Dynamic modeling of stem water content during the dormant period in walnut trees

Guillaume Charrier, Thierry Ameglio

To cite this version:

Guillaume Charrier, Thierry Ameglio. Dynamic modeling of stem water content during the dormant period in walnut trees. Tree Physiology, 2024, 44 (1), 10.1093/treephys/tpad128 . hal-04690255

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

Guillaume Charrier[1*], Thierry Améglio[1]

[1] Université Clermont Auvergne, INRAE, PIAF, F-63000 Clermont-Ferrand, France

*: corresponding author: guillaume.charrier@inrae.fr

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

Summary statement

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

Abstract

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

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

Introduction

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

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

Water status results from the balance between water uptake by the root and evapotranspiration from aboveground tissues. As transpiration through stomata is negligible during winter in deciduous trees, passive evaporation mainly through the bark represents the outflow. Cold temperature (<5°C) inhibits root water uptake by decreasing membrane fluidity and increasing water viscosity (Sachs, 1868; Kramer, 1940; Kaufmann, 1975; Améglio *et al.*, 1990). Although the water can circulate passively in the apoplasmic compartment from the soil to the endoderm, to reach the xylem, water molecules must cross plasma membrane via the symplasmic pathway through the Casparian strip. As temperature decreases, membrane lipids change from a fluid state to a viscous, more rigid, state (Come *et al.*, 1992; Lee *et al.*, 2008), which affects the activity of intrinsic proteins, including aquaporins (Wolfe, 1978; Lee *et al.*, 2012). The movement of water molecules across the membrane is restricted, increasing the resistance to water flow (Lyons, 1973; Lee, 1975; Lee *et al.*, 2008). Water uptake by roots is therefore temperature dependent, with very limited uptake at temperatures below 8°C (Améglio *et al.*, 2002; Mellander *et al.*, 2006). Soil temperatures above 20°C allow for increased uptake by the root system and rehydration of the above-ground parts (Turcotte *et al.*, 2009; Charrier & Améglio, 2011), which can result in faster cell expansion during budbreak (Lockhart, 1965). In addition, rootlet turnover is reduced by cold, further reducing water flux from the root system (Wan *et al.*, 1999; Wang *et al.*, 2018).

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

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

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

To achieve the goal of building a mechanistic but parsimonious model, we conducted a series of experiments to identify the relevant factors and quantify their influence on water status. We hypothesized that the total amount of water in one-year-old stems is composed of a fixed (bound) and a variable (mobile) part. The variations in water content is considered as a balance between influx (*i.e.* water uptake by the root system) and loss (*i.e.* evaporation through the bark and leaf cuticle and transpiration through the stomata). As water is normally not limiting in temperate areas outside the summer, soil temperature is expected to be the main driver of root water uptake and stem rehydration in late winter, which may slightly delay budbreak. We also hypothesized that stem water evaporation would depend on current water status, climatic demand, and leaf area, if present. We conducted two experiments to measure WC dynamics in relation to soil temperature and climatic demand. Finally, the model integrating functions describing the main experimental results was tested against independent winter dynamics in *Juglans regia* orchards.

Material and methods

In situ observations

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

Soil temperature experiment

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

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

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

Stem dehydration experiment

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

Stem water content

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

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

Frost hardiness

Frost hardiness of living cells was assessed using the electrolyte leakage conductivity method (Charrier & Améglio, 2011). Branch samples were split into six 5 cm long pieces and exposed to different temperatures between +5 and –80°C using temperature-controlled boxes connected to a circulator bath (Ministat Huber, Offenburg, Germany). Samples were exposed to one freeze-thaw cycle with one step during one hour at minimum temperature and temperature changes at a rate of $5 K.h^{-1}$. Air and sample temperatures were monitored using type T thermocouples connected to a data logger (CR1000, Campbell, Logan, USA).

