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RESEARCH PAPER

Duration and extension of anatomical changes in wood structure after cambial injury

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Abstract

Cambial injury has been reported to alter wood structure in broad-leaved trees. However, the duration and extension of associated anatomical changes have rarely been analysed thoroughly. A total of 18 young European ash (*Fraxinus excelsior* L.) trees injured on the stem by a spring flood were sampled with the aim of comparing earlywood vessels and rays formed prior to and after the scarring event. Anatomical and hydraulic parameters were measured in five successive rings over one-quarter of the stem circumference. The results demonstrate that mechanical damage induces a decrease in vessel lumen size (up to 77%) and an increase in vessel number (up to 475%) and ray number (up to 115%). The presence of more earlywood vessels and rays was observed over at least three years after stem scarring. By contrast, abnormally narrow earlywood vessels mainly developed in the first ring formed after the event, increasing the thickness-to-span ratio of vessels by 94% and reducing both xylem relative conductivity and the index for xylem vulnerability to cavitation by 54% and 32%, respectively. These vessels accumulated in radial groups in a 30° sector immediately adjacent to the wound, raising the vessel grouping index by 28%. The wound-induced anatomical changes in wood structure express the functional need of trees to improve xylem hydraulic safety and mechanical strength at the expense of water transport. Xylem hydraulic efficiency was restored in one year, while xylem mechanical reinforcement and resistance to cavitation and decay lasted over several years.

Key words: Cambial injury, earlywood vessel, European ash, *Fraxinus excelsior*, ray, ring-porous, wood anatomy.

Introduction

Trees are constantly exposed to environmental stresses that may impair their ability to metabolize normally (Schweingruber, 2007). While both heredity and the environment influence the physiological processes that control tree growth (Kozlowski *et al.*, 1991), it is acknowledged that tree adaptability to the environment can be assessed by analysing xylem cells across series of annual rings (Denn and Dodd, 1981; Sass and Eckstein, 1995; Fonti *et al.*, 2010). When the cambium of trees is injured, normal cambial activity is locally disrupted, which is followed by a series of defence and wound healing processes including compartmentalization of decay and formation of callus tissue (Shigo, 1984; Neely, 1988; Blanchette, 1992; Larson, 1994; Fink, 1999). The healing proceeds from the wound margin inward in order to shield the exposed xylem with new healthy tissue.

Wood anatomical investigations of injured broad-leaved trees have mostly involved diffuse-porous species experimentally wounded by partial girdling, pinning or drilling of the stem (Rier and Shigo, 1972; Sharon, 1973; Bauch *et al.*, 1980; Rademacher *et al.*, 1984; Kuroda and Shimaji, 1985; Lev-Yadun, 1994; Stobbe *et al.*, 2002). Broad-leaved trees with naturally inflicted injuries have only rarely been studied at the cellular level, with the exception of recent research aiming at retrieving environmental information on natural hazards or forest fires from xylem cells (Arbellay et al., 2010; Ballesteros et al., 2010; Bigio et al., 2010; Kames et al., 2011). It is well established that ecologically relevant information can be obtained retrospectively from vessels of broad-leaved trees. Earlywood vessels of ring-porous species, in particular, have yielded successful results when screened for signals induced by climate (Woodcock, 1989; Pumijumnong and Park, 1999; García-González and Eckstein, 2003; Fonti and García-González, 2004; Tardif and Conciatori, 2006; Fonti et al., 2007), drought (Corcuera et al., 2004; Eilmann et al., 2006; Galle et al., 2010), flooding (Yanosky, 1983; Astrade and Bégin, 1997; St George and Nielsen, 2003), and insect defoliation (Huber, 1993; Asshoff et al., 1998–1999). Nevertheless, comparative studies focusing on wounding of ring-porous species and its functional implications are scarce.

The aim of this research is to identify and quantify anatomical changes in the wood structure of European ash (*Fraxinus excelsior* L.) caused by cambial injury and crystallized in the newly formed xylem. Young trees injured on the stem by a spring flood were sampled to compare earlywood vessels and rays formed prior to and after the scarring event. Anatomical and hydraulic parameters were analysed in both radial and tangential directions so as to determine the duration and extension of wound effects.

