Insights into xylem vulnerability to cavitation in *Fagus sylvatica* L.: phenotypic and environmental sources of variability

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**Summary**  Xylem vulnerability to cavitation is a key parameter in understanding drought resistance of trees. We determined the xylem water pressure causing 50% loss of hydraulic conductivity (*P*<sub>50</sub>), a proxy of vulnerability to cavitation, and we evaluated the variability of this trait at tree and population levels for *Fagus sylvatica*. We checked for the effects of light on vulnerability to cavitation of stem segments together with a time series variation of *P*<sub>50</sub>. Full sunlight-exposed stem segments were less vulnerable to cavitation than shade-exposed ones. We found no clear seasonal change of *P*<sub>50</sub>, suggesting that this trait was designed for a restricted period. *P*<sub>50</sub> varied for populations settled along a latitudinal gradient, but not for those sampled along an altitudinal gradient. Moreover, mountainside exposure seemed to play a major role in the vulnerability to cavitation of beech populations, as we observed the differences along north-facing sides but not on south-facing sides. Unexpectedly, both north-facing mountainside and northern populations appeared less vulnerable than those grown on the southern mountainside or in the South of France. These results on beech populations were discussed with respect to the results at within-tree level.

**Keywords:** drought tolerance, European beech, hydraulic, phenotypic plasticity, populations, xylem embolism.

**Introduction**

Global warming models predict that drought will become an increasingly important factor limiting tree growth and survival. Knowledge of factors enabling tree species to occupy different habitats is therefore important. According to *Bazzaz* (1996), a high degree of ecological variation, i.e., a wide range of different habitats potentially occupied by a species, can be achieved by various means: the species can show a high degree of genetic variability, comprising genotypes especially adapted to different habitats, or the tree can exhibit phenotypic plasticity in different environments. European beech (*Fagus sylvatica*) is a widespread species of the European temperate forest with a natural range of distribution extending from southern Sweden to central Italy, western France, northern Portugal and central Spain and eastern to north-western Turkey. It dominates natural forests in dry to humid conditions and requires a humid atmosphere and a well-drained soil (Rose et al. 2009). In the Mediterranean basin, beech grows in mountainous regions where rainfall is high, just barely within the limit of its requirements (García-Plazaola and Becerril 2000). In France, beech forests and/or stands occupy broad geographic and altitudinal ranges from plains at sea level up to 1700 m a.s.l. Because of its broad ecological amplitude, European beech is expected to contain variability for many traits such as resistance to cavitation events by its xylem.

It is now clear that xylem dysfunction induced by drought is a serious threat to plant productivity and survival. Comparisons of species growing in contrasting environments suggest that increased cavitation resistance is a key component of species growing in drought-prone habitats (Tyree and Ewers 1991, Pockman and Sperry 2000, Cochard et al. 2008). Correlations between drought resistance and xylem vulnerability to cavitation have been found in groups of woody species with contrasting ecological preferences (Nardini et al. 2000, Sperry and Hacke 2002, Pita et al. 2003, Tyree et al. 2003, Maherali et al. 2004). Xylem vulnerability to cavitation is usually described through vulnerability curves, i.e., the plot of percent loss of xylem hydraulic conductivity against xylem tension. For more than two decades, vulnerability to cavitation has been seen as an intrinsic property of the conductive elements of the xylem (Tyree and Ewers 1991, Tyree et al. 1994, Cochard 2006) and can be evaluated without submitting the plant material to a long period of drought. Consequently, xylem...
vulnerability to cavitation has widely been documented across species and has been shown to vary greatly between species (Tyree and Ewers 1991, Maherali et al. 2004, Bréda et al. 2006, Cochard 2006, Froend and Drake 2007, Brodribb and Cochard 2009). To date, reports at the intra-specific level are much scarcer, but resistance to cavitation can vary significantly between populations of the same species (Alder et al. 1996, Sparks and Black 1999, Choat et al. 2007) or between closely related genotypes (Vander Willigen and Pammenter 1998, Sangsing et al. 2004, Cochard et al. 2007), and between organs of the same plant (Tyree et al. 1993, Sperry and Saliendra 1994, Hacke and Sauter 1995, Cochard et al. 1999).

We hypothesized that vulnerability to cavitation varies in tree populations according to their water availability. Recently, we demonstrated in a poplar clone that this trait acclimates to drier soil conditions (Awad et al. 2010). Here, we examined the variability of vulnerability to cavitation in beech at the within-tree level and between in situ populations. First, we assessed the within-tree variation in vulnerability to cavitation due to light and seasonal effects. Then, we compared in situ populations along altitudinal and latitudinal gradients for their vulnerability to cavitation. We hypothesized that vulnerability to cavitation should vary in beech forests both with elevation and along a latitudinal gradient as mountains are steep climate gradients, the altitudinal gradient is often claimed to mirror the latitudinal one (Rahbek 1995, Fosaa 2004).

