

1 **Delayed effect of drought on the xylem vulnerability to cavitation in *Fagus sylvatica* L.**

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8 **Abstract**

9 Knowledge on variations of drought resistance traits is needed to predict the potential of trees to adapt to severe
10 drought events expected to be more intense and frequent. Xylem vulnerability to cavitation is among the most
11 important traits related to drought-induced mortality and exhibits a large variability between species. Acclimation of
12 this trait to environmental conditions implies changes in the xylem structure and organization, leading previous studies
13 to investigate its variations under conditions preserving growth. In European beech saplings, we assessed the effect of
14 droughts of on the vulnerability to cavitation in branches that develop during recovery. The newly formed branches
15 displayed lower vulnerability to cavitation in the plants that underwent the severest droughts leading to native
16 embolism; the pressure that induces 50% loss of conductance being of -3.98 MPa in severely droughted plants
17 whereas it was of -3.1 MPa in control plants, respectively. Although unexpected, these results argue for an
18 acclimation, and not a weakening, of this trait to drought events.

19

20 **Keywords :** Cavitation, drought, hydraulic, xylem

21

22 **Key message :** severe water stress make the future developed shoots less vulnerable to xylem cavitation.

23

24 **Authors' contributions :** H.C., S.H. and T.S.B. designed the study and analysed the data. O.C. and T.S.B performed
25 measurements; S.H. contributed to some hydraulics measurements and H.C. performed the model simulations. S.H.
26 wrote the manuscript with H.C. and T.S.B.'s contributions. All authors approved this manuscript.

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28

29 **Introduction**

30 In a context of climate changes, the study of variations among and within species of drought resistance
31 mechanisms is needed to predict their potential of adaptation to drought events expected to be more intense and
32 frequent. For woody species, drought-induced dieback is more likely due to xylem hydraulic failure (Anderegg et al.
33 2015; 2016) caused by the cavitation events in the xylem conduits, even if the carbon starvation can also contribute to
34 this dieback (Hartmann 2015). A recent model (Martin-St Paul et al. 2017) has pointed out the importance of the
35 coordination between the water potential causing stomatal closure and the xylem pressure that provokes cavitation, i.e.
36 xylem vulnerability to cavitation (VC) (Martin-St Paul et al. 2017). This VC is considered as a critical trait for drought
37 tolerance in woody (Choat et al. 2012; Lens et al. 2013) and in herbaceous species (Lens et al. 2016; Volaire et al.
38 2018). Moreover, a global analysis pointed out the narrow hydraulic safety margin at which woody species commonly
39 operate (Choat et al. 2012), inferring that research is needed on the variability for xylem VC under the current climate
40 changes. Whereas a high variability of VC was reported between species in several studies, ranging from more than -1
41 to -19 MPa (Choat et al. 2012; Larter et al. 2015; Martin-St Paul et al. 2017), the extent of variation within species has
42 been reported to be considerably less, in a range usually lower than 1 MPa (e.g. Herbette et al. 2010). However, the
43 studies are rather few, and knowledge is lacking on the drivers of within species variability for VC, and thus on the
44 extent of this variability.

45 The genetic variability for VC is rather limited, in both natural populations (Wortemann et al. 2011; Lamy et
46 al. 2011) and cultivated genotypes (Jinagool et al. 2015; 2018). An uniform selection has been reported to explain the
47 weak variability for VC in a pine species (Lamy et al., 2011). The within species variation in VC is largely attributed
48 to environmental factors (Martinez-Vilalta et al. 2009; Herbette et al. 2010; Worteman et al. 2011). These factors
49 include sunlight exposure (Barigah et al. 2006; Herbette et al. 2010), nutrient availability (Harvey and Van Den
50 Driessche 1999; Plavcová and Hacke 2012), and mostly soil water contents (Awad et al. 2010). Acclimation of xylem
51 VC to environmental conditions implies changes in the xylem structure and organization. This trait relies on conduits
52 properties from intervessel pit to xylem structure (Lens et al. 2013). Changes in xylem anatomy can occur only in the
53 growing period during wood formation, as xylem conduits are made of dead cells with no short-term capacity to
54 acclimatize or adjust to changes in hydraulic demand. For example, no change in VC was observed for beech branches
55 across contrasted seasons along a growing season, or for branch samples put in humid and dark conditions during
56 several weeks (Herbette et al. 2010). A decrease in stem VC related to drier conditions has been observed during the
57 growing period, providing that the growth is maintained (Awad et al. 2010; Fichot et al. 2010; Plavcová and Hacke