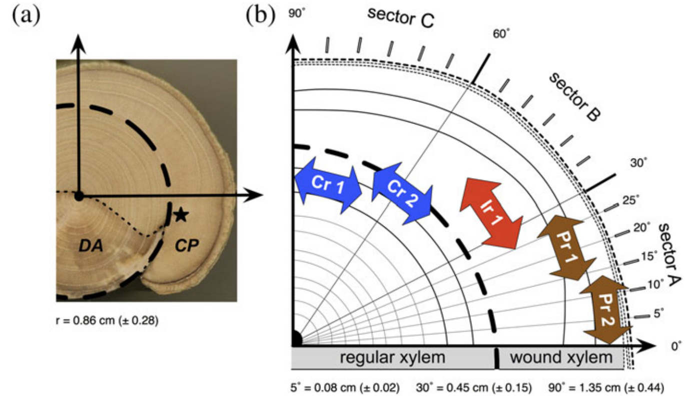

Fig. 1. (a) Location of the disc quarter extracted from each sample. The dashed line shows the position of the cambium at the time of wounding. A callus pad (*CP*) started to grow over the scar from the wound margin level (star). Care was taken to avoid the callus pad and the decayed area (*DA*) so as to obtain an undisrupted and sound time series of xylem cells. Average disc radius (r) is given. (b) Schematic view of the disc quarter with the different rings and sectors used for analysis. The 5° sectors can be grouped in three 30° sectors (A, B, C). Sector A is the closest to the injury. Measurements of the arc length of sectors were made on the outer edge of samples and then averaged. Ring types: Cr, control ring; Ir, injury ring; Pr, post-injury ring. (This figure is available in colour at *JXB* online.)

Materials and methods

Field campaign and sample preparation

Trees were sampled in summer 2009 along the St-Barthélemy torrent (Valais, Swiss Alps, 46°11' N, 7°00' E, 570 m a.s.l.), located in the upper Rhone river valley. The riparian vegetation at the site was predominantly composed of grey alder (*Alnus incana* (L.) Moench) and further included European ash (*Fraxinus excelsior* L.), sycamore maple (*Acer pseudoplatanus* L.), and goat willow (*Salix caprea* L.). Stem cross-sections were collected from 18 young *F. excelsior* trees displaying one elongated scar (140×2.5 cm maximum) up to the xylem and oriented to the flow direction of the torrent. All trees were injured in spring 2007 before the start of the growing season on roughly half of the stem circumference. The average tree age was 17.11±4.46 years and the average stem circumference at breast height was 8.03±2.32 cm. One sample per tree was taken at the mid-length of the injury. The average disc radius was 0.86±0.28 cm.

Each sample was sectioned with a chisel to obtain a disc quarter (Fig. 1a). After dating the rings, 15 μm thick transverse sections of the quarters were cut using a Reichert sliding microtome. The microsections were then stained with a 1% safranin and astrablue solution, rinsed with water, alcohols, and xylol, and permanently mounted on microscope slides using Canada balsam.

Xylem anatomical and hydraulic parameters

Earlywood vessels and rays were studied over the whole disc quarter in five successive rings for each tree (90 rings in total): the injury ring (Ir 1) built during the growing season following wounding, two control rings (Cr 1 and Cr 2) laid down previously and two post-injury rings (Pr 1 and Pr 2) formed subsequently (Fig. 1b). Anatomical measurements of the cells were generally performed from images of the microsections captured at 25× magnification with a digital camera mounted on a light microscope. The software WinCELL Pro V 2004a (Régent Instruments Inc., 2004) was used to measure the number of earlywood vessels and, for each of them, the wall thickness and the lumen area, as well as the radial and tangential lumen diameter. The vessels were then converted into circular conduits following White's (2006) equation $d=[32\ (ab)^3/(a^2+b^2)]^{1/4}$, where d is the circular lumen diameter, and a and b the radial and tangential lumen diameter, respectively. The number of rays was manually surveyed from the images by counting the rays crossing an ideal line in the middle of the rings. The position (coordinates) of cells was used to determine vessel and ray numbers as well as average values of vessel lumen size in 5° sectors with increasing tangential distance from the wound (Fig. 1b). To our knowledge, wood anatomical features in the context of mechanical damage have never been continuously studied in the tangential direction. They have only been locally analysed in the injured area or, at best, in different radial segments (Arbellay et al., 2010; Bigio et al., 2010; Delvaux et al., 2010).

