

Hydraulic properties of naturally regenerated beech saplings respond to canopy opening

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Summary Enhanced sapling growth in advance regeneration requires gaps in the canopy, but is often delayed after canopy opening, because acclimation of saplings to the new environment is gradual and may last for several years. Canopy opening is expected to result in an increased transpiration because of a larger climatic demand and a higher stomatal conductance linked to the higher rates of photosynthesis. Therefore, we focused on the changes in water relations and the hydraulic properties of beech (*Fagus sylvatica* L.) saplings during 2 years after canopy opening. We tested the hypothesis that an increase in leaf-specific hydraulic conductance and a decrease in vulnerability to cavitation occur to sustain an enhanced transpiration. Hydraulic conductance of defoliated shoots, vulnerability to cavitation, size and density of xylem vessels as well as stomatal conductance were recorded on saplings growing in shade (S saplings) or in gaps created by opening the canopy (shade-to-light, SL saplings). Hydraulic conductance per unit cross-sectional area (K_{AS}) did not differ in the shoots of S and SL saplings. But a higher ratio stem cross-sectional area/leaf area resulted in a higher leaf-specific hydraulic conductance of the shoots (K_{AL}) of SL saplings. Contrary to expectations, vulnerability to cavitation increased transiently in stems during the first year after canopy opening and no difference was observed between the two treatments in light-saturated stomatal conductance. During the second year, vulnerability to cavitation was similar in the S and SL saplings and light-saturated stomatal conductance increased in SL saplings. These results demonstrate a release of the hydraulic constraints after canopy opening with an adjustment of the ratio stem

cross-sectional area/leaf area. But the larger vulnerability to cavitation during the first year could limit stomatal opening and therefore the ability of beech saplings to use the available light for photosynthesis and could therefore partly explain why the growth increase was delayed to the second growing season after canopy opening.

Keywords: acclimation, biomass allocation, gap, hydraulic conductance, vulnerability to cavitation.

Introduction

Assisted natural regeneration is a usual regeneration scheme for managed forest ecosystems in Europe. Natural regeneration evolves either from a newly germinated seed or from advance regeneration. Advance regeneration consists of saplings of shade-tolerant species, which are able to establish and persist for long periods under closed canopies and which may grow rapidly upon canopy opening (Canham 1988, Messier et al. 1999). Advance saplings that are able to resume rapid growth immediately after gap opening may outcompete slowly responding neighbours and may eventually reach the upper canopy (Canham 1988, Messier et al. 1999).

When the canopy is opened, the advance saplings are exposed to rapid changes in microclimate. Irradiance rises usually from < 5% (Emborg 1998) to much higher values. Air temperature and vapour pressure deficit (VPD) increase in parallel. Moreover, canopy opening also results in increased soil water and nutrient contents, due to a smaller number of competing trees (Aussenac 2000, Ritter and

Vesterdal 2006). Under the new environment, the growth rates of advance saplings markedly increase for many tree species (Mesquita 2000, Devine and Harrington 2006). However, the growth increase is often delayed by several weeks up to a few years after gap opening (Collet et al. 2001, Aranda et al. 2004, Shimizu et al. 2006). This delay may reflect the time required for the advance saplings to adjust their phenotype towards an acclimated phenotype that allows greater growth and survival under the new environment.

Acclimation of the advance saplings to a sudden increase in irradiance implies short-term increases in photosynthetic performance (Naidu and DeLucia 1997a, Tognetti et al. 1998, Yamashita et al. 2000). But the number of studies carried out over a long term stated that growth conditions in shade the year previous to canopy opening can limit the acclimation of seedlings to increasing irradiance. A rapid increase in leaf mass area and area-based nitrogen content was observed in beech seedlings or branches that were exposed to canopy gap (Kimura et al. 1998, Uemura et al. 2000, Aranda et al. 2004). However, an increase in leaf gas exchange was delayed by several months up to several years after canopy opening in beech seedlings or in mature maples (Reynolds and Frochot 2003, Aranda et al. 2004, Jones and Thomas 2007). This time lag has been linked to the inability of the vascular system that developed under shade conditions to compensate for increased water demand (Maherali et al. 1997, Renninger et al. 2007) and/or to a delayed increase in leaf assimilation capacity (Jones and Thomas 2007). In red oak saplings, Naidu and DeLucia (1997b) showed changes in biomass allocation towards root growth and reserve storage the first year after canopy opening that allowed priming for fast growth and carbon gain the following year. Such a gradual plant acclimation to changes in growth conditions needs yet to be investigated through whole-plant level studies and may explain the frequently observed delay in growth increase after canopy opening.

To efficiently use the available light for carbon gain and resume rapid growth after canopy release, advance saplings need to increase the stomatal conductance, and hence transpiration. However, supporting larger transpiration rates requires a hydraulic system that is able to sustain larger water fluxes. This implies an increased efficiency of water transport from roots to leaves (i.e., an increased leaf-specific hydraulic conductance) as well as the ability to tolerate large water potential differences in the xylem conduits (i.e., low xylem vulnerability to cavitation, Tyree and Ewers 1991). Shade-acclimated saplings usually display a small leaf-specific hydraulic conductance (Schultz and Matthews 1993, Barigah et al. 2006) and a large vulnerability to cavitation (Cochard et al. 1999) as compared to light-acclimated ones. To increase leaf-specific hydraulic conductance, anatomical and morphological adjustments are required (Whitehead et al. 1984, Tyree and Ewers 1991). Changes in xylem anatomy as well as increases in the ratio of absorbing root area or sapwood area versus transpiring leaf area have been reported for the saplings that are exposed to canopy opening (Maherali

et al. 1997, Shimizu et al. 2005) and for branches of mature trees that are exposed to a higher irradiance after thinning (Lemoine et al. 2002b). Hydraulic properties of suppressed saplings in understory could therefore become the main constraint on growth after canopy opening because of a limited transpiration capacity, until morphological and anatomical acclimations are completed.

