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1 **Dynamic modeling of stem water content during the dormant period in**
2 **walnut trees.**

3

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7

8 Running title: Modelling of stem water content during dormant period.

9 **Summary statement**

10 Dehydration and rehydration experiments were conducted to identify relevant factors for
11 modeling stem water content during the dormant period in the deciduous walnut tree.

12

13 **Abstract**

14 Water content is a key variable in plant physiology, even during the winter period. To simulate
15 stem water content (WC) during the dormant season, a series of experiments were carried out
16 on walnut trees under controlled conditions. In the field, WC was significantly correlated with
17 soil temperature at 50cm depth ($R^2=0.526$). In the greenhouse, WC remained low as long as
18 soil temperature was kept cold ($<+5^\circ\text{C}$) and increased after soil temperature was warmed to
19 $+15^\circ\text{C}$, regardless of the date. Stem dehydration rate was significantly influenced by WC and
20 evaporative demand. A parsimonious model with functions describing the main experimental
21 results was calibrated and validated with field data from 13 independent winter dynamics in
22 *Juglans regia* orchards. Three functions of water uptake were tested and gave equivalent
23 accuracies (RMSE=0.127-8; RMSEP=0.116). However, only a sigmoid function describing the
24 relationship between root water uptake and soil temperature gave values in agreement with the
25 experimental results. Finally, the simulated WC provided similar accuracy in predicting frost
26 hardiness compared to the measured WC (RMSE *ca.* 3°C) and was excellent in spring (RMSE
27 *ca.* 2°C). This model may be a relevant tool for predicting the risk of spring frost in walnut
28 trees. Its genericity should be tested in other fruit and forest tree species.

29

30 **Keywords:** Evaporation, Frost hardiness, Root water uptake, Soil temperature, Tree
31 physiology, Water content, Winter biology

32 **Introduction**

33 Water is the most important physiological variable in all life forms. In plants, many processes
34 are affected by changes in the water balance during the growing season: heat energy balance
35 (Monteith, 1972), hydraulic conductance (Sapes *et al.*, 2019; Lamacque *et al.*, 2020),
36 photosynthesis (Williams & Flanagan, 1996), turgidity of living cells (Essiamah and Eschrich,
37 1986). Despite the decrease in metabolic activity, water content is also an important variable
38 during the winter dormant period, controlling embolism formation in the hydraulic system
39 (Charra-Vaskou *et al.*, 2016; Charrier *et al.*, 2017). At the cellular level, low water content
40 (WC) triggers the biosynthesis of abscissic acid which promotes the synthesis of important
41 osmotic compounds such as dehydrins (Welling *et al.*, 1997) and soluble carbohydrates
42 (Charrier & Améglio, 2011).

43 During winter, WC decreases in many growth forms, such as grasses (Lugojan & Ciulca, 2011)
44 and trees (Charrier *et al.*, 2013a). WC decreases in early winter, during cold acclimation
45 (Luoranen *et al.*, 2004) and increases in spring, before growth resumes (Turcotte *et al.*, 2009).
46 Decreasing WC acts as a signal to trigger dormancy induction (Maurya & Bhale Rao 2017) and
47 cold acclimation (Bravo *et al.*, 1997). An artificial increase in WC in autumn reduces the ability
48 of the tree to cold acclimate, by shunting the role of cold temperature ($>15^{\circ}\text{C}$; Charrier &
49 Améglio, 2011). The effect by which increased WC modulates cold acclimation is by
50 maintaining active metabolism resulting in increased respiration and consequently increased
51 sugar consumption (Charrier *et al.*, 2018a). WC is therefore key to predicting changes in
52 carbohydrate (Charrier *et al.*, 2018a) and frost resistance (Charrier *et al.*, 2013b).

53 Water status results from the balance between water uptake by the root and evapotranspiration
54 from aboveground tissues. As transpiration through stomata is negligible during winter in
55 deciduous trees, passive evaporation mainly through the bark represents the outflow. Cold

56 temperature ($<5^{\circ}\text{C}$) inhibits root water uptake by decreasing membrane fluidity and increasing
57 water viscosity (Sachs, 1868; Kramer, 1940; Kaufmann, 1975; Améglio *et al.*, 1990). Although
58 the water can circulate passively in the apoplasmic compartment from the soil to the endoderm,
59 to reach the xylem, water molecules must cross plasma membrane via the symplasmic pathway
60 through the Casparian strip. As temperature decreases, membrane lipids change from a fluid
61 state to a viscous, more rigid, state (Come *et al.*, 1992; Lee *et al.*, 2008), which affects the
62 activity of intrinsic proteins, including aquaporins (Wolfe, 1978; Lee *et al.*, 2012). The
63 movement of water molecules across the membrane is restricted, increasing the resistance to
64 water flow (Lyons, 1973; Lee, 1975; Lee *et al.*, 2008). Water uptake by roots is therefore
65 temperature dependent, with very limited uptake at temperatures below 8°C (Améglio *et al.*,
66 2002; Mellander *et al.*, 2006). Soil temperatures above 20°C allow for increased uptake by the
67 root system and rehydration of the above-ground parts (Turcotte *et al.*, 2009; Charrier &
68 Améglio, 2011), which can result in faster cell expansion during budbreak (Lockhart, 1965). In
69 addition, rootlet turnover is reduced by cold, further reducing water flux from the root system
70 (Wan *et al.*, 1999; Wang *et al.*, 2018).

