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Assessing frost damages using dynamic models in walnut trees: exposure rather than vulnerability controls frost risks.

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1 **Abstract**

2 Frost damages develop when exposure overtakes frost vulnerability. Frost risk assessment
3 therefore needs dynamic simulation of frost hardiness using temperature and photoperiod in
4 interaction with developmental stage. Two models, including or not the effect of photoperiod
5 were calibrated using five years of frost hardiness monitoring (2007-2012), in two locations
6 (low and high elevation) for three walnut genotypes with contrasted phenology and maximum
7 hardiness (*Juglans regia* cv Franquette, *Juglans regia* x *nigra* ‘Early’ and ‘Late’). The
8 Photothermal model predicted more accurate values for all genotypes (Efficiency=0.879;
9 RMSEP=2.55°C) than the Thermal model (Efficiency=0.801; RMSEP=3.24°C). Predicted
10 frost damages were strongly correlated to minimum temperature of the freezing events ($\rho=-$
11 0.983) rather than actual frost hardiness ($\rho=-0.515$), or ratio of phenological stage completion
12 ($\rho=0.336$). Higher frost risks are consequently predicted during winter, at high elevation,
13 whereas spring is only risky at low elevation in early genotypes exhibiting faster dehardening
14 rate. However, early frost damages, although of lower value, may negatively affect fruit
15 production the subsequent year ($R^2=0.381$, $P=0.057$). These results highlight the interacting
16 pattern between frost exposure and vulnerability at different scales and the necessity of intra-
17 organ studies to understand the time course of frost vulnerability in flower buds along the
18 winter.

19 Keywords

20 Frost hardiness, abiotic stress, temperature, photoperiod, dormancy, phenology.

21 Summary statement:

22 To predict critical periods facing frost damages, two dynamic integrated models of frost
23 hardiness were adapted using five years of monitoring in three different genotypes of walnut
24 trees and two locations. Frost hardiness was more accurately predicted when using both
25 temperature and photoperiod as input variables. Higher frost damages (*i.e.* frost risks) were
26 more closely related to temperature of the freezing events (*i.e.* frost exposure) rather than frost
27 hardiness or phenological stage (*i.e.* frost vulnerability). Even though frost risks are higher in
28 winter at high elevation (exposure constrained) and spring at low elevation, especially in early
29 genotypes (exposure and vulnerability constrained), fruit yields observed the following year
30 were correlated to autumn damages.

31

32 Introduction

33 Frost damages are of major importance for perennial crops. Across weather hazards
34 impacting agriculture, frost induces the most important economic losses (Snyder *et al.*, 2005)
35 *e.g.* damages can cost up to several hundreds of millions dollars in fruit and tree production
36 (Attaway, 1997). Frost damages develop when the exposure (*i.e.* freezing event) overtakes the
37 vulnerability (*i.e.* frost hardiness) of the plant (Williams *et al.*, 2008; Charrier *et al.*, 2015a).
38 Freezing events mainly occur when the heat energy balance is negative (*i.e.* radiative frost;
39 Lindkvist *et al.*, 2000). Exposure to radiative frosts, which is observed under clear sky and
40 low wind speed, exhibits temporal pattern in frequency and intensity. Although substantial
41 freezing events could occur at different periods along the year, most studies focused on spring
42 period when vulnerability is high (close to leaf unfolding and blooming dates (*cf.* Rigby &
43 Porporato, 2008; Bennie *et al.*, 2010; Augspurger, 2013). To assess risks of frost damages,
44 three different approaches are commonly used:

45 (i) Probability of freezing event at a given date in the recorded temperature time series
46 from meteorological stations (Cooter & LeDuc, 1995; Easterling, 2002; Robeson, 2002).

47 (ii) Comparison between budburst dates, either observed or simulated by a phenological
48 model and last freezing dates (Cannell & Smith 1986; Scheifinger *et al.*, 2003; Schwartz *et*
49 *al.*, 2006; Bennie *et al.*, 2010; Ferguson *et al.*, 2011; Marino *et al.*, 2011)

50 (iii) Estimation of frost damage using phenological models dynamically coupled to frost
51 hardiness models (Leinonen, 1996; Morin & Chuine, 2014).

52 In the context of global change (IPCC, 2014), these different approaches led to
53 contradictory results, independently of the approach used. Some studies forecasted an
54 increasing vulnerability (Hanninen, 1991; Inouye, 2008; Auspurger, 2013), whereas it was
55 supposed to decrease in other ones (Murray *et al.*, 1989; Scheifinger *et al.*, 2003; Eccel *et al.*,

56 2009; Baraer *et al.*, 2010; Bennie *et al.*, 2010; Dai *et al.*, 2013; Morin & Chuine, 2014).
57 Finally, some authors highlighted the fact that the pattern is complex (Linkosalo *et al.*, 2000;
58 2006; Gu *et al.*, 2008) due to antagonistic effects of increasing temperature in mean and
59 variance (Rigby & Porporato, 2008).

60 One important missing point in most of these studies is that frost hardiness is dynamic
61 exhibiting successive stages of hardening and dehardening (Charrier *et al.*, 2013a), including
62 re-hardening (Kalberer *et al.*, 2006). Frost hardiness varies along the year and within the plant
63 (Charrier *et al.*, 2013b), which therefore drives the plant's frost vulnerability.

64 At the end of the growing season, when photoperiod, and temperature decrease, bud
65 meristems get into endodormancy (Welling & Palva, 2006; Druart *et al.*, 2007), and frost
66 hardening is induced (Charrier *et al.*, 2011). Endodormancy, which is a temporary suspension
67 of meristem activity controlled by intrinsic factors, is released by chilling exposure (Lang *et al.*,
68 1987). Finally, when endodormancy is released, bud goes through the ecodormancy phase
69 during which growth is temperature-limited. Increase of temperature in late winter-early
70 spring promotes both bud growth and dehardening (Greer & Warrington, 1984).
71 Photoperiodic control of the ecodormancy stage has also been evidenced in evergreen
72 conifers, and some deciduous species (*e.g. Fagus sylvatica*; Basler & Körner, 2012).

73 Environmental conditions control both exposure and vulnerability to freezing event, which
74 can therefore explain the complexity in frost risk assessment. Exposure and vulnerability
75 diverging across organs (Charrier *et al.*, 2013b; 2015), bud and shoot frost hardiness indicate
76 both short and long term risks, respectively. Furthermore, frost hardiness being dynamic
77 along the frost exposure period in relation with annual phenological cycle, delayed frost
78 hardening in autumn and premature dehardening in spring can subsequently damage trees
79 (Bannister & Neuner, 2001; Aitken & Hannertz, 2001). An efficient process-based model for

80 frost damage should thus integrate processes, revealing indirect and direct effects of
81 environmental factors (Hänninen & Kramer, 2007; Hänninen, 2016):

- 82 - Indirect effects mediated by the annual phenological cycle *i.e.* time course of endo- and
83 ecodormancy, bud growth and budset,
- 84 - Direct effects *i.e.* modulation of frost hardiness depending on temperature, and
85 eventually photoperiod.

86 Among existing dynamic models (Fuchigami *et al.*, 1982; Leinonen, 1996; Jonsson *et al.*,
87 2004; Ebel *et al.*, 2005; Ferguson *et al.*, 2011), we selected two which were compatible with
88 these pre-requisites: the one from Leinonen (1996, derived from Kellomäki *et al.*, 1992; 1995,
89 thereafter called Photothermal model), including thermal and photoperiodic control of frost
90 hardiness, has already been used to assess frost vulnerability in future climatic conditions of
91 several North American deciduous species (Morin & Chuine, 2014). An alternative model
92 from Ferguson *et al.* (2011, thereafter called Thermal model) predicts frost hardiness under a
93 strict thermal control.

