



**HAL**  
open science

## Altered stomatal dynamics of two Euramerican poplar genotypes submitted to successive ozone exposure and water deficit

Nicolas Dusart, Marie-Noëlle Vaultier, Jean-Charles Olry, Cyril Buré, Joëlle Gérard, Yves Jolivet, Didier Le Thiec

### ► To cite this version:

Nicolas Dusart, Marie-Noëlle Vaultier, Jean-Charles Olry, Cyril Buré, Joëlle Gérard, et al.. Altered stomatal dynamics of two Euramerican poplar genotypes submitted to successive ozone exposure and water deficit. *Environmental Pollution*, 2019, 252, pp.1687-1697. 10.1016/j.envpol.2019.06.110 . hal-02265924

**HAL Id: hal-02265924**

**<https://hal.science/hal-02265924v1>**

Submitted on 25 Oct 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1 Altered stomatal dynamics of two Euramerican poplar genotypes  
2 submitted to successive ozone exposure and water deficit

3

4 Nicolas Dusart, Marie-Noëlle Vaultier, Jean-Charles Olry, Cyril Buré, Joëlle Gérard, Yves  
5 Jolivet, Didier Le Thiec\*

6 Université de Lorraine, AgroParisTech, Inra, Silva, F-54000 Nancy, France

7

8 \*Corresponding author: E-mail: [didier.lethiec@inra.fr](mailto:didier.lethiec@inra.fr)

9 Tel: +33 (0)3 83 39 40 98

10 **ABSTRACT**

11 The impact of ozone (O<sub>3</sub>) pollution events on the plant drought response needs special  
12 attention because spring O<sub>3</sub> episodes are often followed by summer drought. By causing  
13 stomatal sluggishness, O<sub>3</sub> could affect the stomatal dynamic during a subsequent drought  
14 event. In this context, we studied the impact of O<sub>3</sub> exposure and water deficit (in the presence  
15 or in the absence of O<sub>3</sub> episode) on the stomatal closure/opening mechanisms relative to  
16 irradiance or vapour pressure deficit (VPD) variation. Two genotypes of *Populus nigra* x  
17 *deltoides* were exposed to various treatments for 21 days. Saplings were exposed to 80  
18 ppb/day O<sub>3</sub> for 13 days, and then to moderate drought for 7 days. The curves of the stomatal  
19 response to irradiance and VPD changes were determined after 13 days of O<sub>3</sub> exposure, and  
20 after 21 days in the case of subsequent water deficit, and then fitted using a sigmoidal model.  
21 The main responses under O<sub>3</sub> exposure were stomatal closure and sluggishness, but the two  
22 genotypes showed contrasting responses. During stomatal closure induced by a change in  
23 irradiance, closure was slower for both genotypes. Nonetheless, the genotypes differed in  
24 stomatal opening under light. Carpaccio stomata opened more slowly than control stomata,  
25 whereas Robusta stomata tended to open faster. These effects could be of particular interest,  
26 as stomatal impairment was still present after O<sub>3</sub> exposure and could result from imperfect  
27 recovery. Under water deficit alone, we observed slower stomatal closure in response to VPD  
28 and irradiance, but faster stomatal opening in response to irradiance, more marked in  
29 Carpaccio. Under the combined treatment, most of the parameters showed antagonistic  
30 responses. Our results highlight that it is important to take genotype-specific responses and  
31 interactive stress cross-talk into account to improve the prediction of stomatal conductance in  
32 response to various environmental modifications.

33 Capsule : This study indicates that the possible multiple stomatal responses to environmental  
34 stimuli must be taken into account in order to better define the parameters of the stomatal  
35 conductance model.

36 Keywords: ozone, drought, stomata, VPD, light

## 37 1 Introduction

38 Forest health depends at least on our capacities to improve risk assessment. This  
39 evaluation depends on our understanding of tree biological and physiological responses to  
40 multiple environmental stressors (Sicard et al., 2016). Among abiotic stress factors,  
41 tropospheric ozone (O<sub>3</sub>) and drought are detrimental for tree growth and health (Allen et al.,  
42 2010; Wittig et al., 2007). O<sub>3</sub> is a phytotoxic air pollutant that impairs gas exchanges and  
43 reduces plant biomass (Dizengremel et al., 2013; Jolivet et al., 2016; Wittig et al., 2007). It is  
44 a secondary pollutant resulting from a photochemical process in the troposphere, with an  
45 annual cycle: higher daily O<sub>3</sub> concentrations are reported in spring and summer, lower ones in  
46 autumn and winter. Recurrent spring maxima have been reported in the northern hemisphere  
47 (Kalabokas et al., 2017; Monks, 2000; Parrish et al., 2013). On the other hand, drought events  
48 are predicted to increase in the near future. Due to global warming, wet regions are becoming  
49 wetter and dry regions drier (Liu and Allan, 2013). A reduction of primary growth was  
50 observed following the 2003 drought and heat wave (Ciais et al., 2005). Stand mortality was  
51 reported all over the world (Allen et al., 2010). Models integrating temperature, vapour  
52 pressure deficit (VPD) and rainfall predict an increased probability of similar events in the  
53 near future (IPCC, 2014; Lehner et al., 2006; Park Williams et al., 2013). Against this  
54 environmental fluctuation, the main physiological responses of trees and more generally  
55 plants are the control of transpiration by stomata. In the case of O<sub>3</sub>, stomatal closure limits O<sub>3</sub>  
56 entrance. Under drought, stomatal closure prevents water loss. Nevertheless, there is a cost for  
57 carbon assimilation under both constraints. Stomata exert a major control on both the water  
58 and carbon cycles round the world (Hetherington and Woodward, 2003).

59 Meta-analyses of published data indicate that ambient O<sub>3</sub> reduces tree biomass  
60 production (Li et al., 2017; Wittig et al., 2009), while an analysis of survey data showed how  
61 O<sub>3</sub> reduces tree growth (Braun et al., 2014). Different metric indicators have been created to  
62 assess critical levels of O<sub>3</sub> for plants. The simplest ones were only based on cumulative  
63 exposure levels. The USA selected the SUM-index (sum of all hourly average concentrations  
64 over *X* ppb), whereas the EU selected the AOT40 (Accumulated Ozone over a Threshold of  
65 40 ppb) which takes into account hourly O<sub>3</sub> concentrations above 40 ppb *per* hour when  
66 irradiance is 50 W.m<sup>-2</sup> minimum (Fuhrer et al., 1997; Musselman et al., 2006). The advantage  
67 of these metrics is that they make it simple to determine exposure levels only based on O<sub>3</sub>  
68 concentration data. In the early 2000's, the biologically more relevant concept of flux-based  
69 approach emerged, resulting in the scientific adoption of the Phytotoxic Ozone Dose over a

70 threshold of  $Y \text{ nmol.m}^{-2}.\text{s}^{-1}$  (PODy) (Emberson et al., 2007; Hayes and Bangor, 2017;  
71 Karlsson et al., 2000; Mills et al., 2011). This metric is available for risk assessment and takes  
72  $\text{O}_3$  uptake by the leaves through stomata into account. The  $\text{DO}_3\text{SE}$  model (Deposition of  $\text{O}_3$   
73 for Stomatal Exchange) was developed to account for the variation in stomatal opening and  
74 closure with climate, soil, and plant factors (Büker et al., 2012; Emberson et al., 2007); it is  
75 based on the empirical Jarvis-type stomatal conductance model (Jarvis, 1976). This model  
76 was developed using steady-state parameters, and the stomatal dynamic modification  
77 specifically induced by  $\text{O}_3$  was ignored (Hoshika et al., 2013). Tropospheric  $\text{O}_3$  is known to  
78 slow down the stomatal responses to environmental factors, named stomatal sluggishness.  
79 Stomatal sluggishness has been reported in response to changes in light (Dumont et al., 2013;  
80 Paoletti and Grulke, 2010), VPD (Dumont et al., 2013; Grulke et al., 2007), and soil water  
81 stress (Durand et al., 2019; Gérardin et al., 2018; Hoshika et al., 2013). Hoshika et al. (2017)  
82 showed that stomatal sluggishness and closure should be taken into account in stomatal  
83 response modelling in a Jarvis-type model.  $\text{O}_3$ -induced stomatal sluggishness potentially  
84 increases transpiration (Hoshika et al., 2015).  $\text{O}_3$ - and drought-induced loss of stomatal  
85 function may enhance both leaf water loss and  $\text{O}_3$  uptake (Hoshika et al., 2013). In natural  
86 conditions, an  $\text{O}_3$  spring episode is very likely before summer drought in Europe. Plants  
87 subjected to  $\text{O}_3$  stress in spring can be particularly sensitive to drought events in summer  
88 (Pollastrini et al., 2014).  $\text{O}_3$  could affect the stomatal dynamic under drought by causing  
89 stomatal sluggishness. The present study aims to decipher the response of stomatal  
90 closure/opening relative to light or vapour pressure deficit variation in  $\text{O}_3$  and in water deficit  
91 stress conditions (with or without previous  $\text{O}_3$  stress). The two *Populus deltoides x nigra*  
92 (Moench.) genotypes (“Carpaccio” and “Robusta”) were documented in our recent  
93 experiments under 120 ppb  $\text{O}_3$  as being impaired in radial growth and gas exchanges, and  
94 exhibiting increased visible leaf injuries and senescence, and changes in detoxification  
95 capacities (Dghim et al., 2013; Dumont et al., 2013, 2014a, 2014b; Dusart et al., 2018). Better  
96 stomatal control has been observed under  $\text{O}_3$  in the Carpaccio genotype as compared to  
97 Robusta (Dumont et al., 2013), while both genotypes showed efficient stomatal closure under  
98 soil water deficit (Durand et al., 2019; Dusart et al., 2019; Ridolfi and Dreyer, 1997). The  
99 present paper addresses the following questions: i) are any of the differences in stomatal  
100 dynamics linked to environmental variables (light, VPD) between the two poplar genotypes?,  
101 ii) does 80 ppb  $\text{O}_3$  or water deficit induce stomatal sluggishness in poplar?, iii) does  $\text{O}_3$   
102 treatment modify water-deficit-induced stomatal closure?, and iv) does stomatal dynamics  
103 recover after 7 days in the absence of  $\text{O}_3$  treatment?

## 104 2 **Materials and methods**

### 105 2.1 *Plant material and exposure conditions*

106 Cuttings of the two Euramerican poplar genotypes “Carpaccio” and “Robusta” were  
107 grown in growth chambers as already described in Dusart et al. (2019) with slight  
108 modifications. Cuttings were planted in ten-liter pots filled with a sand/peat mixture (1/1, v/v)  
109 and fertilised by adding 15 g of slow-release nutritive granules (Nutricot T-100) and 1 g.L<sup>-1</sup>  
110 CaMg(CO<sub>3</sub>)<sub>2</sub>. For both genotypes, forty-eight plants were randomly distributed in eight  
111 phytotronic chambers, i.e. twenty-four plants in control chambers (charcoal-filtered air), and  
112 twenty-four plants in chambers set for O<sub>3</sub> treatment (80 nmol.mol<sup>-1</sup> for 13 hours, from 09:00  
113 to 22:00). For reasons of space in the culture chambers and length of measurement times, the  
114 experiment was duplicated separately for each genotype. After a 7-day-long acclimation  
115 period, the O<sub>3</sub> treatment started while control saplings were exposed to charcoal-filtered air  
116 for 13 days. After 13 days (d) of fumigation, the total cumulative sum of O<sub>3</sub> flux (SUM00),  
117 the cumulative O<sub>3</sub> dose above a threshold of 40 ppb (AOT 40), and the phytotoxic O<sub>3</sub> dose  
118 above a threshold flux of 0 nmol.m<sup>-2</sup>.s<sup>-1</sup> (POD<sub>0</sub>) (based on measured stomatal conductance, see  
119 Bagard et al., 2015) were determined (Table S1). At the end of the O<sub>3</sub> exposure period, half of  
120 the saplings were submitted to a moderate water deficit for 7 d. Soil Water Content (SWC)  
121 was determined with 24 wireless Time Domain Reflectometry (TDR) probes (CWS655E,  
122 Campbell Scientific Ltd, Antony, France). A calibration between volumetric SWC measured  
123 by TDR and pot weight was performed. The biological available water was expressed as  
124 relative extractable water (REW), as described by Wildhagen et al. (2018) for the same soil.  
125 Poplars were watered with a known volume of water several times a day to maintain the level  
126 of REW stable. For the well-watered treatment, poplars were irrigated at 75 % (±10%) of  
127 REW, whereas for the water deficit treatment, irrigation was set to 45% (± 2%) of REW until  
128 the end of the experiment (Fig. S1). A cumulative sum of the amount of water added for each  
129 treatment for 21 d is presented in Table S1.

