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# Changes in tracheid and ray traits in fire scars of North American conifers and their ecophysiological implications

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- **Background and Aims** Fire scars have been widely used as proxies for the reconstruction of fire history; however, little is known about the impact of fire injury on wood anatomy. This study investigates changes in tracheid and ray traits in fire scars of Douglas fir (*Pseudotsuga menziesii*), western larch (*Larix occidentalis*) and ponderosa pine (*Pinus ponderosa*), and discusses their ecophysiological implications for tree recovery from fire.
- **Methods** Transverse and tangential microsections were prepared for light microscopy and image analysis. Measurements of tracheids and rays were made in the three spatial dimensions: axially (at different section heights), radially (in different rings) and tangentially (with increasing distance from the wound margin).
- **Key Results** Changes were strongest in the first year after fire injury, with a decrease in tracheid size (by 25–30 %) and an increase in tracheid density (by 21–53 %) for the three species. In addition, an increase in ray size (by 5–27 %) and an increase in ray density (by 19–36 %) were found in *P. menziesii* and *L. occidentalis*. Changes were comparable along the fire-injured stem and were often most marked close to the fire scar.
- **Conclusions** The differentiation after fire injury of narrower and more numerous tracheids expresses a trade-off between hydraulic safety and hydraulic efficiency, while that of larger and more numerous rays serves compartmentalization and wound closure, mechanical strength and defence responses. *Pinus ponderosa* does not generally produce more ray tissue after fire injury and thus appears to be more adapted to fire.

**Key words:** Ecophysiology, conifer, fire scar, *Larix occidentalis*, western larch, *Pinus ponderosa*, ponderosa pine, *Pseudotsuga menziesii*, Douglas fir, ray, tracheid, wood anatomy.

## INTRODUCTION

Fire scars form on trees when surface fires of low to moderate intensity heat the cambium to lethal temperatures around a portion of the bole (Gutsell and Johnson, 1996). Similarly to other types of mechanical injury to the cambium, tree response to fire injury includes (1) compartmentalization to resist the spread of decay and (2) production of wound xylem (or woundwood) to close the wound and restore continuity of the cambium (Smith and Sutherland, 1999, 2001). Wound closure occurs from the growth of new, healthy vascular tissues that converge inward from both sides of the wound to shield the exposed xylem (Larson, 1994; Fink, 1999).

Fire scars provide macroscopic – at the sample level – evidence of past forest fires that has been used to determine the occurrence, extent and frequency of events (Heyerdahl *et al.*, 2001; Swetnam *et al.*, 2009; Farris *et al.*, 2010; Falk *et al.*, 2011). Nevertheless, limitations of fire scars (Van Horne and Fulé, 2006) have motivated the exploration of alternative proxies for the reconstruction of fire history. In this context, both scarred and unscarred trees have been studied for microscopic – at the tissue level – evidence of past forest fires, including anomalies in wood anatomy (Bigio *et al.*, 2010; Bravo, 2010; Kames *et al.*, 2011) and wood chemistry (Bukata *et al.*, 2008; Beghin *et al.*, 2011; Pearson *et al.*, 2011). At the same time, a growing body of research on mechanically injured trees has presented results on the wood anatomical response of conifers and broad-leaved trees to flooding (Ballesteros *et al.*, 2010*a*, *b*; Arbellay *et al.*, 2012*b*) and mass-movement processes (Bollschweiler *et al.*, 2008; Stoffel and Hitz, 2008; Schneuwly *et al.*, 2009*a*, *b*; Arbellay *et al.*, 2010, 2012*a*, 2013). However, the impact of fire injury on wood anatomy has been assessed more rarely and conifers have yet to be investigated, as existing studies on the topic have been conducted exclusively with broad-leaved trees (Bigio *et al.*, 2010; Bravo, 2010).

This study elucidates the wood anatomical response of conifers to fire injury. Tracheids and rays were analysed for changes in size and density in fire scars of three widely distributed North American conifers: Douglas fir (*Pseudotsuga menziesii*), western larch (*Larix occidentalis*) and ponderosa pine (*Pinus ponderosa*). Changes were measured axially (at different section heights), radially (in different rings) and tangentially (with increasing distance from the wound margin). This work determines the spatio-temporal extent of these changes, discusses their ecophysiological implications for tree recovery from fire, and evaluates their potential as new proxy evidence for tree exposure to fire.

## MATERIALS AND METHODS

### *Field campaign and sample preparation*

In mid-August 2003, the lightning-ignited Black Mountain fire burned 2800 ha of the Lolo National Forest near Missoula, west central Montana, USA. In late August through September 2011, 32 *Pseudotsuga menziesii* (Mirb.) Franco, 11 *Larix occidentalis* Nutt. and 18 *Pinus ponderosa* P.Lawson & C.Lawson trees with charred bark were sampled in the Dry Gulch area (46°52′N/114°12′W, 1787 m a.s.l.; E. K. Sutherland, USDA Forest Service, Missoula, USA, pers. comm.). Cross-sections were taken at 25 cm intervals up to the complete height of the bark char. For this study, two scarred trees of each species were chosen for wood anatomical analysis (Table 1), which was performed on cross-sections taken at four different section heights (25, 50, 75 and 100 cm above the ground surface) along the fire-injured stem (Fig. 1A). In total, 24 cross-sections were sectioned with a chisel to obtain a wood block where tracheids and rays could be compared between normal xylem and wound xylem within 4 cm from the wound margin (Fig. 1B). Each wood block was further split into two 2 cm wide pieces for preparation of 15 μm thick microsections with a sliding microtome. The more friable *P. ponderosa* microsections were cut 30 μm thick. In a first step, transverse microsections (Fig. 1C) were prepared to analyse earlywood tracheids in the last ring formed pre-fire (ring 0) and in the first, second, fourth and eighth rings formed post-fire (rings 1, 2, 4 and 8). Despite the fact that fire injury affects cells formed pre-fire via necrosis of the directly injured cells and increased metabolic activity of the adjoining uninjured cells (Fink, 1999), fire injury does not affect the size and density of these cells. Nevertheless, preliminary comparative analyses were carried out among rings of normal

TABLE 1. *Characteristics of the six trees analysed*

| | | Age (years) | SH = 25 cm | | SH = 50 cm | | SH = 75 cm | | SH = 100 cm | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | TC (cm) | WS (%) | TC (cm) | WS (%) | TC (cm) | WS (%) | TC (cm) | WS (%) |
| *P. menziesii* | PSM1 | 97 | 71 | 58 | 68 | 53 | 64 | 53 | 60 | 52 |
| | PSM2 | 47 | 61 | 54 | 57 | 47 | 52 | 27 | 51 | 20 |
| *L. occidentalis* | LAO1 | 108 | 49 | 53 | 44 | 64 | 39 | 62 | 42 | 52 |
| | LAO2 | 124 | 71 | 46 | 68 | 50 | 64 | 53 | 62 | 16 |
| *P. ponderosa* | PIP1 | 97 | 68 | 37 | 66 | 42 | 61 | 34 | 59 | 34 |
| | PIP2 | 156 | 71 | 31 | 68 | 46 | 67 | 37 | 62 | 31 |

SH, section height above the ground surface; TC, tree circumference at section height; WS, wound size at section height, i.e. percentage of cambium killed.