71 Water in plant tissues is composed of free and bound water. The bound water fraction
72 constitutes 20 - 35% of the dry matter and cannot evaporate under biological conditions (Sun,
73 1999). Under normal physiological conditions, the amount of water remains well above the
74 fibre saturation point (*ca.* 25%; Abdulqader *et al.*, 2016), although a substantial fraction of
75 water in the stem varies with environmental conditions. As transpiration through stomata is
76 negligible in deciduous trees during winter, passive evaporation mainly through the bark
77 represents the outflow. Mobile free water evaporates passively depending on the atmospheric
78 water potential (Monteith, 1965). In a biological matrix, evaporation also depends on the
79 relative amount of mobile water (Mellander *et al.*, 2006).

80 The integration of physiological variables into predictive models is essential to account for the
81 effect of stresses occurring outside the period of interest (Charrier *et al.*, 2021). A static model
82 has been developed to predict frost resistance in different tree species, based on soluble
83 carbohydrate and water contents (Charrier *et al.*, 2013b; Baffoin *et al.*, 2021). To use this
84 formalism in predictive studies, it is crucial to describe the dynamics of each of the input
85 variables during the dormant season. The dynamic simulation of carbohydrates has recently
86 been developed (Charrier *et al.*, 2018a), but the simulation of water content is lacking. Although
87 several models have been developed to predict tree water relations during the growing season
88 (*e.g.* SUREAU; Cochard *et al.*, 2021), it was not within their scope to simulate water relations
89 during the dormant period, especially for deciduous trees.

90 The aim of this study is to develop a model describing the relevant physiological processes
91 affecting stem water content during the dormant period. To build a realistic model *i.e.* one that
92 predicts the dynamics of a physiological variable that cannot be continuously monitored,
93 reverse modelling is preferable, starting with experimental data to identify potential causal
94 drivers as suggested by the correlations in the dataset. Whether these correlations are causal or
95 simply due to chance defines the mechanistic (*i.e.* process-based) and empirical (*i.e.* statistical)
96 models. Mechanistic models are often preferred for predictive studies in a changing
97 environment because they would predict a realistic response of the simulated process even
98 outside of this calibration range (*i.e.* genericity). However, such an approach often results in
99 over-parameterized models that are difficult to use or extend to other contexts (*e.g.* other
100 species; Cox *et al.*, 2006). The principle of parsimony considers goodness of fit with as few
101 explanatory variables as possible (Gauch, 2002).

102 To achieve the goal of building a mechanistic but parsimonious model, we conducted a series
103 of experiments to identify the relevant factors and quantify their influence on water status. We
104 hypothesized that the total amount of water in one-year-old stems is composed of a fixed

105 (bound) and a variable (mobile) part. The variations in water content is considered as a balance
106 between influx (*i.e.* water uptake by the root system) and loss (*i.e.* evaporation through the bark
107 and leaf cuticle and transpiration through the stomata). As water is normally not limiting in
108 temperate areas outside the summer, soil temperature is expected to be the main driver of root
109 water uptake and stem rehydration in late winter, which may slightly delay budbreak. We also
110 hypothesized that stem water evaporation would depend on current water status, climatic
111 demand, and leaf area, if present. We conducted two experiments to measure WC dynamics in
112 relation to soil temperature and climatic demand. Finally, the model integrating functions
113 describing the main experimental results was tested against independent winter dynamics in
114 *Juglans regia* orchards.

115 **Material and methods**

116 **In situ observations**

117 Branches were sampled from 5 mature walnut trees (10 to 30 year-old *Juglans regia* L. cv.
118 Franquette) growing at the INRAE station in Crouel and at a higher elevation orchard (45°43'N
119 03° 01' E 880 m a.s.l.) during several winters between 1994 and 2012 to measure water content
120 (Tab. S1). Between 2007 and 2012, frost hardiness tests were performed on the same branches
121 using the electrolyte leakage method.