This study was performed on naturally regenerated beech (*Fagus sylvatica* L.) saplings. Beech is a widespread late-successional species in Europe, which tolerates deep shade at the sapling stage (Balandier et al. 2007) and shows a 1- to 2-year delay before switching from slow to fast growth after canopy opening (Collet et al. 2001). We assessed the changes in hydraulic properties (hydraulic conductance of defoliated shoots and vulnerability to cavitation), leaf stomatal conductance, biomass allocation and maximum potential hydraulic conductivity (calculated from vessel size and density) in the advance saplings of beech during 2 years after canopy opening. Tested hypotheses were (1) changes in hydraulic properties of shade-acclimated saplings following canopy opening take 1 or 2 years and are a prerequisite for enhanced sapling growth; (2) the observed changes in hydraulic properties require morphological and anatomical adjustments; and (3) the increase in stomatal conductance after canopy opening is constrained by the hydraulic properties.

Materials and methods

Stand and study site

The study site was located in a deciduous forest of North-Eastern France (49°04'40" N and 6°01'02" E), on a limestone plateau (300 m a.s.l.). The soil was a shallow calcisol (38–60 cm depth, Piboule et al. 2005). The stand was a copice-with-standards broadleaved stand converted into a mixed-species even-aged forest at the beginning of the 1960s. From that date onwards, only a few thinning operations were performed. The canopy was mainly composed of beech (*F. sylvatica*), sycamore (*Acer pseudoplatanus* L.), Norway maple (*Acer platanoides* L.) and oak (*Quercus petraea* Liebl. and *Quercus robur* L.). Two 300 m distant homogeneous plots containing regeneration patches with beech saplings were selected. In the regeneration patches, sapling density was about 20 saplings m⁻² and the height ranged between 5 and 175 cm. Each plot was divided into two homogeneous subplots. In January 2005, all mature trees were felled on one subplot of each plot. The area of the created gaps was 427 and 1979 m² in plots 1 and 2, respectively. Two treatments were considered: shade-to-light (SL) treatment (released saplings in the gaps) and shade (S) treatment (suppressed saplings maintained under closed canopy). The plots were fenced to prevent browsing by deer. Very little scattered herbaceous vegetation was present before gap formation, but it became more abundant after gap formation. It was manually removed to prevent any competition with the saplings.

Microclimate

The photosynthetic photon flux density (PPFD) was measured using amorphous silicon light sensors (CBE, Solems S.A., Palaiseau, France) that were calibrated at the beginning of the measurement period against a LI-191SB sensor (Li-Cor Inc., Lincoln, NE). In each plot, two transects crossing the closed canopy and the gap subplots were defined. Light sensors were installed at 1.5 m height, every 3 m along the four transects. Eighteen and seventeen sensors were used in the S and SL subplots, respectively. Three CBE sensors were installed on a mast to record the above canopy irradiance and were used as a reference. Four probes measuring air temperature and relative humidity simultaneously (HMP35AC and 50Y, Campbell Scientific Ltd., Leicestershire, UK) were set in the centre of each S and SL subplot. Instantaneous values of irradiance, temperature and humidity were recorded every minute, and 15-min average values were stored. These data were collected over two growing seasons. Relative irradiance below the canopy was calculated as the ratio of the sum of PPFD over a growing season in understory versus overstory.

Sapling sampling, plant biomass and leaf area

Immediately after gap opening, about 50 non-sprouted and undamaged (no visible browsing or logging damage) saplings were selected in each S and SL treatment. The saplings were located close to the centre of each subplot and were 0.50–1 m tall. In 2005 and 2006, 18 of these saplings were harvested in the S and SL treatments from end of August to mid September and were used for biomass and hydraulic properties measurements. In addition, annual growth rings at the stem base were counted in 2006 on eight harvested S and SL saplings. Sapling age was found to range between 11 and 18 years.

Leaves were removed from all saplings, stored in an ice-box, and the whole-plant leaf area (A_L) was measured with a leaf area meter (Li-Cor model 3000-A equipped with a Li-Cor Belt Conveyor 3050-A, Li-Cor instruments, Lincoln, NE). Ten saplings of S and SL treatments were used to measure the basal diameter at 5 cm above the ground and the root biomass (BR). After harvesting aboveground biomass, the taproots were excavated and the lateral roots were retrieved from about 0.3 m³ volume of soil. The shoot (leaf, stem and branch) and the BR were weighed after drying at 50 °C for at least 5 days. Stem cross-sectional area (A_S), ratio between cross-sectional area and leaf area (A_S/A_L) and ratio between BR and leaf area (BR/A_L) were calculated.

Hydraulic conductance

Hydraulic conductance was measured in 2005 and 2006 on 10 S and 10 SL saplings using a high-pressure flowmeter (Tyree et al. 1995, Barigah et al. 2006). Measurements were made in situ from end of August to early September. Severed shoots were perfused with distilled and degassed

ultra-pure water filtered through a 0.1-µm filtration membrane (Calyx capsule, Osmonics, Westborough, MA). Water was forced through the shoot at a pressure of 0.3 MPa (P) until water dripped from the stomata. Water flow rate (F) was measured and hydraulic resistance (R) was calculated as the ratio between P and F , assuming that water potential was zero in the perfused leaves. The hydraulic resistance was measured between 7:30 am and 5:00 pm solar time. Tyree et al. (2005) and Cochard et al. (2007) reported rapid irradiance-induced variations in hydraulic resistance of leaves. The hydraulic resistance of defoliated shoots was recorded after severing all leaves following the procedure of Yang and Tyree (1994). The hydraulic conductance of defoliated shoots (K) was calculated as the inverse of the hydraulic resistance.