Materials and methods

Vulnerability to xylem cavitation

We used the Cavitron technique (Cochard et al. 2005) to measure vulnerability to cavitation of stem segments. This technique uses centrifugal force to increase tension in a calibrated stem segment, while variations in its xylem hydraulic conductance are measured. Samples (0.7–0.8 cm in diameter and 28 cm in length) were cut in the air from 50-cm-long stem segments, placed on a custom-built rotor and spun at set velocities. Maximal conductance ($k_{max}$) was determined by setting the xylem pressure to a reference value of ~1.00 Mpa, without having flushed them previously. Xylem pressure was then set to gradually more negative values by 0.50 MPa steps. For each new xylem pressure, a hydraulic conductance ($k_p$) value was measured at equilibrium and the percent loss of conductance (PLC) per sample was computed as $PLC = 100 \times (1 - k_p/k_{max})$. This procedure was repeated until PLC reached at least 90%. The rotor velocity was monitored with an electronic tachometer (10 r.p.m. resolution) and xylem pressure was adjusted to approximately ±0.02 MPa. The dependence of PLC on xylem pressure was used to generate vulnerability curves for each stem segment, which were fitted to the following sigmoid function (Pammenter and Vander Willigen 1998):

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

where $P$ is the water potential, PLC is the corresponding percent loss of conductivity, $P_{50}$ is the negative pressure causing 50% loss of conductance and $s$ is the slope of the curve at its inflection point.

Plant material

Current year plant material from differently aged European beech trees grown in different, contrasting environments were used, according to the questions addressed. Except where specified, we used full sunlight stem segments. They were collected between 9:00 a.m. and 12:00 noon. We used a pruning pole to sample stem segments 50 cm long in the top half of each branch or shoot, according to the tree height. These stem segments were promptly defoliated, wrapped in a wet soft tissue and enclosed in a humid black plastic bag to minimize dehydration and prevent non-steady-state embolism occurring. We brought the stem segments to the laboratory and stored them at 5 °C for up to 30 days, i.e., until measurements were completed.

In August 2006, we collected samples within 2 h from a single 7-m-high tree growing in a deciduous Allagnat forest location, to evaluate the length of time stem segments could be stored without impacting on their $P_{50}$ value. To avoid light acclimation effects, we harvested only full sunlight-exposed branches. The samples were wrapped in humid paper and kept at 5 °C in a sealed black plastic bag. We used a set of six to six stem segments stored for 0, 3, 6, 7, 8 and 10 weeks to determine $P_{50}$ values in this experiment.

To analyse the effects of light together with seasonal variations in vulnerability to cavitation, four mature trees were chosen in a deciduous forest at the Allagnat location (Table 1). The selected trees grew at the edge of the forest with southern branches fully exposed to sunlight, whereas...
northern ones were deeply shaded by upper branches and (or) surrounding trees during the growing season. For each light exposure condition, three to five stem segments were collected at different locations in each tree to avoid spatial positioning effects between sampling periods. In these experiments, we used segments that were more than 1 or 2 years old, as the current year shoots were too short to fit the customized centrifuge rotor, especially the shade-exposed ones. $P_{50}$ data collected from the four trees were pooled, and their means and standard errors (SEs) were calculated. For two out of the four trees, we carried out a survey of vulnerability to cavitation for both light- and shade-exposed stem segments ($n = 4$ or $5$) for 11 sampling dates between April 2007 and August 2008. The mean and SEs of their $P_{50}$ values were calculated for each tree on each sampling date.

During the 2006 growing season, we selected four beech populations located along a latitudinal gradient from northern to southern France (Graouilly, Hesse, Allagnat and Mount Ventoux; Table 1) to investigate the difference in vulnerability to cavitation between in situ populations. The samples, collected from 10 adult tree branches and 9 stems from juveniles in Allagnat, were also used to check for the effects of tree stature on vulnerability to cavitation. As no difference was found between juvenile and adult plants, we pooled the data. We also pooled the data for the Mount Ventoux populations, as a comparison of vulnerability to cavitation made at five elevations along a northern altitudinal gradient revealed no difference in $P_{50}$ for the whole set of samples. One stem segment per tree from 8, 9, 19 and 50 trees in the Graouilly, Hesse, Allagnat and Mount Ventoux sites were, respectively, sampled (Table 1) and the mean $P_{50}$ values with their SEs were calculated. These mean and SE values were used as a proxy for each given site. On 14 June 2008, we again harvested six stem segments from different trees at three elevations on north-facing and south-facing mountainsides at the Mount Ventoux site to check for mountainside exposure effects in variability in vulnerability to cavitation. In this site, the sampling was carried out >900 m a.s.l. because only scattered beech trees were available below this baseline.