### 130 2.2 *Plant growth*

131 The number of leaves and the diameter at the collar and height were recorded twice a  
132 week until the end of the experiment for each individual. At the end of the experiment, leaves,  
133 stems and roots were oven-dried at 60 °C until they reached a constant dry mass.

### 134 2.3 *Gas exchanges and photosynthetic pigment kinetics*

135 Gas exchanges ( $A_n$ , net CO<sub>2</sub> assimilation, and  $g_s$ , stomatal conductance to water vapour)  
136 were measured using a Li-6200 (Li Cor, Lincoln, NE, USA) as described in Dusart et al.  
137 (2019). Non-destructive determination of the chlorophyll pigment content was performed  
138 with a Dualex (Force-A, Orsay, France). For all non-destructive leaf measurements, the same  
139 leaf was used, i.e., the first fully expanded leaf (the 10th leaf from the apex) at the beginning  
140 of the O<sub>3</sub> treatment.

## 141 2.4 Stomatal response to irradiance and vapour pressure deficit

### 142 2.4.1 Gas exchange measurements

143 Gas exchange measurements were performed with a Li-6400 system, as described in  
144 Durand et al. (2019) with some minor modifications. Parameters of the leaf cuvette were for  
145 light: PAR: 800  $\mu\text{mol.m}^{-2}.\text{s}^{-1}$  with 30  $\mu\text{mol.m}^{-2}.\text{s}^{-1}$  of blue irradiance and VPD: 0.8 kPa, until  
146  $g_s$  reached a steady state ( $g_0$ , defined as a variation lower than 5% over 5 minutes). Then light  
147 was turned off (as well as in the phytotronic chamber) until  $g_s$  got to a new steady state ( $g_1$ ),  
148 then turned on to 800  $\mu\text{mol.m}^{-2}.\text{s}^{-1}$  until stomatal conductance reached the last steady state  
149 ( $g_2$ ). The 800  $\mu\text{mol.m}^{-2}.\text{s}^{-1}$  value was chosen to avoid photoinhibition due to excess light  
150 (Niinemets and Kull, 2001). A similar procedure was used to monitor  $g_s$  response to a change  
151 of VPD: it was switched to 3 kPa instead of 0.8 kPa (for a fixed PAR: 800  $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ ). VPD  
152 from leaf tissues to air was controlled with a dew point generator as described in Vialet-  
153 Chabrand et al. (2013).

### 154 2.4.2 Modelling

155 The obtained stomatal response curves were fitted using the following sigmoidal  
156 model (Vialet-Chabrand et al., 2013):

$$157 \quad g_s = g_0 + (G - g_0) e^{-e^{\left(\frac{\lambda-t}{\tau}\right)}}$$

158 where  $g_s$  is the fitted stomatal conductance,  $g_0$  and  $G$  are the steady-state values of  $g_s$  ( $\text{mol.m}^{-2}.\text{s}^{-1}$ ),  
159 respectively at the start and at the end of the curve,  $\tau$  is a time constant (s),  $\lambda$  is the lag  
160 time (s), and  $t$  is time (s). The speed of the stomatal response was estimated by calculating the  
161 maximum slope ( $SL_{\text{max}}$ ), as follows:

$$162 \quad SL_{\text{max}} = \frac{G - g_0}{\tau \cdot e}$$

163 where  $(G-g_0)$  represents the amplitude of the stomatal response and  $e$  is Euler's number ( $e \approx$   
164 2.718). Further information regarding the model parameters and fitting procedure can be  
165 found in Gérardin et al. (2018) and Durand et al. (2019).

## 166 2.5 Statistical analyses

167 Statistical analyses were performed using R 3.1.0 (R Development Core Team) open-  
168 source software. Linear models created from the *nlme* package (Pinheiro et al., 2018) were  
169 used to study growth parameters with ANOVA, including the effects of water deficit,  $O_3$  and  
170 genotype. The growth chamber was also tested and excluded from the models because the  
171 effect was not significant for all the parameters tested throughout the whole experimental  
172 period. Model parameters,  $G$ ,  $g_0$ ,  $\lambda$ ,  $\tau$ ,  $SL_{max}$  were explored in the same way. The *lme4*  
173 package (Bates et al., 2015) was used to fit a linear mixed-effect model on gas exchange and  
174 chlorophyll content data with fixed variables (water deficit and  $O_3$  data) whereas biological  
175 replicas were random variables. Residual plots of the model were used to assumed  
176 heteroscedasticity and variance homogeneity. The *emmeans* package (Lenth, 2016) was used  
177 to perform multiple comparisons. To determine if  $O_3$  and water deficit had an additive,  
178 synergistic or antagonistic impact on  $g_s$ , we compared the observed effects to the expected  
179 additive effects for the saplings exposed to  $O_3$  and then to water deficit (Methods 1 available  
180 in Supplementary data).

## 181 3 Results

### 182 3.1 Effect on growth, chlorophyll contents and gas exchange kinetics

183  $O_3$  impacted only the stem biomass of the two genotypes. Water deficit only slightly  
184 impacted the number of leaves of Robusta genotype, nevertheless leaf surface area decreased  
185 in the Carpaccio genotype only (Table S2).

186 An increase in chlorophyll contents was observed in both genotypes under the WW:FA  
187 modality throughout the 21 days of the experiment (Fig. 1A and B), and Robusta  
188 systematically contained 1.5 times more chlorophyll than Carpaccio. Chlorophyll contents  
189 were significantly lower under  $O_3$  treatment in both genotypes; from 11 days, chlorophyll  
190 levels remained lower than the control treatment (Fig. 1A and B). The chlorophyll content of  
191 the water deficit treatment (D:FA) was significantly lower in Robusta as compared to the  
192 WW:FA modality (Fig. 1A and B). D: $O_3$  impacted chlorophyll contents similarly to WW: $O_3$ .

193 Concomitantly, gas exchanges were recorded twice or three times a week (Fig. 1C and  
194 D). The control treatment values decreased throughout the experiment because the leaves  
195 received less light (because the upper leaves were still growing). Although net CO<sub>2</sub>  
196 assimilation (A<sub>n</sub>) decreased over time, A<sub>n</sub> values were 3 μmol CO<sub>2</sub>.m<sup>-2</sup>.s<sup>-1</sup> higher in the  
197 Robusta genotype. ANOVA on A<sub>n</sub> only showed a significant effect of O<sub>3</sub> on both genotypes  
198 (Fig. 1C and D). O<sub>3</sub> decreased A<sub>n</sub>, from 10 d in Carpaccio and 6 d in Robusta. After the O<sub>3</sub>  
199 treatment was switched off on d 13, Robusta photosynthesis reached the same levels as the  
200 control, *i.e.*, 7.5 μmol.m<sup>-2</sup>.s<sup>-1</sup>.

201 Concerning stomatal conductance values, O<sub>3</sub> decreased g<sub>s</sub> from 6 d in Carpaccio and 2 d  
202 in Robusta, with a significant effect only on Carpaccio (Fig. 1E and F). At the end of the 13 d  
203 of O<sub>3</sub> fumigation, g<sub>s</sub> went back to the control level in both genotypes, *i.e.*, 400 mmol.m<sup>-2</sup>.s<sup>-1</sup>  
204 and 300 mmol.m<sup>-2</sup>.s<sup>-1</sup> in Robusta and Carpaccio, respectively. Water deficit decreased g<sub>s</sub>  
205 (around 230 mmol.m<sup>-2</sup>.s<sup>-1</sup>) in both genotypes (Fig. 1E and F). Carpaccio stomata displayed  
206 similar conductance values under the D:FA and D:O<sub>3</sub> conditions. A difference was observed  
207 in Robusta, *i.e.*, stomata under D:O<sub>3</sub> were less closed than under D:FA (330 mmol.m<sup>-2</sup>.s<sup>-1</sup>, Fig.  
208 1F), resulting in a significant interaction between O<sub>3</sub> exposure and water deficit.

### 209 3.2 Effect of O<sub>3</sub> and recovery of stomatal behaviour

#### 210 3.2.1 Responses to irradiance

211 At the end of the O<sub>3</sub> treatment (13 d), net CO<sub>2</sub> assimilation in steady state 0, *i.e.*, A<sub>0</sub>,  
212 was down from 19.6 to 7.9 μmol.m<sup>-2</sup>.s<sup>-1</sup> in Carpaccio, and from 19.2 to 15.05 μmol.m<sup>-2</sup>.s<sup>-1</sup> in  
213 Robusta (Table S3). The steady states of g<sub>s</sub> after stomatal closure (g<sub>1</sub>) were similar for both  
214 genotypes and treatments (around 100 mmol.mm<sup>-2</sup>.s<sup>-1</sup>). After stomatal closure, net CO<sub>2</sub>  
215 assimilation reached negative values (A<sub>1</sub>) due to dark respiration (Table S3). The respiration  
216 rates in FA conditions were higher in Robusta than in Carpaccio (-1.6 vs. -1.3 μmol.m<sup>-2</sup>.s<sup>-1</sup>).  
217 Under WW:O<sub>3</sub> conditions, an increase of respiration was observed, *i.e.*, +30% and +18% in  
218 Carpaccio and Robusta, respectively. Concerning the stomatal closure phase, the τ and λ  
219 parameters were not modified by O<sub>3</sub> treatment in Carpaccio (Fig. 2A and E). In Robusta, τ  
220 tended to increase under O<sub>3</sub> exposure (1.5 fold) (Fig. 2A) and λ was not modified (Fig. 2E).  
221 Moreover, λ values differed between genotypes, with a higher value for Carpaccio (1.2-fold  
222 higher) (Fig. 2E). SL<sub>max</sub> values highlighted a trend for an O<sub>3</sub> effect: the stomatal closure speed  
223 was reduced by 0.5 fold and 0.4 fold in Carpaccio and Robusta, respectively (Fig. 2I). As  
224 regards the stomatal opening phase, λ was 6 min faster in Carpaccio than in Robusta (Fig.  
225 2F). λ was not impacted by O<sub>3</sub> in Carpaccio, whereas it significantly decreased by 25% in

226 Robusta.  $SL_{max}$  values significantly differed between the two genotypes (Table S4). Stomatal  
227 opening was slower in Carpaccio, with an  $SL_{max}$  0.5 fold lower under  $O_3$ , but changes were  
228 not significant in Robusta (Fig. 2J). The steady states of conductance ( $g_2$ ) after the opening  
229 phase (Table S3) returned to the same levels as the first steady states in Carpaccio. In Robusta  
230 under  $O_3$ ,  $g_2$  was 30% lower than  $g_0$  before stomatal closure (Table S3). Similarly,  
231 assimilation went back to the same levels ( $A_0=A_2$ ) in the control leaves (Table S5).

232 After a week without  $O_3$  exposure (21 d), net  $CO_2$  assimilation ( $A_0$ ) went back to the  
233 same level as the control in Carpaccio, whereas a 20% decrease in  $CO_2$  uptake was observed  
234 in Robusta as compared to the WW:FA modality (Table S3). In parallel,  $g_0$  was the same  
235 under the  $O_3$  and control treatments in Carpaccio. As for Robusta,  $g_0$  was higher under  $O_3$ ,  
236 and stomata opened 31% more (Table S3). During stomatal closure,  $\tau$  and  $\lambda$  values in the  
237 WW: $O_3$  treatments changed non significantly as compared to 13 d (Fig. 2C and G). However,  
238 as compared to the WW:FA treatments,  $\tau$  and  $\lambda$  values were 66% and 38% greater in  
239 Carpaccio, respectively, and 60% and 15% greater in Robusta, respectively (Fig. 2C and G).  
240  $SL_{max}$  decreased by 56% in Carpaccio and by 47% in Robusta (Fig. 2K).