Leaf-specific hydraulic conductance, K_{AL} (K per unit leaf area) and specific hydraulic conductance, K_{AS} (K per unit stem cross-sectional area) were computed, assuming that all the stem section was conductive (Cochard et al. 2001). K_{AL} describes the capacity of a shoot to supply its leaves with water.

Vulnerability to cavitation

Eight saplings of each treatment were used for vulnerability to cavitation measurements. At the end of September 2005 and 2006, a stem segment (~ 30 cm long) was collected from these saplings, wrapped in a moist paper and kept at 4 °C to prevent dehydration and brought to the laboratory. Vulnerability curves [percent loss of hydraulic conductivity (PLC) versus xylem tension] were established using the 'Cavitron' technique (Cochard 2002, Cochard et al. 2005b). Briefly, the principle of the technique is to lower the pressure in a xylem segment by centrifugal force and, at the same time, to measure the decrease in hydraulic conductance. Samples were cut in air to obtain 28-cm-long segments. The minimal segment length had been assessed by the air perfusion method (Barigah and Cochard, unpublished results), which guaranteed that the segments were longer than the longest vessels. Xylem pressure (P) was first set to a reference pressure (−1.0 MPa) and maximal conductance (K_{max}) was recorded. Preliminary experiments showed that there was no difference beginning at −1.0 MPa or at −0.5 MPa (data not shown). The xylem pressure was then set to a more negative pressure for 180 s and subsequently returned to the reference pressure to determine the new conductance K_S . Percent loss conductance was then computed as

$$PLC = 100 \times \left(1 - \frac{K_S}{K_{max}} \right). \quad (1)$$

This procedure was repeated for more negative pressures (typically in −0.25 to −0.50 MPa increments) until PLC reached at least 95%. Rotor velocity was monitored with an electronic tachymeter (10 rpm resolution; Multimeter Probes A2108, Compact Instruments Limited, Bolton, UK), allowing the xylem pressure to be adjusted to about ± 0.02 MPa.

Vulnerability to cavitation was described by two parameters: Ψ_{PLC50} and Ψ_{PLC10} , the xylem water potentials inducing 50% and 10% loss of hydraulic conductivity, respectively (Tyree and Sperry 1988).

Xylem anatomy

Stem segments of 0.02–0.03 m length were cut from four stumps of the S and SL saplings that were used to construct the vulnerability curves and stored at $-20\text{ }^{\circ}\text{C}$ in a freezer. Thin sections were cut with a hand microtome (SM2000, Leica Jung, Nussloch, Germany), double stained with safranin and astra-blue solutions and fixed on a blade with Canada balsam. For each stem section, 4–5 sectors bounded by rays were delimited under an optical microscope (Leica DM-LB, Meyer Instruments, Inc., Houston) in each of the two youngest growth rings (2005 and 2006 growth seasons) and were photographed (Canon PowerShot S80, Canon France, Courbevoie). The pictures were analysed using an image analysis software (Visilog 6.5 Xpert, Noesis, Courtaboeuf, France). Vessel number and cross-sectional area of each vessel were recorded using a homemade macro after adjusting the threshold to separate walls and lumens. The macro allows differentiating fibres and vessels according to their lumen section. From preliminary observations, maximum lumen section was set below $100\text{ }\mu\text{m}^2$ in fibres and above $100\text{ }\mu\text{m}^2$ in vessels.

A theoretical hydraulic conductivity of the mean conduit in a stem section (k) was calculated from the Hagen–Poiseuille law according to Tyree and Zimmermann (1971, in Steppe and Lemeur 2007):

$$k = \left(\frac{\pi\rho}{128\eta} \right) \times \left(\frac{1}{n} \sum_{i=1}^n d_i^4 \right), \quad (2)$$

where ρ is the density of water, η is the viscosity coefficient of water and d_i is the i th vessel diameter of n measured vessel. Specific stem hydraulic conductivity (k_s) was computed by multiplying k by the mean vessel number per square meter.

Gas exchange measurements

Light-saturated stomatal conductance (g_{sat}) was measured during 2 years on the same set of 10 S and SL saplings at the end of the growing season 2005 (early in September) and repeated on the same individuals during summer 2006. Saplings were non-sprouted, undamaged and 0.50–1 m high. All measurements were made between 7:30 am and 3:00 pm solar time on one leaf in the upper part of sapling crown with an open-flow gas exchange system (Li-6400, Li-Cor, Lincoln, NE) using a 6 cm^2 chamber with a red–blue light source. To prevent any bias due to diurnal changes in stomatal conductance, the saplings were randomly measured in each plot. Measurements were made at $25\text{ }^{\circ}\text{C}$, ambient relative humidity and CO_2 concentration. The PPFD was set to the saturating level estimated from

light response curves ($800\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$ for S saplings and $1200\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$ for SL saplings). When stomatal conductance was stable, three records were taken at 15-s intervals and averaged.