Samples were then sliced into 1-2 mm thick slices, immersed into 15 mL of distilled-deionized water (Labwater, Veolia, Le Plessis-Robinson, France) in glass vials and shaken over night at +5°C. After warming the samples back to room temperature, a first measurement of electric conductivity of the solution (C_1) was performed using an electric conduct meter (Portable conductivity meter ProfiLine Cond 3310, Bellingham + Stanley, Tunbridge Wells, UK). Vials were autoclaved at 120°C, 1 bar for 30 min and cooled down to room temperature before a second conductivity measurement was performed (C_2). Relative Electrolytic Leakage (REL) was computed as the ratio between C_1 and C_2 (Zhang & Willison, 1987). The relation between REL and the minimum temperature reached by the sample (θ) was fit assuming a sigmoid relationship between both variables:

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

where t is the temperature (in °C), b the slope at the inflection point c and a and (a + d) the lower and higher asymptotes of the relation, respectively.

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

Simulation of water content

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

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

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

Water uptake

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

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

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

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

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

Water loss

Water loss was simulated as a function of evaporative demand (approximated by PET), transpiration by leaf area (LA) and the amount of mobile water expressed as a variable water pool (MW *i.e.* not taking into account fixed water). The presence of leaf area was simulated by a phenological model simulating the date of budburst in walnut (parameters from Charrier *et al.*, 2011; 2018b) and leaf fall (parameters from Delpierre *et al.*, 2009). Leaves were considered absent (LA = 0) between leaf fall and budbreak and, between budbreak and leaf fall, equal to 1. During the transition between leafy and leafless period, leaf area was considered decreasing from 1 to 0 within 14 days. After budbreak date, leaf area increased from 0 to 1 at the same rate. A linear model, including the three potential variables (PET, LA and WC) and their interactions simulated the amount of water evaporated daily:

$$Ev(t) = \mathrm{c} + \mathrm{d} \cdot MW(t-1) + \mathrm{e} \cdot PET + \mathrm{f} \cdot LA + \mathrm{g} \cdot MW(t-1) \cdot PET + \mathrm{h} \cdot MW(t-1) \cdot LA + \mathrm{i} \cdot PET \cdot LA + \mathrm{j} \cdot MW(t-1) \cdot PET \cdot LA \qquad (6)$$

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

WC was computed on a daily basis according to:

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

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

Frost hardiness model

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

$$FH(t) = \mathrm{a} \cdot \frac{Ln(\mathrm{GFS})}{WC(t)} + \mathrm{b} \qquad (7)$$

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

Optimization of the parameters

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

The amount of fixed water was set according to the minimum WC usually observed during the winter period (*i.e.* $M_{bw} = 0.65$; Charrier *et al.*, 2013b). The parameters used to predict leaf fall and bud break phenology were set as in the original studies (Delpierre *et al.*, 2009 and Charrier *et al.*, 2018b). The remaining parameters ($n = 11$; Tab. S1) were optimized by minimizing the residual sum of square between simulated values and measured data (objective function). For the model describing water losses, all the variables and their interactions are considered to have a positive effect. The parameters of Eq. (6) were therefore constrained to positive values (lower limit ≥ 0). This constraint made it possible to simplify the convergence of the algorithm by setting a zero effect for variables and their interactions that did not have a significant effect on water losses.

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

Statistical analysis

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

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

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

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

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

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

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

Results

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

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

Soil temperature experiment

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

Stem dehydration experiment

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

Modelling changes in water content

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

The optimization of the evaporation function returned null values for the parameters related to PET, LA and any of their interactions with current WC, suggesting that these variables had a minor contribution to WC dynamics compared to current WC, in the period considered. Finally, the more parsimonious model predicted WC with an RMSE of 0.127 $g.g^{-1}$ DM on the calibration dataset (Fig. 4). This model was also robust as the RMSEP obtained on an external dataset was 0.116 $g.g^{-1}$ DM. Overall, the model described the seasonal dynamics observed *in situ* with good accuracy (Fig. 5, 6). However, in autumn and spring, the accuracy was lower than in winter (RMSE = 0.15 vs 0.07 g.g^{-1} DM). When stem rehydration is extremely rapid in spring, the prediction was underestimated in some years (*e.g.* 1996-1997 or 2008-2009).