94 We hypothesized that critical periods facing frost damages could be (i) in autumn, when
95 trees have not acclimated yet, (ii) in the core of the winter, when temperature are minimum, or
96 (iii) in spring when trees have deacclimated. Finally, to identify such critical periods, we
97 calibrated Photothermal and Thermal models to test the relevancy of including photoperiod to
98 model frost vulnerability in a deciduous species. Calibration was performed using a large
99 amount of ecophysiological data (*e.g.* frost hardiness measured over five years in two
100 locations and three genotypes, chilling and forcing requirements for bud dormancy release
101 measured over approx. twenty years), on the walnut tree, *J. regia*, which is the second
102 perennial crop in France (Rodier, 2012), with an extensive leafless period. We selected three
103 walnut genotypes that differed either in phenology or in maximal frost hardiness to perform

104 pair-wise comparison and compare situations with similar phenological behavior, but
105 contrasted maximal frost hardiness (*Juglans regia* L. Cv Franquette and *Juglans regia* x *nigra*
106 cv ‘Early’), and contrasted phenological behavior, but similar maximal frost hardiness
107 (*Juglans regia* x *nigra* cv ‘Early’, and ‘Late’). Finally, we compared the predicted damages at
108 different periods in buds and branches with the temperature of the freezing event, the actual
109 frost hardiness, the ratio of phenological stage completion, the date and also the annual yield
110 of fruits the subsequent growing season.

111 **Material and Methods**

112 **Models description**

113 The computation of frost damages is based on three sub-models: (i) annual phenological
114 cycle, (ii) frost hardiness and (iii) frost damage. For the reasons detailed above, frost
115 hardiness was modeled according to Leinonen (1996) or Ferguson *et al.*, (2011) which shares
116 similar philosophy *i.e.* frost hardening competence of a particular organ (H_C) depends on
117 direct effect (*i.e.* temperature and, eventually, photoperiod) and indirect effect of
118 environmental conditions (*i.e.* phenological stage). For the sake of clarity, details of frost
119 hardiness models are provided in supplementary material, although they did not differ from
120 the previously published versions.

121 ***Annual cycle***

122 The annual cycle is divided into four different phases: endodormancy, ecodormancy,
123 growth and lignification, which occur sequentially (Hanninen & Kramer, 2007).

124 **Endodormancy**

125 Endodormancy is induced by decreasing photoperiod and temperature, and released by
126 chilling temperatures (Lang *et al.*, 1987). Chilling accumulation was modeled according to

127 the inverse of the Richardson function (originally used to predict ecodormancy release;
 128 Richardson *et al.*, 1974) which best described endodormancy release dates in walnut trees and
 129 other fruit species (Chuine *et al.*, 2016). When $CU(t)$ reaches parameter CU_{crit} (arbitrary
 130 chilling units, CU), endodormancy is released and ecodormancy stage initiates.

$$131 \quad CU(t + 1) = CU(t) + \text{Max}(\text{Min}(T_{high} - \theta(t); T_{high} - T_{low}); 0) \quad (1)$$

132 with $CU(t)$, the chilling unit at day t , T_{high} , both the temperature above which CU equals 0 and
 133 the amount of CU when temperature equals T_{low} or lower; CU being linear between T_{low} and
 134 T_{high} .

135 Ecodormancy

136 Ecodormancy stage was modeled according to a sigmoid function, which is one of the
 137 most relevant for this particular stage (Caffarra *et al.*, 2011). When $FU(t)$ reaches parameter
 138 FU_{crit} (arbitrary forcing units, FU), budburst occurs and apparent growth initiates.

$$139 \quad FU(t + 1) = FU(t) + \frac{1}{1 + e^{-slp(\theta(t) - T_{50})}} \quad (2)$$

140 with $FU(t)$, the forcing unit at day t , slp , the slope of the function at the temperature inducing
 141 half of the maximal apparent growth rate T_{50} .

142 Growth

143 Growth stage was modeled with a temperature sum in Degree Days (de Réaumur, 1735).
 144 When $GDD(t)$ reaches GDD_{crit} (1200 DD), growth stage stops and lignification stage initiates
 145 (Dreyer, 1984).

$$146 \quad GDD(t + 1) = GDD(t) + \text{Max}(0; \theta(t) - T_{thG}) \quad (3)$$

147 with GDD_t , the growing degree day at day t , and T_{thG} the threshold temperature for growth.

148 Lignification

149 Lignification stage was considered under photoperiodic control. This stage constitutes a
 150 transition to endodormancy stage, which was arbitrary set to 1st of September (Chuine *et al.*,
 151 2016).

152 ***Frost hardiness***

153 Frost hardiness varied among two limits (FH_{Max} and FH_{Min}), which were set as the mean
 154 lowest and highest measured frost hardiness, respectively. Daily changes in frost hardiness,
 155 caused by environmental factors (*i.e.* additive effect of temperature and photoperiod in the
 156 Photothermal model, or temperature only in the Thermal model) depend on the difference
 157 between the stationary (target) level of frost hardiness and actual frost hardiness
 158 (Photothermal) or the thermal time (Thermal), both modulated by the effect of annual
 159 development of trees. Complete descriptions of the models are available in the supplementary
 160 materials and original publications (Leinonen, 1996; Ferguson *et al.*, 2011).

161 ***Frost damage***

162 Based on the relation between frost hardiness (FH , temperature inducing 50% relative
 163 electrolyte leakage REL , see below) and frost sensitivity (FS , slope at this temperature; Fig.
 164 1B), potential frost damages were calculated on a daily basis. Potential frost damages (FD ,
 165 in % of electrolyte leakage) induced by a freezing event ($\theta_{min} < -4^{\circ}C$) were therefore
 166 calculated as:

$$167 \quad FD = \frac{1}{1 + e^{FS(FH - \theta_{min})}} \quad (4)$$

168 Quantification of damages could therefore be calculated using minimal temperature on a daily
 169 basis. For each year, the maximal predicted damages over a specific period were also
 170 calculated (from September to November, December to February and March to May,
 171 respectively called autumn, winter and spring period in the subsequent parts) as well as the

172 damages induced by the first, maximal (*i.e.* absolute lowest temperature), and last freezing
173 events. The predicted damages were related to temperature, frost hardiness, date, year or ratio
174 of phenological stage completion (*i.e.* $\frac{CU(t)}{CU_{crit}}$ during endo- and $\frac{FU(t)}{FU_{crit}}$ eco-dormancy,
175 respectively).

176 **Data**

177 *Annual cycle*

178 Parameters used for endodormancy release stage were taken from Chuine *et al.* (2016),
179 which was fitted on endodormancy release dates from *Juglans regia* cv Franquette in orchards
180 from France from 1975 until 2013 (18 dates). Parameters driving endodormancy release was
181 fixed similar across genotypes since chilling requirements are similar in these genotypes
182 (Charrier *et al.*, 2011). From this latter study, sigmoid functions describing the response to
183 temperature during the ecodormancy phase was fit for each genotype ('Franquette', 'Early',
184 and 'Late', called Fra, E-Hyb, and L-Hyb, respectively). Finally, GDD_{crit} was taken from
185 Dreyer (1984).

186 *Frost hardiness*

187 Frost hardiness was measured from September until budburst on one-year-old branches
188 along the period 2007-2012 (58 to 61 dates with n=5 replicates per date; supplementary
189 materials) in two different orchards (low : 45° 46' N 03°08' E, 340m a.s.l. and high
190 elevation: 45°43'N 03° 01' E 880m a.s.l.). We used the electrolyte leakage method that
191 provides accurate estimation of frost hardiness along winter, and across organs (Zhang and
192 Willison 1987; Sutinen *et al.* 1992; Charrier & Améglio, 2011). Frost hardiness was
193 concomitantly measured on buds and bearing branch during winter 2008-2009 (10 sampling
194 dates) in low elevation orchard.

195 Samples were cut into six 5-cm long segments without buds. Buds were kept intact
 196 attached to a small piece of branch. Samples were exposed to four different freezing
 197 temperatures among this set of temperatures: -5, -10, -15, -20, -30°C, -40°C. Depending on
 198 the season either the highest or the lowest temperatures were not used. Two supplementary
 199 subsamples were exposed to control temperature (+5°C) and maximal freezing temperature (-
 200 75°C). Frozen and thawed rate was set to 5K.h⁻¹. Details are provided in Charrier and
 201 Améglio (2011).

202 Relative electrolytic leakage (*REL*) was calculated as $(C_1/C_2)*100$ as described in Zhang &
 203 Willison (1987). We assumed the following relationship between *REL* and temperature (θ) for
 204 each sample:

$$205 \quad REL = \frac{a}{(1 + e^{b(c-\theta)})} + d \quad (5)$$

206 where parameters *a* and *d* define asymptotes of the function, and *b* is the slope at the
 207 inflection point *c*.