Statistical analysis

Data were analysed with mixed-effects linear models using the statistical software package R 2.2.0 (R Development Core Team 2007). For all analyses, residual normality and homoscedasticity were graphically checked. In cases where residuals were heteroscedastic, variance was modelled as a power function of predicted values rather than using classical data transformations. For all analyses, a model with canopy opening and year as fixed effects was used, and canopy opening and year effects were evaluated by the contrast analysis with the multcomp R package (Hothorn et al. 2007). In a preliminary analysis, plot effect was included as a random effect in the model and tested by comparing the models with and without random plot effect using the log-likelihood ratio test. No significant effect of plot was found and the factor ‘plot’ was removed from the model. The model used was

$$\text{var} = \text{treat} + \text{year} + \text{treat} \times \text{year}, \quad (3)$$

where var refers to the tested variable (A_L , A_S , BR, A_S/A_L , BR/ A_L , K_{AL} , K_{AS} , Ψ_{PLC50} , Ψ_{PLC10} and g_{sat}), treat refers to treatment effect (levels: S and SL) and year refers to year effect (levels: 2005 and 2006).

To test treatment and year effects on K independently of plant size, either A_L or A_S was added as covariate to the model. Effect of treatment and year on BR and A_S was also tested independently of plant size by adding A_L as covariate in the model. In these models, all variables were log-transformed.

When analysing the radial growth, the year effect was tested by comparing two growth rings of each sapling. Dependencies between these measurements were accounted for by adding a random sapling effect in the model.

Vulnerability curves were fitted with a sigmoid model of the PLC versus xylem water potential (Ψ_x) (Pammenter and Willigen 1998):

$$\text{PLC} = 100 / (1 + \exp(a \times (\Psi_x - \Psi_{\text{PLC50}}))), \quad (4)$$

where Ψ_{PLC50} is the xylem water potential inducing 50% loss of hydraulic conductivity. Ψ_{PLC10} was then derived from a and Ψ_{PLC50} for each plant. The effects of treatment and year on Ψ_{PLC50} and Ψ_{PLC10} were evaluated by contrast analysis.

Results

Microclimate

Relative irradiance and maximum daily VPD reached higher values after gap opening (Figure 1). The average

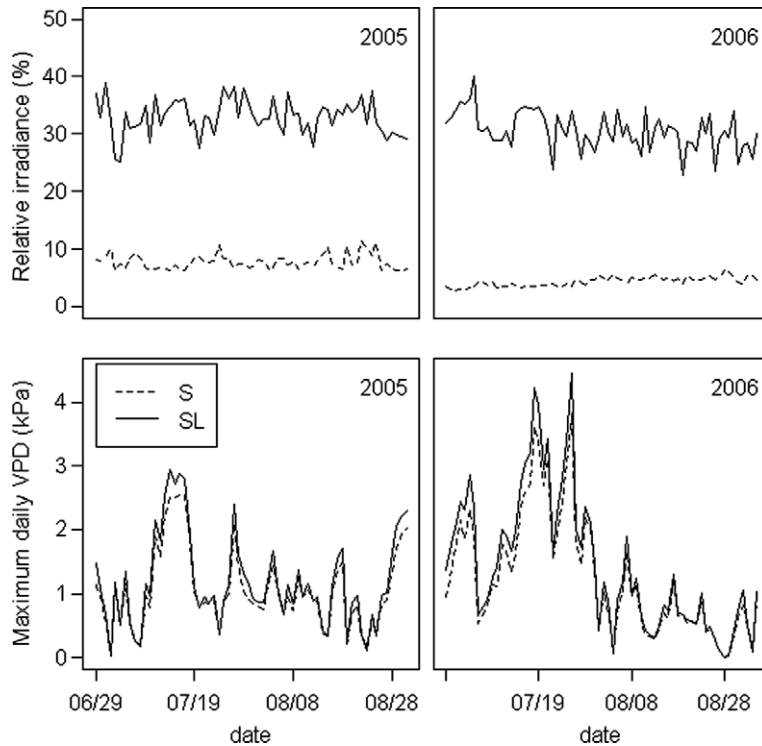


Figure 1. Seasonal pattern of relative irradiance and maximum daily VPD. Data are represented for shade, S (dashed line) and shade-to-light, SL (solid line) beech saplings, 1 year (2005) and 2 years (2006) after canopy opening.

value of maximum daily VPD was significantly higher in SL than in S (1.13 ± 0.04 kPa versus 1.01 ± 0.03 kPa in 2005 and 1.26 ± 0.06 kPa versus 1.45 ± 0.07 kPa in 2006). Mean relative irradiance did not differ between years in the SL treatment ($33.0 \pm 2.43\%$ in 2005 and $31.3 \pm 2.55\%$ in 2006), while it decreased in the S treatment from 7.3% in 2005 to 4% in 2006 ($n = 69$; $P < 0.0001$).

Canopy opening effects on total leaf area and plant biomass

SL and S saplings displayed a similar leaf area (A_L) 1 year after canopy opening while a slight difference (+41% for SL versus S; $P = 0.07$) was observed during year 2 (Table 1). Shoot biomass and stem cross-sectional area (A_S), which were positively correlated ($r^2 = 0.95$, $n = 43$, $P < 0.0001$ and data not shown), were significantly higher in SL than in S saplings at the end of years 1 and 2 (Table 1). A_L and A_S were also positively correlated, and the relationship differed significantly between treatments ($r^2 > 0.74$; $P < 0.0001$) and years for SL saplings ($P < 0.006$; Figure 2A). A_S/A_L was indeed higher in SL saplings after canopy opening (Table 1). BR/A_L was higher in SL than in S saplings during both years after canopy opening ($r^2 > 0.79$, $P < 0.0003$; Table 1; Figure 2B).

Hydraulic conductance

During both years, leaf-specific hydraulic conductance of defoliated shoots (K_{AL}) was larger in SL than in S saplings (Table 2) and the relationship between K and A_L differed

significantly between treatments ($r^2 > 0.7$, $P < 0.0001$; Figure 2C). Shoot hydraulic conductance per stem cross-sectional area (K_{AS}) was similar for both S and SL saplings (Table 2) and no difference was observed between years and treatments for the relationship between K and A_S (Figure 2D).