241 Regarding stomatal opening,  $g_1$  was not significantly affected by  $O_3$  in either genotype  
242 (Table S3). Final steady states were similar under the WW: $O_3$  and WW:FA treatments, *i.e.*,  
243 around 340 mmol.m<sup>-2</sup>.s<sup>-1</sup>.  $\tau$  and  $\lambda$  non significantly decreased under  $O_3$  treatment in both  
244 genotypes (Fig. 2D and 2H). Stomatal opening tends to be faster in both genotypes under  
245 WW: $O_3$  (Fig. 2L).

### 246 3.2.2 Responses to VPD

247 The study of the vapour pressure deficit response curves consisted in measuring  
248 stomatal closure under pressures ranging between 0.8 and 3 kPa. At the end of the  $O_3$   
249 treatment (13 d), the assimilation ( $A_0$ ) and stomatal conductance ( $g_0$ ) values of the initial  
250 steady states of Carpaccio and Robusta were almost the same as those of the light response  
251 curves (Tables S3 and S4). After stomatal closure,  $CO_2$  assimilation ( $A_1$ ) decreased by 16 to  
252 30% as compared to  $A_0$  depending on genotype or treatment (Table S5). The  $\tau$  parameter in  
253 control conditions differed between genotypes (Fig. 3A), as it took Robusta 19 min more than  
254 Carpaccio.  $O_3$  increased  $\tau$  by 17 min and 11 min in Carpaccio and Robusta, respectively (Fig.  
255 3A). The  $\lambda$  parameter also differed between the two genotypes in the control conditions: it  
256 took Robusta saplings 11 min more to reach the inflection point (Fig. 3C).  $\lambda$  increased under  
257  $O_3$  treatment in both genotypes, *i.e.*, by 12 min and 5 min in Carpaccio and Robusta,  
258 respectively (Fig. 3C).

259 After a week without O<sub>3</sub> treatment, stomatal dynamic responses to VPD still showed a  
260 few differences between the control and the O<sub>3</sub> treatment. In Carpaccio, O<sub>3</sub> increased  $\tau$  and  $\lambda$   
261 by 1.8 and 1.6 fold, respectively (Fig. 3B and D). SL<sub>max</sub> decreased by 70% in Carpaccio (Fig.  
262 3F). Robusta was impacted in a different way, as  $\tau$  slightly increased by 1.1 fold under O<sub>3</sub>  
263 treatment, but  $\lambda$  was unaffected (Fig. 3B and D).

### 264 3.3 Effect of water deficit on stomatal behaviour

#### 265 3.3.1 Response to irradiance

266 After 7 days of water deficit treatment, a significant water deficit effect was observed  
267 for some parameters of the model. Regarding stomatal closure under light, the initial steady  
268 state showed stomatal closure under water deficit conditions (Table S3). Water deficit  
269 induced a 45% decrease of  $g_0$  in both genotypes (Table S3). After closure,  $g_1$  showed some  
270 marked differences with the control treatment, *i.e.*, an 84% decrease in Carpaccio and a 47%  
271 decrease in Robusta. Moreover, stomatal closure was greater in Carpaccio than in Robusta (88  
272 *vs.* 122 mmol.m<sup>-2</sup>.s<sup>-1</sup> for  $g_1$ , respectively). During the stomatal closure phase,  $\tau$  and  $\lambda$  were  
273 unaffected by water deficit in either genotype (Fig. 2C and 2G).

274 During the stomatal opening phase due to irradiance, the water deficit effect was  
275 significant on  $\tau$  and SL<sub>max</sub> (Table S4).  $\tau$  decreased by 74% in Carpaccio but increased by  
276 26% in Robusta as compared to the control (Fig. 2D).  $\lambda$  was unaffected in Carpaccio but  
277 increased in Robusta (+ 21%) (Fig. 2H). SL<sub>max</sub> increased under water deficit almost 3 times  
278 faster in Carpaccio (Fig. 2L). These increases differed significantly between the genotypes  
279 (Table S4).

#### 280 3.3.2 Responses to VPD

281 After the VPD closing phase,  $g_1$  were unaffected in Carpaccio, whereas it was 50  
282 mmol.m<sup>-2</sup>.s<sup>-1</sup> below the WW:FA values in Robusta (Table S5). A<sub>0</sub> and A<sub>1</sub> decreased under  
283 water deficit by the same amplitude (around 30%) in both genotypes (Table S5).

284 Water deficit affected the stomatal dynamics through a non significant decrease of the  $\tau$   
285 parameter in both genotypes, by 20% in Carpaccio *vs.* 15% in Robusta (Fig. 3B). The  $\lambda$   
286 parameter was not modified in Carpaccio but decreased in Robusta (-13%) (Fig. 3D). Finally,  
287 a lower SL<sub>max</sub> was observed: -51% and -33% in Carpaccio and Robusta, respectively, as  
288 compared to the control (Fig. 3F).

### 289 3.4 Effects of O<sub>3</sub> and water deficit on stomatal behaviour

### 290 3.4.1 Responses to irradiance

291 After 7 days of water deficit treatment, most of the parameters showed an antagonistic  
292 response in the combined treatment (Fig. 4), except the initial steady state  $g_0$  in Carpaccio. In  
293 this genotype, stomatal closure was greater than under the water deficit treatment alone (Table  
294 S3). During stomatal closing under irradiance,  $\tau$  slightly increased by 6 min in Carpaccio, in-  
295 between the values under water deficit and  $O_3$  exposure, and was unaffected in Robusta as  
296 compared to the control (Fig. 2C).  $\lambda$  showed the same trends as under the D:FA modality  
297 (Fig. 2G).  $SL_{max}$  was significantly affected by the water deficit  $\times$   $O_3$  interaction ( $p=0.05$ ): in  
298 Carpaccio,  $SL_{max}$  was lower than under the D:FA and WW: $O_3$  modalities, by 23% as  
299 compared to the WW:FA modality. In Robusta,  $SL_{max}$  was in the same range as under D:FA  
300 conditions (Fig. 2K). Stomatal opening as a result of irradiance under the combined treatment  
301 revealed no significant effect of the water deficit  $\times$   $O_3$  interaction on any of the parameters of  
302 the models except  $\tau$  (Table S4). In Carpaccio,  $SL_{max}$  values were intermediate between D:FA  
303 and WW: $O_3$  values, almost twice the WW:FA value. In Robusta,  $SL_{max}$  tended to increase as  
304 compared to the other treatments (Fig. 2L), resulting in a synergistic effect (Fig. 4).

### 305 3.4.2 Responses to VPD

306 After 7 days of water deficit treatment on saplings previously submitted to  $O_3$  treatment,  
307 no significant effect was observed (Table S4). There was a synergistic effect on  $G$  ( $g_1$ ), under  
308 the D: $O_3$  modality, the final steady state tended to be “more closed stomata” in Carpaccio vs.  
309 “slightly more open stomata” in Robusta than under the WW: $O_3$  or D:FA modalities (Table  
310 S5). Otherwise, most of the parameters showed an antagonistic effect (Fig. 4). In Robusta, the  
311  $\lambda$  parameter decreased (by 26% as compared to the control) under the combined treatments  
312 (Fig. 3D), more than under water deficit or  $O_3$  alone, resulting in a synergistic effect (Fig. 4).  
313 In Carpaccio,  $SL_{max}$  decreased in a similar way under all three modalities. (Fig. 3F).

## 314 4 Discussion

### 315 4.1 Sluggish stomatal response to $O_3$

316 In our experiment, the first symptom of the daily exposure to 80 ppb  $O_3$  was visible on  
317  $g_s$ : after one week of exposure, both genotypes closed their stomata in response to  $O_3$ . This  
318 stomatal closure was associated with decreased net  $CO_2$  assimilation and chlorophyll  
319 contents. Impairment of gas exchanges under  $O_3$  exposure is well documented (Wittig et al.,  
320 2007). The decrease in net  $CO_2$  assimilation by the two poplar genotypes under  $O_3$  exposure

321 had been mentioned previously and mainly linked to the modification of Rubisco activity and  
322 chlorophyll degradation (Dghim et al., 2013; Guidi et al., 2001). This impact on  
323 photosynthesis was clearly visible for each steady state at  $800 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  of PAR.

324 In addition, stomatal closure went along with decreased steady states. This stomatal  
325 response was reversible: when  $\text{O}_3$  exposure was interrupted, saplings rapidly recovered the  
326 same steady states as in the control treatment. According to the dynamics parameters in  
327 response to irradiance, the lag time ( $\lambda$ ) was not modified by  $\text{O}_3$  during stomatal closure, but a  
328 few constitutive differences between the genotypes were visible. In Robusta,  $\tau$  tended to  
329 increase under  $\text{O}_3$  exposure. The main effect for both genotypes was a trend toward a lower  
330  $\text{SL}_{\text{max}}$ , *i.e.*, the so-called  $\text{O}_3$  sluggishness. In addition, steady states ( $g_1$ ) in the dark were  
331 slightly higher. This might have consequences under natural conditions, when  $\text{O}_3$  entrance  
332 during the night time could be significant (Hoshika et al., 2013) and could enhance water loss  
333 (Grulke et al., 2004). From our results, we can propose a hypothetical model of the stomatal  
334 daily course (Fig. S2) in conditions of stable diurnal irradiance throughout the day, with  
335 maximum VPD at midday. This representation highlights the differences between treatments  
336 and genotypes and the potential impact on the  $\text{O}_3$  flux,  $\text{CO}_2$  assimilation (grey area), or used  
337 water (blue area). This theoretical representation was inspired by a hypothetical model of  
338 Dumont et al. (2013), the results of a Ball-Berry-type model from Tuzet et al. (2003), and  
339 daily conductance measurements from Durand et al. (2019). The differences in  $g_s$  values  
340 between treatments, genotypes, and irradiance variations were conserved. As for stomatal  
341 closure, the model parameters were also modified during stomatal opening.

342 The main atmospheric determinant of stomatal opening at midday is VPD (McAdam  
343 and Brodribb, 2015). Higher VPD differently affected stomatal behaviour among genotypes  
344 in the control. Firstly, the  $\lambda$  parameter differed between the genotypes; Robusta was less  
345 responsive to VPD and needed more time to reach the inflection point. This could be linked to  
346 constitutive differences between the genotypes. Previous works showed that Robusta stomata  
347 were less responsive to VPD variation (Dumont et al., 2013). These genotypic differences  
348 could be as determining as differences between species. Differences between species were  
349 taken into account in the  $\text{O}_3$  flux model (Hayes and Bangor, 2017). It is obvious that the  
350 genotypic specificity could also affect the calculation of the species-specific stomatal  $\text{O}_3$  flux;  
351 moreover,  $\text{O}_3$ -induced stomatal closure and sluggishness could affect water use efficiency and  
352 transpiration calculations (Dumont et al., 2013; Hoshika et al., 2015; Paoletti and Grulke,  
353 2005). These effects could be of particular interest, as stomatal sluggishness was still present

354 after O<sub>3</sub> exposure and could be responsible for a greater water use in the middle and at the end  
355 of the day (Fig. S2). Interestingly, after O<sub>3</sub> exposure was stopped, Robusta recovered the same  
356 assimilation rate as the control whereas Carpaccio photosynthesis remained impaired. Our  
357 results are consistent with stomatal recovery after O<sub>3</sub>-induced stress observed in the literature.  
358 Similar recovery was reported in white clover (Francini et al., 2007). Nonetheless, this  
359 imperfect recovery due to carry-over or ‘memory’ effects could be particularly detrimental  
360 under repeated O<sub>3</sub> exposure (Oksanen, 2003; Oksanen and Saleem, 1999) combined with  
361 other biotic or abiotic stresses (Langebartels et al., 1998), e.g., water deficit conditions.  
362 However, this ‘memory’ could also be responsible for a ‘conditioning’ mechanism through a  
363 hormetic response (Agathokleous et al., 2019) and/or cross-tolerance (Walter et al., 2013). In  
364 both cases, this effect could modify tree resilience and acclimation to a new disturbance.