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

Discussion

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

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

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

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

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

Limited root water uptake is not the only factor explaining seasonal changes in water content during the dormant period. Stem dehydration is driven by potential evapotranspiration (PET), which is actively controlled at the leaf level by stomatal closure and passively controlled by water fluxes through the leaf cuticle and leaky stomata during the leafy period and through the bark throughout the year (Duursma *et al.*, 2019). As water loss is not compensated by root uptake, the amount of free water is gradually reduced. Evaporation from aboveground parts depends on two factors: water availability and climatic demand (Mellander *et al.*, 2006). Stem evaporation was simulated as a linear function of PET, free water in the stem and leaf area, including the interactions between these variables. Parameters related to leaf area were not significant, suggesting that although leaves were still present at some dates in autumn, the amount of transpired water was too low to significantly affect the dynamics of stem water content at this time. Surprisingly, a similar result was observed for PET, although the rate of dehydration was significantly affected by evaporative demand (Fig. 3). The short photoperiod, low light intensity and low VPD observed in autumn do not induce a strong dehydration force as observed in the climate chamber. Furthermore, as water status and evaporative demand show colinear variations (*i.e.* parallel seasonal changes: decrease from autumn to winter and increase from winter to spring), the optimization algorithm cannot distinguish the respective influence of each of these variables. The influence of the evaporative demand on the dehydration rate can also become significant above a substantial threshold, greater than the VPD usually observed in the field during the simulation period. Finally, the change in solute concentration during the simulation period, (interconversion between starch and soluble carbohydrates; Charrier *et al.*, 2013b) could affect the water potential of the stem tissue more strongly than the evaporative demand.

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

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

Since the last decade, more frequent spring frost damages have been recorded (Augspurger, 2013). The results of this model open up new research questions for the prevention of frost damage. Would delaying spring rehydration be a relevant strategy to mitigate frost risks? Wet buds are more vulnerable than dry buds (Charrier *et al.*, 2013b) However, although the soil temperature experiment affected the timing of stem rehydration, the buds were unlikely to be affected as the date of bud break did not differ between treatments. Furthermore, hydrated woody tissues exhibit higher temperature of ice nucleation (Lintunen *et al.*, 2018).

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

References

Abdulqader, A. A., Suliman, H. H., Dawod, N. A. & Hussain, B. J. 2016 Characteristics and some technological properties of *Juglans regia* trees grown in Duhok Province In *The 2nd Scientific Agricultural Conference* (p. 280).

Alves, G., Améglio, T., Guilliot, A., Fleurat-Lessard, P., Lacointe, A., Sakr, S., ... & Julien, J. L. (2004). Winter variation in xylem sap pH of walnut trees: involvement of plasma membrane H+-ATPase of vessel-associated cells. *Tree Physiology*, *24*(1), 99-105.

Alves, G., Decourteix, M., Fleurat-Lessard, P., Sakr, S., Bonhomme, M., Améglio, T., ... & Guilliot, A. (2007). Spatial activity and expression of plasma membrane H+-ATPase in stem xylem of walnut during dormancy and growth resumption. *Tree Physiology*, *27*(10), 1471-1480.

Améglio, T., Bodet, C., Lacointe, A., & Cochard, H. (2002). Winter embolism, mechanisms of xylem hydraulic conductivity recovery and springtime growth patterns in walnut and peach trees. *Tree Physiology*, *22*(17), 1211-1220.

Améglio, T., Morizet, J., Cruiziat, P., & Martignac, M. (1990). The effects of root temperature on water flux, potential and root resistance in sunflower. *Agronomie*, *10*(4), 331-340.

Augspurger, C. K. (2013). Reconstructing patterns of temperature, phenology, and frost damage over 124 years: spring damage risk is increasing. *Ecology*, *94*(1), 41-50.

Baffoin, R., Charrier, G., Bouchardon, A. E., Bonhomme, M., Améglio, T., & Lacointe, A. (2021). Seasonal changes in carbohydrates and water content predict dynamics of frost hardiness in various temperate tree species. *Tree Physiology*, *41*(9), 1583-1600.