Xylem anatomy

Mean vessel area was larger in SL than in S saplings during the 2 years following canopy opening (Table 2). Vessel density was smaller in SL than in S saplings during both years. As a result of this compensation, specific hydraulic conductivity of stems (k_S) was not significantly different between S and SL saplings (Table 2). In addition, and despite a smaller vessel density, the number of vessels was higher in SL saplings because of a larger annual ring area (Table 2).

Vulnerability to cavitation

One year after canopy opening, SL saplings displayed a higher vulnerability to cavitation than S saplings (Figure 3) as shown by higher values of Ψ_{PLC50} and Ψ_{PLC10} (Table 2). Two years after canopy opening, Ψ_{PLC50} and Ψ_{PLC10} no longer differed between treatments. SL saplings showed a higher Ψ_{PLC50} during the first than the second year after canopy opening but no difference in Ψ_{PLC10} was detected between years (Table 2; Figure 3).

Stomatal conductance

Light-saturated stomatal conductance (g_{sat}) was not significantly different between SL and S saplings during the first

Table 1. Total leaf area (A_L), shoot biomass, stem cross-sectional area (A_S), root biomass (BR), ratio of stem cross-sectional area to leaf area (A_S/A_L) and ratio of root biomass to leaf area (BR/A_L) of shade (S) and shade-to-light (SL) beech saplings, 1 and 2 years after canopy opening. Mean \pm SE (for A_L and shoot biomass, $n = 18$ for S and SL saplings; for A_S , A_S/A_L and BR/A_L , $n = 10$ for S and SL saplings). Two different letters, within a year or between the 2 years, indicate a significant difference (contrast analysis, $P < 0.05$).

	Shoot biomass (g)	A_L (m ²)	A_S (cm ²)	BR (g)	A_S/A_L	BR/A_L (g m ⁻²)
<i>One year after canopy opening</i>						
S	10.5 \pm 1.05 a	0.067 \pm 0.007 a	0.277 \pm 0.030 a	4.76 \pm 0.62 a	4.13e ⁻⁴ \pm 2.75e ⁻⁵ a	69.23 \pm 3.62 a
SL	15.3 \pm 1.56 b	0.051 \pm 0.005 a	0.495 \pm 0.043 b	8.43 \pm 1.09 b	8.48e ⁻⁴ \pm 4.62e ⁻⁵ b	136.15 \pm 8.91 b
<i>Two years after canopy opening</i>						
S	13.0 \pm 1.33 a	0.106 \pm 0.013 b	0.292 \pm 0.031 a	6.61 \pm 1.49 a	3.23e ⁻⁴ \pm 3.14e ⁻⁵ c	57.70 \pm 5.05 a
SL	29.9 \pm 2.84 c	0.149 \pm 0.014 b	0.739 \pm 0.090 c	13.4 \pm 1.59 c	5.01e ⁻⁴ \pm 3.01e ⁻⁵ d	91.15 \pm 5.05 c

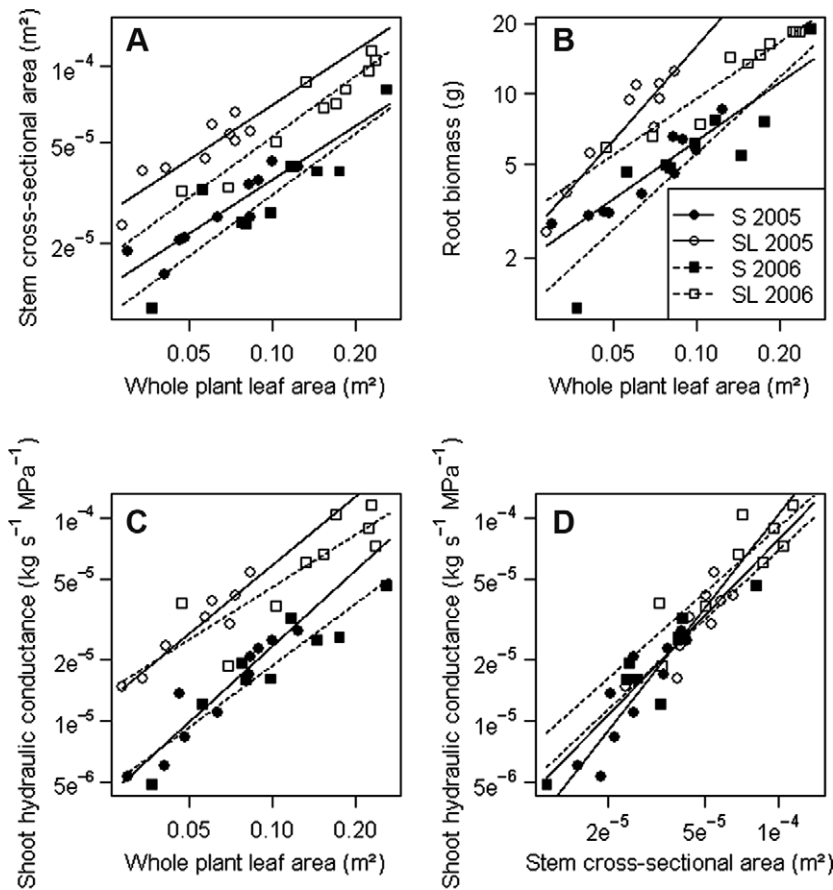


Figure 2. Relationships between (A) whole-plant leaf area and stem cross-sectional area, (B) whole-plant leaf area and BR, (C) whole-plant leaf area and defoliated shoot hydraulic conductance and (D) stem cross-sectional area and hydraulic conductance of defoliated shoots. Data are represented for shade, S (closed symbols) and shade-to-light, SL (open symbols) beech saplings, 1 year (2005; circles and solid line) and 2 years (2006; squares and dashed line) after canopy opening. Each value represents a single seedling and axes are log-scaled.

year after canopy opening, while a large increase was observed in SL saplings during year 2 (Figure 4).