122 **Soil temperature experiment**

123 From January until May 2004, 28 plants growing under natural conditions and with an optimal
124 water supply were distributed among four temperature-controlled systems of seven pots each
125 (Fig. S1). In early February, the plants were transferred to a heated greenhouse (air temperature
126 = 15°C; natural light). The different treatments consisted of maintaining soil temperature cold
127 (*ca.* +5°C) for different durations. On different dates, the cooling system was stopped and the
128 soil temperature reached the air temperature within a few days: early (Feb. 11th: day 1),
129 intermediate (Mar. 2nd: day 21), late (Mar. 23rd Day 42) and very late warming (Apr. 21st: Day

130 71). Control trees were exposed to natural air temperature. Stem water content was measured
131 on five randomly selected stems on each date for each treatment.

132 The phenology of each terminal bud was monitored every 2 days according to the BBCH scale
133 (Meier, 2018). The budbreak date for each tree was computed as the mean date at which the
134 buds reached stage 07.

135 **Stem dehydration experiment**

136 In late autumn (Dec. 1st), one year-old branches (n = 10) were sampled on 15 years old *Juglans*.
137 *regia* L. cv. Franquette growing at the INRAE station in Crouel (45°43'N 03° 01' E 880 m
138 a.s.l.). Branches were immediately weighed, before the lower end was sealed with wax. Two
139 sets of five branch each were stored under constant conditions (constant photoperiod of 12/12
140 D/N) for *ca.* 60 days (until Jan. 26th): low evaporative demand (temperature = $9.43 \pm 0.24^{\circ}\text{C}$;
141 relative humidity $77.9 \pm 1.4 \%$, mean \pm SD) and high evaporative demand (temperature = 20.45
142 $\pm 0.33^{\circ}\text{C}$; relative humidity: $30.6 \pm 7.7 \%$, mean \pm SD). Each branch was weighed every other
143 day. At the end of the experiment, the branches were freeze-dried and the dry weights measured.

144 **Stem water content**

145 Fresh weight (FW) was measured before the samples were frozen in liquid nitrogen. After
146 freeze-drying, dry weight (DW) was measured and water content (WC) was calculated as:

$$147 \quad WC = \frac{(FW-DW)}{DW} \quad (1)$$

148 **Frost hardiness**

149 Frost hardiness of living cells was assessed using the electrolyte leakage conductivity method
150 (Charrier & Améglio, 2011). Branch samples were split into six 5 cm long pieces and exposed
151 to different temperatures between +5 and -80°C using temperature-controlled boxes connected
152 to a circulator bath (Ministat Huber, Offenburg, Germany). Samples were exposed to one

153 freeze-thaw cycle with one step during one hour at minimum temperature and temperature
154 changes at a rate of $5\text{K}\cdot\text{h}^{-1}$. Air and sample temperatures were monitored using type T
155 thermocouples connected to a data logger (CR1000, Campbell, Logan, USA).
156 Samples were then sliced into 1-2 mm thick slices, immersed into 15 mL of distilled-deionized
157 water (Labwater, Veolia, Le Plessis-Robinson, France) in glass vials and shaken over night at
158 $+5^{\circ}\text{C}$. After warming the samples back to room temperature, a first measurement of electric
159 conductivity of the solution (C_1) was performed using an electric conduct meter (Portable
160 conductivity meter ProfiLine Cond 3310, Bellingham + Stanley, Tunbridge Wells, UK). Vials
161 were autoclaved at 120°C , 1 bar for 30 min and cooled down to room temperature before a
162 second conductivity measurement was performed (C_2). Relative Electrolytic Leakage (REL)
163 was computed as the ratio between C_1 and C_2 (Zhang & Willison, 1987). The relation between
164 REL and the minimum temperature reached by the sample (θ) was fit assuming a sigmoid
165 relationship between both variables:

$$166 \quad REL = \frac{a}{1+e^{b*(c-t)}} + d \quad (5)$$

167 where t is the temperature (in $^{\circ}\text{C}$), b the slope at the inflection point c and a and $(a + d)$ the
168 lower and higher asymptotes of the relation, respectively.

169 Parameters were fitted by minimizing the sums of squares using non-linear regression analysis
170 (ExcelStat software ver.2019.3.2). Frost hardness was defined as the temperature at the
171 inflection point c (*i.e.* corresponding to 50% induced damages; Repo & Lappi, 1989).

172 **Simulation of water content**

173 The results from soil temperature and stem dehydration experiments were used to define the
174 architecture of the model simulating changes in stem water content. Different functions were
175 tested to predict seasonal changes in water content using a minimal number of input variables:

176 initial water content, Potential Evapo-Transpiration (PET; see Penman, modified by Monteith;
177 Monteith, 1965) and air and soil temperature at 50cm depth (Fig. S2).

178 The models use as input variable the observed climatic data: the daily soil temperature at 50cm
179 depth, the PET and the daily average temperature monitored by a weather station located in the
180 same orchard. For each year, the initial point was set as the observed water content.