#### 365 4.2 *Water deficit induces slower stomatal closure in response to light and VPD*

366 Water deficit reduced only the total leaf surface of Carpaccio genotype (Table S2)  
367 associated with an expected stomatal closure over time (Bogeat-Triboulot et al., 2007; Chaves  
368 et al., 2002; Dusart et al., 2019). In response to darkness, the time response parameters were  
369 modified, with a few differences between the genotypes. Nevertheless, the lag time and  
370 closing speed were not impacted by water deficit in either genotype. Interestingly, in the dark  
371 both genotypes had their stomata more closed than the control saplings. Moreover, Robusta  
372 stomata were less closed than Carpaccio stomata. This could result in differences in water loss  
373 at night (Fig. S2) (Caird et al., 2007). By contrast, stomatal opening following irradiance  
374 affected all the parameters of the models. The opening speed increased in both genotypes,  
375 especially in Carpaccio. Faster stomatal opening under water deficit has been reported in  
376 *Phaseolus vulgaris* (Barradas et al., 1994). Water loss might increase due to faster stomatal  
377 opening, but may also increase the leaf carbon assimilation (Barradas et al., 1994). In  
378 response to VPD, water deficit affected stomatal dynamics through a decrease of the  $\tau$   
379 parameter in both genotypes. The lag time parameter was not modified in Carpaccio and  
380 decreased in Robusta. There was great sluggishness in the response to VPD. Stomatal closure  
381 sluggishness in response to irradiance and/or VPD has been reported in different species, after  
382 leaf excision in *Populus trichocarpa* x *deltoides* (Reich and Lassoie, 1984), under soil water  
383 deficit conditions in *Phaseolus vulgaris* (Hoshika et al., 2013), *Nicotiana tabacum* (Gérardin  
384 et al., 2018), *Populus nigra*, and *Populus euramericana* (Durand et al., 2019). Stomatal  
385 closure under soil water deficit or increased VPD was found closely related to ion transport  
386 and abscisic acid perception (McAdam and Brodribb, 2015; Pospíšilová, 2003). These

387 mechanisms could be modified by O<sub>3</sub> through ABA synthesis (McAdam et al., 2017) or  
388 through other phytohormones such as ethylene (Wilkinson and Davies, 2010, 2009).

#### 389 4.3 *Antagonistic effect of the successive exposure to O<sub>3</sub> and water deficit*

390 When O<sub>3</sub> exposure was followed by water deficit, we observed a combination of the  
391 effects of O<sub>3</sub> or water deficit alone. The impact on height and radial growth was similar to the  
392 effect of water deficit alone in both genotypes. Nevertheless, total biomass decreased more  
393 following the successive stresses (Table S2). As seen above, water deficit and O<sub>3</sub> separately  
394 modified stomatal behaviour in response to VPD or irradiance fluctuations. The interaction  
395 between the two stress factors may have modified stomatal responses (Hoshika et al., 2013;  
396 Wilkinson and Davies, 2009). Concerning gas exchanges, stomatal closure was observed in  
397 both genotypes, with a decreased steady state. Carpaccio stomata tended to be more closed  
398 than under O<sub>3</sub> exposure or water deficit alone, whereas Robusta stomata were slightly less  
399 closed than under water deficit alone (Fig. S2). Under the combined treatment, most of the  
400 parameters showed an antagonistic response, *i.e.*, a weaker response than the expected  
401 additive effect (Bansal et al., 2013; Dusart et al., 2019; Pellegrini et al., 2019).  $\tau$  seemed to  
402 increase as compared to the control and drought treatment alone, but less than under O<sub>3</sub>  
403 exposure. The lag time and final steady state were modified in the same way as under water  
404 deficit alone. In Carpaccio, stomatal sluggishness increased as compared to the separate  
405 constraints, and this may have increased transpiration under water deficit. In Robusta, the  
406 slower closure was in the same range as under water deficit alone. Regarding stomatal  
407 opening due to irradiance, the response was the same as for water deficit alone on all the  
408 parameters of the models, without any significant interaction of O<sub>3</sub>. In response to VPD, most  
409 of the parameters showed an antagonistic effect, except a synergistic effect on the lag time for  
410 Robusta.  $\lambda$  parameters indeed decreased under the combined treatment, more than under water  
411 deficit or O<sub>3</sub> exposure alone, as compared to the control. This effect could result from a better  
412 detection of environmental variation. The different stomatal behaviours under successive  
413 stresses in the two genotypes could result in different water losses (Fig. S2) at the leaf scale.  
414 Robusta may have lost more water during the day when exposed to the combined stress than  
415 to water deficit alone. These differences in leaf scale between genotypes could be particularly  
416 detrimental for a water-deficit sensitive genotype. Nevertheless, in order to model  
417 physiological processes, it is important to take constitutive differences in total leaf area  
418 between genotypes into account, as this difference could impact extrapolation at the whole  
419 tree scale.

#### 420 4.4 Consequences for modelling

421 Modelling  $g_s$  is an important issue to use flux-based metrics for forest  $O_3$  risk assessment  
422 (Fares et al., 2013). Scientific literature about  $g_s$  models at the leaf level is abundant (see  
423 Damour et al., 2010 for a review). Most current models used for  $O_3$ , such as the  $DO_3SE$  model  
424 (Emberson et al., 2000), are based on Jarvis multiplicative model (Jarvis, 1976) and do not  
425 take  $O_3$ -induced stomatal sluggishness into account (Hayes and Bangor, 2017). Hoshika et al.  
426 (2017) proposed to take stomatal sluggishness into account through a single parameter ( $s$ ) and  
427 directly on steady states through an  $O_3$  function ( $f_{O_3}$ ). The main competitor of the Jarvis-type  
428 model is based on the Ball-Woodrow-Berry model (BWB) (Ball et al., 1987). This model  
429 integrates a more physiological approach and considers the relationship between  $g_s$  and  
430 photosynthesis. Its modified version should be preferred for  $O_3$ - induced decoupling between  
431 photosynthesis and stomatal conductance (Cailleret et al., 2018; Lombardozzi et al., 2012).  
432 Moreover, this model requires adding a soil water function. Contrasting results have been  
433 found between the Jarvis or BWB models (Hoshika et al., 2017a). If these models are  
434 properly parameterised, they can both accurately predict  $g_s$  in complex ecosystems (Fares et  
435 al., 2013). This will depend on available data for model parameterisation and calibration  
436 (Fares et al., 2013), but could be crucial for studying  $O_3$ -sensitive tree species. Moreover, our  
437 results highlight that it is important to take genotype-specific responses into account, *e.g.*  
438 Carpaccio stomata closed faster than Robusta stomata under VPD, and opened faster under  
439 irradiance. Nonetheless, these observations at the leaf scale might not be easily extrapolated at  
440 the tree scale because of multiple obstacles: i) scaling up from leaf to canopy is tricky  
441 (Ollinger et al., 1997; Zhou et al., 2017), ii) the impact of phenology, aging, and enhanced  
442 leaf senescence should be taken into account (Anav et al., 2018), iii) leaf stomatal density is  
443 modified in newly formed leaves (Durand et al., 2019; Pääkkönen et al., 1997), iv)  
444 detoxification and repair processes occur (Tuzet et al., 2011), and v) conditioning  
445 (Agathokleous et al., 2019) or cross-tolerance processes (Tausz et al., 2007) occur too. All  
446 these points could challenge the implementation of larger models and the scaling up to whole  
447 tree or forest ecosystems.

#### 448 5 Conclusion

449 Despite constitutive differences between genotypes,  $O_3$  and water deficit induced  
450 stomatal closure and closing sluggishness. 80 ppb  $O_3$  exposure followed by water deficit  
451 modified stomatal closure differently between the two genotypes: Carpaccio stomata closed  
452 more than under water deficit alone, whereas Robusta stomata closed less than under water

453 deficit alone. These modifications could have a non-negligible effect on O<sub>3</sub> uptake, carbon  
454 storage, and water use efficiency. The underlying mechanisms still need investigations into  
455 the active or passive physiological regulation induced by environmental fluctuations  
456 (irradiance, VPD, O<sub>3</sub>, soil water deficit, etc.). Previous studies suggested different stomatal  
457 closure/opening mechanisms, *i.e.*, i) passive hydric regulation of guard cells (Buckley and  
458 Mott, 2002), ii) mediation by a cross-talk between phytohormones (Daszkowska-Golec and  
459 Szarejko, 2013), with a particular implication of abscisic acid or ethylene (McAdam and  
460 Brodribb, 2015; Wilkinson and Davies, 2010, 2009), iii) O<sub>3</sub> and/or drought-induced stomatal  
461 closure through modification of guard cell homeostasis *via* direct modulation of K<sup>+</sup> channels  
462 (Geiger et al., 2009; Vahisalu et al., 2010), alteration of Ca<sup>2+</sup>/H<sup>+</sup> vacuolar antiporters (Dumont  
463 et al., 2014a), and production of reactive oxygen species such as H<sub>2</sub>O<sub>2</sub> (Damour et al., 2010).  
464 Understanding these mechanisms and the cross-talks between the O<sub>3</sub> and water deficit  
465 responses (as successive stresses or in combination) could allow for a better prediction of g<sub>s</sub>  
466 in response to various environmental modifications.

467 **Acknowledgements**

468           Nicolas Dusart was supported by a PhD grant from the Ministère de l'Enseignement  
469 Supérieur et de la Recherche. The team was supported by the French National Research  
470 Agency through the Laboratory of Excellence ARBRE (ANR-12-LABXARBRE-01). We  
471 thank S. Martin for taking care of the O<sub>3</sub> facilities and the nursery of Guéméné-Penfao for  
472 providing the cuttings. This research did not receive any specific grant from funding agencies  
473 in the public, commercial, or not-for-profit sectors.