58 2012). Yet, the climate change models predict further increases in the frequency and the severity of extreme drought
59 events (IPCC 2013), and such drought events occur mainly during the dry season when there is few or no more
60 growth, and if there is, it stops the growth. Knowing the effects of severe drought conditions on the VC, as well as on
61 other drought-resistance mechanisms, may help us to predict the response of woody species impact to climate
62 changes.

63 In this study, we assessed the impact of different duration of water deprivation on VC of branches developed
64 after resumption from water shortage. The “Cavitron” centrifugal technique (Cochard et al. 2005) is an effective,
65 rapid method for large-scale investigation of VC (Lamy et al. 2011; Wortemann et al. 2011; Cochard et al. 2016),
66 especially for beech samples (Herbette et al. 2010; Wortemann et al. 2011; Barigah et al., 2013 Stojnic et al. 2017),
67 and could thus be used in this study. We carried out the experiment on European beech (*Fagus sylvatica* L.) saplings
68 with water stress applied at the end of spring i.e. after the growth has stopped. It is a widespread species of the
69 European forest and known to be sensitive to drought (Bréda et al.2006). We thus assumed that a severe drought
70 would make the new branches more vulnerable to cavitation as the carbon reserve would be impaired and thus would
71 impact the development of new axes. Indeed, it is well established that there is a carbon cost involved in developing
72 cavitation-resistant xylem (Hacke and Sperry 2001).

73

74 **Materials and Methods**

75 *Plant material and growth conditions*

76 We carried out the experiment in a greenhouse at the INRA research station of Clermont-Ferrand in France
77 (45877'N, 3814'E; altitude of 300 m) during 2008 and 2009. Plant material and growth conditions were previously
78 described in Barigah et al. (2013). We excavated from a nursery stand and transplanted saplings of European beech
79 (*Fagus sylvatica* L.) of two-years old in November 2007 into 20 liters pots filled with a 1:2 (v/v) mixture of peat and
80 local forest soil. The 159 saplings grew up in a greenhouse with daily irrigation to field capacity (FC) from planting to
81 the beginning of the water-stress treatment. We set up a drip irrigation controlled by a timer to supply the plants with
82 tap water. We applied a severe water-stress to 144 individuals from June 30th 2008 by ceasing watering them for 1 to
83 12 weeks (12 individuals per week treatment), except to a set of 15 control plants we watered at field capacity. At the
84 end of each of the 12 drought periods, the plants were re-watered to FC after data collection completed (Barigah et al.
85 2013). Then, we kept all the plants at FC after the resumption from the water shortage, in the same growth conditions
86 than before the drought. While most of the plants were used for sampling in Barigah et al. (2013) or died because of

37 the drought severity, 6 plants per treatment were kept from any cutting and recovered in 7 treatments: control and
38 drought duration from 1 to 6 weeks. Among these latter, 4 to 6 plants per treatment developed new branches of at least
39 0.3m long after the drought that were harvested on July 2009 for measuring their VC.

30 *Stomatal conductance and native embolism*

31 Leaf conductance to water vapour (g_s) was measured each week during the drought treatment with a steady-
32 state porometer (Li-1600, Li-Cor, Lincoln, Ne, USA) equipped with a broad leaf chamber. Measurements were
33 performed on leaves from 5 to 40 different individual saplings per water treatment. Minimal g_s was measured between
34 12:00 and 14:00 solar time on sunny days. Depending on the climatic conditions when measuring g_s , we could observe
35 variations in data, leading us to increase the number until 40 individual saplings per condition.