Statistical analysis

We carried out analysis of variance (ANOVA) to determine whether $P_{50}$ means are significantly different between populations settled along latitudinal and altitudinal gradients. In case of significant differences, we refer to the Tukey’s multiple range test at $P < 0.05$ to compare the mean values. To analyse the light effects together with intra-annual variations in $P_{50}$, a repeated ANOVA was performed based on the factors such as ‘light’ and ‘time’. Then, for each factor, the mean values were compared using the Tukey’s multiple range test at $P < 0.05$. We carried out regression analysis to check whether the slope is significantly different from zero at $P < 0.05$ to test samples for any age effect and the conservation delay.

All the measured and derived data underwent statistical analysis using the Statgraphics plus 4.1 software package.

Results

Age effect and conservation delay of severed samples

No significant difference in $P_{50}$ values was observed regarding the age effect of the stem segments (Figure 1a). We also found that beech stem segments stored for 10 weeks at 5 °C in humid dark conditions were still suitable for $P_{50}$ determination (Figure 1b), probably because xylem conduits and the bulky xylem tissue are made of dead cells. Thus, storing stem segments for weeks at low temperature constitutes a great issue for large-scale screening campaigns of vulnerability to cavitation, such as those carried out in this study.

Phenotypic variation of vulnerability to cavitation

Figure 2 shows that the full sunlight-exposed stem segments were significantly less vulnerable to cavitation than the shade-exposed ones. When both types of stem segment of a given tree were considered separately, they differed significantly in their vulnerability to cavitation over the timespan of the survey, except for a few sampling dates (Figure 3). Nevertheless, the full sunlight-exposed stem segments
Variability in vulnerability to cavitation between populations along altitudinal and latitudinal gradients

Values of $P_{50}$ differed significantly between most of the populations (Figure 4) growing in France along a north-east to south-east latitudinal gradient (Table 1). The population from the centre of France had intermediate values of $P_{50}$ when compared with both northern ($-3.77$ MPa) and southern ($-2.40$ MPa) ones. The largest difference in $P_{50}$ (1.37 MPa) was found between the populations of the Mount Ventoux (southern) and Graouly (northern) sites. Unexpectedly, the $P_{50}$ values of beech harvested at the southern site were lower than those from the central and southern sites. At the central site, vulnerability to cavitation was similar for stems of juveniles and branches of adult trees. Hence, plant stature probably plays only a minor role in determining beech $P_{50}$.

Figure 5 shows that populations located on south-facing mountainsides along an altitudinal gradient of Mount Ventoux displayed no statistical changes in their vulnerability to cavitation. In contrast, populations from the north-facing mountainside differed in $P_{50}$ in some cases.

Discussion

Phenotypic plasticity of vulnerability to cavitation

We have shown that vulnerability to cavitation in $F. sylvatica$ varies with the exposure of the shoots to sunlight, the shade-exposed stem being more vulnerable to cavitation than the sun-exposed one (Figures 2 and 3). Our results are in line with those previously reported (Cochard et al. 1999; Barigah et al. 2006). To grow, European beech, as a shade-tolerant tree species, can take advantage of changes in irradiance, such as those associated with mortality of overstory trees (Stickan and Zhang 1992). Consequently, the tree needs to acclimatize its hydraulic features to use the available light efficiently for carbon gain and resume rapid growth. A high transpiration rate requires a hydraulic system able to sustain large water fluxes, together with the ability to tolerate large water potential differences in the xylem conduits (i.e., low xylem vulnerability to cavitation, Tyree and Ewers 1991). Thus, full sunlight-exposed stems need to be designed with low vulnerability to cavitation so as to support increases in the stomatal conductance and transpiration rate of their leaves.