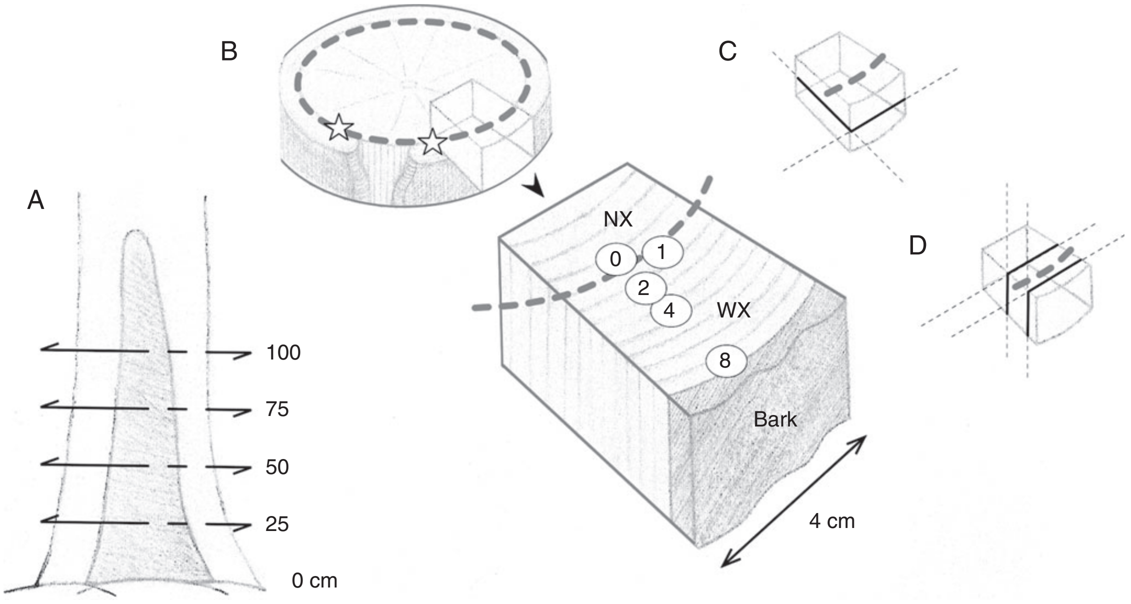

FIG. 1. Study design for wood anatomical analysis. (A) Tracheids and rays were analysed in cross-sections taken at four different section heights (25, 50, 75 and 100 cm above the ground surface) along the fire-injured stem. (B) Cross-sections were sectioned with a chisel to obtain a wood block where tracheids and rays could be compared between normal xylem (NX) and wound xylem (WX) within 4 cm from the wound margin, indicated by a star. The dashed line shows the position of the cambium at the time of fire injury. (C) Transverse microsections were prepared to analyse earlywood tracheids in the last ring formed pre-fire (ring 0) and in the first, second, fourth and eighth rings formed post-fire (rings 1, 2, 4 and 8). (D) Tangential microsections were prepared to analyse rays, requiring one cut through normal xylem and one cut through wound xylem.

xylem to establish ring 0 as a suitable control. In a second step, tangential microsections (Fig. 1D) were prepared to analyse rays, requiring two radial cuts (one through normal xylem and one through wound xylem). The latter cut was performed about 2 mm inside the wound xylem, i.e. within ring 1 or 2. All microsections (144 in total) were stained with a 1 % safranin and astrablue solution, rinsed with water, alcohols and xylol, and mounted permanently on microscope slides using Canada balsam.

### *Wood anatomical analysis*

Images of the transverse and tangential microsections were captured at ×200 and ×100 magnification, respectively, with a digital camera mounted on a light microscope. WinCELL software (Régent Instruments Inc., 2004) was used to measure average tracheid lumen area (ATLA), tracheid density (TD), average ray height (ARH), average ray width (ARW) and ray density (RD). Ray size was based on uniseriate rays, whereas ray density was derived from both uniseriate and fusiform rays. Measurements were made at the four section heights and in the five rings mentioned previously, and at 0·5 cm intervals along the 4 cm wide tangential window. A total of 48 000 earlywood tracheids were recorded: 4 section heights × 5 rings × 8 tangential measurements × 50 tracheids at each location = 8000 in each tree. The unit area considered at each location on the transverse microsections was about 0·01 mm$^2$. In addition, a total of 3840 rays were examined: 4 section heights × 2 radial cuts × 8 tangential measurements × 10 rays at each location = 640 in each tree. The unit area considered at each location on the tangential

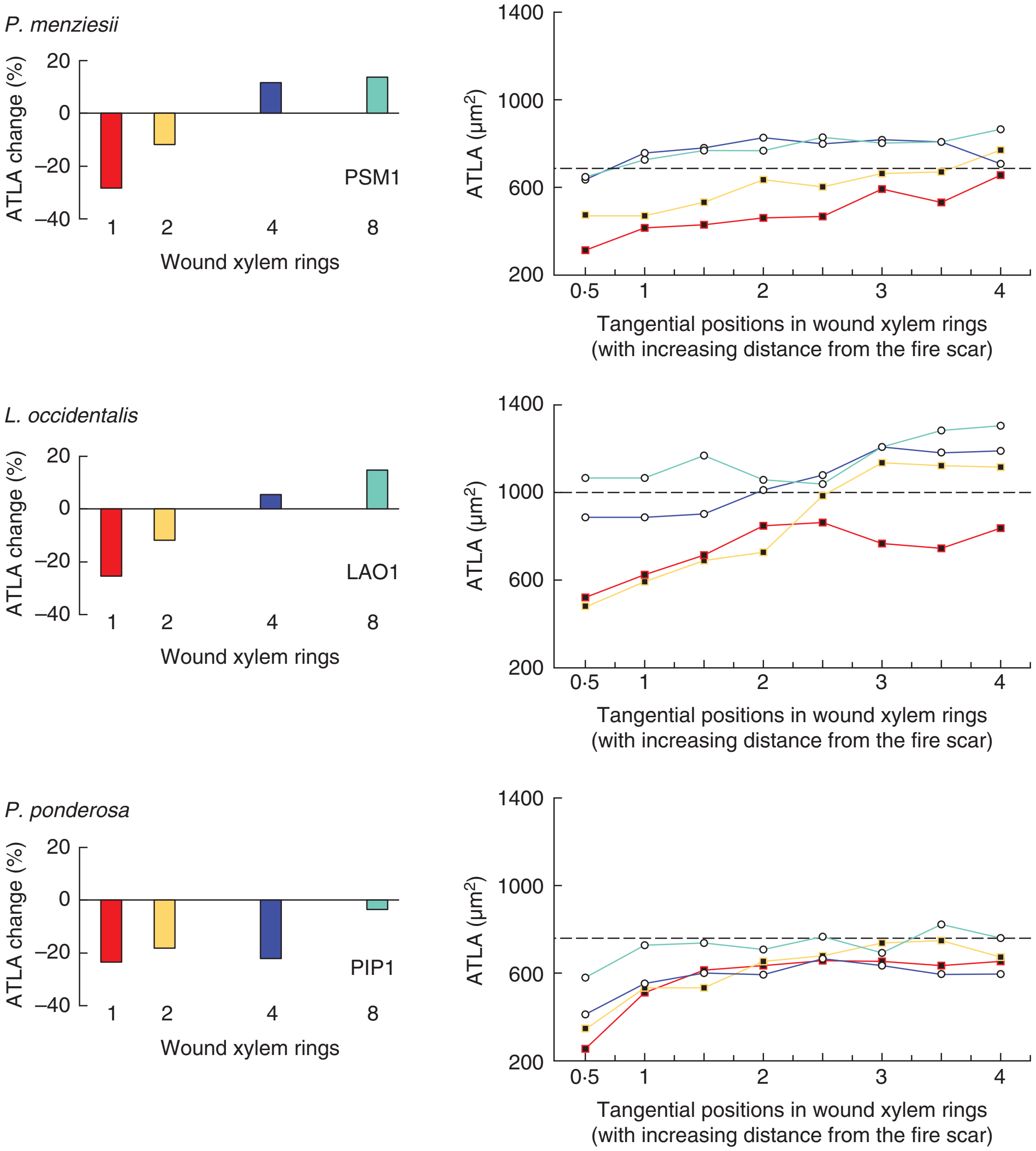

FIG. 2. Changes in average tracheid lumen area (ATLA) between normal xylem (ring 0) and wound xylem (rings 1, 2, 4 and 8), presented for one tree each of *P. menziesii*, *L. occidentalis* and *P. ponderosa*. Data used are from all section heights. ATLA was measured in each ring at 0·5 cm intervals along the 4 cm wide tangential window. The dashed line indicates the mean value in ring 0.

microsections was about 1 mm$^2$. One-way analysis of variance (ANOVA) was used to determine whether there were significant ($P < 0{\cdot}05$) changes in tracheid and ray traits between normal xylem and wound xylem. Changes were comparable along the fire-injured stem. As a consequence, and for ease of comparison, data from the four section heights were pooled together in the results.