In addition, to evaluate the impact of wounding on tree hydraulic architecture, xylem relative conductivity (Zimmermann, 1983) and three indicators of xylem vulnerability to cavitation were calculated. Xylem relative conductivity (*REC*), i.e. xylem hydraulic efficiency per unit area, was obtained using a Hagen-Poiseuille modified equation (Van den Oever et al., 1981): $REC=VF\ (AVLD/2)^4$, where $AVLD$ is the average vessel (circular) lumen diameter and VF the vessel frequency, i.e. the total number of vessels per (earlywood) unit area. Xylem vulnerability to hydraulic failure was determined by calculating the xylem vulnerability index (VUL), the thickness-to-span ratio (THS) of vessels, and the vessel grouping index (VG). The xylem vulnerability index was considered to assess xylem safety from embolism, as proposed by Carlquist (1977): $VUL=AVLD/VF$. The thickness-to-span ratio of vessels, i.e. the intervessel wall thickness divided by the vessel (circular) lumen diameter, is an indicator of cell mechanical support against implosion (Hacke et al., 2001). *THS* was measured at 50× magnification on 30 vessels for each ring (150 vessels in total). Finally, the vessel grouping index, i.e. the total number of vessels divided by the total number of vessel groups (including solitary and grouped vessels), was used as an indicator of alternative water pathways in case of hydraulic failure (Carlquist, 2001).

Results

Table 1 provides information on xylem anatomical and hydraulic parameters for the five rings investigated. Average vessel lumen area (*AVLA*) and average vessel lumen diameter (*AVLD*) were respectively greater than 1000 μm^2 and 150 μm in all rings, except in the injury ring (Ir 1) where both values were lower. The three rings built after cambial injury (Ir 1, Pr 1, and Pr 2) counted more earlywood vessels and rays than the two control rings (Cr 1 and Cr 2) (Table 1). No significant anatomical and hydraulic changes were statistically detected between the two control rings (Table 2, ANOVA test, $P < 0.05$). However, significant ($P < 0.05$) to highly significant ($P < 0.001$) differences were found between Cr 2 and the three rings of the callus tissue.

Ir 1 showed the most evident response to wounding, with lower values for *AVLA* (35%) and *AVLD* (22%) as well as greater values for vessel number (119%) and ray number (33%). The formation of more and narrower earlywood vessels substantially reduced xylem relative conductivity (*REC*) and the xylem vulnerability index (*VUL*) by 54% ($P < 0.001$) and 32% ($P < 0.01$), respectively (Table 2). The thickness-to-span ratio (*THS*) of vessels increased by 94% ($P < 0.01$) and the vessel grouping index (*VG*) by 28% ($P < 0.001$). The wound-induced anatomical changes in wood structure were stronger close to the wound margin and were approximately limited to the extent of sector A (Fig. 2). *AVLA* decreased up to 77% in this 30° sector, whereas vessel number increased up to 475% and ray number up to 115%. Earlywood vessels with a lumen diameter less than 80 μm predominantly accumulated in sector A (Fig. 3).

Pr 1 and Pr 2, similarly to Ir 1, were composed of more earlywood vessels and rays in comparison with the control rings (Table 2). Vessel number was significantly larger in Pr 1 (50%) and Pr 2 (41%) though less than in Ir 1 (119%). In both post-injury rings, it displayed a tangential constant pattern as opposed to the sharp increase observed in sector A of the injury ring (Fig. 2). Ray number was also larger in Pr 1 (48%) and Pr 2 (46%) (Table 2), showing a rather steady increase toward the wound margin (Fig. 2). After careful examination of the callus tissue, most of the rays counted in the middle of Pr 1 were seen to originate in the late portion of Ir 1. Finally, it was noteworthy that narrower earlywood vessels almost exclusively developed in sector A of the injury ring (Fig. 2). The post-injury rings, by contrast, recovered from the diminution of vessel lumen size in sector A and were then built of wider conduits in sectors