36 Native embolism measurements were detailed in *Barigah et al. (2013)*.

37 *Vulnerability to cavitation*

38 Intact stems of at least 0.3m long were defoliated and then harvested from 4 to 6 individual saplings for each
39 drought duration. Stems were wrapped in moist paper, placed in plastic bags to avoid dehydration, and cold-stored
40 (4 °C) for not more than 7 d before analysis of their VC. Segments of 0.28 m-long were recut using a razor blade to
41 refresh the ends, just before the measurement of their VC by using the Cavitrone technique (Cochard et al. 2005).
42 Xylem pressure (Ψ_x) was first set to a reference pressure (-0.5 MPa) and the sample maximal conductance (K_{max}) was
43 determined. The xylem pressure was then set to a more negative pressure for 2 min and the new conductance (K) was
44 determined. The procedure was repeated for more negative pressures (with -0.25 or -0.50 MPa increments) until PLC
45 reached at least 90%. Vulnerability curves were generated for each species as plots of PLC versus Ψ_x . Data were then
46 fitted with the following exponential–sigmoidal functions:

$$PLC = \frac{100}{1 + \exp(s/25(P - P_{50}))}$$

37 Where P_{50} is the pressure causing 50% loss of conductance and s is the slope value at this point.

38 *Model simulations*

39 To simulate the impact of the plasticity of P_{50} on plant drought resistance, we used the mechanistic SurEau model
40 (Martins St-Paul et al 2017, Cochard 2019) to predict the timing of hydraulic failure under standardized conditions.

41 The model was parametrized to match as much as possible the characteristics of the plant material used in this study
42 and the environmental growth conditions. In more details, the key plant parameters of the simulation were the
43 following: leaf area: 0.1 m²; shoot length: 0.6 m; basal diameter: 0.6 cm; maximum stomatal conductance: 300

14 mmol/s/m²; minimum cuticular conductance: 2 mmol/s/m²; Leaf Π_0 : -2.0 MPa; maximum whole plant conductance:
15 0.28 mmol/s/m²/MPa. The P₅₀ and s parameters were identical for all the plant organs, and P₅₀ was allowed to vary
16 between -3 to -4MPa (see results). The environmental conditions were set as follow: night/day air temperature:
17 15/25°C; night/day RH: 80/40%; PAR: 650 μ mol/s/m²; wind speed: 1m/s; soil volume: 20 dm³; available soil water
18 content: 7.2 kg. We assumed that stomata closed during water stress in response to changes in leaf turgor pressure
19 (Martin St-Paul et al 2017). The stimulation started at midnight with a soil at full water capacity and the plant were let
20 to transpired until 90% of embolism was formed in the stem, that was considered as the point of hydraulic failure.

21 *Statistics*

22 An analysis of variance (ANOVA) was used to test the effects of watering regimes on VC. Means were
23 compared with Tukey's multiple range with P-value < 0.05, after checking homogeneity and normality of the data. All
24 the measured and derived data were subjected to the statistical analysis using a software package (XLSTAT v7.5.3,
25 Addinsoft, Paris, France).

26

27 **Results and discussion**

28 The beech sapling were submitted to a drought by ceasing watering for up to 12 weeks. The stomatal
29 conductance declined starting from the first week of the drought, then it reached very low and constant values after 3
30 weeks (fig. 1), indicating a stomatal closure starting at this time. We could not measure the g_s beyond 7 weeks of
31 drought as the leaves dessicated. The rate of native embolism started to increase 3 weeks after the water-stress
32 inception and reached their maximal values starting from the 9th week (fig. 1). After each week of drought, a set of
33 plants were rewatered to full capacity up to the following year. This experimental device allows assessing the delayed
34 effects of a wide range of water stress from the weak decrease in g_s to the death, through the stomatal closure point
35 and the accumulation of embolism.