## RESULTS

### *Changes in tracheid traits*

The ATLA values pre-fire were 695 μm$^2$ in PSM1, 1013 μm$^2$ in LAO1 and 764 μm$^2$ in PIP1 (Fig. 2). In all trees and in equivalent magnitude in the three species, ATLA decreased significantly in wound xylem, by 25–30 % in ring 1 and by 13–25 % in ring 2 (Table 2). This decrease was most marked close to the fire scar. In ring 1, ATLA values at 0·5 cm from the scar were 314 μm$^2$ in PSM1, 523 μm$^2$ in LAO1 and 256 μm$^2$ in PIP1 (Fig. 2). PSM1, LAO1 and LAO2 showed a significant increase in ATLA by ring 8 (by 10–13 %), apparent within the entire tangential window and originating from the base of the tree. In comparison, PSM2 and PIP1 still showed a significant decrease in ATLA by ring 8 (by 5–8 %), although signs of recovery started to appear in the lower section heights. PIP2 still produced narrower tracheids in ring 8 (by 32 %) with no evident signs of recovery.

The TD values pre-fire were 1426 mm$^{-2}$ in PSM1, 977 mm$^{-2}$ in LAO1 and 1266 mm$^{-2}$ in PIP1 (Fig. 3). TD increased significantly in wound xylem, in all trees and in equivalent magnitude in the three species (Table 2). This increase was strongest in ring 1 (21–53 %) and was most marked close to the fire scar. In ring 1, TD values at 0·5 cm from the scar were 2406 mm$^{-2}$ in PSM1, 1906 mm$^{-2}$ in LAO1 and 2219 mm$^{-2}$ in PIP1 (Fig. 3). TD increase post-fire was no longer significant by ring 4 in PSM1 and by ring 8 in PSM2, LAO1, LAO2 and PIP1. Pre-fire values were regained within the entire tangential window in these rings. PIP2 was again slower in terms of recovery, still producing more numerous tracheids in ring 8 (by 21 %).

### *Changes in ray traits*

The ARH and ARW values pre-fire were respectively 71 and 7 μm in PSM1, 122 and 7 μm in LAO1 and 204 and 20 μm in PIP1. ARH and ARW increased significantly in wound xylem in *P. menziesii* (by 14–27 %) and *L. occidentalis* (by 5–21 %) (Table 3). These increases showed local fluctuations as opposed to localized peaks of intensity (Fig. 4). In contrast, *P. ponderosa* exhibited no increases in wound xylem: ARH and ARW values were significantly lower in PIP1 (by 5–8 %) and not significantly different in PIP2 (Table 3).

The RD values pre-fire were 35 mm$^{-2}$ in PSM1, 31 mm$^{-2}$ in LAO1 and 27 mm$^{-2}$ in PIP1. RD increased significantly in wound xylem in *P. menziesii* (by 19–25 %) and *L. occidentalis* (by 33–36 %) (Table 3). This increase was most marked close to the fire scar. At 0·5 cm from the scar, RD increased by 52 % in PSM1 and by 56 % in LAO1 (Fig. 4). In *P. ponderosa*, however, RD values were not significantly different in wound xylem (Table 3), although more numerous rays were produced close to the scar (Fig. 4).

## DISCUSSION

### *Changes in tracheid traits*

*Pseudotsuga menziesii*, *L. occidentalis* and *P. ponderosa* all produced narrower and more numerous tracheids after fire injury (Fig. 5C), most conspicuously in the first post-fire ring (Fig. 5A, B). Similar responses were observed in some other species exposed to fire. Nicholls (1972) described a ‘fire ring’ made of smaller vessels in *Eucalyptus marginata*. Moreover, narrower and more numerous vessels were detected within the fire ring in *Castanea sativa* (Bigio *et al.*, 2010) and in the first post-fire ring in *Fraxinus nigra* (Kames *et al.*, 2011).

The decrease in conduit size caused by fire injury corresponds to that associated with other types of mechanical injury to the cambium induced by other natural hazards (flooding and mass-movement processes) or human actions (partial girdling, pinning and drilling). Wound xylem has shown narrower tracheids in *Pinus halepensis*, *Larix decidua* and *Pinus pinaster* (Fahn and Zamski, 1970; Stoffel and Hitz, 2008; Ballesteros *et al.*, 2010*a*) and narrower vessels in various broad-leaved tree species (Bauch *et al.*, 1980; Aloni and Zimmermann, 1984; Rademacher *et al.*, 1984; Lowerts *et al.*, 1986; Lev-Yadun and Aloni, 1993; Arbellay *et al.*, 2010, 2012*a*, *b*, 2013; Ballesteros *et al.*, 2010*b*). The suggestion of Aloni and Zimmermann (1983) that conduit

Table 2. *Changes in tracheid traits between normal xylem (ring 0) and wound xylem (rings 1, 2, 4 and 8) for the six trees analysed*

| | | Rings 0–1 | | Rings 0–2 | | Rings 0–4 | | Rings 0–8 | |
|---|---|---|---|---|---|---|---|---|---|
| | | *P*-value | Δ (%) | *P*-value | Δ (%) | *P*-value | Δ (%) | *P*-value | Δ (%) |
| PSM1 | ATLA | **<0·001** | **−30** | **<0·001** | **−13** | **<0·001** | **+10** | **<0·001** | **+12** |
| | TD | **<0·001** | **+32** | **<0·001** | **+14** | 0·627 | +1 | 0·929 | +1 |
| PSM2 | ATLA | **<0·001** | **−27** | **<0·001** | **−25** | **<0·001** | **−18** | **<0·001** | **−8** |
| | TD | **<0·001** | **+21** | **0·008** | **+16** | **0·008** | **+14** | 0·608 | +6 |
| LAO1 | ATLA | **<0·001** | **−27** | **<0·001** | **−14** | 0·091 | +2 | **<0·001** | **+13** |
| | TD | **<0·001** | **+39** | **<0·001** | **+28** | **<0·001** | **+19** | 0·864 | +1 |
| LAO2 | ATLA | **<0·001** | **−28** | **<0·001** | **−16** | 0·125 | −2 | **<0·001** | **+10** |
| | TD | **<0·001** | **+53** | **<0·001** | **+28** | **0·001** | **+22** | 0·903 | +1 |
| PIP1 | ATLA | **<0·001** | **−25** | **<0·001** | **−20** | **<0·001** | **−24** | **<0·001** | **−5** |
| | TD | **0·001** | **+23** | **0·002** | **+20** | **0·005** | **+14** | 0·778 | +1 |
| PIP2 | ATLA | **<0·001** | **−25** | **<0·001** | **−19** | **<0·001** | **−31** | **<0·001** | **−32** |
| | TD | **<0·001** | **+37** | **<0·001** | **+25** | **<0·001** | **+23** | **<0·001** | **+21** |

ATLA, average tracheid lumen area; TD, tracheid density.One-way ANOVA test. Significant results appear in bold. Data used are from all section heights.