208 Frost hardness level was estimated as the temperature of the inflection point (*c*) of the
 209 adjusted logistic sigmoid function Eq. (1) (Repo and Lappi, 1989), whereas frost sensitivity is
 210 considered to be estimated by the parameter *b* in % Damage.°C⁻¹. Parameter estimation was
 211 performed by nonlinear regression using ExcelStat ver. 7.5.2.

212 **Calibration and validation**

213 Parameters driving the annual phenological cycle were taken from literature data and
 214 therefore not fitted. In the frost hardness and frost damage sub-models, some parameters
 215 were fixed according to observed values (FH_{MinBch} , FH_{MinBud} , FH_{MaxBch} , α , β , and γ ; Table I).
 216 However, in the Photothermal model, FH_{Max} has two components *i.e.* FH_{PMax} and FH_{TMax}
 217 corresponding to photoperiodic and thermal control, respectively. The parameter δ was

218 introduced to split FH_{Max} into its thermal (δ) and photoperiodic ($1 - \delta$) components, for each
219 genotype. Finally, 9 and 6 parameters were fitted in the Photothermal and the Thermal model,
220 respectively. Optimization of these parameters was assessed by minimization of the mean
221 squared error between simulated values and observed data from the calibration dataset. We
222 used Generalized Reduced Gradient algorithm with different initial values of parameters to
223 avoid local minima problems. The complete dataset was split into two datasets of similar size,
224 one for calibration (low elevation from autumn 2007 to spring 2009 and high elevation from
225 autumn 2008 to spring 2009: 34, 32 and 33 sampling dates for Fra, E-Hyb and L-Hyb,
226 respectively), and the other one for validation (low elevation from autumn 2009 to spring
227 2012: 26, 26 and 28 sampling dates for Fra, E-Hyb and L-Hyb, respectively; Tab. S1). Table
228 1 presents the values of the different parameters. Frost damage predicted at different period
229 were finally compared to the yield measured in the Walnut research facility in Creysse (44°
230 52' N, 0°30' W, 45 m a.s.l.).

231 **Climate input data**

232 For frost risk simulation, meteorological data (daily mean and minimal temperatures,
233 photoperiod) from the closest weather station *i.e.* 2 km away from the orchards were used.
234 Data were spread from September 1970 until August 2015, from September 1970 until
235 August 2015, and from September 1990 until August 2015 for low elevation, high elevation
236 and research facility orchard, respectively.

237 **Statistical analysis**

238 Statistical analyses were performed using R software (R Development Core Team, 2015).
239 The normality of the potential damage distribution was tested using Shapiro-Wilk test. Most
240 of the distributions of predicted damages being not normally distributed, the comparison

241 between seasons, genotypes, location, organs were performed using the Kruskal-Wallis non
 242 parametric test and using Spearman's rank correlation coefficient ρ .

243 **Results**

244 **Parameters value estimated from data**

245 Frost hardiness and frost damage sub-models included parameters that have been estimated
 246 from the dataset (*i.e.* FH_{MinBud} , FH_{MinBch} , FH_{MaxBch} , α , β , and γ ; Tab. 1). Frost hardiness
 247 measured for buds (FH_{Bud}) was strongly and significantly correlated to frost hardiness
 248 measured in the bearing branch (FH_{Bch}) through a logarithmic function ($R^2 = 0.904$; $P < 0.001$;
 249 Fig. 1A). Frost hardiness data were mostly measured on branches (56 different dates). We
 250 therefore calibrated the frost hardiness model on branch data and extrapolated to buds (10
 251 common dates) such as:

$$252 \quad FH_{\text{Bud}} = a \times \text{Ln}(-FH_{\text{Bch}}) - b \quad (6)$$

253 with $a = -14.4$, and $b = -28.5^\circ\text{C}$.

254 Frost sensitivity (FS in % Damage. $^\circ\text{C}^{-1}$) was related to frost hardiness in all genotypes and
 255 organs ($R^2 = 0.517$; $P < 0.0001$; Fig. 1B) such as:

$$256 \quad FS = \alpha + \beta \cdot e^{\frac{\gamma}{FH}} \quad (7)$$

257 with $\alpha = 0.106$, $\beta = -0.135$, and $\gamma = -13.57$ (Tab. 1).

258 Frost sensitivity was thus high for low frost hardiness, and almost constant for the
 259 maximum frost hardiness. Minimum frost hardiness in branch ($FH_{\text{MinBch}} = -7.7 \pm 0.2^\circ\text{C}$, mean
 260 \pm SE) were not significantly different similar across genotypes ($P = 0.104$) or locations
 261 ($P = 0.766$). For buds, frost hardiness was only measured in Franquette and was minimum at
 262 budburst date $FH_{\text{MinBch}} = -4.4 \pm 0.2^\circ\text{C}$, mean \pm SE). These parameters were therefore fixed to

263 -7.7°C and -4.4°C for branches and buds, respectively (Tab. 1). However, maximal
264 resistances (FH_{MaxBch}) significantly differed between Franquette (Fra, $FH_{MaxBch} = -32.2 \pm$
265 1.1°C , mean \pm SE) and hybrids (Early: $FH_{MaxBch} = -36.6 \pm 0.7^\circ\text{C}$, mean \pm SE and Late:
266 $FH_{MaxBch} = -37.6 \pm 1.0^\circ\text{C}$, mean \pm SE; $P < 0.01$), but not across hybrids (Early vs Late;
267 $P = 0.725$). Different values were therefore fixed: -32.2, and -37.1°C, for Fra and hybrids,
268 respectively (Tab. 1).

269 **Model validation**

270 The calibrated Photothermal model predicted accurate values of frost hardiness in the three
271 genotypes on the calibration dataset (Efficiency = 0.951, 0.909, 0.948, and RMSE = 1.45,
272 2.64, and 2.12°C in Fra, E-Hyb, and L-Hyb, respectively; Fig. 2A; Fig. S1). On the validation
273 dataset, accuracy was also high (Efficiency = 0.855, 0.879, 0.904, and RMSEP = 2.28, 2.91,
274 and 2.47°C in Fra, E-Hyb and L-Hyb, respectively; Fig. 2B).

275 The Photothermal model split maximal frost hardiness (FH_{Max}) into two components: one
276 driven by photoperiod (FH_{Pmax}) and another one driven by temperature (FH_{Tmax}). Whereas Fra
277 and E-Hyb predominantly responded to temperature ($\delta = 0.517$ and 0.578 , respectively), in L-
278 Hyb, frost hardiness seemed to be under higher photoperiodic control than other genotypes (δ
279 = 0.293). Differences across genotypes were also observed in the extent of effective
280 nyctiperiod, *i.e.* $NL_2 - NL_1$: from 2h in E-Hyb to 4h in L-Hyb. Extent of effective
281 temperature, *i.e.* $T_2 - T_1$, also exhibited genotypic differences: from 18.9°C (-6.9 to 12.0°C) in
282 Fra to 35.2°C (-9.3 to 25.9°C) in L-Hyb. Another difference lied in the time constant ($\tau = 5$
283 days in Fra; 16 and 15 days in E-Hyb and L-Hyb, respectively).

284 The Thermal model (under strict temperature control), more accurately predicted frost
285 hardiness than the Photothermal model on calibration dataset (Efficiency = 0.928, and 0.929
286 and RMSE = 1.75 and 2.33°C in Fra and E-Hyb, respectively; Fig. S1), but accuracy was

287 lower on the validation dataset (Efficiency = 0.760 and 0.751 and RMSEP = 2.94 and 4.16°C
288 in Fra and E-Hyb, respectively). However, in L-Hyb, this model more accurately predicted
289 frost hardiness in the calibration dataset (Efficiency = 0.980 and RMSE = 1.30°C), but not in
290 the validation dataset (Efficiency = 0.891 and RMSEP = 2.63°C).