474 **References**

- 475 Agathokleous, E., Belz, R.G., Calatayud, V., De Marco, A., Hoshika, Y., Kitao, M., Saitanis,  
 476 C.J., Sicard, P., Paoletti, E., Calabrese, E.J., 2019. Predicting the effect of ozone on  
 477 vegetation via linear non-threshold (LNT), threshold and hormetic dose-response  
 478 models. *Sci. Total Environ.* 61–74. <https://doi.org/10.1016/j.scitotenv.2018.08.264>
- 479 Allen, C.D., Macalady, A.K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M.,  
 480 Kitzberger, T., Rigling, A., Breshears, D.D., Hogg, E.H. (Ted), Gonzalez, P.,  
 481 Fensham, R., Zhang, Z., Castro, J., Demidova, N., Lim, J.-H., Allard, G., Running,  
 482 S.W., Semerci, A., Cobb, N., 2010. A global overview of drought and heat-induced  
 483 tree mortality reveals emerging climate change risks for forests. *For. Ecol. Manag.*  
 484 259, 660–684. <https://doi.org/10.1016/j.foreco.2009.09.001>
- 485 Anav, A., Liu, Q., De Marco, A., Proietti, C., Savi, F., Paoletti, E., Piao, S., 2018. The role of  
 486 plant phenology in stomatal ozone flux modeling. *Glob. Change Biol.* 24, 235–248.  
 487 <https://doi.org/10.1111/gcb.13823>
- 488 Bagard, M., Jolivet, Y., Hasenfratz-Sauder, M.-P., Gérard, J., Dizengremel, P., Le Thiec, D.,  
 489 2015. Ozone exposure and flux-based response functions for photosynthetic traits in  
 490 wheat, maize and poplar. *Environ. Pollut.* 206, 411–420.  
 491 <https://doi.org/10.1016/j.envpol.2015.07.046>
- 492 Ball, J.T., Woodrow, I.E., Berry, J.A., 1987. A model predicting stomatal conductance and its  
 493 contribution to the control of photosynthesis under different environmental conditions.  
 494 *Prog. Photosynth. Res.* 221–224. [https://doi.org/10.1007/978-94-017-0519-6\\_48](https://doi.org/10.1007/978-94-017-0519-6_48)
- 495 Bansal, S., Hallsby, G., Löfvenius, M.O., Nilsson, M.-C., 2013. Synergistic, additive and  
 496 antagonistic impacts of drought and herbivory on *Pinus sylvestris*: leaf, tissue and  
 497 whole-plant responses and recovery. *Tree Physiol.* 33, 451–463.  
 498 <https://doi.org/10.1093/treephys/tpt019>
- 499 Barradas, V.L., Jones, H.G., Clark, J.A., 1994. Stomatal responses to changing irradiance in  
 500 *Phaseolus vulgaris* L. *J. Exp. Bot.* 45, 931–936. <https://doi.org/10.1093/jxb/45.7.931>
- 501 Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects Models  
 502 Using lme4. *J. Stat. Softw.* 67, 1–48. <https://doi.org/10.18637/jss.v067.i01>
- 503 Bogeat-Triboulot, M.-B., Brosché, M., Renaut, J., Jouve, L., Le Thiec, D., Fayyaz, P.,  
 504 Vinocur, B., Witters, E., Laukens, K., Teichmann, T., Altman, A., Hausman, J.-F.,  
 505 Polle, A., Kangasjärvi, J., Dreyer, E., 2007. Gradual soil water depletion results in  
 506 reversible changes of gene expression, protein profiles, ecophysiology, and growth  
 507 performance in *Populus euphratica*, a poplar growing in arid regions. *Plant Physiol.*  
 508 143, 876–892. <https://doi.org/10.1104/pp.106.088708>
- 509 Braun, S., Schindler, C., Rihm, B., 2014. Growth losses in Swiss forests caused by ozone:  
 510 Epidemiological data analysis of stem increment of *Fagus sylvatica* L. and *Picea*  
 511 *abies* Karst. *Environ. Pollut.* 192, 129–138.  
 512 <https://doi.org/10.1016/j.envpol.2014.05.016>
- 513 Buckley, T.N., Mott, K.A., 2002. Dynamics of stomatal water relations during the humidity  
 514 response: implications of two hypothetical mechanisms. *Plant Cell Environ.* 25, 407–  
 515 419. <https://doi.org/10.1046/j.0016-8025.2001.00820.x>
- 516 Büker, P., Morrissey, T., Briolat, A., Falk, R., Simpson, D., Tuovinen, J.-P., Alonso, R.,  
 517 Barth, S., Baumgarten, M., Grulke, N., Karlsson, P.E., King, J., Lagergren, F.,  
 518 Matyssek, R., Nunn, A., Ogaya, R., Peñuelas, J., Rhea, L., Schaub, M., Uddling, J.,

- 519 Werner, W., Emberson, L.D., 2012. DO<sub>3</sub>SE modelling of soil moisture to determine  
520 ozone flux to forest trees. *Atmospheric Chem. Phys.* 12, 5537–5562.  
521 <https://doi.org/10.5194/acp-12-5537-2012>
- 522 Cailleret, M., Ferretti, M., Gessler, A., Rigling, A., Schaub, M., 2018. Ozone effects on  
523 European forest growth—Towards an integrative approach. *J. Ecol.* 106, 1377–1389.  
524 <https://doi.org/10.1111/1365-2745.12941>
- 525 Caird, M.A., Richards, J.H., Donovan, L.A., 2007. Nighttime stomatal conductance and  
526 transpiration in C<sub>3</sub> and C<sub>4</sub> plants. *Plant Physiol.* 143, 4–10.  
527 <https://doi.org/10.1104/pp.106.092940>
- 528 Chaves, M.M., Pereira, J.S., Maroco, J., Rodrigues, M.L., Ricardo, C.P.P., Osório, M.L.,  
529 Carvalho, I., Faria, T., Pinheiro, C., 2002. How plants cope with water stress in the  
530 field? Photosynthesis and growth. *Ann. Bot.* 89, 907–916.  
531 <https://doi.org/10.1093/aob/mcf105>
- 532 Ciais, Ph., Reichstein, M., Viovy, N., Granier, A., Ogée, J., Allard, V., Aubinet, M.,  
533 Buchmann, N., Bernhofer, Chr., Carrara, A., Chevallier, F., De Noblet, N., Friend,  
534 A.D., Friedlingstein, P., Grünwald, T., Heinesch, B., Keronen, P., Knohl, A., Krinner,  
535 G., Loustau, D., Manca, G., Matteucci, G., Miglietta, F., Ourcival, J.M., Papale, D.,  
536 Pilegaard, K., Rambal, S., Seufert, G., Soussana, J.F., Sanz, M.J., Schulze, E.D.,  
537 Vesala, T., Valentini, R., 2005. Europe-wide reduction in primary productivity caused  
538 by the heat and drought in 2003. *Nature* 437, 529–533.  
539 <https://doi.org/10.1038/nature03972>
- 540 Damour, G., Simonneau, T., Cochard, H., Urban, L., 2010. An overview of models of  
541 stomatal conductance at the leaf level. *Plant Cell Environ.* 33, 1419–1438.  
542 <https://doi.org/10.1111/j.1365-3040.2010.02181.x>
- 543 Daszkowska-Golec, A., Szarejko, I., 2013. Open or close the gate – stomata action under the  
544 control of phytohormones in drought stress conditions. *Front. Plant Sci.* 4, 1–16.  
545 <https://doi.org/10.3389/fpls.2013.00138>
- 546 Dghim, A.A., Dumont, J., Hasenfrazz-Sauder, M.-P., Dizengremel, P., Le Thiec, D., Jolivet,  
547 Y., 2013. Capacity for NADPH regeneration in the leaves of two poplar genotypes  
548 differing in ozone sensitivity. *Physiol. Plant.* 148, 36–50. <https://doi.org/10.1111/j.1399-3054.2012.01686.x>
- 550 Dizengremel, P., Jolivet, Y., Tuzet, A., Ranieri, A., Le Thiec, D., 2013. Integrative leaf-level  
551 phytotoxic ozone dose assessment for forest risk modelling. *Clim. Change Air Pollut.*  
552 *Glob. Chall. Underst. Perspect. For. Res.* 13, 267–288. [https://doi-org/10.1016/B978-  
553 0-08-098349-3.00013-X](https://doi-org/10.1016/B978-0-08-098349-3.00013-X)
- 554 Dumont, J., Cohen, D., Gérard, J., Jolivet, Y., Dizengremel, P., Le Thiec, D., 2014a. Distinct  
555 responses to ozone of abaxial and adaxial stomata in three Euramerican poplar  
556 genotypes. *Plant Cell Environ.* 37, 2064–2076. <https://doi.org/10.1111/pce.12293>
- 557 Dumont, J., Keski-Saari, S., Keinänen, M., Cohen, D., Ningre, N., Kontunen-Soppela, S.,  
558 Baldet, P., Gibon, Y., Dizengremel, P., Vaultier, M.-N., Jolivet, Y., Oksanen, E., Le  
559 Thiec, D., 2014b. Ozone affects ascorbate and glutathione biosynthesis as well as  
560 amino acid contents in three Euramerican poplar genotypes. *Tree Physiol.* 34, 253–  
561 266. <https://doi.org/10.1093/treephys/tpu004>
- 562 Dumont, J., Spicher, F., Montpied, P., Dizengremel, P., Jolivet, Y., Le Thiec, D., 2013.  
563 Effects of ozone on stomatal responses to environmental parameters (blue light, red

- 564 light, CO<sub>2</sub> and vapour pressure deficit) in three *Populus deltoides* × *Populus nigra*  
565 genotypes. *Environ. Pollut.* 173, 85–96. <https://doi.org/10.1016/j.envpol.2012.09.026>
- 566 Durand, M., Brendel, O., Buré, C., Le Thiec, D., 2019. Altered stomatal dynamics induced by  
567 changes in irradiance and vapour-pressure deficit under drought: impact on the whole  
568 plant transpiration efficiency of poplar species. *New Phytol.*  
569 <https://doi.org/10.1111/nph.15710>
- 570 Dusart, N., Gérard, J., Le Thiec, D., Collignon, C., Jolivet, Y., Vaultier, M.-N., 2019.  
571 Integrated analysis of the detoxification responses of two Euramerican poplar  
572 genotypes exposed to ozone and water deficit: Focus on the ascorbate-glutathione  
573 cycle. *Sci. Total Environ.* 2365–2379. <https://doi.org/10.1016/j.scitotenv.2018.09.367>
- 574 Emberson, L.D., Ashmore, M.R., Cambridge, H.M., Simpson, D., Tuovinen, J.-P., 2000.  
575 Modelling stomatal ozone flux across Europe. *Environ. Pollut.* 109, 403–413.  
576 [https://doi.org/10.1016/S0269-7491\(00\)00043-9](https://doi.org/10.1016/S0269-7491(00)00043-9)
- 577 Emberson, L.D., Büker, P., Ashmore, M.R., 2007. Assessing the risk caused by ground level  
578 ozone to European forest trees: A case study in pine, beech and oak across different  
579 climate regions. *Environ. Pollut., Air Pollution and Climate Change: A Global  
580 Overview of the Effects on Forest Vegetation* 147, 454–466.  
581 <https://doi.org/10.1016/j.envpol.2006.10.026>
- 582 Fares, S., Matteucci, G., Scarascia Mugnozza, G., Morani, A., Calfapietra, C., Salvatori, E.,  
583 Fusaro, L., Manes, F., Loreto, F., 2013. Testing of models of stomatal ozone fluxes  
584 with field measurements in a mixed Mediterranean forest. *Atmos. Environ.* 67, 242–  
585 251. <https://doi.org/10.1016/j.atmosenv.2012.11.007>
- 586 Francini, A., Nali, C., Picchi, V., Lorenzini, G., 2007. Metabolic changes in white clover  
587 clones exposed to ozone. *Environ. Exp. Bot.* 60, 11–19.  
588 <https://doi.org/10.1016/j.envexpbot.2006.06.004>
- 589 Fuhrer, J., Skärby, L., Ashmore, M.R., 1997. Critical levels for ozone effects on vegetation in  
590 Europe. *Environ. Pollut.* 97, 91–106. [https://doi.org/10.1016/S0269-7491\(97\)00067-5](https://doi.org/10.1016/S0269-7491(97)00067-5)
- 591 Geiger, D., Scherzer, S., Mumm, P., Stange, A., Marten, I., Bauer, H., Ache, P., Matschi, S.,  
592 Liese, A., Al-Rasheid, K.A.S., Romeis, T., Hedrich, R., 2009. Activity of guard cell  
593 anion channel SLAC1 is controlled by drought-stress signaling kinase-phosphatase  
594 pair. *Proc. Natl. Acad. Sci.* 106, 21425–21430.  
595 <https://doi.org/10.1073/pnas.0912021106>
- 596 Gérardin, T., Douthe, C., Flexas, J., Brendel, O., 2018. Shade and drought growth conditions  
597 strongly impact dynamic responses of stomata to variations in irradiance in *Nicotiana*  
598 *tabacum*. *Environ. Exp. Bot.* 153, 188–197.  
599 <https://doi.org/10.1016/j.envexpbot.2018.05.019>
- 600 Grulke, N.E., Alonso, R., Nguyen, T., Cascio, C., Dobrowolski, W., 2004. Stomata open at  
601 night in pole-sized and mature ponderosa pine: implications for O<sub>3</sub> exposure metrics.  
602 *Tree Physiol.* 24, 1001–1010. <https://doi.org/10.1093/treephys/24.9.1001>
- 603 Grulke, N.E., Neufeld, H.S., Davison, A.W., Roberts, M., Chappelka, A.H., 2007. Stomatal  
604 behavior of ozone-sensitive and -insensitive coneflowers (*Rudbeckia laciniata* var.  
605 *digitata*) in Great Smoky Mountains National Park. *New Phytol.* 173, 100–109.  
606 <https://doi.org/10.1111/j.1469-8137.2006.01872.x>
- 607 Guidi, L., Nali, C., Lorenzini, G., Filippi, F., Soldatini, G.F., 2001. Effect of chronic ozone  
608 fumigation on the photosynthetic process of poplar clones showing different