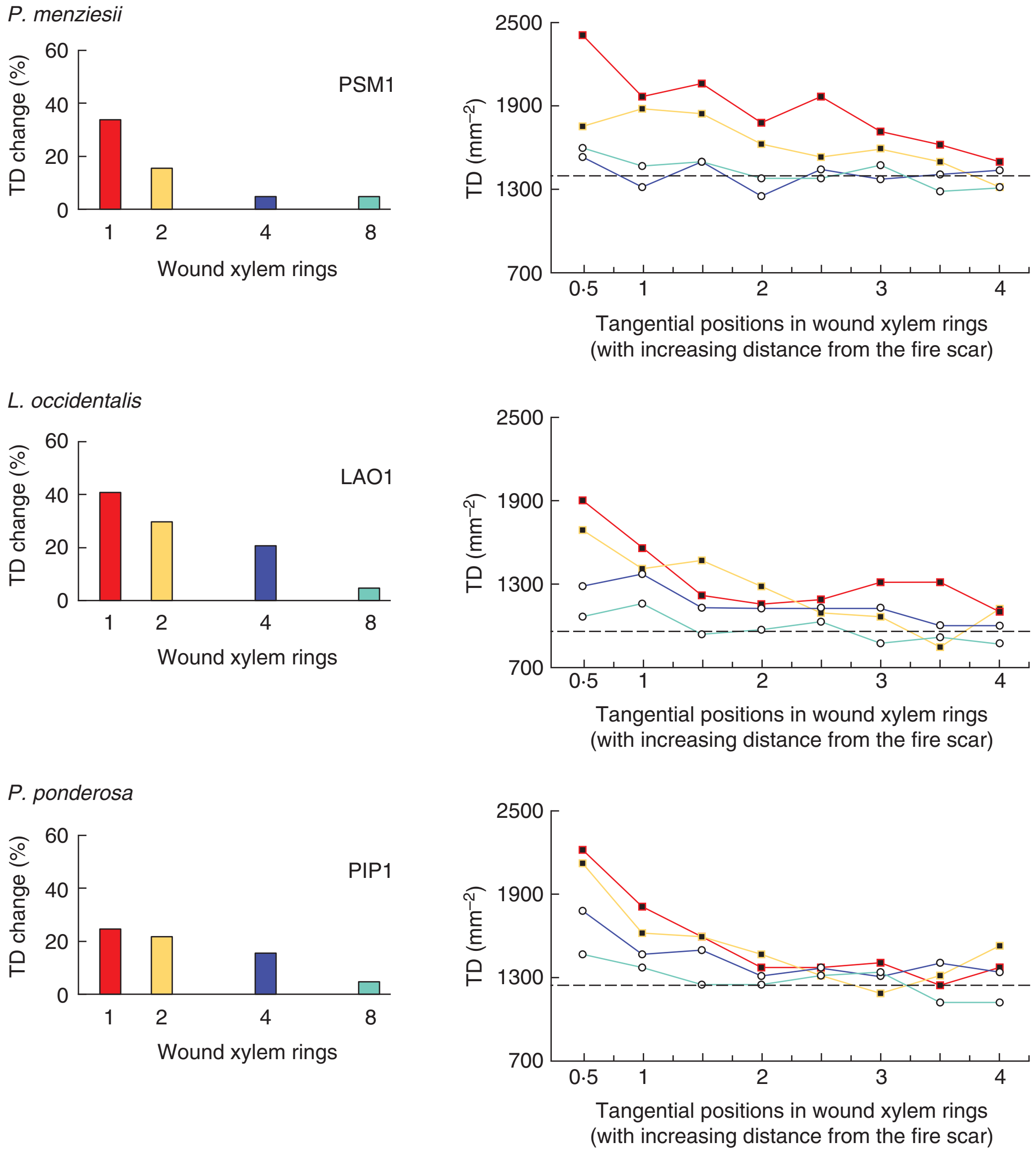

FIG. 3. Changes in tracheid density (TD) between normal xylem (ring 0) and wound xylem (rings 1, 2, 4 and 8), presented for one tree each of *P. menziesii*, *L. occidentalis* and *P. ponderosa*. Data used are from all section heights. TD was measured in each ring at 0·5 cm intervals along the 4 cm wide tangential window. The dashed line indicates the mean value in ring 0.

size is related negatively to auxin concentration and to the rate of conduit differentiation has been confirmed repeatedly (Leitch, 2001; Lovisolo *et al.*, 2002; Anfodillo *et al.*, 2012). The formation of abnormally narrow tracheids in *P. menziesii*, *L. occidentalis* and *P. ponderosa* after fire injury would thus be accounted for by a local obstruction of the basipetal flow of auxin by the fire scar, consequently raising the auxin concentration in the injured area and causing rapid tracheid differentiation (Aloni and Zimmermann, 1983, 1984). Furthermore, ethylene is synthesized following wounding (Imaseki, 1985) and is also likely to influence tracheid formation by interfering with the flow of auxin (Abeles *et al.*, 1992; Aloni *et al.*, 2000). Ethylene stimulated the development of numerous abnormally narrow vessels in *Ulmus americana* (Yamamoto *et al.*, 1987) and increased the density of tracheids in *Pinus halepensis* (Yamamoto and Kozlowski, 1987).

Knowledge about conduit density after cambial injury is scarce. The barrier zone in broad-leaved trees is known to contain fewer vessels than normal xylem (Mulhern *et al.*, 1979; Bauch *et al.*, 1980; Rademacher *et al.*, 1984; Lowerts *et al.*, 1986; Stobbe *et al.*, 2002). Wound xylem (or woundwood), as the broader band of wood associated with injury, has been little investigated. Nevertheless, and in line with the increase in conduit density caused by fire injury, wound xylem contained more vessels than normal xylem in *Acer rubrum* after partial girdling (Aloni and Zimmermann, 1984) and in *Fraxinus excelsior* after flood-induced wounding (Arbellay *et al.*, 2012*b*).

### *Changes in ray traits*

*Pseudotsuga menziesii* and *L. occidentalis* produced larger and more numerous rays after fire injury (Fig. 5D), which concurs with results on mechanically injured trees where fire was not the cause of injury (Carmi *et al.*, 1972; Bauch *et al.*, 1980; Kuroda and Shimaji, 1984; Rademacher *et al.*, 1984; Lowerts *et al.*, 1986; Lev-Yadun and Aloni, 1992, 1993; Lev-Yadun, 1994; Arbellay *et al.*, 2012*b*). In contrast, *P. ponderosa* did not generally produce more ray tissue (radial parenchyma) after fire injury. Changes in ray size and density can vary among species of different genera (Bravo, 2010) as well as among species of the same genus. In contrast to *P. ponderosa*, pinning increased ray size in *Pinus taeda* (Kuroda and Shimaji, 1984), while partial girdling increased both ray size and density in *Pinus halepensis* (Lev-Yadun and Aloni, 1992).

TABLE 3. *Changes in ray traits between normal xylem (NX) and wound xylem (WX) for the six trees analysed*

| | | NX – WX | |
|---|---|---|---|
| | | *P*-value | Δ (%) |
| PSM1 | ARH | **<0·001** | **+26** |
| | ARW | **<0·001** | **+27** |
| | RD | **<0·001** | **+25** |
| PSM2 | ARH | **<0·001** | **+20** |
| | ARW | **<0·001** | **+14** |
| | RD | **0·021** | **+19** |
| LAO1 | ARH | **<0·001** | **+11** |
| | ARW | **<0·001** | **+5** |
| | RD | **<0·001** | **+33** |
| LAO2 | ARH | **<0·001** | **+7** |
| | ARW | **<0·001** | **+21** |
| | RD | **<0·001** | **+36** |
| PIP1 | ARH | **<0·001** | **−8** |
| | ARW | **<0·001** | **−5** |
| | RD | 1·000 | 0 |
| PIP2 | ARH | 0·105 | +1 |
| | ARW | 0·383 | −1 |
| | RD | 0·517 | +3 |

ARH, average ray height; ARW, average ray width; RD, ray density. One-way ANOVA test. Significant results appear in bold. Data used are from all section heights.