181 ***Water uptake***

182 Water uptake capacity Up was simulated as function of soil temperature between 0 (complete
183 inhibition) and 1 (full capacity). Different functions were tested to simulate the relationship
184 between water uptake and soil temperature: linear (3), exponential (4) and sigmoid (5):

$$185 \quad Up(t) = a \cdot t_{soil} + b \quad (3)$$

$$186 \quad Up(t) = a \cdot e^{b \cdot t_{soil}} \quad (4)$$

$$187 \quad Up(t) = \frac{1}{1 + e^{a \cdot (t_{soil} - b)}} \quad (5)$$

188 where Up is the amount of absorbed water, t_{soil} the soil temperature at 50cm depth and a and b
189 the parameters.

190 ***Water loss***

191 Water loss was simulated as a function of evaporative demand (approximated by PET),
192 transpiration by leaf area (LA) and the amount of mobile water expressed as a variable water
193 pool (MW *i.e.* not taking into account fixed water). The presence of leaf area was simulated by
194 a phenological model simulating the date of budburst in walnut (parameters from Charrier *et*
195 *al.*, 2011; 2018b) and leaf fall (parameters from Delpierre *et al.*, 2009). Leaves were considered
196 absent (LA = 0) between leaf fall and budbreak and, between budbreak and leaf fall, equal to
197 1. During the transition between leafy and leafless period, leaf area was considered decreasing
198 from 1 to 0 within 14 days. After budbreak date, leaf area increased from 0 to 1 at the same

199 rate. A linear model, including the three potential variables (PET, LA and WC) and their
200 interactions simulated the amount of water evaporated daily:

$$201 \quad Ev(t) = c + d \cdot MW(t - 1) + e \cdot PET + f \cdot LA + g \cdot MW(t - 1) \cdot PET + h \cdot MW(t - 1) \cdot LA + i \cdot \\ 202 \quad PET \cdot LA + j \cdot MW(t - 1) \cdot PET \cdot LA \quad (6)$$

203 where Ev is the amount of evaporated water, MW, PET and LA the explaining variables and
204 letters from c to j the parameters.

205 WC was computed on a daily basis according to:

$$206 \quad WC(t) = WC(t - 1) + Ab \cdot Up(t) - Ev(t) \quad (6)$$

207 where WC is the water content, Up the amount of absorbed water, Ev is the amount of
208 evaporated water and Ab a coefficient.

209 **Frost hardiness model**

210 The ability of the simulated WC to predict frost hardiness (FH) was tested using the unified
211 osmo-hydric model developed by Charrier *et al.* (2013b) in different organs of walnut trees,
212 with the same dataset as Charrier *et al.* (2018a) on stems only (Tab. S1). The architecture of the
213 model reflects the ability of solutes to lower the freezing point of cell sap. An increase in solutes
214 and/or a decrease in water content favors the crystallization of liquid water in the apoplastic
215 compartment, where the low water potential of ice subsequently dehydrates further the living
216 cells. This model, developed for different organs (from fine roots to buds) and tissues (bark,
217 and xylem) of walnut trees *J. regia* describes the freezing point depression and the associated
218 FH induced by the increase in GFS (Glucose + Fructose + Sucrose, major part of winter solutes
219 in this species) and the decrease in WC.

$$220 \quad FH(t) = a \cdot \frac{\ln(\text{GFS})}{WC(t)} + b \quad (7)$$

221 To compute FH, the GFS data were fixed according to measurements whereas WC was
222 simulated according to the best model. FH was compared to the FH simulated using actual WC
223 values.

224 **Optimization of the parameters**

225 To calibrate the model, a set of measured water content dynamics were obtained from 5 mature
226 walnut trees (10 to 30 year-old *Juglans regia* L. cv. Franquette) growing at the INRAE station
227 in Crouel and at a higher elevation orchard (45°43'N 03° 01' E 880 m a.s.l.) during several
228 winters between 1994 and 2012. As successive observations were not independent during the
229 same winter dynamic, entire winter dynamics, without the initial point, were either attributed
230 to the calibration or to the validation dataset. The winter dynamics were assigned to the
231 calibration dataset to maximize the variability of the meteorology, based on the minimum,
232 maximum and highest range between minimum and maximum value for each of the climatic
233 variables. Measured data from nine different winter dynamics, corresponding to 74
234 observations (83 data points minus 9 initial observations) were used as the calibration dataset
235 and compared to the remaining 4 winter dynamics (39 data points minus 4 initial observations,
236 35 data points) as the external validation dataset (Tab. S1).

237 The amount of fixed water was set according to the minimum WC usually observed during the
238 winter period (*i.e.* $M_{bw} = 0.65$; Charrier *et al.*, 2013b). The parameters used to predict leaf fall
239 and bud break phenology were set as in the original studies (Delpierre *et al.*, 2009 and Charrier
240 *et al.*, 2018b). The remaining parameters ($n = 11$; Tab. S1) were optimized by minimizing the
241 residual sum of square between simulated values and measured data (objective function). For
242 the model describing water losses, all the variables and their interactions are considered to have
243 a positive effect. The parameters of Eq. (6) were therefore constrained to positive values (lower
244 limit ≥ 0). This constraint made it possible to simplify the convergence of the algorithm by

245 setting a zero effect for variables and their interactions that did not have a significant effect on
246 water losses.