Table 1. Xylem anatomical and hydraulic variables analysed over the whole disc quarter for the five rings investigated

	Cr 1 Mean ±SD	Cr 2 Mean ±SD	Ir 1 Mean ±SD	Pr 1 Mean ±SD	Pr 2 Mean ±SD
AVLA (μm^2)	1191.84±229.42	1267.27±193.43	825.74±217.08	1350.51±239.45	1377.75±432.56
AVLD (μm)	173.44±18.31	179.72±15.54	140.20±20.26	185.48±16.58	184.29±30.60
VN	82.17±35.75	82.83±39.42	181.11±101.39	124.47±58.70	116.50±52.12
VF (mm^{-2})	34.86±6.73	33.13±6.63	43.12±16.47	35.71±5.90	36.20±8.25
REC (mm^2)	2.03E-03±7.76E-04	2.18E-03±6.10E-04	1.01E-03±3.85E-04	2.68E-03±8.80E-04	2.68E-03±1.25E-03
VUL (mm^3)	5.22E-03±1.04E-03	5.69E-03±1.47E-03	3.85E-03±1.88E-03	5.37E-03±1.24E-03	5.51E-03±2.16E-03
THS (μm)	0.06±0.03	0.06±0.01	0.11±0.10	0.06±0.03	0.06±0.03
VG	1.20±0.07	1.20±0.08	1.53±0.35	1.28±0.10	1.25±0.10
RN	117.50±41.70	126.56±46.95	167.89±53.07	187.12±52.57	184.50±57.75

Variables: *AVLA*, average vessel lumen area; *AVLD*, average vessel lumen diameter; *VN*, vessel number; *VF*, vessel frequency; *REC*, xylem relative conductivity; *VUL*, xylem vulnerability index; *THS*, thickness-to-span ratio of vessels; *VG*, vessel grouping index; *RN*, ray number. Ring types: Cr, control ring; Ir, injury ring; Pr, post-injury ring. Values are averaged over 18 trees.

Table 2. One-way ANOVA results when comparing xylem anatomical and hydraulic variables between rings

	Cr 1–Cr 2 *P*-value	Change (%)	Cr 2–Ir 1 *P*-value	Change (%)	Cr 2–Pr 1 *P*-value	Change (%)	Cr 2–Pr 2 *P*-value	Change (%)
AVLA (μm^2)	0.294	+6	**<0.001**	**–35**	0.265	+7	0.347	+9
AVLD (μm)	0.275	+4	**<0.001**	**–22**	0.297	+3	0.593	+3
VN	0.958	+1	**0.001**	**+119**	**0.019**	**+50**	**0.044**	**+41**
VF (mm^{-2})	0.702	–5	**0.023**	**+30**	0.234	+8	0.269	+9
REC (mm^2)	0.279	+7	**<0.001**	**–54**	0.057	+23	0.159	+23
VUL (mm^3)	0.534	+9	**0.003**	**–32**	0.495	–6	0.791	–3
THS (μm)	0.226	–11	**0.006**	**+94**	0.864	+2	0.709	–4
VG	0.946	+1	**<0.001**	**+28**	**0.016**	**+7**	0.125	+5
RN	0.545	+8	**0.018**	**+33**	**0.001**	**+48**	**0.005**	**+46**

Variables: *AVLA*, average vessel lumen area; *AVLD*, average vessel lumen diameter; *VN*, vessel number; *VF*, vessel frequency; *REC*, xylem relative conductivity; *VUL*, xylem vulnerability index; *THS*, thickness-to-span ratio of vessels; *VG*, vessel grouping index; *RN*, ray number. Ring types: Cr, control ring; Ir, injury ring; Pr, post-injury ring. Significant results appear in bold.