*Pinus* sp. are also different from other conifers in their resin duct formation following wounding (Fahn and Zamski, 1970; Lewinsohn *et al.*, 1991; Stoffel, 2008). Resin ducts are also parenchyma, as they are lined with a layer of secretory, parenchymatic epithelial cells (Wu and Hu, 1997). It is possible that the amount of parenchyma in wound xylem is determined by species-specific sensitivity to wound-induced ethylene, which would explain the different amount of ray tissue between *P. menziesii*, *L. occidentalis* and *P. ponderosa*. Ethylene promotes ray enlargement as well as resin duct formation (Barker, 1979; Yamamoto and Kozlowski, 1987; Yamamoto *et al.*, 1987; Fahn, 1988; Lev-Yadun and Aloni, 1992; Hudgins and Franceschi, 2004). A recent study on resin duct formation in fire scars of *P. menziesii* and *L. occidentalis* yielded contrasting results between the two species (E. Arbellay, University of British Columbia, Canada, unpubl. res.), supporting the above hypothesis.

### *Spatio-temporal extent of changes in tracheid and ray traits*

In *P. menziesii*, *L. occidentalis* and *P. ponderosa*, changes in tracheid size and density were most obvious in the first year

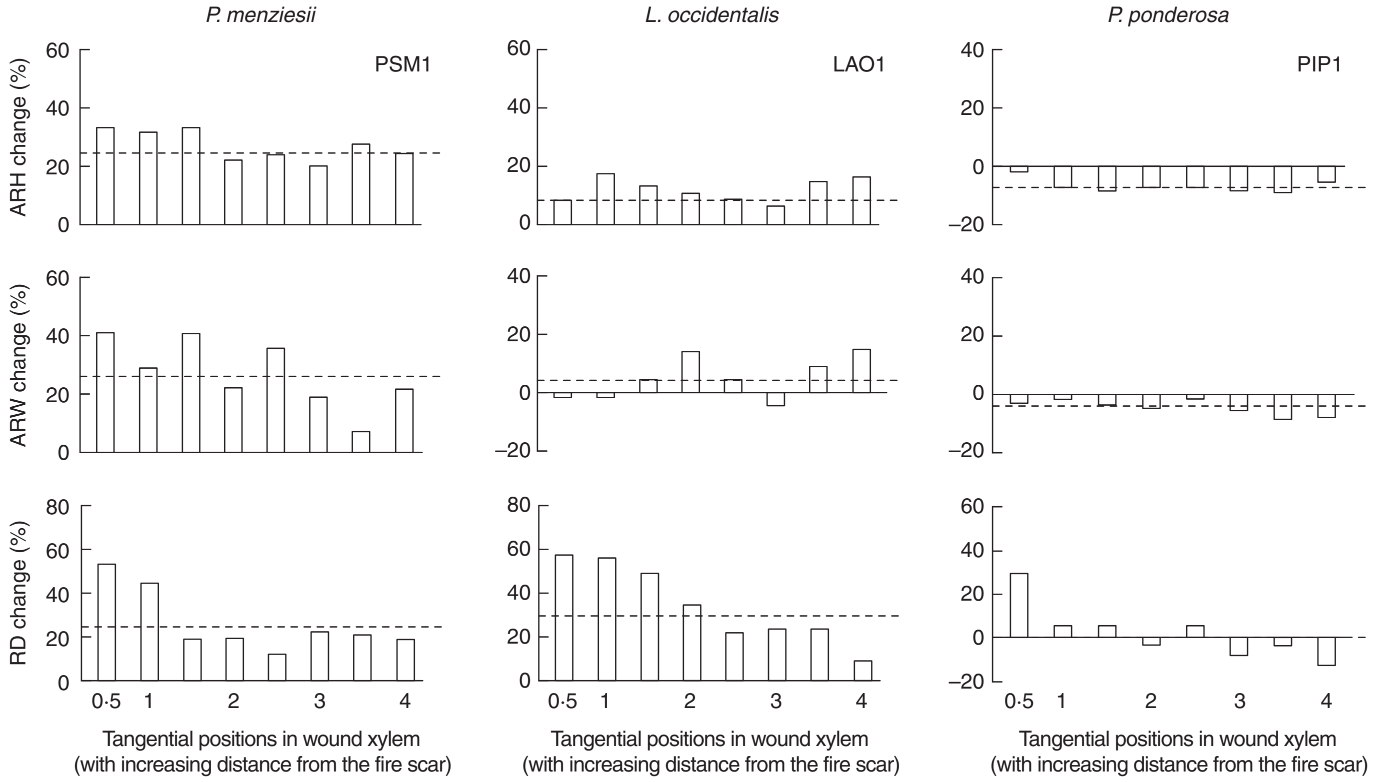

FIG. 4. Changes in average ray height (ARH), average ray width (ARW) and ray density (RD) between normal xylem and wound xylem, presented for one tree each of *P. menziesii*, *L. occidentalis* and *P. ponderosa*. Data used are from all section heights. ARH, ARW and RD were measured at 0·5 cm intervals along the 4 cm wide tangential window. The dashed line indicates the mean change value.