247 The optimization was performed using the Nelder Mead algorithm (package *nloptr* in R; R Core
248 Team, 2019). The algorithm was run up to 50,000 times until convergence (relative tolerance =
249 10^{-10}) starting from 121 (11^2) sets of initial values, a combination of individual parameters
250 selected from the lower and upper part of the realistic range of values for each parameter.

251 **Statistical analysis**

252 The performance of the optimized models were assessed by computing the following indexes:
253 Efficiency (Eff), Root Mean Square Error (RMSE), Predictive Root Mean Square Error
254 (RMSEP) and Akaike Index Criterion (AIC_C):

$$255 \text{ Efficiency: } Eff = \frac{(SS_t - SS_{res})}{SS_{tot}} \quad (8)$$

256 where SS_{tot} and SS_{res} are the total and residual sums of square, respectively.

$$257 \text{ Root Mean Standard Error: } RMSE(P) = \sqrt{\frac{\sum_{i=1}^n (\hat{y}_i - y_i)^2}{n}} \quad (9)$$

258 with \hat{y}_i the predicted values for an observation i and y_i the observed values for an observation i

$$259 \text{ AIC: } AIC_C = 2n \left[\log(RMSE) + \frac{k}{n-k-1} \right] \quad (10)$$

260 with k the number of parameters, n the number of observations.

261 **Results**

262 Stem water content, measured in the field from 1994 to 2012, exhibited a wide range of
263 variation, from 0.68 to 1.66 $\text{g}\cdot\text{g}^{-1}$ DM (Fig. 1). In autumn, stems were well hydrated (WC =
264 $1.153 \pm 0.049 \text{ g}\cdot\text{g}^{-1}$ DM in September – October). Water content progressively decreased to a
265 minimum during the coldest month (WC = $0.865 \pm 0.012 \text{ g}\cdot\text{g}^{-1}$ DM in January and February).

266 With the increase in temperature in spring, stems progressively rehydrated to reach maximum
267 values near the bud break date ($1.078 \pm 0.041 \text{ g.g}^{-1} \text{ DM}$ in April and May). Greater variability
268 was observed during the transition periods (autumn and spring) than during winter. In autumn
269 and spring, soil temperature and PET had 1.5 to 2 times larger standard deviation than during
270 winter. A significant linear correlation was observed between aboveground stem water content
271 and soil temperature at 50 cm depth ($R^2 = 0.526$; $P < 0.001$; Fig. 1). The sigmoid and exponential
272 functions were also significantly correlated with similar AIC (-156.8, -157.1 and -155.2, for
273 linear, exponential and sigmoid function, respectively) and were not significantly different (P
274 > 0.137). Higher variability was observed at approximately $+10^\circ\text{C}$. These outliers corresponded
275 to measurements performed after the budburst date (May to early June) and the correlation was
276 slightly stronger when these measurements were removed ($R^2 = 0.537$; Fig. 1).

277 *Soil temperature experiment*

278 The soil temperature experiment consisted of cold to warm temperature transitions at different
279 times between winter to spring (Feb 2nd to Apr 21st). The cold ($+5^\circ\text{C}$) and warm ($+15^\circ\text{C}$)
280 treatments were consistent with the correlation between stem water content and soil temperature
281 (Fig. 1). Stem water content was relatively low and similar in all treatments at the beginning of
282 the experiment ($\text{WC} = 0.835 \pm 0.011 \text{ g.g}^{-1} \text{ DM}$; $P > 0.324$). As long as soil temperature was
283 kept cold ($+5^\circ\text{C}$), stem water content remained lower than in treatments with warmer soil
284 temperature ($P < 0.001$ and $P = 0.002$ for warmed and ambient soil temperature, respectively;
285 Fig. 2A-B). When soil temperature was warmed to $+15^\circ\text{C}$, stem water content increased from
286 0.85 to $1.0 \text{ g.g}^{-1} \text{ DM}$ in less than three weeks regardless of the date of soil warming. However,
287 it should be noted that the increase in soil temperature had no effect on bud break date of the
288 different treatments (Apr 26th; $P = 0.131$).

289 *Stem dehydration experiment*

290 Under constant conditions, stems dehydrated gradually over 50 days (Fig. 3). The change in
291 water content was strongly correlated with the duration of the experiment by an exponential
292 function ($R^2 > 0.99$; Figure 3A). The rate of dehydration was also affected by the evaporative
293 demand. Stems under higher evaporative demand (temperature = 25°C, VPD = 1.67 kPa)
294 dehydrated to a water content of 0.5 in 20 days whereas under lower evaporative demand
295 (temperature = 15°C, VPD = 0.26 kPa), the WC did not reach this value after 50 days (WC =
296 0.6). However, the daily change in WC decreased throughout the experiment in both treatments,
297 which would lead to a longer-term stabilization of WC.