291 **Predicting frost damages**

292 The frost damages were, finally, predicted using the Photothermal model because, in the
293 Thermal model, the decrease in number of parameters did not compensate the loss in accuracy
294 in predicting frost hardiness (BIC = 383.9 and 418.3, in Photothermal and Thermal version,
295 respectively). However, dynamics were qualitatively similar in both versions of the model
296 (Fig. S1). Maximal potential frost damages per season predicted for the three genotypes (Fra,
297 E-Hyb, and L-Hyb) were calculated for buds and branches, at low and high elevation in
298 autumn, winter, and spring (Fig. 3). Approximately two times less damages were predicted in
299 branches compared to buds: maximum potential damages were relatively moderate in
300 branches (median *ca.* 10-20% and lower than 35%, even in extreme cases), and higher in buds
301 (median *ca.* 10-30%, reaching levels higher than 50% during coldest winters). Significantly
302 higher damages were predicted at high than in low elevation ($P < 0.001$; *e.g.* 8.9 and 4.1% of
303 days inducing more than 10% damage in buds from Fra, in high and low elevation orchard,
304 respectively), and in winter than in spring and autumn, for all genotypes, organs and locations
305 ($P < 0.001$; *e.g.* 2.2, 14.6 and 2.8% of days inducing more than 10% damage in buds from Fra,
306 in autumn, winter, and spring, respectively). In spring compared to autumn, significantly
307 higher damages were only predicted in branches from Fra at low elevation.

308 Frost risk is dynamic along the year and differs across orchards. Along the year, the safety
309 margin (*i.e.* difference between actual frost hardiness and minimal temperature) calculated on
310 a daily basis was relatively wide for all genotypes and locations *i.e.* between 10 and 20°C

311 along the whole year for buds and between 15 and 30°C for branches (Fig. 4). However, risky
312 periods for buds (gray areas in Fig.4) are expected when frost exposure (frost probability > 0)
313 could meet frost vulnerability at least once per decade ($SM_{10} > -10^{\circ}\text{C}$). Frost risk was almost
314 negligible in low elevation orchard except in spring for early genotypes (Fra and E-Hyb), and
315 autumn and winter in Fra only. In high elevation orchard, frost risk was higher in October
316 (only in Fra), December and January (Fra and E-Hyb) and in February for all genotypes.
317 Higher frost risk was thus predicted in high elevation for all genotypes because the maximal
318 level of bud frost hardiness achieved during winter could be overtaken by minimal
319 temperature, even though maximum frost hardiness observed during leafless period was
320 tightly correlated to the absolute minimal temperature observed along winter (Fig.5A-C).
321 Although branches showed much lower frost hardiness than the minimal temperature they
322 were exposed to, buds can suffer more than 50% cell lyses (4.3% of years in Fra and 2.2% in
323 Hybrids during the considered period). Finally, early genotypes (Fra and E-Hyb) may suffer
324 frost damage during spring in low elevation orchard, whereas Fra may be affected by
325 autumnal freezing events, especially at high elevation.

326 Among the numerous freezing events recorded along the 1970-2015 period ($n = 7630$),
327 predicted frost damages were primarily and negatively correlated to freezing temperature ($\rho =$
328 -0.983 ; $P < 0.0001$) through a polynomial function ($R^2 = 0.979$; Fig. 6A). Relatively weaker
329 correlations, although significant, were observed with actual frost hardiness ($\rho = -0.515$; $P <$
330 0.0001 ; Fig. 6B) and ratio of phenological stage completion ($\rho = 0.336$; $P < 0.0001$; Fig. 6C).
331 The predicted damages induced by the first and last freezing events with potential injuring
332 temperature (*i.e.* $\theta_{\min} < -4^{\circ}\text{C}$ occurred) exhibited different correlations. The correlation with
333 temperature of the freezing event was very similar and also highly significant ($\rho = -0.939$; P
334 < 0.0001 ; Fig. 6D), whereas, reversely oriented correlations were observed with actual frost
335 hardiness ($\rho = 0.243$; $P = 0.0009$; Fig. 6E) or ratio of phenological stage completion ($\rho = -$

336 0.268; $P = 0.0002$; Fig. 6F). Also, first frosts occurred on average, ten days earlier at high
337 (Nov. 13th) than in low elevation (Nov. 23rd; $P = 0.012$), and more than three weeks later for
338 last freezing event *e.g.* Mar. 13th and Apr. 5th in low and high elevation respectively ($P <$
339 0.001). The potential frost damages generated by the first and the last freezing events were
340 similar in other genotypes, although exhibiting lower absolute values. Finally, no significant
341 trend were observed with years ($\rho = -0.037$; $\rho = 0.032$, for the complete set or only first and
342 last freezing events, respectively).

343 Yield in nuts production, obtained in an independent orchard, exhibited a wide range over
344 a ten years period (2005-2014) from 1 to 25 kg per tree (Fig. 7). Damages predicted during
345 the previous autumn were lower (9.1%) than during the previous winter, or spring periods
346 (16.2, and 9.6%, respectively). However, these autumn damages were more strongly
347 correlated to fruit production the subsequent year ($R^2 = 0.381$), although only significant at
348 the 10% level ($P = 0.057$). On the contrary, no significant correlation was observed with yield
349 in winter ($R^2 = 0.005$; $P = 0.841$), nor spring ($R^2 = 0.089$; $P = 0.402$).

350 **Discussion**

351 Frost damage, under field conditions, develops when frost exposure overtakes frost
352 hardiness. Although frost exposure is relatively easy to measure using, as a proxy, air
353 temperature from weather station, the access to accurate frost hardiness data is rather limited.
354 Here, a large dataset of frost hardiness seasonality over five years in two locations was used
355 to calibrate integrated models predicting frost damages for three cultivars of walnut trees.
356 Three sub-models interact, namely the annual phenological cycle, the frost hardiness and the
357 frost damage sub-models. The frost damage sub-model was based on empirical observations
358 of the sigmoidal function between frost hardiness and frost sensitivity, over a wide range
359 (from -39.2 to -3.9°C). The annual phenological cycle sub-model was mostly based on

360 experimental observations of temperature response during endodormancy, ecodormancy
361 (Charrier *et al.*, 2011) and growth stages (Dreyer, 1984). The parameter estimates of the
362 response function to temperature during endodormancy were statistically inferred using a
363 unique dataset of 20 years of endodormancy release date. The onset of endodormancy was
364 arbitrarily set to the beginning of September. Using a dynamic date instead of a constant date
365 *e.g.* calculated from leaf fall model (Delpierre *et al.*, 2007) might have delayed frost
366 hardening and, consequently, over-estimated potential damages. However, the annual cycle
367 sub-model has proven robust in previous dormancy and budburst simulations (Chuine *et al.*,
368 2016).

369 The frost hardiness sub-model rested upon parameter optimization and comparison across
370 two alternative models: Photothermal, using photoperiod and temperature as input variables
371 (Leinonen, 1996), and Thermal, using only temperature (Ferguson *et al.*, 2011). The
372 photothermal model used here was based on many experimental results on *Pinus sylvestris*
373 (Valkonen *et al.*, 1990; Repo *et al.*, 1990; 1996; Repo, 1991; Kellomäki *et al.*, 1992; 1995;
374 Leinonen *et al.*, 1995; Leinonen, 1996). The Thermal model, developed on *Vitis sp.*, shares
375 similar philosophy *i.e.* environmental conditions influence frost hardiness through direct and
376 indirect (*i.e.* via annual cycle) effects. These models thus seem relevant for other perennial
377 species, such as deciduous angiosperms, because of the similarity in controlling factors and
378 processes involved: frost hardiness and annual cycle are related (Charrier *et al.*, 2011),
379 hardening and dehardening are under temperature control (Charrier & Améglio, 2011) and
380 reversible (*e.g.* dehardening after warm spells, rehardening after cold period: Kalberer *et al.*,
381 2006; Augspurger, 2013; Arora & Taulavuori, 2016), and the variability in frost hardiness is
382 observed at the intra-individual scale (*e.g.* buds *vs* branches; Charrier *et al.*, 2013b).

383 Even though Thermal model more accurately predicted frost hardiness in the calibration
384 dataset (RMSE = 1.79, and 2.07°C, in the Thermal and Photothermal model, respectively), its
385 predictive value was lower (RMSEP = 3.24, and 2.55°C, in the Thermal and Photothermal
386 model, respectively). Furthermore, it does not properly simulate frost dehardening and
387 rehardening (Fig. S1; Kalberer *et al.*, 2006; Arora & Taulavuori, 2016). Due to its higher
388 number of parameters, Photothermal model more accurately predicts changes in frost
389 hardiness. Photoperiodic control has been presented as an adaptative strategy to prevent frost
390 damages and budburst to occur during atypically warm periods (Basler & Körner, 2012).
391 While the influence of photoperiod has been found relevant to predict phenological cycle in
392 some species, including *Juglans regia* (Laube *et al.*, 2014), the additive effect of photoperiod
393 and temperature on frost hardening has not been observed during controlled experiments
394 (Zhang *et al.*, 2003). However, frost hardening has been observed under short day lengths in
395 evergreen species (Schwartz, 1970) and, even in *Juglans regia* (Charrier & Améglio, 2011).
396 Although recent studies tend to include photoperiodic control in phenological stages (Caffarra
397 & Donnelly, 2011; Caffarra *et al.*, 2011), the exact mechanism involved for frost hardening is
398 unclear, even though it might involve photoreceptors in buds (Zohner & Renner, 2015) or
399 cambium in species performing cortical photosynthesis such as beech trees (Berveiller *et al.*
400 2007). If photosystems or phytochroms are probably involved in this response, the exact
401 signaling pathway still has to be clarified.