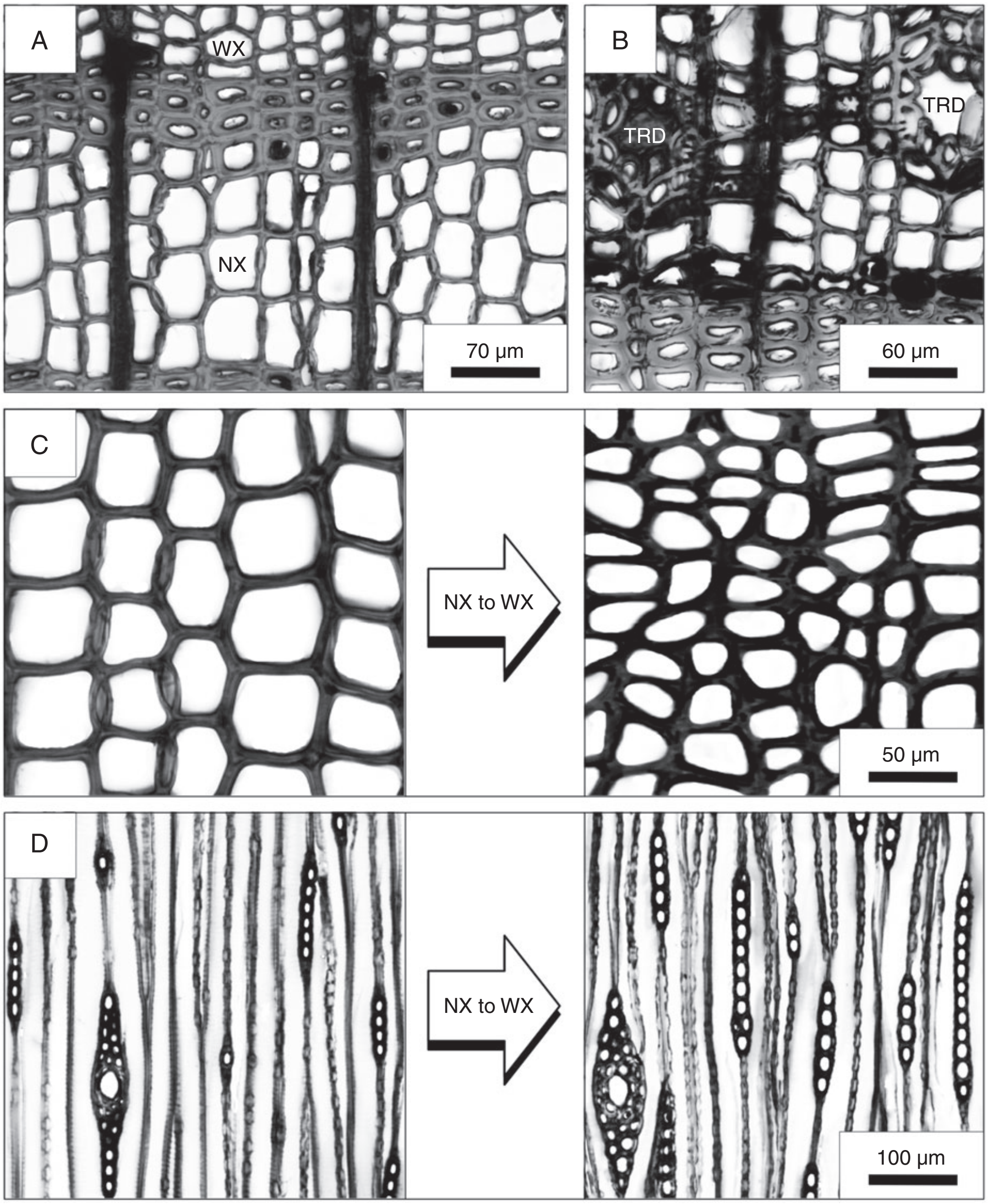

FIG. 5. Changes in wood anatomy in fire-scarred *P. menziesii*, *L. occidentalis* and *P. ponderosa*. (A) Narrower earlywood tracheids were produced in wound xylem (WX) compared with normal xylem (NX) (*L. occidentalis*). (B) Wound xylem exhibited narrower earlywood tracheids as well as tangential rows of traumatic resin ducts (TRD) (*P. menziesii*). (C) Tracheids decreased in size but increased in density between NX and WX (*P. ponderosa*). (D) In *P. menziesii*, as in *L. occidentalis*, rays increased in both size and density between NX and WX.

after fire injury and in the immediate vicinity of the fire scar. Arbellay *et al.* (2012*b*) made similar observations in *Fraxinus excelsior* after flood-induced wounding. In *P. menziesii* and *L. occidentalis*, changes in ray density were also most pronounced in the immediate vicinity of the fire scar, whereas changes in ray size were distributed more evenly with increasing distance from the wound margin. Changes in tracheid and ray traits were comparable along the fire-injured stem, although signs of recovery appeared over time in the form of lesser reduced or larger tracheids toward the base of the tree, seemingly more highly plastic.

In each tree, several factors act in concert to determine the severity of cambial injury (Table 1) as well as the magnitude of subsequent changes in wood anatomy (Tables 2 and 3). These factors include those that influence the rate of wound closure, i.e. wound size, tree species, tree vigour (or growth rate), tree age and tree diameter (Martin and Sydnor, 1987; Neely, 1988; Schneuwly-Bollschweiler and Schneuwly, 2012). Fire-scar formation initially depends on tree vigour, tree age and tree diameter (Guyette and Stambaugh, 2004), bark thickness (Hengst and Dawson, 1994; Pinard *et al.*, 1997) and fuel conditions at the base of the tree (Kolb *et al.*, 2007; Stephens *et al.*, 2010).

Moreover, the trees of this study had portions of their crown scorched (E. K. Sutherland, USDA Forest Service, Missoula, USA, pers. comm.). Both bole and crown damage affect post-fire growth (Wyant *et al.*, 1986; Ryan and Reinhardt, 1988; Peterson *et al.*, 1991) and thereby tree recovery from fire.

### *Ecophysiological implications of changes in tracheid and ray traits*

Fire and associated heating cause tree mortality directly by reducing xylem conductivity (Ducrey *et al.*, 1996; Balfour and Midgley, 2006) via cavitation and conduit wall deformation (Michaletz *et al.*, 2012). North-temperate conifers increase their resistance to cavitation by narrowing tracheid diameter rather than by thickening tracheid walls (Pittermann *et al.*, 2006*b*). This narrowing of conduits for cavitation resistance and the need for mechanical support constrain the hydraulic efficiency of the xylem (Hacke *et al.*, 2001; Pittermann *et al.*, 2006*a*, *b*; Sperry *et al.*, 2006). *Pseudotsuga menziesii*, *L. occidentalis* and *P. ponderosa* would have evolved narrower tracheids after fire injury as a safety measure against cavitation, to the benefit of mechanical support, but at the expense of hydraulic efficiency. The three species of this study also built more numerous tracheids after fire injury. A substantial number of reduced-size tracheids were therefore created, making the xylem porous enough to prevent embolism and provide conductance (Sperry *et al.*, 2008). This trade-off between hydraulic safety and hydraulic efficiency has been confirmed for *P. menziesii*, *L. occidentalis* and *P. ponderosa* (Piñol and Sala, 2000; Domec and Gartner, 2001, 2003).

*Pseudotsuga menziesii* and *L. occidentalis* increased the amount of parenchyma in wound xylem by producing larger and more numerous rays. This shift in tissue composition facilitates compartmentalization and wound closure (Shigo, 1984; Smith and Sutherland, 1999, 2001), reduces notch stresses at the wound margin and reinforces mechanical strength (Mattheck, 1998; Burgert *et al.*, 1999). An increased amount of parenchyma is also important for the refilling of embolized conduits (Hölttä *et al.*, 2006; Nardini *et al.*, 2011) and for the induction of defence responses, i.e. the formation of traumatic resin ducts and the activation of polyphenolic parenchyma cells (Nagy *et al.*, 2000; Hudgins and Franceschi, 2004). Interestingly, *P. ponderosa* did not generally produce more ray tissue after fire injury despite the higher vulnerability of *Pinus* sp. to water stress-induced embolism compared with other conifers (Piñol and Sala, 2000; Martínez-Vilalta *et al.*, 2004). This suggests *P. ponderosa* to be more adapted to fire.

Changes in wood anatomy in fire-scarred *P. menziesii*, *L. occidentalis* and *P. ponderosa* reflect the phenotypic plasticity and structural adaptations of the xylem to respond to multiple, competing functional necessities (Baas *et al.*, 2004; Chave *et al.*, 2009). They provide new ecophysiological insights into the spatio-temporal responsiveness of conifers to fire injury. Moreover, these features could constitute alternative proxies for the reconstruction of fire history and expand our understanding of fire as an evolutionary force.

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## LITERATURE CITED

**Abeles FB, Morgan PW, Saltveit ME Jr. 1992.** *Ethylene in plant biology*. San Diego: Academic Press.

**Aloni R, Zimmermann MH. 1983.** The control of vessel size and density along the plant axis: a new hypothesis. *Differentiation* **24**: 203–208.

**Aloni R, Zimmermann MH. 1984.** Length, width and pattern of regenerative vessels along strips of vascular tissue. *Botanical Gazette* **145**: 50–54.

**Aloni R, Feigenbaum P, Kalev N, Rozovsky S. 2000.** Hormonal control of vascular differentiation in plants: the physiological basis of cambium ontogeny and xylem evolution. In: Savidge RA, Barnett JR, Napier R, eds. *Cell and molecular biology of wood formation.* Oxford: BIOS Scientific Publishers, 223–236.

**Anfodillo T, Deslauriers A, Menardi R, Tedoldi L, Petit G, Rossi S. 2012.** Widening of xylem conduits in a conifer tree depends on the longer time of cell expansion downwards along the stem. *Journal of Experimental Botany* **63**: 837–845.

**Arbellay E, Stoffel M, Bollschweiler M. 2010.** Wood anatomical analysis of *Alnus incana* and *Betula pendula* injured by a debris-flow event. *Tree Physiology* **30**: 1290–1298.

**Arbellay E, Corona C, Stoffel M, Fonti P, Decaulne A. 2012*a*.** Defining an adequate sample of earlywood vessels for retrospective injury detection in diffuse-porous species. *PLoS One* **7**: e38824.