- 609 sensitivity. *Environ. Pollut.* 113, 245–254. [https://doi.org/10.1016/S0269-7491\(00\)00194-9](https://doi.org/10.1016/S0269-7491(00)00194-9)  
610
- 611 Hayes, F., Bangor, C., 2017. Manual on Methodologies and Criteria for Modelling and  
612 Mapping Critical Loads & Levels and Air Pollution Effects, Risks and Trends,  
613 Chapter III : mapping critical levels for vegetation. Presented at the Convention on  
614 Long-range Transboundary Air Pollution.
- 615 Hetherington, A.M., Woodward, F.I., 2003. The role of stomata in sensing and driving  
616 environmental change. *Nature* 424, 901–908. <https://doi.org/10.1038/nature01843>
- 617 Hoshika, Y., Fares, S., Savi, F., Gruening, C., Goded, I., De Marco, A., Sicard, P., Paoletti,  
618 E., 2017a. Stomatal conductance models for ozone risk assessment at canopy level in  
619 two Mediterranean evergreen forests. *Agric. For. Meteorol.* 234–235, 212–221.  
620 <https://doi.org/10.1016/j.agrformet.2017.01.005>
- 621 Hoshika, Y., Katata, G., Deushi, M., Watanabe, M., Koike, T., Paoletti, E., 2015. Ozone-  
622 induced stomatal sluggishness changes carbon and water balance of temperate  
623 deciduous forests. *Sci. Rep.* 5, 1–8. <https://doi.org/10.1038/srep09871>
- 624 Hoshika, Y., Omasa, K., Paoletti, E., 2013. Both ozone exposure and soil water stress are able  
625 to induce stomatal sluggishness. *Environ. Exp. Bot.* 88, 19–23.  
626 <https://doi.org/10.1016/j.envexpbot.2011.12.004>
- 627 Hoshika, Y., Watanabe, M., Carrari, E., Paoletti, E., Koike, T., 2017b. Ozone-induced  
628 stomatal sluggishness changes stomatal parameters of Jarvis-type model in white birch  
629 and deciduous oak. *Plant Biol.* 20–28. <https://doi.org/10.1111/plb.12632>
- 630 IPCC, 2014. Climate change 2013: the physical science basis: Working Group I contribution  
631 to the Fifth assessment report of the Intergovernmental Panel on Climate Change.  
632 Cambridge University Press, New York.
- 633 Jarvis, P.G., 1976. The interpretation of the variations in leaf water potential and stomatal  
634 conductance found in canopies in the field. *Philos. Trans. R. Soc. Lond. B Biol. Sci.*  
635 273, 593–610. <https://doi.org/10.1098/rstb.1976.0035>
- 636 Jolivet, Y., Bagard, M., Cabané, M., Vaultier, M.-N., Gandin, A., Afif, D., Dizengremel, P.,  
637 Le Thiec, D., 2016. Deciphering the ozone-induced changes in cellular processes: a  
638 prerequisite for ozone risk assessment at the tree and forest levels. *Ann. For. Sci.* 73,  
639 923–943. <https://doi.org/10.1007/s13595-016-0580-3>
- 640 Kalabokas, P., Hjorth, J., Foret, G., Dufour, G., Eremenko, M., Siour, G., Cuesta, J.,  
641 Beekmann, M., 2017. An investigation on the origin of regional springtime ozone  
642 episodes in the western Mediterranean. *Atmos Chem Phys* 17, 3905–3928.  
643 <https://doi.org/10.5194/acp-17-3905-2017>
- 644 Karlsson, P.E., Pleijel, H., Karlsson, G.P., Medin, E.L., Skärby, L., 2000. Simulations of  
645 stomatal conductance and ozone uptake to Norway spruce saplings in open-top  
646 chambers. *Environ. Pollut.* 109, 443–451. [https://doi.org/10.1016/S0269-7491\(00\)00047-6](https://doi.org/10.1016/S0269-7491(00)00047-6)  
647
- 648 Langebartels, C., Heller, W., Führer, G., Lippert, M., Simons, S., Sandermann, H., 1998.  
649 Memory effects in the action of ozone on conifers. *Ecotoxicol. Environ. Saf.* 41, 62–  
650 72. <https://doi.org/10.1006/eesa.1998.1668>
- 651 Lehner, B., Döll, P., Alcamo, J., Henrichs, T., Kaspar, F., 2006. Estimating the Impact of  
652 Global Change on Flood and Drought Risks in Europe: A Continental, Integrated  
653 Analysis. *Clim. Change* 75, 273–299. <https://doi.org/10.1007/s10584-006-6338-4>

- 654 Lenth, R.V., 2016. Least-Squares Means: The R Package lsmeans. *J. Stat. Softw.* 69, 1–33.  
655 <https://doi.org/10.18637/jss.v069.i01>
- 656 Li, P., Feng, Z., Catalayud, V., Yuan, X., Xu, Y., Paoletti, E., 2017. A meta-analysis on  
657 growth, physiological, and biochemical responses of woody species to ground-level  
658 ozone highlights the role of plant functional types. *Plant Cell Environ.* 40, 2369–2380.  
659 <https://doi.org/10.1111/pce.13043>
- 660 Liu, C., Allan, R.P., 2013. Observed and simulated precipitation responses in wet and dry  
661 regions 1850–2100. *Environ. Res. Lett.* 8, 034002. <https://doi.org/10.1088/1748-9326/8/3/034002>
- 662
- 663 Lombardozzi, D., Levis, S., Bonan, G., Sparks, J.P., 2012. Predicting photosynthesis and  
664 transpiration responses to ozone: decoupling modeled photosynthesis and stomatal  
665 conductance. *Biogeosciences Discuss.* 9, 4245–4283. <https://doi.org/10.5194/bgd-9-4245-2012>
- 666
- 667 McAdam, E.L., Brodribb, T.J., McAdam, S.A.M., 2017. Does ozone increase ABA levels by  
668 non-enzymatic synthesis causing stomata to close? *Plant Cell Environ.* 40, 741–747.  
669 <https://doi.org/10.1111/pce.12893>
- 670 McAdam, S.A.M., Brodribb, T.J., 2015. The evolution of mechanisms driving the stomatal  
671 response to vapor pressure deficit. *Plant Physiol.* 167, 833–843.  
672 <https://doi.org/10.1104/pp.114.252940>
- 673 Mills, G., Pleijel, H., Braun, S., Büker, P., Bermejo, V., Calvo, E., Danielsson, H., Emberson,  
674 L., Fernández, I.G., Grünhage, L., Harmens, H., Hayes, F., Karlsson, P.-E., Simpson,  
675 D., 2011. New stomatal flux-based critical levels for ozone effects on vegetation.  
676 *Atmos. Environ.* 45, 5064–5068. <https://doi.org/10.1016/j.atmosenv.2011.06.009>
- 677 Monks, P.S., 2000. A review of the observations and origins of the spring ozone maximum.  
678 *Atmos. Environ.* 34, 3545–3561. [https://doi.org/10.1016/S1352-2310\(00\)00129-1](https://doi.org/10.1016/S1352-2310(00)00129-1)
- 679 Musselman, R.C., Lefohn, A.S., Massman, W.J., Heath, R.L., 2006. A critical review and  
680 analysis of the use of exposure-and flux-based ozone indices for predicting vegetation  
681 effects. *Atmos. Environ.* 40, 1869–1888.  
682 <https://doi.org/10.1016/j.atmosenv.2005.10.064>
- 683 Niinemets, Ü., Kull, O., 2001. Sensitivity of photosynthetic electron transport to  
684 photoinhibition in a temperate deciduous forest canopy: Photosystem II center  
685 openness, non-radiative energy dissipation and excess irradiance under field  
686 conditions. *Tree Physiol.* 21, 899–914. <https://doi.org/10.1093/treephys/21.12-13.899>
- 687 Oksanen, E., 2003. Physiological responses of birch (*Betula pendula*) to ozone: a comparison  
688 between open-soil-grown trees exposed for six growing seasons and potted seedlings  
689 exposed for one season. *Tree Physiol.* 23, 603–614.  
690 <https://doi.org/10.1093/treephys/23.9.603>
- 691 Oksanen, E., Saleem, A., 1999. Ozone exposure results in various carry-over effects and  
692 prolonged reduction in biomass in birch (*Betula pendula* Roth). *Plant Cell Environ.*  
693 22, 1401–1411. <https://doi.org/10.1046/j.1365-3040.1999.00501.x>
- 694 Ollinger, S.V., Aber, J.D., Reich, P.B., 1997. Simulating ozone effects on forest productivity:  
695 interactions among leaf-, canopy-, and stand-level processes. *Ecol. Appl.* 7, 1237.  
696 <https://doi.org/10.2307/2641211>
- 697 Pääkkönen, E., Holopainen, T., Kärenlampi, L., 1997. Differences in growth, leaf senescence  
698 and injury, and stomatal density in birch (*Betula pendula* Roth.) in relation to ambient

- 699 levels of ozone in Finland. *Environ. Pollut.* 96, 117–127.  
700 [https://doi.org/10.1016/S0269-7491\(97\)00034-1](https://doi.org/10.1016/S0269-7491(97)00034-1)
- 701 Paoletti, E., Grulke, N.E., 2010. Ozone exposure and stomatal sluggishness in different plant  
702 physiognomic classes. *Environ. Pollut.* 158, 2664–2671.  
703 <https://doi.org/10.1016/j.envpol.2010.04.024>
- 704 Paoletti, E., Grulke, N.E., 2005. Does living in elevated CO<sub>2</sub> ameliorate tree response to  
705 ozone? A review on stomatal responses. *Environ. Pollut.* 137, 483–493.  
706 <https://doi.org/10.1016/j.envpol.2005.01.035>
- 707 Park Williams, A., Allen, C.D., Macalady, A.K., Griffin, D., Woodhouse, C.A., Meko, D.M.,  
708 Swetnam, T.W., Rauscher, S.A., Seager, R., Grissino-Mayer, H.D., Dean, J.S., Cook,  
709 E.R., Gangodagamage, C., Cai, M., McDowell, N.G., 2013. Temperature as a potent  
710 driver of regional forest drought stress and tree mortality. *Nat. Clim. Change* 3, 292–  
711 297. <https://doi.org/10.1038/nclimate1693>
- 712 Parrish, D.D., Law, K.S., Staehelin, J., Derwent, R., Cooper, O.R., Tanimoto, H., Volz-  
713 Thomas, A., Gilge, S., Scheel, H.-E., Steinbacher, M., Chan, E., 2013. Lower  
714 tropospheric ozone at northern midlatitudes: Changing seasonal cycle. *Geophys. Res.*  
715 *Let.* 40, 1631–1636. <https://doi.org/10.1002/grl.50303>
- 716 Pellegrini, E., Hoshika, Y., Dusart, N., Cotrozzi, L., Gérard, J., Nali, C., Vaultier, M.-N.,  
717 Jolivet, Y., Lorenzini, G., Paoletti, E., 2019. Antioxidative responses of three oak  
718 species under ozone and water stress conditions. *Sci. Total Environ.* 647, 390–399.  
719 <https://doi.org/10.1016/j.scitotenv.2018.07.413>
- 720 Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., Team, R.C., 2018. nlme: Linear and Nonlinear  
721 Mixed Effects Models. R Package Version 3.1-137. [https://CRAN.R-](https://CRAN.R-project.org/package=nlme)  
722 [project.org/package=nlme](https://CRAN.R-project.org/package=nlme).
- 723 Pollastrini, M., Desotgiu, R., Camin, F., Ziller, L., Gerosa, G., Marzuoli, R., Bussotti, F.,  
724 2014. Severe drought events increase the sensitivity to ozone on poplar clones.  
725 *Environ. Exp. Bot.* 100, 94–104. <https://doi.org/10.1016/j.envexpbot.2013.12.016>
- 726 Pospíšilová, J., 2003. Participation of phytohormones in the stomatal regulation of gas  
727 exchange during water stress. *Biol. Plant.* 46, 491–506.
- 728 Reich, P.B., Lassoie, J.P., 1984. Effects of low level O<sub>3</sub> exposure on leaf diffusive  
729 conductance and water-use efficiency in hybrid poplar. *Plant Cell Environ.* 7, 661–  
730 668. <https://doi.org/10.1111/1365-3040.ep11571645>
- 731 Ridolfi, M., Dreyer, E., 1997. Responses to water stress in an ABA-unresponsive hybrid  
732 poplar (*Populus koreanaxtrichocarpa* cv. Peace) III. Consequences for photosynthetic  
733 carbon assimilation. *New Phytol.* 135, 31–40. [https://doi.org/10.1046/j.1469-](https://doi.org/10.1046/j.1469-8137.1997.00624.x)  
734 [8137.1997.00624.x](https://doi.org/10.1046/j.1469-8137.1997.00624.x)
- 735 Sicard, P., Augustaitis, A., Belyazid, S., Calfapietra, C., de Marco, A., Fenn, M.,  
736 Bytnerowicz, A., Grulke, N., He, S., Matyssek, R., Serengil, Y., Wieser, G., Paoletti,  
737 E., 2016. Global topics and novel approaches in the study of air pollution, climate  
738 change and forest ecosystems. *Environ. Pollut.*  
739 <https://doi.org/10.1016/j.envpol.2016.01.075>
- 740 Tausz, M., Grulke, N.E., Wieser, G., 2007. Defense and avoidance of ozone under global  
741 change. *Environ. Pollut.* 147, 525–531. <https://doi.org/10.1016/j.envpol.2006.08.042>