402 Parameter estimates of the Photothermal model suggest that both temperature and
403 photoperiod do explain the variability in frost hardiness (Tab. 1). The L-Hyb genotype
404 exhibits lower δ parameter (*i.e.* FH_{Max} mainly under photoperiod control) and, consequently, a
405 shallower slope than other genotypes in the relation between FH_{Max} and absolute minimal
406 temperature of the winter (Fig. 5). Furthermore, branches also exhibit steeper slopes than
407 buds. This would imply that branches would support even lower temperature (down to -35.4, -

408 38.2 and -36.9°C in Fra, E-Hyb and L-Hyb, respectively), whereas buds already reached their
409 theoretical limit (-21.1, -22.4, -22.9°C in Fra, E-Hyb and L-Hyb, respectively). High
410 temperature dependence ($\delta > 0.5$) could be critical under sudden temperature changes when
411 trees are not yet hardened (autumn), or already dehardened (spring). This is especially true for
412 Fra which presents a lower FH_{Max} and τ parameters (*i.e.* more reactive to environmental
413 changes). The slope of the relation between minimum temperature and FH_{Max} thus highlights
414 the plasticity of frost hardiness under changing environment and would imply a trade-off
415 between higher safety at the margin of frost-exposed period (L-Hyb) and higher plasticity to
416 respond to rapid temperature changes (Fra and E-Hyb). Early hybrid (E-Hyb) indeed mainly
417 differed from late hybrids (L-Hyb) during dehardening period in relation with their faster rate
418 of ontogenic development and therefore exhibit higher spring risks (Charrier *et al.*, 2011).
419 However, higher FU_{critR} (*i.e.* ability to reacclimate for a longer period after budburst), as
420 observed in Fra, may compensate too high plasticity and prevent this genotype to endure too
421 much late frost damages, especially under warmer springs.

422 According to the model, maximum frost hardiness was considered to be achieved every
423 winter (Larcher & Mair, 1968; Charrier *et al.*, 2011). This was probably the case during the
424 considered period, but may not always happen with warmer winter temperatures, as observed,
425 for instance, during Spanish warm autumns (Guardia *et al.*, 2013). However, this parameter
426 may be shunt by the photoperiodic control of frost hardening (Schwartz *et al.*, 1970; Charrier
427 & Améglio, 2011). This parameter should be useful to determine potential altitudinal limit
428 (Charrier *et al.*, 2013). In conclusion, parameter estimates for the three genotypes differed
429 from each other (Tab. 1) and differed from that found for *Pinus sylvestris*. However, this
430 highlights the necessity to use specific parameter sets for each particular species and/or
431 genotypes (as discussed in Morin & Chuine, 2014), especially given the potential plasticity

432 affecting thermal prerequisites involved in frost hardiness and phenological cycles (Kramer *et*
433 *al.*, 2017).

434 The Photothermal and Thermal models both accurately predict frost hardiness and
435 sensitivity of the calibration dataset. Potential frost damages were consequently assessed
436 using the Photothermal model, because of its better accuracy (crucial for a predictive
437 purpose), its ability to simulate dehardening and rehardening and the sensitivity of *J. regia* to
438 photoperiod for annual cycle (Laube *et al.*, 2014), even though any causal involvement of
439 photoperiod for frost hardiness has been proved yet.

440 The factors inducing frost damages are manifold, *i.e.* related to environmental conditions
441 (frost exposure), to genotypic differentiation (maximal frost hardiness, sensitivity to
442 photoperiod), and their interaction (acclimation, chilling and forcing accumulation). However,
443 our results highlighted the major role of frost exposure (using minimum temperature as a
444 proxy) on frost damage throughout the whole winter leafless period ($\rho = -0.983$; Fig. 6A),
445 compared to frost vulnerability, using actual frost hardiness ($\rho = -0.515$; Fig. 6B) or
446 phenological stage ($\rho = 0.336$; Fig. 6C), as proxies. These latter correlations indeed indicate
447 that more damage are predicted when the plant is fully hardened, at the end of endodormancy,
448 or beginning of ecodormancy. According to the hypothesis (ii), maximal damages are
449 predicted during winter months, for colder location (Fig. 3), when minimal temperature could
450 overtake bud frost hardiness (Fig. 4 - 5). Potential damages generated by the first and last
451 freezing events are much lower and also depend on frost exposure ($\rho = -0.939$; Fig. 6D),
452 whereas not to vulnerability (frost hardiness: $\rho = -0.243$, phenological stage completion $\rho = -$
453 0.268 ; Fig. 6E-F). Finally, frost damages are more likely to happen during autumn and winter
454 season in colder orchards, whereas in spring in warmer orchards, especially in early
455 genotypes. Among the parameters of this model, the difference in ∂ and τ revealed the

456 plasticity of frost vulnerability, which could be critical after warm spells. However, FU_{critR}
457 may mitigate frost risks in early genotypes.

458 Surprisingly, even though predicted frost damages generated by first freezing events were
459 lower than in other periods, it was significantly correlated to next growing season's fruit
460 production (Fig. 7). Whereas, many studies focused either on winter or spring frost
461 hardiness's (e.g. Rodrigo, 2000; Mc Kenney *et al.*, 2014), only a few of them characterized
462 autumnal damages which can be of similar intensities and frequencies (Charrier & Améglio,
463 2011; Guardia *et al.*, 2013). Under extended warm conditions during autumn, frost damages
464 may be critical (Guardia *et al.*, 2013; 2016). Frost hardening, which is tightly connected to
465 chilling exposure, may be subsequently delayed until the moment of first freezing event
466 (Kalberer *et al.*, 2006; Arora & Taulavuori, 2016). However, the correlation between frost
467 damage and fruit yield were relatively weak because of the numerous abiotic and biotic
468 factors influencing fruit production, especially during the growing period (Fig.7). In mountain
469 plants, reproductive buds are more vulnerable than vegetative buds (Ladinig *et al.*, 2013), and
470 leave primordia than xylem parenchyma or cambium (Andergassen & Bauer, 2002). The
471 exact temporal pattern of frost hardiness in flower buds would deserve further studies during
472 autumn period using methods that could quantify frost damages in different cellular types
473 (e.g. Infra-Red differential analysis; Hacker & Neuner, 2007; Charrier *et al.*, 2015b; in press).
474 At the organ scale, meristem temperature may also diverge from air temperature of several
475 degrees (Savvides *et al.*, 2013). Although damages on vegetative bud may be compensated by
476 the development of vegetative latent buds, flower buds are generated the previous growing
477 season. Resiliency of the vegetative system would therefore insure survival of the tree, if
478 these events do not occur every year. Frost damages on buds still accounts for major losses
479 for crop production, more than any other abiotic stress (Rieger, 1989; Snyder *et al.*, 2005),
480 also during autumn period (Fig. 7).

481 Under future climate, many disturbances are expected to affect the biology of trees during
482 winter, even though such a trend has not been observed in the conditions of the study. Global
483 warming may displace the risk at a given location (Mc Kenney *et al.*, 2014), through both
484 exposure and vulnerability modulations. In relation with minimal temperature, frost damages
485 should decrease if the number and intensity of freezing events decrease. Winter damages
486 might remain similar, whereas autumn or spring period could become frost-free for a longer
487 extent. However, many uncertainties remain about the frequency and intensity of future
488 freezing events when microclimatic variability is not taken into account into the projections
489 *i.e.* actual organ temperature (Charrier *et al.*, 2015a). Autumn period with warmer conditions
490 therefore deserve a particular attention in upcoming studies (Charrier & Améglio, 2011;
491 Guardia *et al.*, 2013; 2016).