Discussion

Anatomical and morphological adjustments in beech saplings after canopy opening

After canopy opening, acclimation of beech saplings to the new irradiance in the gaps was very gradual and changes in sapling growth rate and hydraulic properties were not

immediate but took place over at least two growing seasons. The total hydraulic conductance of defoliated shoots (K) increased but K reported to stem cross-sectional area (K_{AS}) remained unaffected, despite the large changes in stem diameter and xylem structure. Measured K values may differ from the field values because cavitated xylem would be refilled with water by a pressure of 0.3 MPa. However, native embolism was certainly weak. The assumptions that the whole stem cross-section was conductive (Cochard et al. 2001) might also not be valid and could bias the comparison between S and SL saplings. However, the lack of difference in K_{AS} was reinforced when computing

Table 2. Leaf-specific (reported to total leaf area, K_{AL}) and specific (reported to stem cross-sectional area, K_{AS}) hydraulic conductance of defoliated shoots, xylem anatomy (mean vessel area, vessel density, annual ring area and specific stem hydraulic conductivity computed from vessel cross-sectional area, k_S) and parameter describing vulnerability to cavitation [xylem water potential inducing 50% (Ψ_{PLC50}) and 10% (Ψ_{PLC10}) loss of hydraulic conductivity] in shade (S) and shade-to-light (SL) beech saplings, 1 and 2 years after canopy opening. Mean \pm SE ($n = 10$ for hydraulic conductance parameter, $n = 4$ for xylem anatomy parameter and $n = 8$ for vulnerability to cavitation parameter). Two different letters, within a year or between the 2 years, indicate a significant difference (contrast analysis, $P < 0.05$).

	One year after canopy opening		Two years after canopy opening	
	S	SL	S	SL
K_{AL} ($\text{kg s}^{-1} \text{m}^{-2} \text{MPa}^{-1}$)	$2.15e^{-4} \pm 1.50e^{-5}$ a	$5.48e^{-4} \pm 2.41e^{-5}$ b	$1.93e^{-4} \pm 1.56e^{-5}$ a	$4.26e^{-4} \pm 6.10e^{-5}$ b
K_{AS} ($\text{kg s}^{-1} \text{m}^{-2} \text{MPa}^{-1}$)	$5.43e^{-1} \pm 5.24e^{-2}$ a	$6.69e^{-1} \pm 5.42e^{-2}$ a	$6.19e^{-1} \pm 4.78e^{-2}$ a	$8.45e^{-1} \pm 1.08e^{-1}$ a
Mean vessel area (μm^2)	225 ± 20 a	315 ± 13 b	272 ± 38 a	428 ± 42 c
Vessel density (mm^{-2})	1001 ± 53 a	586 ± 95 b	819 ± 103 a	476 ± 94 c
Annual ring area (mm^2)	3.03 ± 0.64 a	12.68 ± 3.12 b	5.30 ± 0.77 a	20.52 ± 4.78 b
k_S ($\text{kg s}^{-1} \text{MPa}^{-1} \text{m}^{-3}$)	$5.06e^{-6} \pm 8.19e^{-7}$ a	$5.56e^{-6} \pm 1.15e^{-6}$ a	$5.67e^{-6} \pm 8.95e^{-7}$ a	$8.55e^{-6} \pm 1.51e^{-6}$ a
Ψ_{PLC50} (MPa)	-4.07 ± 0.10 a	-3.00 ± 0.11 b	-3.81 ± 0.23 a	-3.58 ± 0.11 a
Ψ_{PLC10} (MPa)	-2.67 ± 0.19 a	-1.76 ± 0.14 b	-2.30 ± 0.10 a	-1.81 ± 0.11 ab

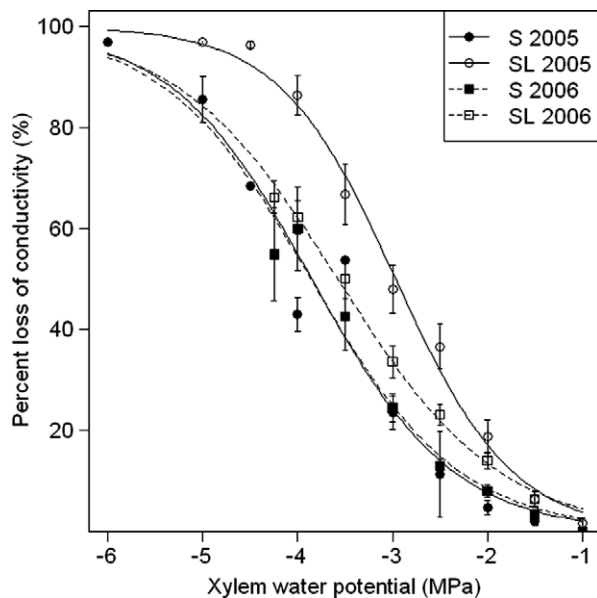


Figure 3. Percent loss of hydraulic conductivity versus xylem water potential for shade, S (closed symbol) and shade-to-light, SL (open symbol) beech saplings, 1 year (2005; circles and solid line) and 2 years (2006; squares and dashed line) after canopy opening. Vertical bars represent standard errors (SE) of the mean ($n = 8$ for S and SL saplings).

the theoretical-specific hydraulic conductivity of the stem (k_S) from xylem vessel size and distribution. The increase in mean vessel cross-sectional area was counterbalanced by a decrease in vessel density. Because of this compensating effect, the increase of K was primarily due to a larger sapwood area in SL saplings. This is in contrast to the concomitant increase of vessel diameter and density observed in 1-year-old shade twigs that were suddenly exposed to full sunlight (Lemoine et al. 2002b).