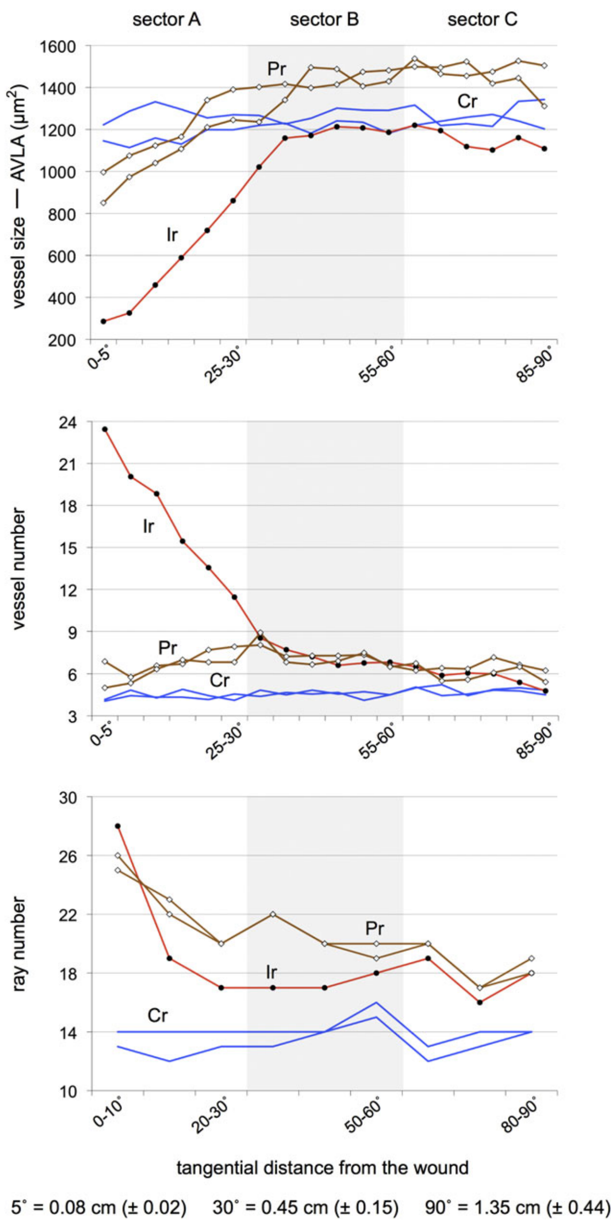

Fig. 2. Line plots displaying radial and tangential variations in xylem anatomical parameters. The five rings investigated were examined in distinct sectors with increasing tangential distance from the wound: in 5° sectors for vessel features (size and number) and in 10° sectors for ray number. Measurements of the arc length of sectors were made on the outer edge of samples and then averaged. Values are averaged over 18 trees. Variable: *AVLA*, average vessel lumen area. Ring types: Cr, control ring; Ir, injury ring; Pr, post-injury ring. (This figure is available in colour at *JXB* online.)

B and C. ANOVA results were not significant when comparing *AVLA* and *AVLD* between Cr 2 and the two post-injury rings (Table 2). They indicated a slight increase in vessel lumen size comparable with that between the two control rings. Moreover, the majority of conduits in both ring types had a lumen diameter ranging from 160–280 µm (Fig. 3). Earlywood vessels in Pr 1 and Pr 2 enhanced xylem relative conductivity (*REC*) and reduced the xylem

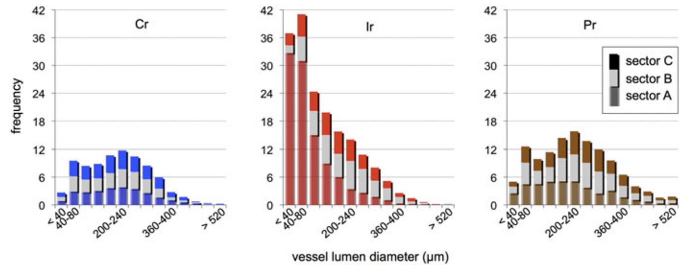

Fig. 3. Histograms of vessel size distribution for each ring type. Data were split into equal-sized classes of vessel lumen diameter. This variable was analysed in three 30° sectors (A, B, C). Sector A is the closest to the injury. Values are averaged over 18 trees. For the control rings and post-injury rings, values are averaged over the two rings that were considered in each ring type. Ring types: Cr, control ring; Ir, injury ring; Pr, post-injury ring. (This figure is available in colour at *JXB* online.)

vulnerability index (*VUL*) though not significantly. The thickness-to-span ratio (*THS*) of vessels reached pre-wounding values in both post-injury rings, while the vessel grouping index (*VG*) was significantly higher by 7% ($P < 0.05$) in Pr 1, but was no longer different from Cr 1 and Cr 2 in Pr 2 (Table 2).