298 *Modelling changes in water content*

299 Fitting the different functions for water uptake provided similar results to predict *in situ*
300 observations of WC (RMSE between 0.127 and 0.128 g.g⁻¹ DM; RMSEP = 0.116 g.g⁻¹ DM,
301 Efficiency = 0.95; Tab. S1). The AIC_C parameter, taking into account the number of parameters,
302 discriminated the linear and exponential functions (AIC_C = -121.2) and the sigmoid function
303 (AIC_C = -124.1). The optimized parameters for these two functions do not provide a realistic
304 relationship between water uptake and soil temperature (Fig. S3). In contradiction with the soil
305 temperature experiment, the linear function simulates significant water uptake at temperatures
306 below 8°C. The exponential and the sigmoid functions were extremely close below 15°C but
307 diverged above. Finally, the sigmoid function for root water uptake was the most accurate with
308 a temperature inducing 50% of water uptake capacity at 8.7°C and the best AIC_C value (-124.1).

309 The optimization of the evaporation function returned null values for the parameters related to
310 PET, LA and any of their interactions with current WC, suggesting that these variables had a
311 minor contribution to WC dynamics compared to current WC, in the period considered. Finally,
312 the more parsimonious model predicted WC with an RMSE of 0.127 g.g⁻¹ DM on the calibration
313 dataset (Fig. 4). This model was also robust as the RMSEP obtained on an external dataset was
314 0.116 g.g⁻¹ DM. Overall, the model described the seasonal dynamics observed *in situ* with good

315 accuracy (Fig. 5, 6). However, in autumn and spring, the accuracy was lower than in winter
316 (RMSE = 0.15 vs 0.07 g.g⁻¹ DM). When stem rehydration is extremely rapid in spring, the
317 prediction was underestimated in some years (*e.g.* 1996-1997 or 2008-2009).

318 Finally, the ability to help predicting frost hardiness of the *in situ* observations was assessed by
319 replacing observed WC with simulated values in the osmo-hydric model ($FH = \ln(GFS)/WC$).
320 Compared with the measured frost hardiness, the accuracy of the simulated frost hardiness was
321 approximately 3.4°C, without significant bias (fig 7A). Compared to the original osmo-hydric
322 model, based on point measurement of the variables, the simulated FH was also extremely
323 accurate (RMSE = 2.9°C). However, the accuracy was also seasonally variable. In autumn,
324 RMSE was greater than 4°C (4.6 and 4.4°C for direct and simulated frost hardiness,
325 respectively; n= 8). In winter, similar accuracy was observed with respect to direct
326 measurements (4.0°C), whereas accuracy was higher compared to simulated water content
327 (2.8°C; n =19). The prediction of frost hardiness during the spring period was much more
328 accurate (1.7 and 2.1°C for direct and simulated frost hardiness, respectively; n= 21).

329 **Discussion**

330 A series of experiments allowed the identification of relevant factors controlling the stem water
331 content of dormant walnut trees. We showed experimentally that soil temperature is an
332 important factor in root water uptake as rehydration was only observed when the soil warmed
333 up. A second experiment showed that stem evaporation depends on the amount of free water
334 and the climatic demand. Finally, only soil temperature and current water content were
335 sufficient variables to simulate water content under natural conditions. The simulated water
336 content values, combined with a static model of frost hardiness, provided accurate results that
337 could be useful for dynamic physiological simulations of frost hardiness.

338 In the field, the strong correlation between stem water content and soil temperature is mainly
339 explained by the limitation of root water uptake by cold temperature (Fig. 1), as observed on
340 leafy plants (Sachs, 1868) or in evergreen conifers (Running & Reid, 1980). Low soil
341 temperature severely limits water uptake by the root system, creating an imbalance between
342 water loss by evapotranspiration and water uptake, as observed by a decrease in leaf water
343 potential (Améglio *et al.*, 1990).

344 The control of soil temperature confirmed its causal role on the timing of stem rehydration in
345 late winter (Fig. 2). Such an effect has been observed in evergreen conifers (Running & Reid,
346 1980). However, even when the soil-plant-atmosphere *continuum* is disrupted, the root system
347 can promote distal stem rehydration. In walnut trees, an increase in xylem pressure allows stem
348 rehydration, in relation to the ability to concentrate nitrogen from the rhizosphere (Ewers *et al.*,
349 2001). The transfer of nitrate from soil water to root cells against a concentration gradient is an
350 active process based on a symport with H⁺ (Miller & Smith, 1996). The catalytic activity of the
351 proton pump (H⁺-ATPase), which is therefore crucial for the generation of an H⁺ gradient, is
352 limited at temperatures below 8°C, as shown in walnut stems (Alves *et al.*, 2004; 2007). Whether
353 such dynamics would be observed in a deciduous species unable to generate root pressure
354 remains an open question.