492 Frost damage occurs at the crossing of high frost exposure and vulnerability. The modeling
493 approach used here highlighted the major role of frost exposure rather than vulnerability on
494 frost damages along the dormant periods for walnut trees. However, flower buds, which could
495 endure >50% cellular damages during the harshest winters, may be more impacted by the first
496 freezing events regarding fruit production the subsequent growing season.

Reference

- Aitken S.N. & Hannerz M. (2001) Genecology and gene resource management strategies for conifer cold hardiness. In *Conifer cold hardiness* (pp. 23-53). Springer Netherlands.
- Andergassen S. & Bauer H. (2002) Frost hardiness in the juvenile and adult life phase of ivy (*Hedera helix* L.). *Plant Ecology* 161, 207-213.
- Arora R. & Taulavuori K. (2016) Increased risk of freeze damage in woody perennials vis-à-vis climate change: importance of deacclimation and dormancy response. *Frontiers in Environmental Science* 4, 44.
- Attaway J.A. (1997) A history of Florida citrus freezes. Lake Alfred, Florida: Florida Science Source, Inc.
- Augspurger C.K. (2013) Reconstructing patterns of temperature, phenology, and frost damage over 124 years: Spring damage risk is increasing. *Ecology* 94, 41-50.
- Bannister P. & Neuner G. (2001) Frost resistance and the distribution of conifers. In *Conifer cold hardiness* (pp. 3-21). Springer Netherlands.
- Baraer M., Madramootoo C.A. & Mehdi B.B. (2010) Evaluation of winter-freeze damage risk to apple trees in global warming projections. *Transactions of the Asabe* 53, 1387-1397.
- Basler D. & Koerner C. (2012) Photoperiod sensitivity of bud burst in 14 temperate forest tree species. *Agricultural and Forest Meteorology* 165, 73-81.
- Bennie J., Kubin E., Wiltshire A., Huntley B. & Baxter, R. (2010) Predicting spatial and temporal patterns of bud-burst and spring frost risk in north-west Europe: the implications of local adaptation to climate. *Global Change Biology* 16, 1503-1514.
- Berveiller D., Kierzkowski D. & Damesin C. (2007) Interspecific variability of stem photosynthesis among tree species. *Tree Physiology* 27, 53-61.
- Caffarra A. & Donnelly A. (2011) The ecological significance of phenology in four different tree species: effects of light and temperature on bud burst. *International Journal of Biometeorology* 55, 711-721.
- Caffarra A., Donnelly A. & Chuine I. (2011) Modelling the timing of *Betula pubescens* budburst. II. Integrating complex effects of photoperiod into process-based models. *Climate Research* 46, 159-170.
- Cannell M.G.R. & Smith R.I. (1986) Climatic warming, spring budburst, and frost damage on trees. *Journal of Applied Ecology* 23, 177-191.
- 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, 351-357.
- Charrier G., Bonhomme M., Lacoite 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, 763-774.

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

Charrier G., Poirier M., Bonhomme M., Lacoïnte A. & Améglio T. (2013b) Frost acclimation in different organs of walnut trees *Juglans regia* L.: How to link physiology and modelling? *Tree Physiology* 33, 1229–1241.

Charrier G., Ngao J., Saudreau M. & Améglio T. (2015a) Effects of environmental factors and management practices on microclimate, winter physiology, and frost resistance in trees. *Frontiers in Plant Science* 6, 259.

Charrier G., Pramsohler M., Charra-Vaskou K., Saudreau M., Améglio T., Neuner G. & Mayr S. (2015b). Ultrasonic emissions during ice nucleation and propagation in plant xylem. *New Phytologist*, 207, 570–578.

Charrier G., Nolf M., Leitinger G., Charra-Vaskou K., Tappeiner U., Améglio T., Mayr S. in press Freezing in timberline trees: a simple phase shift causes complexity. *Plant Physiology*.

Chuine I., Bonhomme M., Legave J.-M., García de Cortázar-Atauri I., Charrier G., Lacoïnte A. & Améglio T. (2016) Can phenological models predict tree phenology accurately in the future? The unrevealed hurdle of endodormancy break. *Global Change Biology* 22, 3444–3460.

Cooter E. & LeDuc S. (1995) Recent frost date trends in the northeastern United States. *International Journal of Climatology* 15, 65–75.

Dai J., Wang H. & Ge Q. (2013) The decreasing spring frost risks during the flowering period for woody plants in temperate area of eastern China over past 50 years. *Journal of Geographical Sciences* 23, 641–652.

de Reaumur R. (1735) Observation du thermomètre, faites à Paris pendant l'année 1735, comparées avec celles qui ont été faites sous la ligne, à l'Isle de France, à Alger et en quelques-unes de nos isles de l'Amérique. Paris : Mémoires de l'Académie des Sciences, 1735.

Delpierre N., Dufrêne E., Soudani K., Ulrich E., Cecchini S., Boe J. & Francois C. (2009) Modelling interannual and spatial variability of leaf senescence for three deciduous tree species in France. *Agricultural and Forest Meteorology* 149, 938–948.

Dreyer E. (1984) Comportement d'une plante pérenne soumise à des contraintes hydriques: réponses physiologiques de jeunes Noyers à des périodes de sécheresse (Doctoral dissertation, University Clermont-Ferrand).

Druart N., Johansson A., Baba K., Schrader J., Sjödin A., Bhalerao R.R., Resman L., Trygg J., Moritz T., Bhalerao R.P. (2007) Environmental and hormonal regulation of the activity-dormancy cycle in the cambial meristem involves stage-specific modulation of transcriptional and metabolic networks. *Plant Journal* 50, 557–573.

Easterling D.R. (2002) Recent changes in frost days and the frost-free season in the United States. *Bulletin of the American Meteorological Society* 83, 1327–1332.

Ebel R.C., Campbell B.L., Nesbitt M.L., Dozer W.A., Linsey J.K., Wilkins B.S. (2005) A temperature index model to estimate long-term freeze-risk of Satsuma mandarins grown on the northern coast of the Gulf of Mexico. *Journal of the American Society for Horticultural Science* 130, 500–507

Eccel E., Rea R., Caffarra A. & Crisci A. (2009) Risk of spring frost to apple production under future climate scenarios: the role of phenological acclimation. *International Journal of Biometeorology* 53, 273-286.

Ferguson J.C., Tarara J.M., Mills L.J., Groove G.G., Keller M. (2011) Dynamic thermal time model of cold hardiness for dormant grapevine buds. *Annals of Botany* 107, 389-396.

Fuchigami L.H., Weiser C.J., Kobayashi K., Timmis R. & Gusta L.V. (1982) A degree growth stage (degree GS) model and cold acclimation in temperate woody plants. In *Plant cold hardiness and freezing stress. Mechanisms and crop implications*. Vol. 2 Ed. S.A. Li P.H. Academic Press, New York, pp. 93-116.

Greer D.H. & Warrington I.J. (1982) Effect of photoperiod, night temperature, and frost incidence on development of frost hardiness in *Pinus radiata*. *Australian Journal of Plant Physiology* 9, 333-342.

Gu L., Hanson P.J., Mac Post W., Kaiser D.P., Yang B., Nemani R., Pallardy S.G. & Meyers T. (2008) The 2007 eastern US spring freezes: Increased cold damage in a warming world? *Bioscience* 58, 253-262.

Guàrdia M., Diaz R., Savé R., & Aletà N. (2013) Autumn frost resistance on several walnut species: methods comparison and impact of leaf fall. *Forest Science* 59, 559-565.

Guàrdia M., Charrier G., Vilanova A., Savé R., Améglio T., & Aletà N. (2016) Genetics of frost hardiness in *Juglans regia* L. and relationship with growth and phenology. *Tree Genetics & Genomes*, 12, 83.

Hacker J. & Neuner G. (2007) Ice propagation in plants visualized at the tissue level by infrared differential thermal analysis (IDTA). *Tree Physiology*, 27, 1661.

Hanninen H. (1991) Does climatic warming increase the risk of frost damage in northern trees? *Plant, Cell and Environment* 14, 449-454.

Hanninen H. & Kramer K. (2007) A framework for modelling the annual cycle of trees in boreal and temperate regions. *Silva Fennica* 41, 167.

Hänninen H. (2016) *Boreal and temperate trees in a changing climate: modelling the ecophysiology of seasonality*. Springer Science+Business Media, Dordrecht. 342 p. ISBN 978-94-017-7549-6.