36 We selected plants that recovered and that were not previously cut for sampling. All plants were let to finish
37 their growth period, with watering at field capacity, to ensure that there is no delay in growth between sets of plants.
38 Then, VC was measured on branches that newly formed under watering at field capacity. For control plants, the VC
39 increased from -3.43 MPa (+/- 0.17) just before the drought experiment to -3.10 MPa (+/- 0.30) in newly formed
40 stems in the following year. Such difference can be explained by difference in growth conditions and physiological
41 status of the plants between the 2 years. To avoid such effects, the drought effect was analyzed by comparing newly
42 formed stems of the different treatments.

43 The figure 2 shows the P_{50} mean values for these branches depending on the drought duration the plants
44 underwent on the previous year. The P_{50} decreased from -3.10 to -3.98 MPa for 6 weeks of drought, and mean values
45 became significantly different from control plants after 4 weeks of drought. Our results clearly demonstrate that beech
46 sapling developed new branches with lower VC after the plants underwent severe drought condition that leads to
47 hydraulic failure. The lowest VC was obtained after 6 weeks of drought and no death was observed in this condition
48 (Barigah et al. 2013). Thus, a selection effect of the drought can be rule out and led us conclude that the change in VC
49 was only due to phenotypic plasticity. We could not analyzed the VC for longer drought periods as part or most of the
50 plants died (Barigah et al. 2013). Our results would explain how marginal beech populations have a lower VC and are
51 drought-tolerant (Stojnic et al. 2017; Bolte et al. 2017), whereas there is little or no difference in VC between core
52 populations. Indeed, marginal populations in drier conditions are more prone to undergo severe water deficit that
53 would induce a decrease in VC. The extent of this decrease in P_{50} is rather large (0.88 MPa or 28% of the control
54 value) when compared to variations observed for this species due to other environmental conditions (Barigah et al.
55 2006; Herbette et al. 2010), and would have a significant impact on the drought resistance of this species. We tested
56 this impact using the hydraulic soil-plant model proposed by Martin-St Paul et al. (2017) and Cochard (2019) that
57 predicts the time to plant hydraulic failure. The model parameters were first turned to match the dynamics of PLC and
58 g_s shown in figure 1 and considering a P_{50} of -3.1 MPa. We then ran simulations with the same set of parameters,
59 allowing only P_{50} to change according to the values reported in figure 2. Simulations showed that a decrease of P_{50}
60 from -3.10 to -4 MPa would delay by 17 days the timing of hydraulic failure, and thus shoot mortality (fig. 3). The
61 next step will be to assess the delayed effect of drought on several drought-related mechanisms, in order to investigate
62 the potential of adaptation to drought events using the same model.

63 This drought-induced decrease in VC is rather surprising, as we assumed that drought would increase the VC
64 as the carbon assimilation is impaired. The droughted plants had a lower growth (data not shown) when compared to
65 control, suggesting that the acclimation for VC was prioritized for carbon allocation over the growth. The challenge
66 will be to elucidate the mechanism underlying this delayed effect, especially because this acclimation occurs in newly
67 formed branches that did not underwent the drought.

68

69 **Conflict of interest:** The authors declare that they have no conflict of interest.

70 **References**

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41 Europe. *Tree Physiol* 31: 1175-1182.

42 **Figure Legends**

43 **Fig. 1 Evolutions of leaf stomatal conductance (g_s) and embolism level (PLC) during the drought**

44 Surveys of g_s (black square) and PLC (open circle) were carried on leaves and stems, respectively, of beech saplings
45 that underwent a drought by irrigation stop up to 12 weeks. No g_s could be measured beyond 7 weeks as the leaves
46 were completely dry. PLC data were from Barigah et al. (2013). Data are mean values ($n = 5$ to 40 for g_s and $n = 6$ or
47 7 for PLC) and errors bars are S.E.