355 Optimization of the model resulted in several functions for water uptake that gave results of
356 similar accuracy. However, the sigmoid function was the most realistic, although the
357 exponential function gave realistic results for soil temperatures below 15°C, as observed in
358 Scots pine by Mellander *et al.* (2006). The temperature at which 50% of the root water uptake
359 capacity was predicted by the sigmoid function at 8.7°C (Fig. S3). This value is consistent with
360 previous observations of very limited uptake at temperatures below 8°C and a maximum rate
361 at temperatures above 20°C (Améglio *et al.*, 2002; Mellander *et al.*, 2006).

362 The greater variation in WC in autumn and spring than in winter suggests that the fluctuating
363 environmental conditions during this period are likely to have a greater effect on WC than the
364 relatively stable conditions in mid-winter. Soil temperatures of *ca.* 10°C were observed during
365 the bud break period (late April – May; Fig. 1). The timing of budbreak, by creating a flow of
366 water towards the expanding organs through the enlargement of apoplastic pores from the stem
367 to the bud (Rinne *et al.*, 2001; Signorelli *et al.*, 2020) and the influx of solutes into the buds
368 (Bonhomme *et al.*, 2009), is likely to influence the relationship between soil temperature and
369 water content. Indeed, differential budbreak in the canopy would induce greater variability
370 between stems, explaining why these points deviate from the regression (Fig. 1).

371 Limited root water uptake is not the only factor explaining seasonal changes in water content
372 during the dormant period. Stem dehydration is driven by potential evapotranspiration (PET),
373 which is actively controlled at the leaf level by stomatal closure and passively controlled by
374 water fluxes through the leaf cuticle and leaky stomata during the leafy period and through the
375 bark throughout the year (Duursma *et al.*, 2019). As water loss is not compensated by root
376 uptake, the amount of free water is gradually reduced. Evaporation from aboveground parts
377 depends on two factors: water availability and climatic demand (Mellander *et al.*, 2006). Stem
378 evaporation was simulated as a linear function of PET, free water in the stem and leaf area,
379 including the interactions between these variables. Parameters related to leaf area were not
380 significant, suggesting that although leaves were still present at some dates in autumn, the
381 amount of transpired water was too low to significantly affect the dynamics of stem water
382 content at this time. Surprisingly, a similar result was observed for PET, although the rate of
383 dehydration was significantly affected by evaporative demand (Fig. 3). The short photoperiod,
384 low light intensity and low VPD observed in autumn do not induce a strong dehydration force
385 as observed in the climate chamber. Furthermore, as water status and evaporative demand show
386 colinear variations (*i.e.* parallel seasonal changes: decrease from autumn to winter and increase

387 from winter to spring), the optimization algorithm cannot distinguish the respective influence
388 of each of these variables. The influence of the evaporative demand on the dehydration rate can
389 also become significant above a substantial threshold, greater than the VPD usually observed
390 in the field during the simulation period. Finally, the change in solute concentration during the
391 simulation period, (interconversion between starch and soluble carbohydrates; Charrier *et al.*,
392 2013b) could affect the water potential of the stem tissue more strongly than the evaporative
393 demand.

394 This model provided accurate results for the period considered, capturing most of the variability
395 in the dataset through a parsimonious approach. The model developed in this study is relatively
396 effective in predicting seasonal changes in water content over several years under field
397 conditions (Fig. 5-7). Very few model have attempted to simulate winter water content. To our
398 knowledge, only one focuses on winter drought in conifers (Boyce *et al.*, 1991). Although using
399 a similar approach, their model led to opposite conclusions. The transpiration rate was strongly
400 dependent on the atmospheric water deficit and cuticular losses, whereas the recharge rate (i.e.
401 water flow from the wood tissues) was constant at temperatures above -4°C .

402 Coupled to carbohydrate measurements, the accuracy of the model to predict frost hardiness
403 was similar to that of the direct osmohydric model (RMSE = 3.4 and 2.9°C , using measured
404 and simulated water content values, respectively; Fig. 7). When comparing seasons, the
405 prediction was less accurate in autumn (RMSE *ca.* 4.5°C). At this time, the water balance can
406 be altered by various factors, such as delayed drought, delayed or earlier leaf fall during the
407 warm autumn or an early freezing event. In spring, the prediction of frost hardiness was the
408 most accurate (RMSE *ca.* 2°C), despite rapidly changing environmental conditions. Spring frost
409 risks cause major disturbances in agroecosystems.

