{vessels} + 1)$	
$K_{vac}$	VAC volume without sugar	$m^3$	$0.1V_{w,vac}^0$	Rajashekar and Burke (1996)
$K_{bark\ cell}$	same for bark cell	$m^3$	$0.1V_{w,bark\ cell}^0$	Rajashekar and Burke (1996)
$V_{w,vac}^0$	Init. VAC water volume	$m^3$	$\pi R_{vac}^2 l_{vac}$	
$V_{w,bark\ cell}^0$	Init. bark cell water volume	$m^3$	$\pi R_{bark\ cell}^2 l_{bark\ cell}$	
$V_{bark}^0$	Initial bark volume	$m^3$	$\pi (-\delta_{bark}^2 + 2\delta_{bark} R_{branch}) L_z$	
$V_{w,bark}^0$	Initial bark water volume	$m^3$	$BWF \times V_{bark}^0$	
Physical and physiological parameters				
$P_{ray/vac}^{ray}$	Solute permeability coeff.	m/s	$3 \times 10^{-9}$	Gunning (1977); Tyree et al. (1994)
$D_s^{vac}$	VAC-vessel sugar diff. coeff.	$m^2/s$	$P^{vac} W$	
$D_s^{ray}$	Ray sugar diff. coeff.	$m^2/s$	$P_s^{ray} \Delta l_v$	
$C_s^{vac}(0)$	VAC init. sug. conc.	$mol/m^3$	1200	Estimation for mid winter
$C_s^{bark\ cell}(0)$	Bark cell init. sug. conc.	$mol/m^3$	1200	Estimation for mid winter
$BWF$	Bark water fraction	-	$1.5R_{branch} + 0.08$	Bozonnet et al. (2023)
$k_{vac}$	Vessel-VAC membrane water permeability	$m^2$	$3.63 \times 10^{-21}$	Petty and Palin (1983) on Maple tree
$k_{ray}$	Ray water permeability	$m^2$	$3.63 \times 10^{-17}$	
$B_{vac,bark\ cell}^t$	Elastic mod. for turgid cell	Pa	$10 \times 10^6$	Steudle et al. (1977); Dumais and Forterre (2012)
$f_g^v(0)$	Init. vessel gas vol. frac.	-	0.2	Estimation
$r_g^v(0)$	Init. vessel gas bubble rad.	m	$\sqrt{f_g^v(0)} R_v$	
$C_s^v(0)$	Init. vessel sug. conc.	$mol/m^3$	50	Améglio et al. (2001)
$p_w^v(0)$	Initial vessel water pressure	Pa	$2 \times 10^4$	Améglio et al. (2001)
$p_t^{vac,bark\ cell}(0)$	Init. living cell turg. press.	Pa	$p_w^v(0) + (C_s^{vac}(0) - C_s^v(0)) R_g T_{init}$	Mechanical equilibrium
$p_g^v(0)$	Initial vessel gas pressure	Pa	$p_w^v(0) + \sigma_{gw}/r_g^v(0)$	Laplace law
$n_s^{vac,bark\ cell}(0)$	Sugar quantity in living cells	mol	Eq. (16)	Applied with initial values
$n_s^v(0)$	Sugar quantity in vessels	mol	Eq. (21)	Applied with initial values
$n_g^v$	Gas quantity in vessels	mol	Eq. (18)	Applied with initial values
Environmental conditions				
$T_{ext}$	External temperature	K	$T_{mean} - A \sin(\omega t + \phi)$	
$T_{init}$	Initial temperature	K	$T_c + 5$	
$T_{min}$	Minimal temperature	K	$T_c - 10$	
$T_{max}$	Maximal temperature	K	$T_c + 5$	
$T_{mean}$	Mean temperature	K	$1/2(T_{min} + T_{max})$	
$A$	Temperature amplitude	K	$1/2(T_{max} - T_{min})$	
$\omega$	Pulsation for sinus law	$s^{-1}$	$2\pi/(24 \times 3600)$	
$\phi$	Phase lag for sinus law	-	$\text{asin}((T_{mean} - T_{init})/A)$	
Physical constants				
$T_c$	Zero Celsius degree point	K	273.15	
$k_{th,i}/k_{th,w}$	Thermal conductivities	W/m/K	$10 \times 2.22/10 \times 0.556$	Bozonnet et al. (2023)
$c_i/c_w$	Specific heat capacities	J/K/kg	2100/4180	
$\rho_i/\rho_w$	Densities	$kg \cdot m^{-3}$	917/1000	
$H_i/H_w$	Enthalpies	J/kg	$574 \times 10^3/907 \times 10^3$	
$L$	Latent heat of fusion	J/kg	$H_w - H_i$	
$R_g$	Ideal gas constant	J/K/mol	8.314	
$\sigma_{gw}$	gas-water surface tension	N/m	0.072	

Table 2: Parameter list, description & values. Measurements and estimations were done for *Juglans regia* stems.