- 742 Tuzet, A., Perrier, A., Leuning, R., 2003. A coupled model of stomatal conductance,  
743 photosynthesis and transpiration. *Plant Cell Environ.* 26, 1097–1116.  
744 <https://doi.org/10.1046/j.1365-3040.2003.01035.x>
- 745 Tuzet, A., Perrier, A., Loubet, B., Cellier, P., 2011. Modelling ozone deposition fluxes: The  
746 relative roles of deposition and detoxification processes. *Agric. For. Meteorol.* 151,  
747 480–492. <https://doi.org/10.1016/j.agrformet.2010.12.004>
- 748 Vahisalu, T., Puzõrjova, I., Brosché, M., Valk, E., Lepiku, M., Moldau, H., Pechter, P., Wang,  
749 Y.-S., Lindgren, O., Salojärvi, J., Loog, M., Kangasjärvi, J., Kollist, H., 2010. Ozone-  
750 triggered rapid stomatal response involves the production of reactive oxygen species,  
751 and is controlled by SLAC1 and OST1. *Plant J.* 62, 442–453.  
752 <https://doi.org/10.1111/j.1365-313X.2010.04159.x>
- 753 Walter, J., Jentsch, A., Beierkuhnlein, C., Kreyling, J., 2013. Ecological stress memory and  
754 cross stress tolerance in plants in the face of climate extremes. *Environ. Exp. Bot.* 94,  
755 3–8. <https://doi.org/10.1016/j.envexpbot.2012.02.009>
- 756 Wildhagen, H., Paul, S., Allwright, M., Smith, H.K., Malinowska, M., Schnabel, S.K., Paulo,  
757 M.J., Cattonaro, F., Vendramin, V., Scalabrin, S., Janz, D., Douthe, C., Brendel, O.,  
758 Buré, C., Cohen, D., Hummel, I., Le Thiec, D., van Eeuwijk, F., Keurentjes, J.J.B.,  
759 Flexas, J., Morgante, M., Robson, P., Bogeat-Triboulot, M.-B., Taylor, G., Polle, A.,  
760 2018. Genes and gene clusters related to genotype and drought-induced variation in  
761 saccharification potential, lignin content and wood anatomical traits in *Populus nigra*.  
762 *Tree Physiol.* 38, 320–339. <https://doi.org/10.1093/treephys/tpx054>
- 763 Wilkinson, S., Davies, W.J., 2010. Drought, ozone, ABA and ethylene: new insights from cell  
764 to plant to community. *Plant Cell Environ.* 33, 510–525.  
765 <https://doi.org/10.1111/j.1365-3040.2009.02052.x>
- 766 Wilkinson, S., Davies, W.J., 2009. Ozone suppresses soil drying- and abscisic acid (ABA)-  
767 induced stomatal closure via an ethylene-dependent mechanism. *Plant Cell Environ.*  
768 32, 949–959. <https://doi.org/10.1111/j.1365-3040.2009.01970.x>
- 769 Wittig, V.E., Ainsworth, E.A., Long, S.P., 2007. To what extent do current and projected  
770 increases in surface ozone affect photosynthesis and stomatal conductance of trees? A  
771 meta-analytic review of the last 3 decades of experiments. *Plant Cell Environ.* 30,  
772 1150–1162. <https://doi.org/10.1111/j.1365-3040.2007.01717.x>
- 773 Wittig, V.E., Ainsworth, E.A., Naidu, S.L., Karnosky, D.F., Long, S.P., 2009. Quantifying the  
774 impact of current and future tropospheric ozone on tree biomass, growth, physiology  
775 and biochemistry: a quantitative meta-analysis. *Glob. Change Biol.* 15, 396–424.  
776 <https://doi.org/10.1111/j.1365-2486.2008.01774.x>
- 777 Zhou, P., Ganzeveld, L., Rannik, Ü., Zhou, L., Gierens, R., Taipale, D., Mammarella, I., Boy,  
778 M., 2017. Simulating ozone dry deposition at a boreal forest with a multi-layer canopy  
779 deposition model. *Atmospheric Chem. Phys.* 17, 1361–1379.  
780 <https://doi.org/10.5194/acp-17-1361-2017>
- 781
- 782

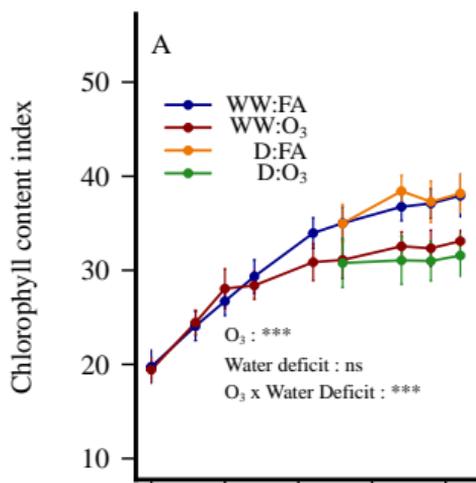
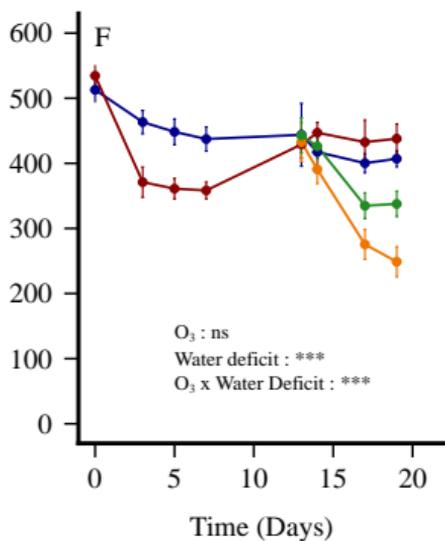
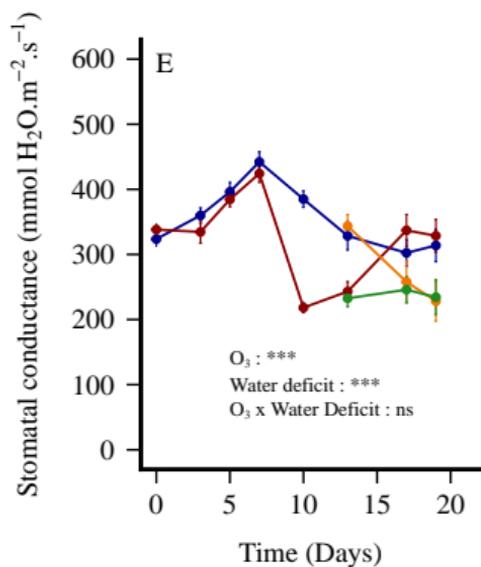
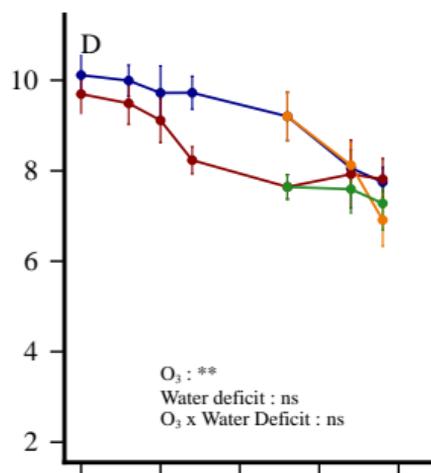
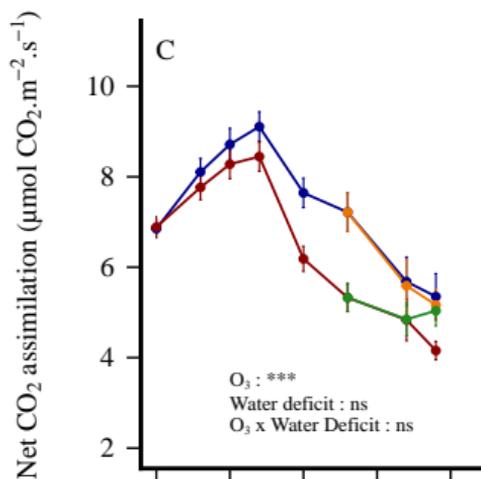
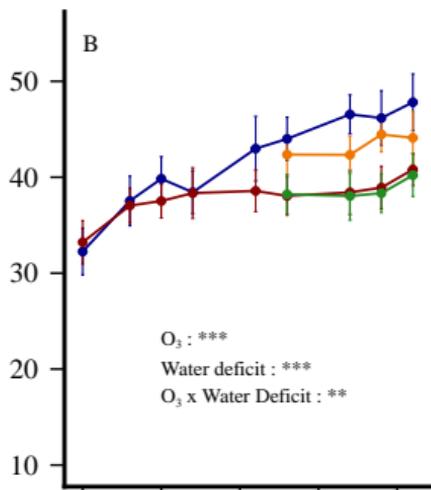
783 **Figure legends**

784 Figure 1: Impact of O<sub>3</sub> or/and water deficit on total chlorophylls (A, B), net CO<sub>2</sub> assimilation  
785 (C, D) and stomatal conductance to water vapour (E, F). Measurements were conducted on  
786 leaves of the Carpaccio and Robusta genotypes two to three times a week. For chlorophyll  
787 contents, results are presented in arbitrary units obtained with Dualex. Means ± se, n ≥ 4.  
788 Blue, WW:FA; orange, D:FA; red, WW:O<sub>3</sub>; green, D:O<sub>3</sub>. FA: filtered air; WW, well-watered;  
789 D, water deficit; O<sub>3</sub>, ozone. Asterisks indicate the significance of the factors or their  
790 interactions tested by a linear mixed-effect model: '\*\*\*\*'P ≤ 0.001, '\*\*'P ≤ 0.01, '\*'P ≤ 0.05,  
791 'ns' non-significant.

792 Figure 2: Sigmoidal model parameters of stomatal dynamics in response to irradiance changes  
793 in the Carpaccio and Robusta poplar genotypes after 13 days (A, B, E, F, I, J), or 21 days (C,  
794 D, G, H, K, L) when submitted to 80 ppb of O<sub>3</sub> for 13 days and/or water deficit for an  
795 additional week. (A, B, C, D): τ, response time(s); (E, F, G, H): λ, lag time(s); (I, J, K, L):  
796 SL<sub>max</sub>, maximum slope (mmol.m<sup>-2</sup>.s<sup>-2</sup>). Means ± se, n ≥ 4. Letters show the significance levels  
797 between treatments and genotype; ns, not significant. FA: filtered air; WW, well-watered; D,  
798 water deficit; O<sub>3</sub>, ozone. ANOVA, P-values are available in Table S4.

799 Figure 3: Sigmoidal model parameters of stomatal dynamics in response to VPD changes in  
800 the Carpaccio and Robusta poplar genotypes after 13 days (A, C, E) or 21 days (B, D, F)  
801 when submitted to 80 ppb of O<sub>3</sub> for 13 days and/or water deficit for an additional week. (A,  
802 B): τ, response time(s); (C, D): λ, lag time(s); (E, F): SL<sub>max</sub>, maximum slope (mmol.m<sup>-2</sup>.s<sup>-2</sup>).  
803 Means ± se, n ≥ 4. Letters show the significance levels between genotype and treatments (p <  
804 0.05); ns, not significant. FA: filtered air; WW, well-watered; D, water deficit; O<sub>3</sub>, ozone.  
805 ANOVA, P-values are available in Table S4.