**Arbellay E, Fonti P, Stoffel M. 2012*b*.** Duration and extension of anatomical changes in wood structure after cambial injury. *Journal of Experimental Botany* **63**: 3271–3277.

**Arbellay E, Stoffel M, Decaulne A. 2013.** Dating of snow avalanches by means of wound-induced vessel anomalies in sub-arctic *Betula pubescens*. *Boreas* **42**: 568–574.

**Baas P, Ewers FW, Davis SD, Wheeler EA. 2004.** Evolution of xylem physiology. In: Hemsley AR, Poole I, eds. *The evolution of plant physiology.* London: Elsevier Academic Press, 273–295.

**Balfour DA, Midgley JJ. 2006.** Fire induced stem death in an African acacia is not caused by canopy scorching. *Austral Ecology* **31**: 892–896.

**Ballesteros JA, Stoffel M, Bodoque JM, Bollschweiler M, Hitz O, Díez-Herrero A. 2010*a*.** Changes in wood anatomy in tree rings of *Pinus pinaster* Ait. following wounding by flash floods. *Tree-Ring Research* **66**: 93–103.

**Ballesteros JA, Stoffel M, Bollschweiler M, Bodoque JM, Díez-Herrero A. 2010*b*.** Flash-flood impacts cause changes in wood anatomy of *Alnus glutinosa*, *Fraxinus angustifolia* and *Quercus pyrenaica*. *Tree Physiology* **30**: 773–781.

**Barker JE. 1979.** Growth and wood properties of *Pinus radiata* in relation to applied ethylene. *New Zealand Journal of Forest Science* **9**: 15–19.

**Bauch J, Shigo AL, Starck M. 1980.** Wound effects in the xylem of *Acer* and *Betula* species. *Holzforschung* **34**: 153–160.

**Beghin R, Cherubini P, Battipaglia G, Siegwolf R, Saurer M, Bovio G. 2011.** Tree-ring growth and stable isotopes ($^{13}$C and $^{15}$N) detect effects of wildfires on tree physiological processes in *Pinus sylvestris* L. *Trees* **25**: 627–636.

**Bigio E, Gärtner H, Conedera M. 2010.** Fire-related features of wood anatomy in a sweet chestnut (*Castanea sativa*) coppice in southern Switzerland. *Trees* **24**: 643–655.

**Bollschweiler M, Stoffel M, Schneuwly DM, Bourqui K. 2008.** Traumatic resin ducts in *Larix decidua* stems impacted by debris flows. *Tree Physiology* **28**: 255–263.

**Bravo S. 2010.** Anatomical changes induced by fire-damaged cambium in two native tree species of the Chaco region, Argentina. *IAWA Journal* **31**: 283–292.

**Bukata AR, Kyser TK, Al TA. 2008.** Nitrogen isotopic composition and elemental concentration of tree-rings may help map the extent of historic fire events. *Fire Ecology* **4**: 101–107.

**Burgert I, Bernasconi A, Eckstein D. 1999.** Evidence for the strength function of rays in living trees. *Holz als Roh- und Werkstoff* **57**: 397–399.

**Carmi A, Sachs T, Fahn A. 1972.** The relation of ray spacing to cambial growth. *New Phytologist* **71**: 349–353.

**Chave J, Coomes D, Jansen S, Lewis SL, Swenson NG, Zanne AE. 2009.** Towards a worldwide wood economics spectrum. *Ecology Letters* **12**: 351–366.

**Domec J-C, Gartner BL. 2001.** Cavitation and water storage capacity in bole xylem segments of mature and young Douglas-fir trees. *Trees* **15**: 204–214.

**Domec J-C, Gartner BL. 2003.** Relationship between growth rates and xylem hydraulic characteristics in young, mature and old-growth ponderosa pine trees. *Plant, Cell and Environment* **26**: 471–483.

**Ducrey M, Duhoux F, Huc R, Rigolot E. 1996.** The ecophysiological and growth responses of Aleppo pine (*Pinus halepensis*) to controlled heating applied to the base of the trunk. *Canadian Journal of Forest Research* **26**: 1366–1374.

**Fahn A. 1988.** Secretory tissues and factors influencing their development. *Phyton* **28**: 13–26.

**Fahn A, Zamski E. 1970.** The influence of pressure, wind, wounding and growth substances on the rate of resin duct formation in *Pinus halepensis* wood. *Israel Journal of Botany* **19**: 429–446.

**Falk DA, Heyerdahl EK, Brown PM, *et al.* 2011.** Multi-scale controls of historical forest-fire regimes: new insights from fire-scar networks. *Frontiers in Ecology and the Environment* **9**: 446–454.

**Farris CA, Baisan CH, Falk DA, Yool SR, Swetnam TW. 2010.** Spatial and temporal corroboration of a fire-scar-based fire history in a frequently burned ponderosa pine forest. *Ecological Applications* **20**: 1598–1614.

**Fink S. 1999.** Pathological and regenerative plant anatomy. *Encyclopedia of plant anatomy*, Vol. 14, Part 6. Berlin: Gebrüder Bornträger.

**Gutsell SL, Johnson EA. 1996.** How fire scars are formed: coupling a disturbance process to its ecological effect. *Canadian Journal of Forest Research* **26**: 166–174.

**Guyette RP, Stambaugh MC. 2004.** Post-oak fire scars as a function of diameter, growth, and tree age. *Forest Ecology and Management* **198**: 183–192.

**Hacke UG, Sperry JS, Pockman WT, Davis SD, McCulloh KA. 2001.** Trends in wood density and structure are linked to prevention of xylem implosion by negative pressure. *Oecologia* **126**: 457–461.

**Hengst GE, Dawson JO. 1994.** Bark properties and fire resistance of selected tree species from the central hardwood region of North America. *Canadian Journal of Forest Research* **24**: 688–696.

**Heyerdahl EK, Brubaker LB, Agee JK. 2001.** Spatial controls of historical fire regimes: a multiscale example from the interior west, USA. *Ecology* **82**: 660–678.

**Hölttä T, Vesala T, Perämäki M, Nikinmaa E. 2006.** Refilling of embolised conduits as a consequence of 'Münch water' circulation. *Functional Plant Biology* **33**: 949–959.

**Hudgins JW, Franceschi VR. 2004.** Methyl jasmonate-induced ethylene production is responsible for conifer phloem defense responses and reprogramming of stem cambial zone for traumatic resin duct formation. *Plant Physiology* **135**: 2134–2149.

**Imaseki H. 1985.** Hormonal control of wound-induced responses. In: Pharis RP, Reid DM, eds. *Encyclopedia of plant physiology*, New Series, Vol. 11, Part 3. Berlin: Springer, 485–512.

**Kames S, Tardif JC, Bergeron Y. 2011.** Anomalous earlywood vessel lumen area in black ash (*Fraxinus nigra* Marsh.) tree rings as a potential indicator of forest fires. *Dendrochronologia* **29**: 109–114.

**Kolb TE, Agee JK, Fulé PZ, *et al.* 2007.** Perpetuating old ponderosa pine. *Forest Ecology and Management* **249**: 141–157.

**Kuroda K, Shimaji K. 1984.** Wound effects on xylem cell differentiation in a conifer. *IAWA Bulletin* **5**: 295–305.

**Larson PR. 1994.** *The vascular cambium. Development and structure*. Berlin: Springer.

**Leitch MA. 2001.** Vessel-element dimensions and frequency within the most current growth increment along the length of *Eucalyptus globulus* stems. *Trees* **15**: 353–357.

**Lev-Yadun S. 1994.** Experimental evidence for the autonomy of ray differentiation in *Ficus sycomorus* L. *New Phytologist* **126**: 499–504.

**Lev-Yadun S, Aloni R. 1992.** The role of wounding and partial girdling in differentiation of vascular rays. *International Journal of Plant Sciences* **153**: 348–357.

