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Resin duct size and density as ecophysiological traits in fire scars of *Pseudotsuga menziesii* and *Larix occidentalis*

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• **Background and Aims** Resin ducts (RDs) are features present in most conifer species as defence structures against pests and pathogens; however, little is known about RD expression in trees following fire injury. This study investigates changes in RD size and density in fire scars of Douglas fir (*Pseudotsuga menziesii*) and western larch (*Larix occidentalis*) as a means to evaluate the ecophysiological significance of traumatic resinosis for tree defence and survival.

• **Methods** Transverse and tangential microsections were prepared for light microscopy and image analysis in order to analyse axial and radial RDs, respectively. Epithelial cells of RDs and fusiform rays associated with radial RDs were also examined. RDs were compared between normal xylem and wound xylem at four different section heights along the fire-injured stem.

• **Key Results** Following fire injury, *P. menziesii* axial RDs narrowed by 38–43 % in the first year after injury, and the magnitude of this change increased with stem height. *Larix occidentalis* axial RDs widened by 46–50 % in the second year after injury. Radial RDs were of equivalent size in *P. menziesii*, but widened by 162–214 % in *L. occidentalis*. Fusiform rays were larger following fire injury, by 4–14 % in *P. menziesii* and by 23–38 % in *L. occidentalis*. Furthermore, axial RD density increased in both species due to the formation of tangential rows of traumatic RDs, especially in the first and second years after injury. However, radial RD density did not change significantly.

• **Conclusions** These results highlight traumatic resinosis as a species-specific response. *Pseudotsuga menziesii* produce RDs of equivalent or reduced size, whereas *L. occidentalis* produce wider RDs in both the axial and radial duct system, thereby increasing resin biosynthesis and accumulation within the whole tree. *Larix occidentalis* thus appears to allocate more energy to defence than *P. menziesii*.

Key words: Ecophysiology, conifer, fire scar, *Larix occidentalis*, western larch, *Pseudotsuga menziesii*, Douglas fir, resin duct, tree defence, wood anatomy.

INTRODUCTION

Most conifer species possess resin-secreting structures for defence against pests and pathogens (Zulak and Bohlmann, 2010). These structures range from simple, isolated resin cells, to multicellular resin blisters, to networked resin ducts (RDs; Lewinsohn *et al.*, 1991*a*). These RDs build a three-dimensional anastomosing system that connects axial RDs in wood and bark tissues to radial RDs within fusiform rays (Chattaway, 1951; Bosshard and Hug, 1980). They are lined with a layer of secretory, parenchymatic epithelial cells (Wu and Hu, 1997). In *Picea*, *Pinus*, *Pseudotsuga* and *Larix*, RDs are produced constitutively (Bannan, 1936; Wu and Hu, 1997) and as an induced response to insect attack, fungal invasion and mechanical wounding (Nagy *et al.*, 2000; Byun-McKay *et al.*, 2003; Poulin *et al.*, 2006; Bollschweiler *et al.*, 2008; Martín-Rodrigues *et al.*, 2013). In *Abies*, *Cedrus*, *Tsuga* and *Pseudolarix*, RDs are only produced as a result of injury to the cambium (Bannan, 1936;

Wu and Hu, 1997). Induced RDs are commonly referred to as traumatic RDs.

Fire scars occur through lethal heating of the cambium around a portion of the bole (Gutsell and Johnson, 1996). They include the killed tissues of normal xylem as well as the new, healthy tissues of wound xylem (or woundwood) produced to close the wound and restore continuity of the cambium (Smith and Sutherland, 1999, 2001). While traumatic RDs have been used as proxies for the reconstruction of fire history (Brown and Swetnam, 1994; Margolis *et al.*, 2007, 2011), their expression (in terms of size and density) in trees following fire injury is less clear. The wood anatomy of fire scars has been studied in broad-leaved trees (Bigio *et al.*, 2010; Bravo, 2010) and only recently in conifers where changes in tracheid and ray traits were quantified (Arbellay *et al.*, 2014). Changes in RD traits have yet to be identified and quantified.

In this study, axial and radial RDs were analysed for changes in size and density in fire scars of Douglas fir (*Pseudotsuga*

menziesii) and western larch (*Larix occidentalis*). Epithelial cells of RDs and fusiform rays associated with radial RDs were also examined. Resin ducts were compared between normal xylem and wound xylem at four different section heights along the fire-injured stem. The extension of traumatic RDs has been shown to be quite significant in the upward direction (Lev-Yadun, 2002; Bollschweiler *et al.*, 2008; Schneuwly *et al.*, 2009*b*). The eco-physiological significance of traumatic resinosis for tree defence and survival is addressed.