The increase in cambial growth, which leads to an increase in A_S/A_L , was one of the first reactions of suppressed

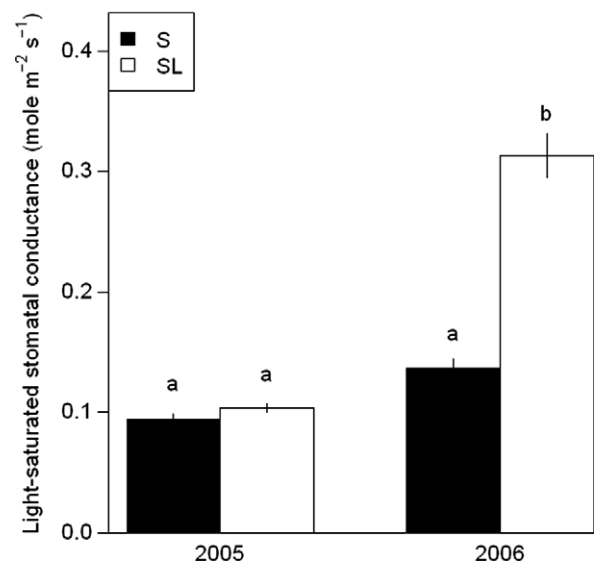


Figure 4. Light-saturated stomatal conductance (g_{sat}) of shade (S, black bars) and shade-to-light (SL, white bars) beech saplings, 1 year (2005) and 2 years (2006) after canopy opening. Vertical bars represent SE of the mean ($n = 10$ for S and SL saplings). Two different letters, within a year or between the 2 years, indicate a significant difference (contrast analysis, $P < 0.05$).

saplings to canopy opening with increased irradiance and evaporative demand. High VPD is indeed known to favour high A_S/A_L , as reported for Scots pines (Mencuccini and Grace 1994). The increase in A_S/A_L can be ascribed to an increased cambial activity and to morphogenetic constraints on leaf development. The number of leaf primordia in beech buds is known to be controlled by hydraulic conductance of the shoot bearing the bud (Cochard et al. 2005a) and all leaves are preformed in the bud during the summer before canopy opening. This is likely the cause for the lack of leaf area increase during the first year. Increased stem hydraulic

conductance due to a larger annual ring in the first year may have stimulated primary-meristem activity, resulting in a larger number of leaf primordia available for the second year after canopy opening. This is consistent with the increase in diameter growth observed during the first year after thinning and the formation of long shoots in the following year (Collet et al. 2001, 2002).

This rapid adjustment towards enhanced cambial growth led to a higher leaf-specific hydraulic conductance in shoots (K_{AL}), i.e., to an increase in water transport capacity of stems, which is already able to sustain a higher transpiration rate during the first year after canopy opening. In *Acer rubrum* L. saplings 7 years after canopy opening, a larger K_{AL} and a higher A_S/A_L were observed with no change in specific hydraulic conductance (Maherali et al. 1997). The release of hydraulic constraints was less rapid in *Acer pennsylvanicum* L., which showed no change in A_S/A_L nor in specific hydraulic conductance (Maherali et al. 1997). In released Douglas-fir and Western hemlock saplings 7 years after thinning, a lower A_S/A_L was counterbalanced by a larger specific conductivity with no change in leaf-specific conductance. This contributed to a stomatal limitation of carbon gain (Renninger et al. 2007). Thus, the release of hydraulic constraints after canopy opening implied changes either in the ratio between A_S and A_L or in shoot anatomy. However, a lower vulnerability to cavitation, which was not measured in these earlier studies, could also allow the support of higher transpiration rates, despite the lack of change in K_{AL} .

Increase in stomatal conductance remains limited after canopy opening

Tyree and Zimmermann (2002) underlined that a large leaf-specific hydraulic conductance (K_{AL}) resulted usually in smaller pressure gradients in the conducting tissues for a given transpiration rate. After canopy opening, SL saplings are exposed to a higher VPD and the increase in K_{AL} may therefore allow an increased transpiration per unit leaf area without a rise in the water potential gradient that limits the risk of cavitation (Sperry 2000). However, no change was observed in light-saturated stomatal conductance (g_{sat}) during the first growing season after canopy opening. This is consistent with the low stomatal conductance recorded on naturally regenerated beech saplings after a windstorm-induced canopy opening (Reynolds and Frochot 2003). The low value of g_{sat} recorded in SL saplings is probably not due to a low stomatal density (not measured in this study) as stomatal density is thought to acclimate rapidly to the new light environment in beech (Aranda et al. 2001a). An imbalance between root water uptake and transpiration is a putative explanation for stomatal limitation after canopy opening (Clearwater et al. 1999). Barigah et al. (2006) reported that irradiance had no detectable effect on the root hydraulic conductance scaled to BR in beech seedlings that were grown under different light

regimes although significant species-specific effects were evident. In contrast, a decrease in root hydraulic conductivity has been shown in some tropical seedlings after shade to gap transfer (Shimizu et al. 2005). Because we did not measure the root hydraulic conductance, it is rather difficult to make statements about the effects of canopy removal with regard to this parameter. However, the rapid adjustment of the BR/ A_L ratio in SL beech saplings, similar to that observed by the latter authors, could compensate for a possible decrease in root hydraulic conductivity (Shimizu et al. 2005) and would also allow an enhanced transpiration per unit leaf area. Further investigations would be needed to determine the ability of seedlings of *Fagus* to modify their root hydraulic conductance in response to the thinning out.