Discussion

Wound-induced anatomical changes in wood structure

The results of this study confirm that wounding induces the formation of narrower vessels (Aloni and Zimmermann, 1984; Rademacher *et al.*, 1984; Kuroda and Shimaji, 1985; Lowerts *et al.*, 1986; Lev-Yadun and Aloni, 1993; Arbellay *et al.*, 2010; Ballesteros *et al.*, 2010). Regenerative earlywood vessels were found to be much narrower in sector A of the injury ring (Fig. 4a). The wider conduits in sectors B and C of the post-injury rings presumably reflect juvenile tree growth. Heliñska-Raczkowska and Fabisiak (1999) determined that, in juvenile wood, earlywood vessel lumen diameter increases with cambial age. Therefore, these wider conduits observed in our young trees possibly attest to the resumption of normal juvenile tree growth.

Abnormally narrow as well as more numerous earlywood vessels developed as a consequence of cambial injury, which is consistent with recent wood anatomical investigations of fire scars (Bigio *et al.*, 2010; Kames *et al.*, 2011). Vessel number increase was also prevalent in sector A of the injury ring, where radial groups of vessels were clearly visible (Fig. 4a). The presence of more vessels, however, was noted over at least three years after stem scarring. Several authors have stated that fewer to no vessels are formed following wounding when actually only describing the barrier zone, i.e. the callus tissue initially built at the wound surface (Sharon, 1973; Moore, 1978; Mulhern *et al.*, 1979; Rademacher *et al.*, 1984; Lowerts *et al.*, 1986; Stobbe *et al.*, 2002; Schwarze *et al.*, 2007).

The initiation of more rays was also noted over at least three years after stem scarring. Ray number was larger in

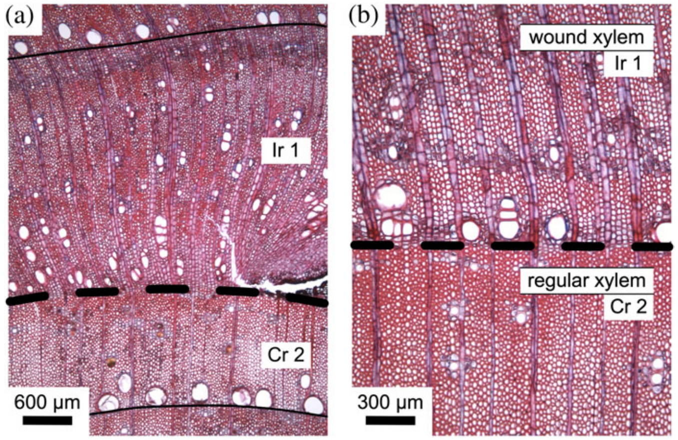

Fig. 4. Microscopic views of *F. excelsior* wood anatomy prior to and after cambial injury. The dashed line shows the position of the cambium at the time of wounding. (a) Xylem cells from the control ring Cr 2 and from the injury ring (Ir 1) as seen close to the wound margin (sector A). In response to mechanical damage, more and narrower earlywood vessels were formed during the growing season following wounding. They accumulated in radial groups. (b) Wound xylem also differed from regular xylem through the enlargement of pre-existing rays. (This figure is available in colour at *JXB* online.)

the post-injury rings, increasing toward the wound margin. It should be emphasized, however, that most of the rays originated in the late portion of the injury ring. Moreover, despite the fact that ray size was not measured in this study, pre-existing rays were noticed to enlarge following wounding (Fig. 4b). These findings further highlight that broad-leaved trees adjust to mechanical damage through ray number increase (Carmi *et al.*, 1972; Rademacher *et al.*, 1984; Lowerts *et al.*, 1986; Lev-Yadun and Aloni, 1992) and ray size increase (Carmi *et al.*, 1972; Sharon, 1973; Mulhern *et al.*, 1979; Bauch *et al.*, 1980; Rademacher *et al.*, 1984; Lev-Yadun and Aloni, 1992, 1993; Lev-Yadun, 1994).