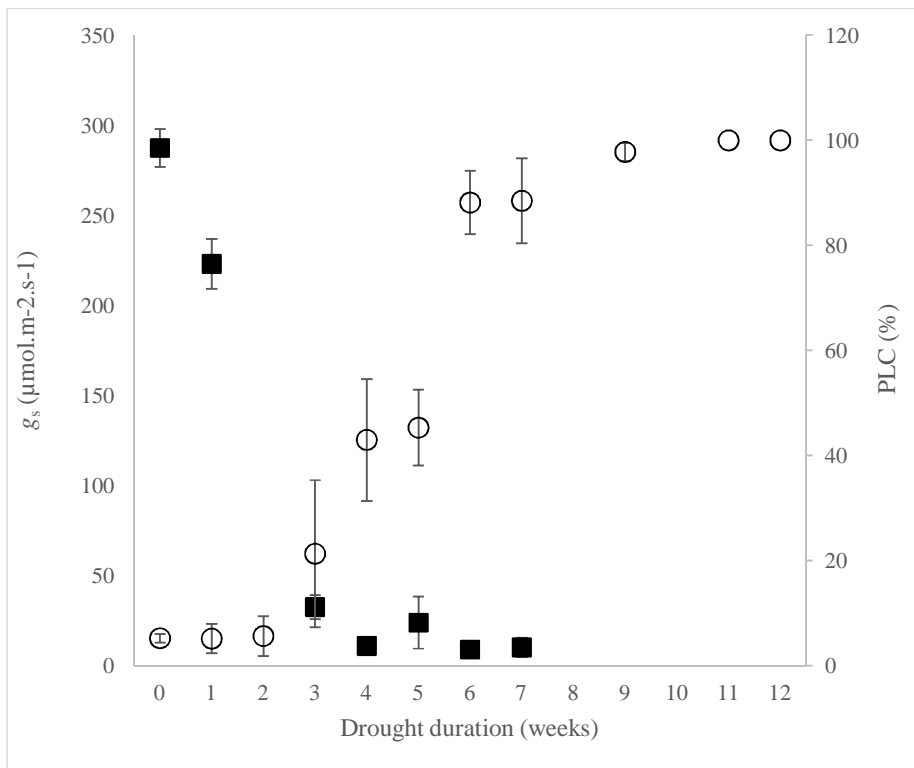
48 **Fig 2 Vulnerability to cavitation (P_{50}) of branch developed after droughts of different duration**

49 Vulnerability to cavitation was measured on branches that developed the year after the beech saplings underwent a
50 drought by withholding up to 6 weeks. No data were recorded for saplings assigned to more than 6 weeks of water
51 stress since some or most of the plants did not recover. Data are mean values ($n = 4$ to 6) and errors bars are S.E. Data
52 were analyzed using ANOVA tests and Tukey's tests ($P < 0.05$). Different letters indicate significant differences for
53 P_{50} between the drought spans.

54 **Fig. 3 Model simulation of the time to reach hydraulic failure for the range of VC observed in this study.**