Inouye D. (2008) Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers. *Ecology* 89, 353-362.

IPCC, 2014: Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp.

Jonsson A.M., Linderson L.J., Stjernquist I., Schlyter P., Barring L. (2004) Climate change and the effect of temperature backlashes causing frost damage in *Picea abies*. *Global and Planetary Change* 44, 195–207.

Kalberer S.R., Wisniewski M. & Arora R. (2006) Deacclimation and reacclimation of cold-hardy plants: Current understanding and emerging concepts. *Plant Science* 171, 3-16.

Kellomäki S., Väisänen H., Hänninen H., Kolström T., Lauhanen R., Mattila U. & Pajari B. (1992) A simulation model for the succession of the boreal forest ecosystem. *Silva Fennica*, 26, 1–18.

Kellomaki S., Hanninen H. & Kolstrom M. (1995) Computations on frost damage to Scots pine under climatic warming in boreal conditions. *Ecological Applications*, 5, 42-52.

Kramer K., Ducouso A., Gömöry D., Hansen J. K., Ionita L., Liesebach M., ... & von Wühlisch G. (2017) Chilling and forcing requirements for foliage bud burst of European beech (*Fagus sylvatica* L.) differ between provenances and are phenotypically plastic. *Agricultural and Forest Meteorology*, 234, 172-181.

Ladinig U., Hacker J., Neuner G. & Wagner J. (2013) How endangered is sexual reproduction of high-mountain plants by summer frosts? Frost resistance, frequency of frost events and risk assessment. *Oecologia*, 171, 743-760.

Lang G.A., Early J.D., Martin G.C. & Darnell R.L. (1987) Endo-, para-, and ecodormancy: physiological terminology and classification for dormancy research. *HortScience*, 22, 371-377.

Larcher W. & Mair B. (1968) Das kälteresistenzverhalten von *Quercus pubescens*, *Ostrya carpinifolia* und *Fraxinus ornus* auf drei thermisch unterschiedlichen standorten. *Oecol Planta* 3, 255–270.

Laube J., Sparks T.H., Estrella N., Höfler J., Ankerst D.P. & Menzel A. (2014) Chilling outweighs photoperiod in preventing precocious spring development. *Global Change Biology*, 20, 170-182.

Leinonen I. (1996) A simulation model for the annual frost hardiness and freezing damage of Scots pine. *Annals of Botany* 78, 687-693.

Leinonen I., Repo T., Hanninen H. (1995) A 2nd-order dynamic model for the frost hardiness of trees. *Annals of Botany* 76, 89-95.

Leinonen I., Repo T., Hanninen H. (1996) Testing of frost hardiness models for *Pinus sylvestris* in natural conditions and in elevated temperature. *Silva Fennica* 30, 159-168.

Lindkvist L., Gustavsson T. & Bogren J. (2000) A frost assessment method for mountainous areas. *Agricultural and Forest Meteorology* 102, 51-67.

Linkosalo T., Carter T.R., Hakkinen R. & Hari P. (2000) Predicting spring phenology and frost damage risk of *Betula spp.* under climatic warming: a comparison of two models. *Tree Physiology* 20, 1175-1182.

Linkosalo T., Hakkinen R. & Hanninen H. (2006) Models of the spring phenology of boreal and temperate trees: is there something missing? *Tree Physiology* 26, 1165-1172.

Marino G.P., Kaiser D.P., Gu L. & Ricciuto D.M. (2011) Reconstruction of false spring occurrences over the southeastern United States, 1901–2007: an increasing risk of spring freeze damage? *Environmental Research Letters* 6, 024015.

Mc Kenney D.W., Pedlar J.H., Lawrence K., Papadopol P., Campbell K. & Hutchinson M.F. (2014) Change and evolution in the plant hardiness zones of Canada. *Bioscience* 64, 341-350.

Morin X. & Chuine I. (2014) Will tree species experience increased frost damage due to climate change because of changes in leaf phenology? *Canadian Journal of Forest Research* 44, 1555-1565.

Murray M.B., Cannell M.G.R. & Smith R.I. (1989) Date of budburst of fifteen species in Britain following climate warming. *Journal of Applied Ecology* 26, 693-700.

R Development Core Team (2015) R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, <https://www.R-project.org>

Repo T. & Lappi J. (1989) Estimation of standard error of impedance-estimated frost resistance. *Scandinavian Journal of Forest Research* 4, 67-74.

Repo T., Makela A. & Hanninen H. (1990) Modelling frost resistance of trees. Modelling to understand forest functions. *Silva Carelica* 15, 61-74.

Repo T., Hanninen H., Kellomaki S. (1996) The effects of long-term elevation of air temperature and CO₂ on the frost hardiness of Scots pine. *Plant, Cell and Environment* 19, 209-216.

Repo T. (1991) Rehardening potential of Scots pine seedlings during dehardening. *Silva Fennica* 25, 13-22.

Richardson E.A., Seeley S.D. & Walker D.R. (1974) A model for estimating the completion of rest for Redhaven and Elberta peach trees. *Hortscience* 9, 331–332.

Rieger M. (1989) Freeze protection for horticultural crops. *Horticultural Reviews*, 11, 45-109.

Rigby J.R. & Porporato A. (2008) Spring frost risk in a changing climate. *Geophysical Research Letters* 35, L12703.

Robeson S.M. (2002) Increasing growing-season length in Illinois during the 20th century. *Climate Change* 52, 219–238.

Rodier D. (2012) Exploitations arboricoles et vergers: Les noyers sont désormais le deuxième verger français. *Agriste Primeur*, 277.

Rodrigo J. (2000) Spring frosts in deciduous fruit trees—morphological damage and flower hardiness. *Scientia Horticulturae* 85, 155-173.

Savvides A., Van Ieperen W., Dieleman J.A. & Marcelis L.F.M. (2013) Meristem temperature substantially deviates from air temperature even in moderate environments: is the magnitude of this deviation species-specific? *Plant, Cell and Environment* 36, 1950-1960.

Scheifinger H., Menzel A., Koch E. & Peter C. (2003) Trends of spring time frost events and phenological dates in Central Europe. *Theoretical and Applied Climatology* 74, 41-51.

Schwarz W.V. (1970) Der einfluss der photoperiode auf das austreiben, die frosthärte und die hitzeresistenz von zirben und alpenrozen. *Flora* 159, 258–285.

Schwartz M.D., Ahas R. & Aasa A. (2006) Onset of spring starting earlier across the Northern Hemisphere. *Global Change Biology* 12, 343–351.

Snyder R.L., Melo-Abreu J.P. de & Matulich S. (2005) Frost protection: fundamentals, practice and economics. Volume 2. Environment and Natural Resources Series - Food and Agriculture Organization of the United Nations Issue 10, 64 pp.

Sutinen M.L., Palta J.P., Reich P.B. (1992) Seasonal differences in freezing stress resistance of needles of *Pinus nigra* and *Pinus resinosa*: evaluation of the electrolyte leakage method. *Tree Physiology* 11, 241–254.

Valkonen M.L., Hanninen H., Pelkonen P. & Repo T. (1990) Frost hardiness of Scots pine seedlings during dormancy. *Silva Fennica* 24, 335-340.

Welling A., Palva E.T. (2006) Molecular control of cold acclimation in trees. *Physiologia Plantarum* 127, 167–181.

Williams S.E., Shoo L.P., Isaac J.L., Hoffmann A.A. & Langham G. (2008) Towards an integrated framework for assessing the vulnerability of species to climate change. *PLoS Biology*, 6, e325.

Zhang M.I.N., Willison J.H.M. (1986) Ultrastructure of the cell-wall of bromegrass (*Bromus Inermis Leyss*) cell-suspension culture. *Plant Cell Reports* 5, 448-451.

Zhang G., Ryyppo A., Vapaavuori E., & Repo T. (2003) Quantification of additive response and stationarity of frost hardiness by photoperiod and temperature in Scots pine. *Canadian Journal of Forest Research*, 33, 1772–1784.

Zohner C.M. & Renner, S.S. (2015) Perception of photoperiod in individual buds of mature trees regulates leaf-out. *New Phytologist*, 208, 1023-1030.