Bonhomme, M., Peuch, M., Améglio, T., Rageau, R., Guilliot, A., Decourteix, M., ... & Lacointe, A. (2010). Carbohydrate uptake from xylem vessels and its distribution among stem tissues and buds in walnut (Juglans regia L.). *Tree physiology*, 30(1), 89-102.

Boyce, R. L., Friedland, A. J., Webb, E. T., & Herrick, G. T. (1991). Modeling the effect of winter climate on high-elevation red spruce shoot water contents. *Forest science*, *37*(6), 1567-1580.

Bravo, L. A., Zúñiga, G. E., Alberdi, M., & Corcuera, L. J. (1998). The role of ABA in freezing tolerance and cold acclimation in barley. *Physiologia Plantarum*, *103*(1), 17-23.

Charra-Vaskou, K., Badel, E., Charrier, G., Ponomarenko, A., Bonhomme, M., Foucat, L., ... & Améglio, T. (2016). Cavitation and water fluxes driven by ice water potential in Juglans regia during freeze–thaw cycles. *Journal of Experimental Botany*, *67*(3), 739-750.

Charrier, G., & Améglio, T. (2011). The timing of leaf fall affects cold acclimation by interactions with air temperature through water and carbohydrate contents. *Environmental and Experimental Botany*, *72*(3), 351-357.

Charrier, G., Bonhomme, M., Lacointe, A., & Améglio, T. (2011). Are budburst dates, dormancy and cold acclimation in walnut trees (Juglans regia L.) under mainly genotypic or environmental control?. *International Journal of Biometeorology*, *55*(6), 763-774.

Charrier, G., Lacointe, A., & Améglio, T. (2018a). Dynamic modeling of carbon metabolism during the dormant period accurately predicts the changes in frost hardiness in walnut trees Juglans regia L. *Frontiers in Plant Science*, 1746.

Charrier, G., Chuine, I., Bonhomme, M., & Améglio, T. (2018b). Assessing frost damages using dynamic models in walnut trees: exposure rather than vulnerability controls frost risks. *Plant, Cell & Environment*, *41*(5), 1008-1021.

Charrier, G., Cochard, H., & Améglio, T. (2013a). Evaluation of the impact of frost resistances on potential altitudinal limit of trees. *Tree Physiology*, *33*(9), 891-902.

Charrier, G., Poirier, M., Bonhomme, M., Lacointe, A., & Améglio, T. (2013b). Frost hardiness in walnut trees (Juglans regia L.): how to link physiology and modelling?. *Tree Physiology*, *33*(11), 1229-1241.

Charrier, G., Martin-Stpaul, N., Damesin, C., Delpierre, N., Hänninen, H., Torres-Ruiz, J. M., & Davi, H. (2021). Interaction of drought and frost in tree ecophysiology: rethinking the timing of risks. *Annals of Forest Science*, *78*(2), 1-15.

Charrier, G., Nolf, M., Leitinger, G., Charra-Vaskou, K., Losso, A., Tappeiner, U., ... & Mayr, S. (2017). Monitoring of freezing dynamics in trees: a simple phase shift causes complexity. *Plant Physiology*, *173*(4), 2196-2207.

Cochard, H., Pimont, F., Ruffault, J., & Martin-StPaul, N. (2021). SurEau: a mechanistic model of plant water relations under extreme drought. *Annals of Forest Science*, *78*(2), 1-23.

Côme, D. (1992). Les végétaux et le froid.

Cox, G. M., Gibbons, J. M., Wood, A. T. A., Craigon, J., Ramsden, S. J., & Crout, N. M. J. (2006). Towards the systematic simplification of mechanistic models. *Ecological Modelling*, *198*(1-2), 240-246.

Delpierre, N., Dufrêne, E., Soudani, K., Ulrich, E., Cecchini, S., Boé, J., & François, C. (2009). Modelling interannual and spatial variability of leaf senescence for three deciduous tree species in France. *agricultural and forest meteorology*, *149*(6-7), 938-948.