**Lev-Yadun S, Aloni R. 1993.** Effect of wounding on the relations between vascular rays and vessels in *Melia azedarach* L. *New Phytologist* **124**: 339–344.

**Lewinsohn E, Gijzen M, Croteau R. 1991.** Defense mechanisms of conifers. Differences in constitutive and wound-induced monoterpene biosynthesis among species. *Plant Physiology* **96**: 44–49.

**Lovisolo C, Schubert A, Sorce C. 2002.** Are xylem radial development and hydraulic conductivity in downwardly-growing grapevine shoots influenced by perturbed auxin metabolism? *New Phytologist* **156**: 65–74.

**Lowerts G, Wheeler EA, Kellison RC. 1986.** Characteristics of wound-associated wood of yellow poplar (*Liriodendron tulipifera* L.). *Wood and Fiber Science* **18**: 537–552.

**Martin JM, Sydnor TD. 1987.** Differences in wound closure rates in 12 tree species. *HortScience* **22**: 442–444.

**Martínez-Vilalta J, Sala A, Piñol J. 2004.** The hydraulic architecture of Pinaceae – a review. *Plant Ecology* **171**: 3–13.

**Mattheck C. 1998.** *Design in nature – learning from trees*. Berlin: Springer.

**Michaletz ST, Johnson EA, Tyree MT. 2012.** Moving beyond the cambium necrosis hypothesis of post-fire tree mortality: cavitation and deformation of xylem in forest fires. *New Phytologist* **194**: 254–263.

**Mulhern J, Shortle WC, Shigo AL. 1979.** Barrier zones in red maple: an optical and scanning microscope examination. *Forest Science* **25**: 311–316.

**Nagy NE, Franceschi VR, Solheim H, Krekling T, Christiansen E. 2000.** Wound-induced traumatic resin duct development in stems of Norway spruce (*Pinaceae*): anatomy and cytochemical traits. *American Journal of Botany* **87**: 302–313.

**Nardini A, Lo Gullo MA, Salleo S. 2011.** Refilling embolized xylem conduits: is it a matter of phloem unloading? *Plant Science* **180**: 604–611.

**Neely D. 1988.** Tree wound closure. *Journal of Arboriculture* **14**: 148–152.

**Nicholls JWP. 1972.** Effect of prescribed burning in a forest on wood characteristics of jarrah. *Australian Forestry* **36**: 178–189.

**Pearson CL, Dale D, Lombardo K. 2011.** An investigation of fire scars in *Pseudotsuga macrocarpa* by scanning X-ray fluorescence microscopy. *Forest Ecology and Management* **262**: 1258–1264.

**Peterson DL, Arbaugh MJ, Pollock GH, Robinson LJ. 1991.** Postfire growth of *Pseudotsuga menziesii* and *Pinus contorta* in the Northern Rocky Mountains, USA. *International Journal of Wildland Fire* **1**: 63–71.

**Pinard MA, Huffman J. 1997.** Fire resistance and bark properties of trees in a seasonally dry forest in eastern Bolivia. *Journal of Tropical Ecology* **13**: 727–740.

**Piñol J, Sala A. 2000.** Ecological implications of xylem cavitation for several Pinaceae in the Pacific Northern USA. *Functional Ecology* **14**: 538–545.

**Pittermann J, Sperry JS, Hacke UG, Wheeler JK, Sikkema EH. 2006*a*.** Inter-tracheid pitting and the hydraulic efficiency of conifer wood: the role of tracheid allometry and cavitation protection. *American Journal of Botany* **93**: 1265–1273.

**Pittermann J, Sperry JS, Wheeler JK, Hacke UG, Sikkema EH. 2006*b*.** Mechanical reinforcement of tracheids compromises the hydraulic efficiency of conifer xylem. *Plant, Cell and Environment* **29**: 1618–1628.

**Rademacher P, Bauch J, Shigo AL. 1984.** Characteristics of xylem formed after wounding in *Acer*, *Betula* and *Fagus*. *IAWA Bulletin* **5**: 141–151.

**Régent Instruments Inc. 2004.** WinCELL Pro V 2004a. www.regentinstruments.com.

**Ryan KC, Reinhardt ED. 1988.** Predicting postfire mortality of seven western conifers. *Canadian Journal of Forest Research* **18**: 1291–1297.

**Schneuwly DM, Stoffel M, Bollschweiler M. 2009*a*.** Formation and spread of callus tissue and tangential rows of resin ducts in *Larix decidua* and *Picea abies* following rockfall impacts. *Tree Physiology* **29**: 281–289.

**Schneuwly DM, Stoffel M, Dorren LKA, Berger F. 2009*b*.** Three-dimensional analysis of the anatomical growth response of European conifers to mechanical disturbance. *Tree Physiology* **29**: 1247–1257.

**Schneuwly-Bollschweiler M, Schneuwly DM. 2012.** How fast do European conifers overgrow wounds inflicted by rockfall? *Tree Physiology* **32**: 968–975.

**Shigo AL. 1984.** Compartmentalization: a conceptual framework for understanding how trees grow and defend themselves. *Annual Review of Phytopathology* **22**: 189–214.

**Smith KT, Sutherland EK. 1999.** Fire-scar formation and compartmentalization in oak. *Canadian Journal of Forest Research* **29**: 166–171.

**Smith KT, Sutherland EK. 2001.** Terminology and biology of fire scars in selected central hardwoods. *Tree-Ring Research* **57**: 141–147.

**Sperry JS, Hacke UG, Pittermann J. 2006.** Size and function in conifer tracheids and angiosperm vessels. *American Journal of Botany* **93**: 1490–1500.

**Sperry JS, Meinzer FC, McCulloh KA. 2008.** Safety and efficiency conflicts in hydraulic architecture: scaling from tissues to trees. *Plant, Cell and Environment* **31**: 632–645.

**Stephens SL, Fry DL, Collins BM, Skinner CN, Franco-Vizcaíno E, Freed TJ. 2010.** Fire-scar formation in Jeffrey pine–mixed conifer forests in the Sierra San Pedro Mártir, Mexico. *Canadian Journal of Forest Research* **40**: 1497–1505.

**Stobbe H, Schmitt U, Eckstein D, Dujesiefken D. 2002.** Developmental stages and fine structure of surface callus formed after debarking of living lime trees (*Tilia* sp.). *Annals of Botany* **89**: 773–782.

**Stoffel M. 2008.** Dating past geomorphic processes with tangential rows of traumatic resin ducts. *Dendrochronologia* **26**: 53–60.

**Stoffel M, Hitz OM. 2008.** Rockfall and snow avalanche impacts leave different anatomical signatures in tree rings of juvenile *Larix decidua*. *Tree Physiology* **28**: 1713–1720.

**Swetnam TW, Baisan CH, Caprio AC, *et al* 2009.** Multi-millennial fire history of the Giant Forest, Sequoia National Park, California, USA. *Fire Ecology* **5**: 120–150.

**Van Horne ML, Fulé PZ. 2006.** Comparing methods of reconstructing fire history using fire scars in a southwestern United States ponderosa pine forest. *Canadian Journal of Forest Research* **36**: 855–867.

**Wu H, Hu Z-H. 1997.** Comparative anatomy of resin ducts of the Pinaceae. *Trees* **11**: 135–143.

**Wyant JG, Omi PN, Laven RD. 1986.** Fire induced tree mortality in a Colorado ponderosa pine/Douglas-fir stand. *Forest Science* **32**: 49–59.

**Yamamoto F, Kozlowski TT. 1987.** Effect of ethrel on growth and stem anatomy of *Pinus halepensis* seedlings. *IAWA Bulletin* **8**: 11–19.

**Yamamoto F, Angeles G, Kozlowski TT. 1987.** Effect of ethrel on stem anatomy of *Ulmus americana* seedlings. *IAWA Bulletin* **8**: 3–10.