Nevertheless, the larger vulnerability to cavitation that was observed in the SL saplings 1 year after canopy opening suggests a potential hydraulic limitation to transpiration that would contribute to maintain low g_{sat} during the first year after canopy opening. Stomatal control of xylem embolism is indeed well documented in several species including beech (Lemoine et al. 2002a).

The transitional increase of xylem vulnerability to cavitation in SL saplings was unexpected, as earlier studies all evidenced an inverse relationship between irradiance and vulnerability to cavitation in beech (Cochard et al. 1999, Barigah et al. 2006). Recent studies showed a negative relationship between xylem mechanical strength (modulus of rupture) and vulnerability to cavitation (Hacke et al. 2001). We then speculate that the increase in cambial growth during the first spring after canopy opening in plants with a small amount of stored carbohydrates (Dillaway et al. 2007) promoted the growth of vessels with thinner cell walls or pit membranes, more fragile and then more sensitive to cavitation. The fibre wall area could also be affected and increase the vulnerability to cavitation (Jacobsen et al. 2005). This increase in xylem vulnerability would only be transient because the amount of available carbohydrate probably significantly increased the following years and helped sustain an enhanced diameter.

The low vulnerability to cavitation in saplings grown in understory

Vulnerability to cavitation was low in the shade-acclimated saplings. Ψ_{PLC50} was close to levels observed 2 years after canopy opening, as well as to those measured on saplings that grew in old forest gaps in a nearby stand ($\Psi_{PLC50} = -3.52 \pm 0.17$, unpublished results) or on sun-exposed twigs of mature trees ($\Psi_{PLC50} = -3.22 \pm 0.07$, Cochard et al. 1999). Actually, similar levels of vulnerability to cavitation were observed between gap and shade grown *Rhododendron maximum* L. (Lipp and Nilsen 1997). These results contrast with those obtained on potted beech sapling grown at full or low irradiance and on sun and shade branches of adult trees (Cochard et al. 1999, Barigah et al. 2006). In both cases, light-acclimated twigs or saplings

were less vulnerable than shade-acclimated ones. Similarly, sudden exposure of shaded branches to full irradiance resulted in an intermediate vulnerability as compared to shaded and light-acclimated twigs (Lemoine et al. 2002b). Our result could be attributed to two factors:

- (i) Vulnerability to cavitation was not recorded on 1-year-old twigs as in the earlier studies but on stems, i.e., on a set of functional growth rings (Cochard et al. 2001); the difference between shade- and light-acclimated saplings could therefore be smaller than in 1-year-old twigs.
- (ii) Shade-acclimated saplings in the field might experience a pronounced soil water deficit because of the competition with the surrounding saplings and mature trees (Aussenac 2000, Robson et al. 2008), which is in contrast to those grown in the controlled conditions and to adult trees (Rice et al. 2004). In line with Aranda et al. (2004) and Robson et al. (2008), a few measurements during July and early August 2006 showed that predawn water potential of S sapling reached -1.66 versus -0.6 MPa in SL saplings (data not shown). Because the low VPD and transpiration rates in understory were counterbalanced by a higher soil water deficit, xylem tension experienced by the shaded saplings may be similar to that experienced by saplings growing in gaps. As xylem tensions that occurred during pit pore differentiation are assumed to affect xylem vulnerability to cavitation allowing the pores to acclimate to these conditions (Cochard et al. 1999, Lemoine et al. 2002b), this could also explain the lack of difference in vulnerability to cavitation between saplings acclimated to shade and saplings in gaps. Robson et al. (2008) observed a larger difference in water potential between soil and leaf in beech saplings growing in gap than in shaded saplings. In addition to low vulnerability to cavitation, leaf osmotic adjustment could allow supporting the higher gradient in water potential and then maintaining leaf turgor and consequently high stomatal conductance in gap saplings (Aranda et al. 2001b, Robson et al. 2008).

In addition, and contrary to adult trees for which carbon gain depends mostly on upper crown branches, carbon acquisition during sunflecks might largely contribute to the seasonal carbon gain of shaded saplings (Cavender-Bares and Bazzaz 2000, Leakey et al. 2005). A lower vulnerability to xylem embolism in shade-acclimated saplings than in shaded potted seedlings or in shaded branches of adult trees, and higher K_{AL} values (8.22×10^{-6} in Barigah et al. 2006 versus 2.15×10^{-4} in this study), suggests that these saplings may sustain a higher transpiration flux and a higher water potential gradient. Such an acclimation together with the maintenance of leaf turgor at more negative water potentials by high cell wall elasticity (Aranda

et al. 2001b, Robson et al. 2008) might help the stomata of shaded saplings to remain open during and between sunflecks, enabling maximal rates of photosynthesis to fulfil their carbon requirements.

Conclusion

Vulnerability to xylem embolism increased transitorily in beech saplings after canopy opening. This precluded any increase in light-saturated stomatal conductance during the first growing season after canopy opening, despite a larger hydraulic conductance per unit leaf area due to a larger growth ring in stems. These results suggest that the ability of beech sapling to fully use the newly available light for photosynthesis is delayed at least to the second growing season after canopy opening, concurrent with the delay in growth increase. For forest managers, this may indicate that beech saplings may in the short-term benefit less from large canopy opening than other co-occurring species that may take a competitive advantage during the first growing season.

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