Functional meaning and ecophysiological origin of the anatomical anomalies

More and narrower earlywood vessels substantially reduced xylem relative conductivity and xylem vulnerability to cavitation in the injury ring, attesting to the functional need of trees to balance xylem hydraulic efficiency against xylem safety from embolism (Zimmermann, 1983; Tyree *et al.*, 1994; Sperry *et al.*, 2008). Moreover, wood composed of narrower vessels is deemed mechanically stronger because of increased cross-sectional area available for fibres, which may be construed as a trade-off between xylem hydraulic efficiency and xylem mechanical strength (Wagner *et al.*, 1998; McCulloh *et al.*, 2004). The diminution of vessel lumen size actually led to a greater thickness-to-span ratio of vessels, which translates into higher wood density, greater mechanical reinforcement, and greater resistance to cavitation (Hacke *et al.*, 2001; Lens *et al.*, 2011). In addition, vessel grouping further increased xylem safety from embolism due to a greater redundancy of water pathways (Carlquist, 1984; Lens *et al.*, 2011). The ray anomalies (more and larger rays) detected in the callus tissue are also of great adaptive value because rays enhance compartmentalization of decay (Shigo, 1984) and strengthen wood to better adjust to radial mechanical stress (Mattheck and Kubler, 1995; Burgert and Eckstein, 2001). In fact, rays in ring-porous wood reinforce radial wood cohesion acting as radial pins preventing layers with different stiffness (i.e. earlywood and latewood) from slipping past each other (Reiterer *et al.*, 2002).

Stem scarring resulted in the occurrence of strong vessel anomalies in the injury ring, presumably because wounding locally obstructed the basipetal flow of auxin, consequently raising auxin concentration in the injured area and causing rapid vessel differentiation (Aloni and Zimmermann, 1983, 1984; Roberts *et al.*, 1988). Furthermore, ethylene is synthesized following wounding (Imaseki, 1985; Abeles *et al.*, 1992) and is believed to influence, along with radial and axial signal flows, the number and size of regenerative cells (Lev-Yadun and Aloni, 1995). Ethylene applied on the stem of unscarred *Ulmus americana* seedlings already stimulated the development of numerous abnormally narrow earlywood vessels as well as the enlargement of rays (Yamamoto *et al.*, 1987). It is possible that the observed wound-induced anatomical changes in wood structure may also be accounted for by ethylene interfering with the axial flow of auxin (Jaffe, 1980; Abeles *et al.*, 1992).

Impact of wounding on tree metabolism

As pointed out by Schweingruber (2007), in times of environmental stresses, tree metabolism is subject to economical principles. Tree priorities following wounding include the re-establishment of xylem mechanical strength and xylem safety from embolism, which were found to occur through an increase in the callus mass, but at the expense of water transport and hence future tree growth. The higher proportion of radial parenchyma in the callus tissue corresponds to a considerable effort for defence against pathogens (compartmentalization) and wound healing. Cambial injury stimulates the production of parenchyma around the wound in order to protect the living tissue (Schmitt and Leise, 1990).

The young trees examined demonstrated high resistance to mechanical damage. On the one hand, the negative wound effects due to the reduced xylem hydraulic efficiency lasted only one year and were restrained to a relatively small sector (30°) immediately adjacent to the wound. An equally prompt recovery in vessel lumen size has been observed in *Quercus pyrenaica* and *F. angustifolia* (Ballesteros *et al.*, 2010). On the other hand, the increased investment in fixing carbohydrates seems to last over a longer period of at least three years. It is probable that, similarly to the time required for wound closure, the strength and persistence of wound-induced anatomical anomalies strongly vary according to tree species, tree vigour, and wound size (Neely, 1988; Delvaux *et al.*, 2010).

In conclusion, this study presents wounding as an environmental force moulding the wood structure of broad-leaved trees and provides detailed information on the duration and extension of associated anatomical changes, thus improving knowledge on temporal (radial) and spatial (tangential) cambial activity in response to mechanical damage.

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