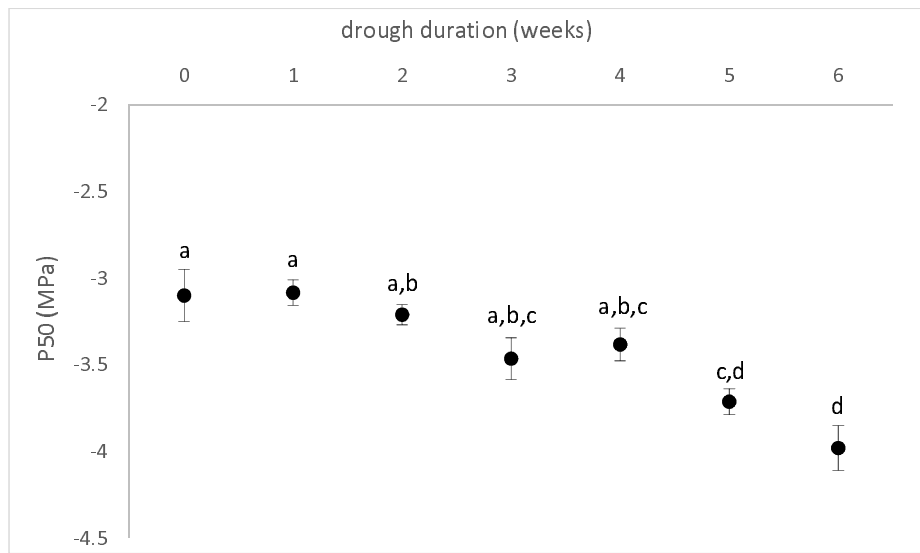
55 The model developed by Martin St Paul et al. (2017) was used to estimate the time to reach 90 % loss of conductance
56 (leading to death according to Barigah et al. 2013) depending on the VC the branch developed in this study. With the
57 exception of the changes in P_{50} made to satisfy the hypothesis tested, all other parameters were kept constant.

58 Figure 1



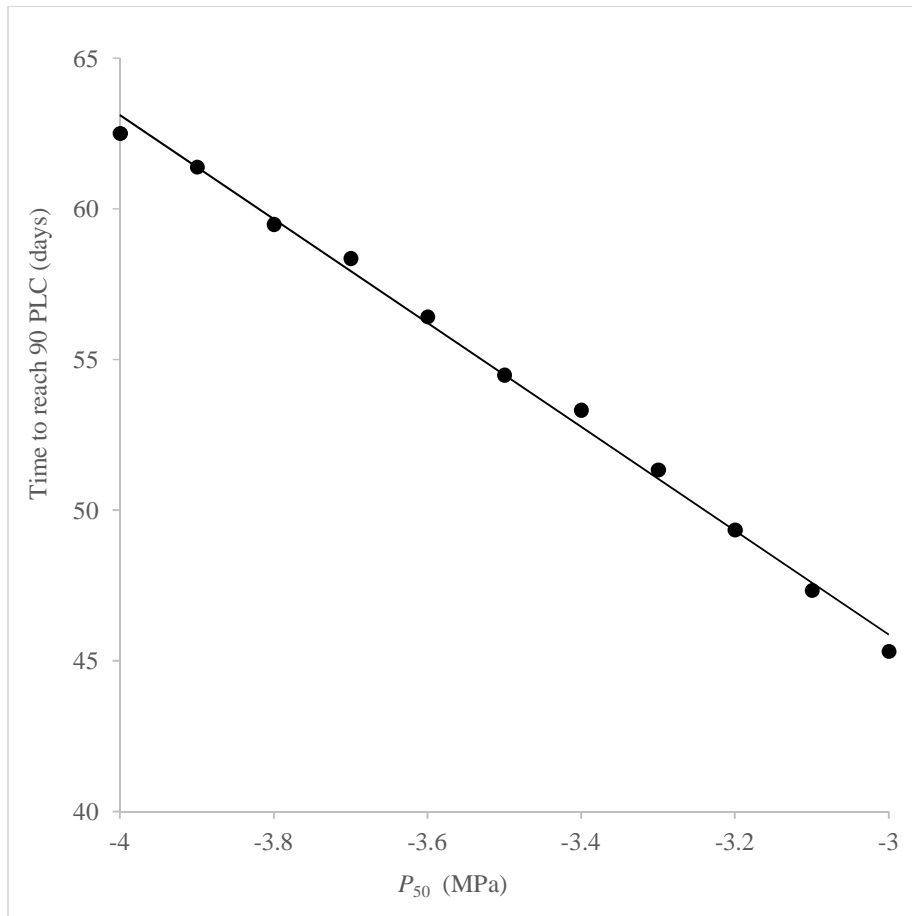
59

50 **Figure 2**



51

52 **Figure 3**



53