Duursma, R. A., Blackman, C. J., Lopéz, R., Martin-StPaul, N. K., Cochard, H., & Medlyn, B. E. (2019). On the minimum leaf conductance: its role in models of plant water use, and ecological and environmental controls. *New Phytologist*, *221*(2), 693-705.

Essiamah, S., & Eschrich, W. (1986). Water uptake in deciduous trees during winter and the role of conducting tissues in spring reactivation. *IAWA journal*, *7*(1), 31-38.

Ewers, F. W., Améglio, T., Cochard, H., Beaujard, F., Martignac, M., Vandame, M., ... & Cruiziat, P. (2001). Seasonal variation in xylem pressure of walnut trees: root and stem pressures. *Tree physiology*, *21*(15), 1123-1132.

Gauch Jr, H. (2002). Parsimony and efficiency. In Scientific Method in Practice (pp. 269-326). Cambridge: Cambridge University Press. doi:10.1017/CBO9780511815034.010

Hänninen, H., Kramer, K., Tanino, K., Zhang, R., Wu, J., & Fu, Y. H. (2019). Experiments are necessary in process-based tree phenology modelling. *Trends in Plant Science*, *24*(3), 199-209.

Kaufmann, M. R. (1975). Leaf water stress in Engelmann spruce: influence of the root and shoot environments. *Plant Physiology*, *56*(6), 841-844.

Kramer, P. J. (1940). Root resistance as a cause of decreased water absorption by plants at low temperatures. *Plant physiology*, *15*(1), 63.

Lamacque, L., Charrier, G., Farnese, F. D. S., Lemaire, B., Améglio, T., & Herbette, S. (2020). Drought-induced mortality: Branch diameter variation reveals a point of no recovery in lavender species. *Plant physiology*, *183*(4), 1638-1649.

Lee, A.G. (1975). Interactions with biological membranes. *Endeavour*, 31, 67-71.

Lee, S. H., Zwiazek, J. J., & Chung, G. C. (2008). Light-induced transpiration alters cell water relations in figleaf gourd (Cucurbita ficifolia) seedlings exposed to low root temperatures. *Physiologia Plantarum*, *133*(2), 354-362.

Lee, S. H., Chung, G. C., Jang, J. Y., Ahn, S. J., & Zwiazek, J. J. (2012). Overexpression of PIP2; 5 aquaporin alleviates effects of low root temperature on cell hydraulic conductivity and growth in Arabidopsis. *Plant Physiology*, *159*(1), 479-488.

Lintunen, A., Mayr, S., Salmon, Y., Cochard, H., & Hölttä, T. (2018). Drivers of apoplastic freezing in gymnosperm and angiosperm branches. *Ecology and evolution*, *8*(1), 333-343.

Lockhart, J. A. (1965). An analysis of irreversible plant cell elongation. *Journal of theoretical biology*, *8*(2), 264-275.

Lugojan, C., & Ciulca, S. (2011). Evaluation of relative water content in winter wheat. *Journal of Horticulture, Forestry and Biotechnology*, *15*(2), 173-177.

Luoranen, J., Repo, T., & Lappi, J. (2004). Assessment of the frost hardiness of shoots of silver birch (Betula pendula) seedlings with and without controlled exposure to freezing. *Canadian Journal of Forest Research*, *34*(5), 1108-1118.

Lyons, J. M. (1973). Chilling injury in plants. *Annual review of plant physiology*, *24*(1), 445-466.

Maurya, J. P., & Bhalerao, R. P. (2017). Photoperiod-and temperature-mediated control of growth cessation and dormancy in trees: a molecular perspective. *Annals of botany*, *120*(3), 351-360.

Mellander, P. E., Stähli, M., Gustafsson, D., & Bishop, K. (2006). Modelling the effect of low soil temperatures on transpiration by Scots pine. *Hydrological Processes: An International Journal*, *20*(9), 1929-1944.

Miller, A. J., & Smith, S. J. (1996). Nitrate transport and compartmentation in cereal root cells. *Journal of Experimental Botany*, 47(7), 843-854.