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 each of *P. menziesii* and *L. occidentalis* 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, 16 cross-sections were sectioned with a chisel to obtain a wood block where RDs 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. In a first step, transverse microsections (Fig. 1C) were prepared to analyse axial RDs and their epithelial cells in several rings formed pre-fire and in the first and second rings formed post-fire (rings 1 and 2). Several rings were investigated in normal xylem in order to constitute an adequate population of control RDs for comparison between normal xylem and wound xylem. Only the first and second rings formed post-fire

TABLE 1. *Characteristics of the four 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

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.
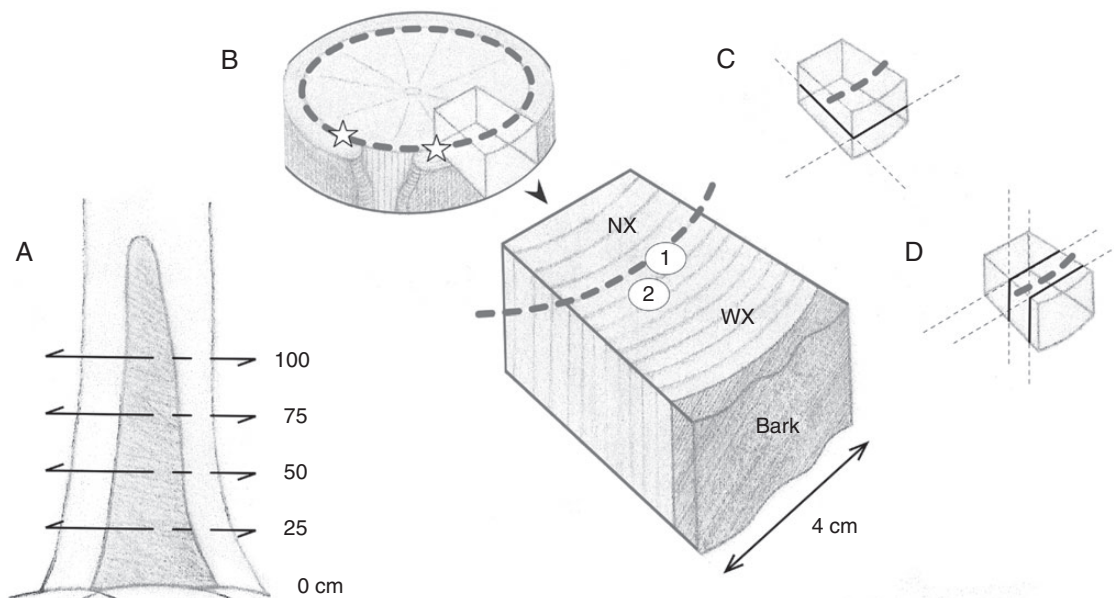

FIG. 1. Study design for wood anatomical analysis. (A) Resin ducts (RDs) were analysed in cross-sections taken at four different section heights (25, 50, 75 and 100 cm above the ground surface) along the fire-injured stem. (B) Cross-sections were sectioned with a chisel to obtain a wood block where RDs 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 axial RDs and their epithelial cells in several rings formed pre-fire and in the first and second rings formed post-fire (rings 1 and 2). (D) Tangential microsections were prepared to analyse radial RDs, their epithelial cells and their associated fusiform rays, requiring one cut through normal xylem and one cut through wound xylem.

TABLE 2. *Changes in the size of axial resin ducts (ADLA$_a$) between normal xylem (NX) and wound xylem (WX, rings 1 and 2) for the four trees analysed*

	SH = 25 cm		SH = 50 cm		SH = 75 cm		SH = 100 cm		All SH	
	P-value	Δ (%)	*P*-value	Δ (%)	*P*-value	Δ (%)	*P*-value	Δ (%)	*P*-value	Δ (%)
PSM1										
NX – WX ring 1	**<0·001**	**−19**	**<0·001**	**−26**	**<0·001**	**−43**	**<0·001**	**−67**	**<0·001**	**−38**
NX – WX ring 2	**<0·001**	**+30**	0·412	−5	0·135	+8	**<0·001**	**−61**	0·398	−4
PSM2										
NX – WX ring 1	**<0·001**	**−21**	**<0·001**	**−25**	**<0·001**	**−43**	**<0·001**	**−43**	**<0·001**	**−43**
NX – WX ring 2	**<0·001**	**−36**	**<0·001**	**−40**	**<0·001**	**−61**	**<0·001**	**−67**	**<0·001**	**−46**
LAO1										
NX – WX ring 1	0·243	+6	**<0·001**	**−49**	**0·007**	**−17**	**0·002**	**−21**	**<0·001**	**−20**
NX – WX ring 2	**<0·001**	**+50**	**<0·001**	**+50**	**<0·001**	**+78**	0·986	−1	**<0·001**	**+50**
LAO2										
NX – WX ring 1	0·327	+5	**0·010**	**+20**	**0·012**	**−16**	**0·001**	**−22**	0·468	−3
NX – WX ring 2	**<0·001**	**+29**	**<0·001**	**+62**	**<0·001**	**+55**	**<0·001**	**+24**	**<0·001**	**+46**