410 Since the last decade, more frequent spring frost damages have been recorded (Augspurger,
411 2013). The results of this model open up new research questions for the prevention of frost

412 damage. Would delaying spring rehydration be a relevant strategy to mitigate frost risks? Wet
413 buds are more vulnerable than dry buds (Charrier *et al.*, 2013b) However, although the soil
414 temperature experiment affected the timing of stem rehydration, the buds were unlikely to be
415 affected as the date of bud break did not differ between treatments. Furthermore, hydrated
416 woody tissues exhibit higher temperature of ice nucleation (Lintunen *et al.*, 2018).

417 The model developed in this study provides a simple tool for predicting water content and frost
418 hardness of deciduous walnut tree *Juglans regia*. The relatively simple modeling approach,
419 considering one input variable, was built based on experimental results, which should result in
420 a relatively robust model (Hanninen *et al.*, 2019). The dataset was acquired in an environment
421 where the soil humidity was not limiting. This model, acquired in conditions where soil
422 moisture is not limiting, should generally work well in temperate zones. In Mediterranean areas,
423 where the soil can be much drier in autumn, it would require specific calibration, although these
424 are not the areas most exposed to cold. At higher elevation, it would be necessary to take into
425 account the change in water phase at freezing temperatures and therefore its unavailability for
426 part of the winter (Charrier *et al.*, 2017). Particular attention should also be paid to mild autumn
427 conditions, where the forces of dehydration could be relatively significant in the future, but
428 should not delay cold acclimation (Sierra Almeida *et al.*, 2016). Finally, if this model could
429 account for WC dynamics in species with different biology, *i.e.* without root pressure or with
430 thinner bark thickness, this would provide a framework to study the effect of changing climate
431 on winter stress such as snow cover duration and root exposure to low temperature (Zhu *et al.*,
432 2000), or to integrate the effect of sublethal stress by coupling it to dynamic changes in
433 carbohydrate contents (Charrier *et al.*, 2021).

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Author Contribution

Data acquisition, analysis, writing and editing original manuscript: G.C. and T.A..

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Figure caption

Figure 1. Water content depending on the soil temperature at 50 cm depth from autumn to spring in stems of walnut trees. Gray dots represent measurements performed during or after budbreak (May-June). The linear regressions including (gray dotted line) or excluding these points (gray dashed line) are indicated. Symbols and bars represent the mean and the standard errors from 5 individual trees.

Figure 2. A. Time course of the water content in walnut trees exposed to extended soil chilling. Soil was warmed from 5°C to 15°C after various duration (0, 21, 42 and 71 days for early, intermediate, late and very late treatments; x-axis). The control treatment without any temperature control had soil temperature in balance with air temperature. Symbols and bars represent the mean and the standard errors from 5 individual trees. **B.** Time course of soil and air temperature in the different treatments.

Figure 3. A. Time course of the water content in excised stems of walnut exposed to low and high evaporative demand (average VPD = 0.26 and 1.67 kPa, respectively). Symbols and bars represent the mean and the standard errors from 5 individual stems. **B.** Rate of change in water content depending on current water content under low and high evaporative demand.

Figure 4. Predicted water content depending on observed water content in the calibration dataset (solid dots) and the validation dataset (open dots). Symbols and bars represent the mean and the standard errors of observed values from 5 individual stems.

Figure 5. Observed and simulated stem water contents in walnut tree during different autumn-spring dynamics in lowland and mountain (M) orchards for the calibration dataset. The initial water content appears as a gray symbol. Symbols and bars represent the mean and the standard errors of observed values from 5 individual stems.

Figure 6. Observed and simulated stem water contents in walnut tree during different autumn-spring dynamics in lowland and mountain (M) orchards for the validation dataset. The initial water content appears as a gray symbol. Symbols and bars represent the mean and the standard errors of observed values from 5 individual stems.

Figure 7. Simulated values of frost hardiness using the direct osmo-hydric model combined with the model simulating water content metabolism vs. measured values (A) or simulated values using the direct osmo-hydric model alone (B). The model used to calculate frost hardiness ($FH = a \cdot + \ln(GFS) / WC + b$; Charrier *et al.*, 2013b) used the parameters $a = -5.77$ and $b = 2.89$. Symbols represent the mean from five replicates (measured values). In both panels, dashed line represent the linear regression and regression coefficient (R^2).

Supplementary material

Figure S1. Experimental set up used for the soil temperature experiment. The pots were introduced in six different cells in contact with refrigerated solution. The upper part of the pot was covered by polystyrene cap (in white) to insulate from air temperature.

Figure S2. Mean daily temperature (A) and soil temperature at 50 cm depth (B) in the field during different winter dynamics in lowland and mountain orchards.

Figure S3. Simulated water uptake depending on soil temperature at 50cm depth. These functions used the best set of parameters after optimization. Linear and sigmoid function are plotted on the left y-axis, whereas exponential function the right y-axis.

Table S1. Number of data points, types of data and use acquired during different winter dynamics in lowland and mountain orchards.

Table S2. Parameters of the three different models calibrated according to different water uptake functions.