Regarding the seasonal variations in vulnerability to cavitation, we observed many occurrences of differences between stem segments with different light exposure (Figure 3a and b). These observations suggest that the light exposure of the stems and/or branches played a major role in their hydraulic architecture. Full sunlight- and shade-exposed stem segments generally differed in their $P_{50}$. However, this observation was unexpected given the environmental conditions, which were prone to significant changes between leafy and leafless periods. During leafless periods, the evaporative demand of the air was assumed to be low and the soil water availability was assumed to be high. The changes in xylem anatomy can occur only in the growing season during wood formation, as xylem conduits are made of dead cells with no short-term capacity to acclimatize or adjust to changes in hydraulic demand. This would explain why no variation in vulnerability to cavitation was observed for samples kept for 5 weeks in humid and dark conditions (Figure 1). Thus, an environmental event such as a frost or a spell of drought that occurs in spring at the time of early wood vessel formation can directly impact on cells undergoing differentiation and thus leave an imprint inside xylem conduits.

We also observed the variations in vulnerability to cavitation in 2007, but the patterns were not similar for the two trees in question (Figure 3). This difference was difficult to explain, as the trees were close to each other and so were assumed to be growing under the same climatic conditions. The observed discrepancy may result from soil heterogeneity, but this did not fit the assumption that in time series of different individuals, the existence of common variability is caused by the influence of a given environmental factor (Fonti et al. 2010).
on two different sites (Figure 5). Such a within-species variability can be attributed to the large ecological amplitude of the beech. In contrast, *Pinus sylvestris*, which is roughly as sensitive as beech, showed limited variability in vulnerability to cavitation between and within populations (Martinez-Vilalta et al. 2009). Such between-population differences in beech can be driven by phenotypic plasticity depending on the environmental conditions and genetic differentiation. According to Hamrick (2004), most of the genetic variation for most traits resides within populations, with few differences between populations. This assumption suggests that environmental factors play a major role in the observed variations between populations for vulnerability to cavitation. We found that beech populations from north-east France were more drought resistant to cavitation than those from the south-east (Figure 4). Although the genetic origins of our beech populations were not fully known, these results are unexpected. Indeed, the southern French populations occurred at the natural southern limit of beech distribution in a Mediterranean climate (Parelle et al. 2006). This area is characterized by its long droughts, high evaporative demand and energy balance in summer, which would be expected to be detrimental to beech growth and survival. In *P. sylvestris*, the vulnerability to cavitation was not associated with global climate dryness (Martinez-Vilalta et al. 2009). There are two explanations for such a discrepancy between vulnerability to cavitation and the locations of beech populations. First, growth processes may have been favoured for drought resistance instead of vulnerability to cavitation. Many studies have investigated the drought sensitivity of beech populations along geographical gradients of precipitation (Garcia-Plazaola and Becerril 2000, Nielsen and Jorgensen...
which take place frequently in these areas. Secondly, we observed, on the one hand, that vulnerability to cavitation did not vary much throughout the seasons (Figure 2) and, on the other hand, that no change in vulnerability to cavitation occurs over the 10 weeks the severed stems were stored in humid and dark conditions (Figure 1b). Because vulnerability to cavitation might rely on the environmental conditions prevailing during xylem vessel formation, differences in vulnerability to cavitation between populations depend on environmental conditions of both limited time and their respective phenology. To support this, we recently showed that vulnerability to cavitation acclimates to drier soil conditions if poplar trees are grown under different water regimes (Awad et al. 2010).

Variability in vulnerability to cavitation due to mountainside exposure seemed to be typically higher than that due to altitudinal distribution. Light exposure seemed to play a major role, as populations growing at 1520 m a.s.l. on the north-facing mountainside had a vulnerability to cavitation similar to that of beeches distributed over the entire south-facing mountainside. Both mountainside effects and altitudinal gradients are complex and involve many different co-varying factors, such as topography, soil and climatic conditions especially the water availability. The correlation between vulnerability to cavitation and environmental conditions such as light exposure, probably those prevailing during wood formation and xylem conduit differentiation. This hinders the interpretation of the relationship prevailing during wood formation and xylem conduit differentiation. This hinders the interpretation of the relationship between vulnerability to cavitation and environmental conditions especially the water availability. The correlation between vulnerability to cavitation and drought resilience found between species appears to be less relevant at the within-species level. From a methodological point of view, the weak variations through the seasons and the conservation delay of the samples make large-scale analyses of the vulnerability to cavitation feasible.

Conclusions

Our results show that the changes in vulnerability to cavitation occur both within trees and between in situ populations. This trait seems to be at least partially governed by environmental conditions such as light exposure, probably those prevailing during wood formation and xylem conduit differentiation. This hinders the interpretation of the relationship between vulnerability to cavitation and environmental conditions especially the water availability. The correlation between vulnerability to cavitation and drought resilience found between species appears to be less relevant at the within-species level. From a methodological point of view, the weak variations through the seasons and the conservation delay of the samples make large-scale analyses of the vulnerability to cavitation feasible.

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