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Table 1. Parameters of the different sub-models of the study. Parameters driving the annual cycle sub-model were taken from Chuine *et al.* (2016), Charrier *et al.* (2011) and Dreyer (1984), for endodormancy, ecodormancy and growth stages respectively. The parameters of the frost damage sub-model were fixed according to our own observations, as well as FH_{MinBch} , FH_{MinBud} , and FH_{MaxBch} (frost hardiness sub-model). Other parameters driving the frost hardiness sub-models were fitted.

Sub-model	Description	Value			Source
Annual cycle					
Endodormancy release					
T_0 (DOY)	Beginning of endodormancy	244			Chuine <i>et al.</i> (2016)
T_{low} (°C)	Threshold temperature below which CU is maximum	0.1			Chuine <i>et al.</i> (2016)
T_{high} (°C)	Threshold temperature above which CU is null	25.0			Chuine <i>et al.</i> (2016)
CU_{crit} (CU)	Amount of Chilling Units to complete endodormancy stage	2170.0			Chuine <i>et al.</i> (2016)
Ecodormancy release					
		Fra	E-Hyb	L-Hyb	
Slp	Slope at the inflexion point T_{50}	0.244	0.134	0.156	Charrier <i>et al.</i> (2011)
T_{50} (°C)	Temperature inducing half of the maximal apparent growth rate	13.5	17.9	18.2	Charrier <i>et al.</i> (2011)
FU_{crit} (FU)	Amount of Forcing Units to complete the ecodormancy stage	21.2	20.5	23.1	Charrier <i>et al.</i> (2011)
Growth					
T_{thG}	Threshold temperature for growth	5			Dreyer (1984)
GDD_{crit} (d°C)	Amount of Growth Degree Days to complete the stage	1200			Dreyer (1984)
Frost Hardiness					
		Fra	E-Hyb	L-Hyb	
FH_{MinBch} (°C)	Minimum level of frost hardiness in branches		-7.7		Fixed
FH_{MinBud} (°C)	Minimum level of frost hardiness in buds		-4.0		Fixed
FH_{MaxBch} (°C)	Maximum level of frost hardiness in branches	-32.2	-37.1	-37.1	Fixed
Photothermal					
T_1 (°C)	Upper limit of the efficient temperature range	12.0	17.8	25.9	Fitted
T_2 (°C)	Lower limit of the efficient temperature range	-6.9	-6.0	-9.3	Fitted
NL_1 (h)	Lower limit of the efficient nyctiperiod range	12	12	11	Fitted
NL_2 (h)	Upper limit of the efficient nyctiperiod range	15	14	15	Fitted
δ	Part of FH_{Max} under temperature control	0.517	0.578	0.293	Fitted
FH_{TMax} (°C)	Maximum level of the increase of FH induced by temperature	-12.7	-17.0	-8.6	Deducted from δ
FH_{PMax} (°C)	Maximum level of the increase of FH induced by photoperiod	-11.8	-12.4	-20.8	Deducted from δ
τ (days)	Time constant	5	16	15	Fitted
FU_{critR} (FU)	Amount of forcing units for hardening competence	31.8	23.4	24.5	Fitted

Thermal**Endodormancy**

$T_{th\ endo}$ (°C)	Threshold temperature during endodormancy stage	17.7	14.1	19.2	Fitted
$k_{a\ endo}$ (°C)	Acclimation rate constant during endodormancy stage	3.45E-2	8.29E-2	5.33E-2	Fitted
$k_{d\ endo}$ (°C)	Deacclimation rate constant during endodormancy stage	1E-8	1E-8	1.61E-5	Fitted

Ecodormancy

$T_{th\ eco}$ (°C)	Threshold temperature during ecodormancy stage	-3.4	-3.5	-36.0	Fitted
$k_{a\ eco}$ (°C)	Acclimation rate constant during ecodormancy stage	2.87E-3	3.72E-3	6.49E-3	Fitted
$k_{d\ eco}$ (°C)	Deacclimation rate constant during ecodormancy stage	2.89E-2	3.54E-2	2.02E-3	Fitted

Frost Damage

α (°C ⁻¹)		0.106			Fixed
β (°C ⁻¹)		-0.135			Fixed
γ (°C)		-13.57			Fixed

Figure caption:

Figure 1. A) Frost hardiness measured in bud depending on frost hardiness in its bearing branch along the leafless period. B) Frost sensitivity (% damage. $^{\circ}\text{C}^{-1}$) depending on frost hardiness ($^{\circ}\text{C}$) in buds and branches from different genotypes of walnut trees: *J. regia* cv Franquette (Fra) and hybrids *J. regia* x *nigra* (E-Hyb: early and L-Hyb: late). Each point represents the mean of 5 replicates sampled in the same orchard at the same date. Dotted line was fitted according to all points, R^2 and p-values of the fits are indicated.

Figure 2. A) Simulated versus observed frost hardiness ($^{\circ}\text{C}$) in branches from different walnut genotypes: *J. regia* cv Franquette (Fra), early (E-Hyb) and late hybrids *J. regia* x *nigra* (L-Hyb) in the calibration (A) and validation (B) datasets. Symbols and bars represent mean and standard errors from 5 replicates, respectively. Root Mean Standard Errors are indicated for each genotype and dataset.

Figure 3. Distribution of maximal frost damages per season (A: autumn, W: winter and S: spring) in buds (upper panel) or branches (lower panel); in low (left side) or high elevation orchards (right side) for three genotypes of walnut trees (*J. regia* cv Franquette (Fra), early (E-Hyb) and late hybrids *J. regia* x *nigra* (L-Hyb)) during the 1970-2015 period. Thick bar represents the median, extremities of the box 1st and third quartile, extremities of the dotted lines 1st and 9th decile, and open circles outliers. For each organ, same letters indicate non-significantly different distribution according to Kruskal-Wallis test ($P > 0.05$).

Figure 4. Safety margin (*i.e.* difference between bud frost hardiness and minimum temperature) for buds: median (green line), 10th (red line) and 90th centile (blue line) and branches: median only (brown line) and probability of freezing event (black line), along the year in buds in *J. regia* cv. Franquette (A-B), early (C-D) and late hybrids (E-F), from low (left) and high elevation orchards (right). Gray areas indicate when safety margin for buds falls below 10 $^{\circ}\text{C}$ with a probability higher than 10% (SM_{10}) when probability of freezing exposure is not null (*i.e.* frost risk).

Figure 5. Maximum frost hardiness predicted per year depending on the absolute minimum temperature observed during the same period for buds and branches in low and high elevation orchard for different walnut genotypes: *J. regia* cv Franquette (A), *J. regia* x *nigra* Early (B) and Late (C). Slopes did not differ across location ($P = 0.139$), but across organs and genotypes ($P < 0.001$): Branches: 0.287, 0.252, 0.108 $^{\circ}\text{C} \cdot ^{\circ}\text{C}^{-1}$ in Fra, E-Hyb and L-Hyb,

respectively; Buds: 0.142, 0.113, 0.045°C.°C⁻¹ in Fra, E-Hyb and L-Hyb, respectively. R² of the linear regressions are indicated.

Figure 6. Potential damages on buds in *J. regia* cv Franquette generated by all the freezing events during the 1970-2015 period (A-C) or by the first and last freezing events (D-F) in low and high elevation orchards depending on (A, D) the temperature of the freezing event, (B, E) the actual frost hardiness, or (C, F) the ratio of phenological stage completion in arbitrary units (A.U.) from the theoretically most vulnerable (0) to the most resistant state (1), calculated as $\frac{CU(t)}{CU_{crit}}$ during endodormancy and $1 - \frac{FU(t)}{FU_{crit}}$ during ecodormancy. R² (A, C) and ρ (Spearman's rank correlation coefficient) and p-values according to Spearman are indicated.

Figure 7. Fruit production per tree observed in an independent orchard depending on maximal damages predicted during the previous autumn (white, short dashed line), winter (gray; dotted line) or spring (black; long dashed line). R² of the linear regressions are indicated.

Figure S1. Simulated frost hardiness according to Photothermal (Black) and Thermal (Gray) models during six different winter dynamics. Within the gray area is represented a dynamic in high elevation orchard, whereas the other dynamics were from low elevation orchard. Symbols represent observed frost hardiness (mean \pm SE; n =5) in branches from different walnut genotypes: *J. regia* cv Franquette (Fra), early (E-Hyb) and late hybrids *J. regia* x *nigra* (L-Hyb) in the calibration (filled symbols) and validation (open symbols) datasets.