806 Figure 4: Combined successive impacts of O<sub>3</sub> exposure and moderate water deficit on the  
807 parameters of the sigmoidal model for irradiance and VPD responses for the Carpaccio and  
808 Robusta genotypes. τ, response time; λ, lag time, and SL<sub>max</sub>, maximum slope; g<sub>0</sub> and G,  
809 steady-state values of stomatal conductance at the beginning and the end of the experiment.  
810 Bars represent the mean difference (± 95% confidence interval) between the observed and  
811 expected additive effects of the combined two stressors. The zero line represents the expected  
812 additive effects of the combined stressors. Additive effects are in white; when the means were  
813 greater or lower than zero, they were considered as synergistic (black) or antagonistic (grey),  
814 respectively. (Bansal et al., 2013).

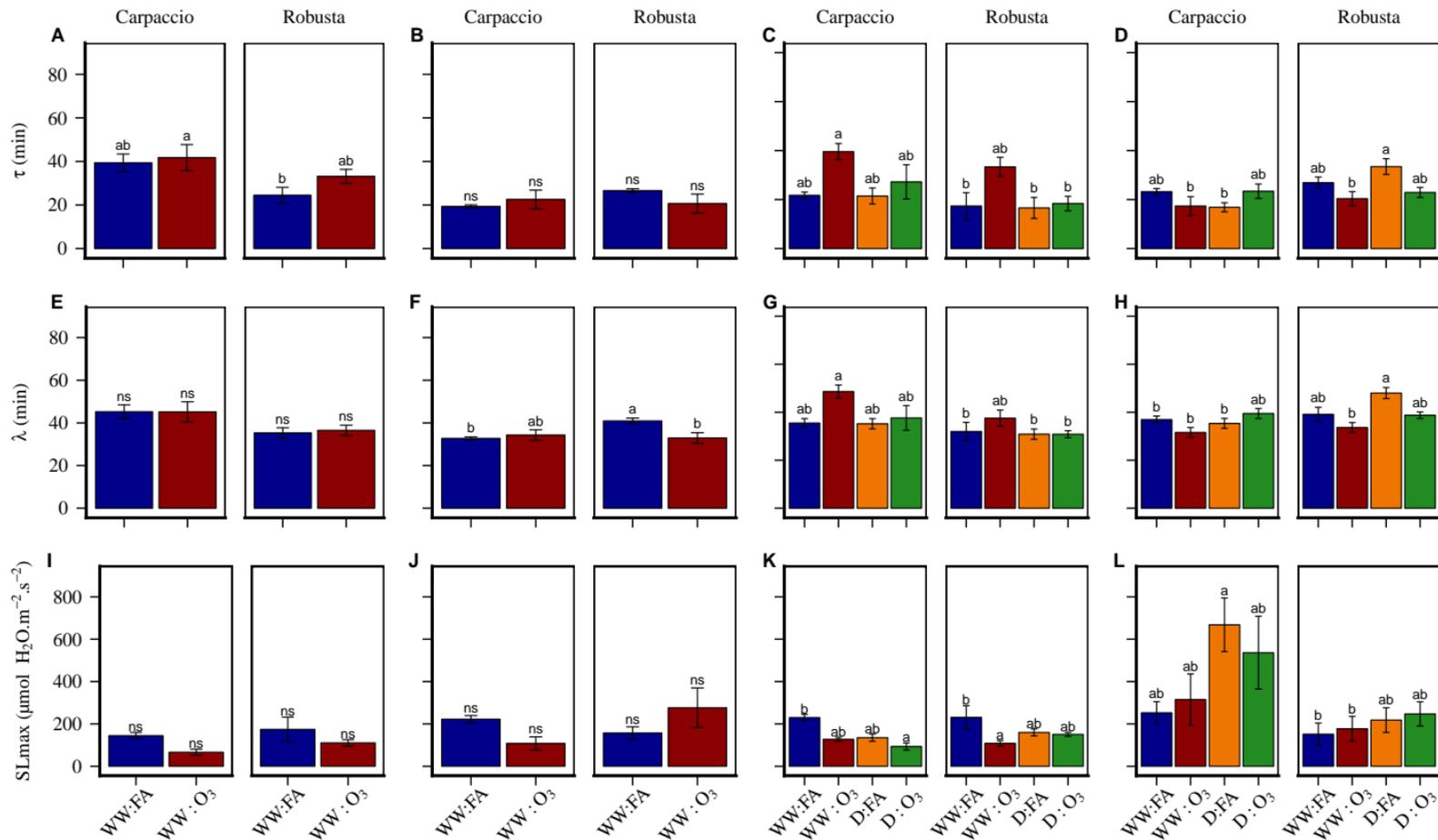
**Carpaccio****Robusta**

## Day 13 : Closing

## Day 13 : Opening

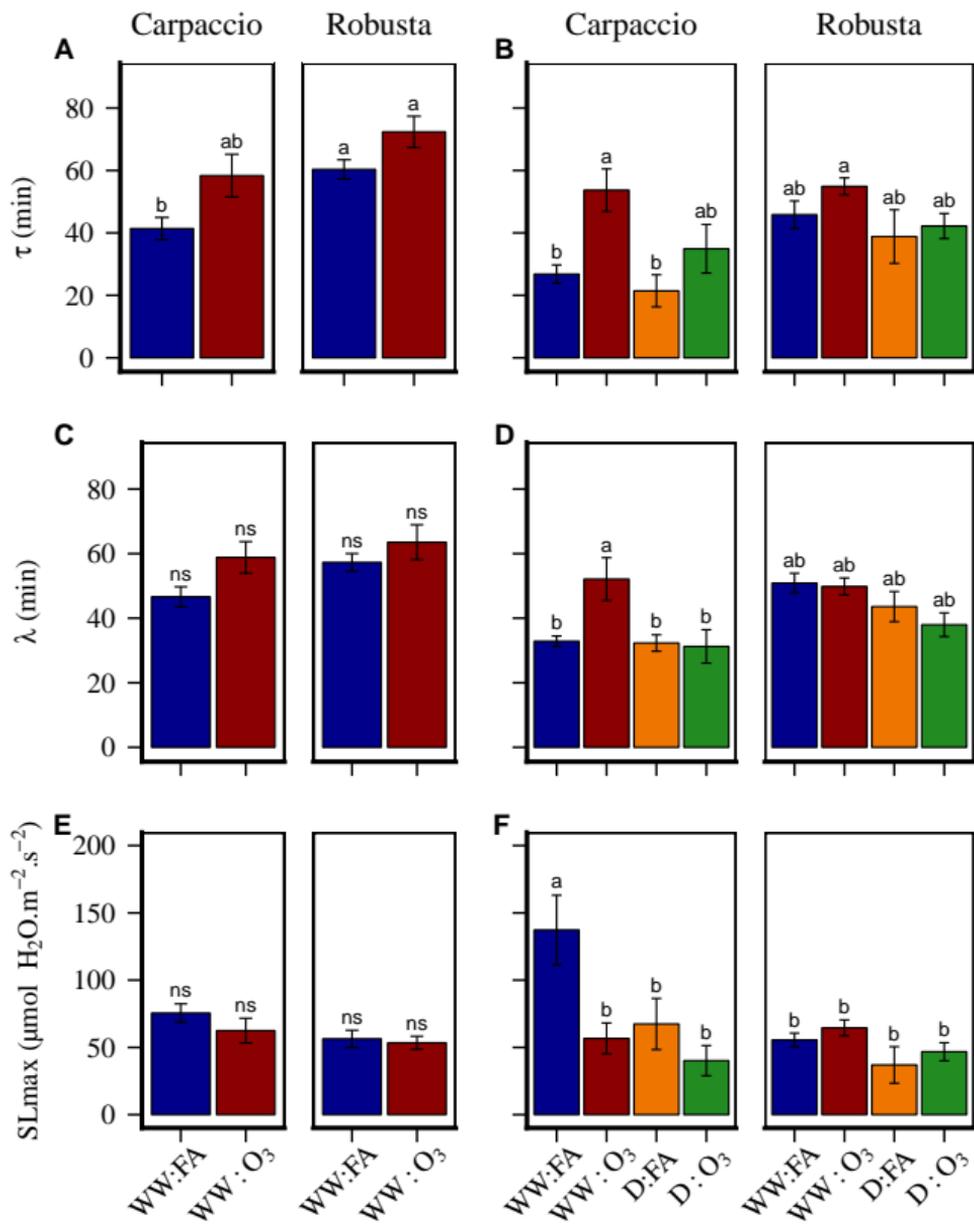
## Day 21 : Closing

## Day 21 : Opening



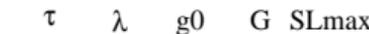
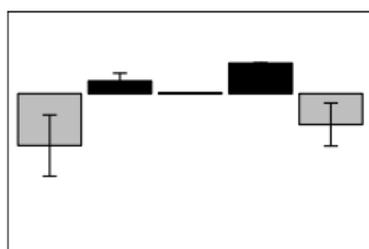
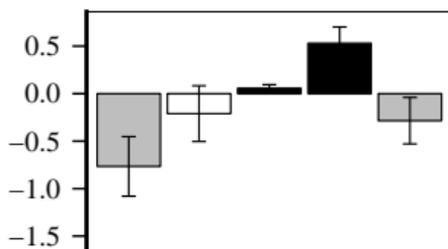
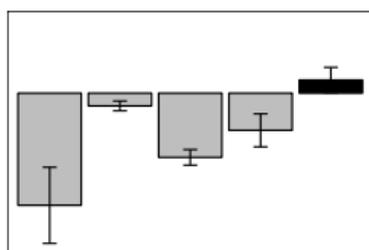
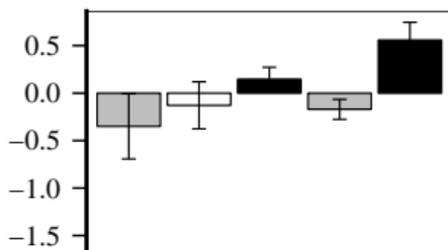
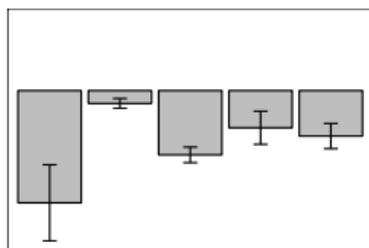
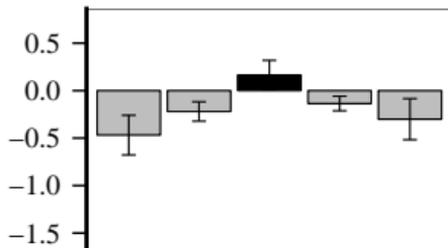
Day 13

Day 21



**Carpaccio****Robusta**

Difference between observed and expected effects



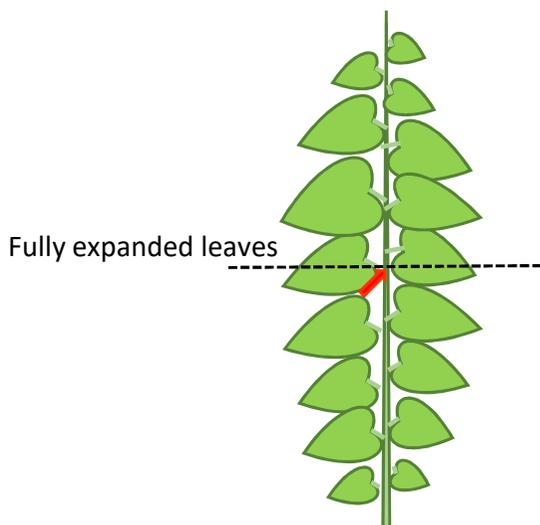
Closing light

Opening light

Closing VPD

Sigmoidal model parameters

*Populus euramericana*



Carpaccio      Robusta

Stomatal dynamics