ADLA$_a$, average duct lumen area of axial resin ducts; SH, section height above the ground surface.
One-way ANOVA. Significant results appear in bold.

were examined, as traumatic RDs were mostly produced in those rings. In a second step, tangential microsections (Fig. 1D) were prepared to analyse radial RDs, their epithelial cells and their associated fusiform rays, requiring two radial cuts (one through normal xylem and one through wound xylem). The latter cut was performed about 2 mm inside wound xylem, i.e. within ring 1 or 2. All microsections (96 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 ×25 and ×100 magnification, respectively, with a digital camera mounted on a light microscope. The number and density of RDs as well as their average number of epithelial cells (ANEC) were obtained from direct observation of the captured images. WinCELL software (Régent Instruments Inc., 2004) was used to measure average duct lumen area (ADLA), average ray height (ARH) and average ray width (ARW); ADLA$_a$ and ADLA$_r$ designate the lumen size of axial and radial RDs, respectively. Measurements were made at the four section heights and in the rings mentioned previously. Within the 4 cm wide tangential window, all axial RDs were recorded, whereas radial RDs and fusiform rays were measured at 0·5 cm intervals along that window, within about 1 mm² at each location. One-way analysis of variance (ANOVA) was used to determine whether there were significant ($P < 0.05$) changes in RD traits between normal xylem and wound xylem. For ease of comparison, results refer to data from the four section heights unless otherwise specified.

RESULTS

Axial RDs in P. menziesii

The ADLA$_a$ values pre-fire were 1696 and 2112 μm² in PSM1 and PSM2, respectively. Results were similar in the two rings of wound xylem. ADLA$_a$ decreased significantly in ring 1, by 38 % in PSM1 and by 43 % in PSM2 (Table 2). ADLA$_a$ also decreased significantly in ring 2, by 46 % in PSM2 (PSM1 only showed that reaction at 100 cm above the ground surface). This narrowing of RDs increased with stem height (Fig. 2A, B). For example, in ring 1 of PSM1, the decrease ranged from 19 % at 25 cm to 67 % at 100 cm (Table 2). The ANEC decreased from ten to six cells in both rings. Moreover, the number of RDs increased in wound xylem compared with normal xylem (Fig. 2A, B). The density of RDs decreased by 44 % from ring 1 to ring 2.

Axial RDs in L. occidentalis

The ADLA$_a$ values pre-fire were 3062 and 3388 μm² in LAO1 and LAO2, respectively. Results differ between the two rings of wound xylem. ADLA$_a$ decreased significantly in ring 1, by 20 % in LAO1 (LAO2 only showed that reaction at 75 and 100 cm above the ground surface). In contrast, ADLA$_a$ increased significantly in ring 2, by 50 % in LAO1 and by 46 % in LAO2 (Table 2). These responses did not increase with stem height (Fig. 2C, D). The ANEC decreased from 11 to nine cells in ring 1 and increased from 11 to 17 cells in ring 2. As in *P. menziesii*, the number of RDs increased in wound xylem compared with normal xylem (Fig. 2C, D). The density of RDs decreased by 53 % from ring 1 to ring 2.