Monteith, J. L. (1965). Evaporation and environment. In *Symposia of the society for experimental biology* (Vol. 19, pp. 205-234). Cambridge University Press (CUP) Cambridge.

Monteith, J. L. (1972). Latent heat of vaporization in thermal physiology. *Nature New Biology*, *236*(64), 96-96.

Rinne, P.L.H., Kaikuranta, P.M., & van der Schoot, C. (2001). The shoot apical meristem restores its symplasmic organization during chilling-induced release from dormancy. *Plant Journal*, 26, 249–264.

Running, S. W., & Reid, C. P. (1980). Soil temperature influences on root resistance of Pinus contorta seedlings. *Plant physiology*, *65*(4), 635-640.

Sachs, J. (1868). *Lehrbuch der Botanik: nach dem gegenwärtigen Stand der Wissenschaft*. Wilhelm Engelmann.

Sapes, G., Roskilly, B., Dobrowski, S., Maneta, M., Anderegg, W. R., Martinez-Vilalta, J., & Sala, A. (2019). Plant water content integrates hydraulics and carbon depletion to predict drought-induced seedling mortality. *Tree physiology*, *39*(8), 1300-1312.

Sierra-Almeida, A., Reyes-Bahamonde, C., & Cavieres, L. A. (2016). Drought increases the freezing resistance of high-elevation plants of the Central Chilean Andes. *Oecologia*, *181*(4), 1011-1023.

Signorelli, S., Shaw, J., Hermawaty, D., Wang, Z., Verboven, P., Considine, J. A., & Considine, M. J. (2020). The initiation of bud burst in grapevine features dynamic regulation of the apoplastic pore size. *Journal of experimental botany*, *71*(2), 719-729.

Turcotte, A., Morin, H., Krause, C., Deslauriers, A., & Thibeault-Martel, M. (2009). The timing of spring rehydration and its relation with the onset of wood formation in black spruce. *Agricultural and Forest Meteorology*, *149*(9), 1403-1409.

Wan, X., Landhäusser, S. M., Zwiazek, J. J., & Lieffers, V. J. (1999). Root water flow and growth of aspen (Populus tremuloides) at low root temperatures. *Tree physiology*, *19*(13), 879-884.

Wang, Y., Mao, Z., Bakker, M. R., Kim, J. H., Brancheriau, L., Buatois, B., ... & Stokes, A. (2018). Linking conifer root growth and production to soil temperature and carbon supply in temperate forests. *Plant and Soil*, *426*(1), 33-50.

Welling, A., Kaikuranta, P., & Rinne, P. (1997). Photoperiodic induction of dormancy and freezing tolerance in Betula pubescens. Involvement of ABA and dehydrins. *Physiologia Plantarum*, *100*(1), 119-125.

Williams, T. G., & Flanagan, L. B. (1996). Effect of changes in water content on photosynthesis, transpiration and discrimination against 13 CO 2 and C 18 O 16 O in Pleurozium and Sphagnum. *Oecologia*, *108*(1), 38-46

Wolfe, J. (1978). Chilling injury in plants. The role of membrane lipid fluidity. *Plant, Cell and Environment*, 1, 241-247.

Zhu, X.B., R.M. Cox and P.A. Arp (2000). Effects of xylem cavitation and freezing injury on dieback of yellow birch (*Betula alleghaniensis*) in relation to a simulated winter thaw. *Tree Physiology*, 20, 541-547.

Author Contribution

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

Acknowledgements

The authors want to acknowledge the help of Christian Bodet, Brigitte Girard, Brigitte Saint-Joanis, Christophe Serre and Marc Vandamme for help in data acquisition. This work has been carried out with financial support from the Pack Ambition Recherche program “Doux-Glace” from the Auvergne-Rhône Alpes region. This work was also supported by the program ANR Acoufollow (ANR-20-CE91-0008) funded by the French National Agency for Research and the Austrian Science Fund, the Frostdefend program (LIFE20 CCA/GR/001747) funded by the European union through the Life program.

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 budbureak (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.