Radial RDs and fusiform rays in P. menziesii

The ADLA$_r$ values pre-fire were 360 and 315 μm² in PSM1 and PSM2, respectively. The ARH and ARW values pre-fire were respectively 264 and 274 μm in PSM1 and PSM2, and 44 and 43 μm in PSM1 and PSM2. The size and density of RDs did not change significantly in wound xylem compared with normal xylem (Fig. 3A; Table 3). The ANEC mostly remained constant with six cells. However, fusiform rays were larger in wound xylem, by 9–14 % for ARH and by 4–13 % for ARW (Table 3).
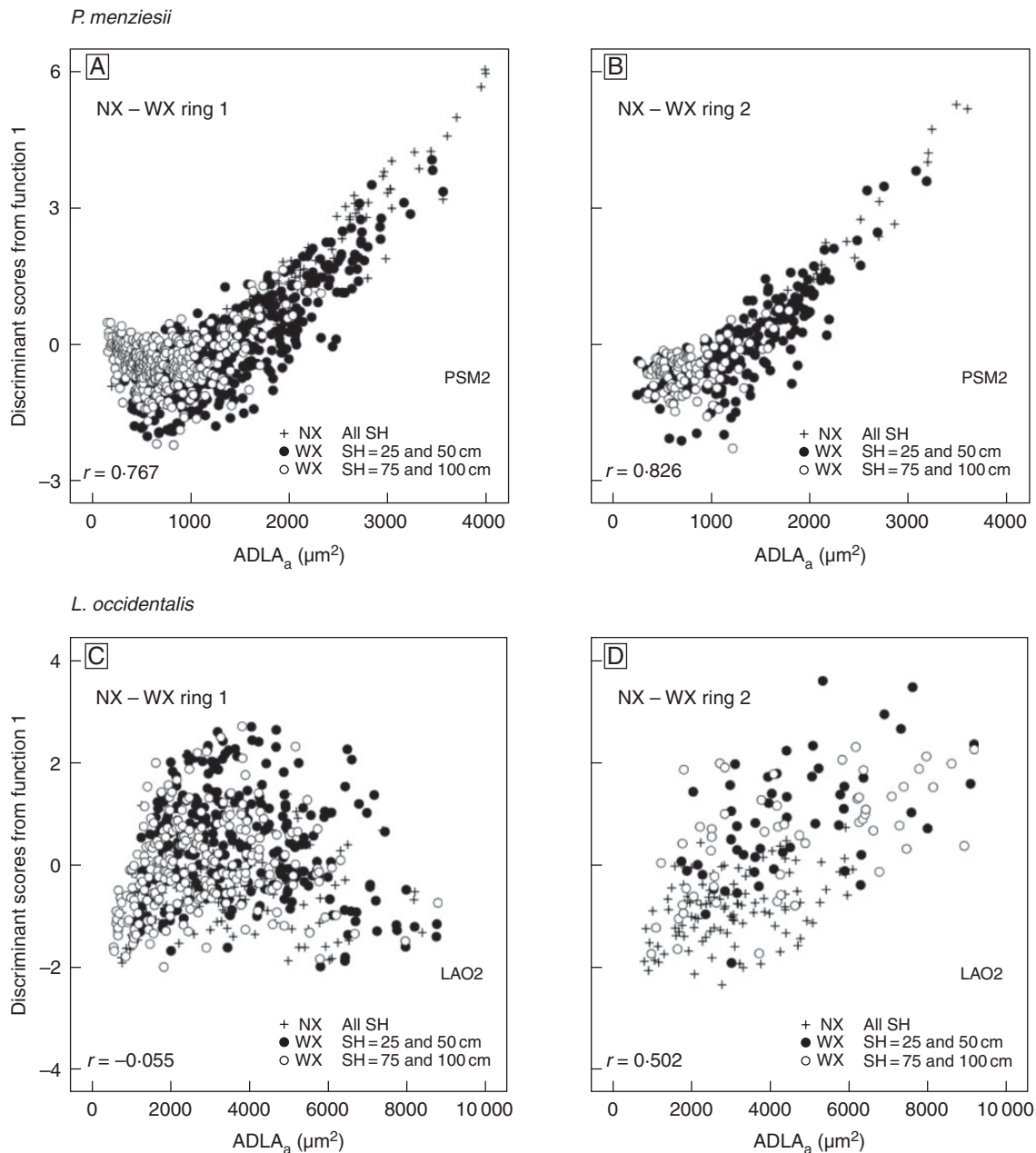
FIG. 2. Changes in average duct lumen area of axial resin ducts (ADLA$_a$), presented for (A, B) one tree of *P. menziesii* and (C, D) one tree of *L. occidentalis*. Discriminant analysis plots allow comparison between normal xylem (NX) and wound xylem (WX, rings 1 and 2). Section heights (SH) were paired in WX and considered all together in NX.

Radial RDs and fusiform rays in L. occidentalis

The ADLA$_r$ values pre-fire were 583 and 705 μm^2 in LAO1 and LAO2, respectively. The ARH and ARW values pre-fire were respectively 373 and 403 μm in LAO1 and LAO2, and 46 and 49 μm in LAO1 and LAO2. In contrast to *P. menziesii*, ADLA$_r$ increased significantly in wound xylem, by 162 % in LAO1 and by 214 % in LAO2 (Table 3). This widening of RDs did not increase with stem height (Fig. 3B). The ANEC increased from nine to 11 cells. As in *P. menziesii*, the density of RDs did not change significantly in wound xylem compared with normal xylem (Fig. 3B), and

fusiform rays were larger in wound xylem, by 23 % for ARH and by 26–38 % for ARW (Table 3).

DISCUSSION

Changes in RD size

Following fire injury, *P. menziesii* and *L. occidentalis* showed some changes in the lumen size of axial and radial RDs. The number of epithelial cells adjusted to the new RD size, which
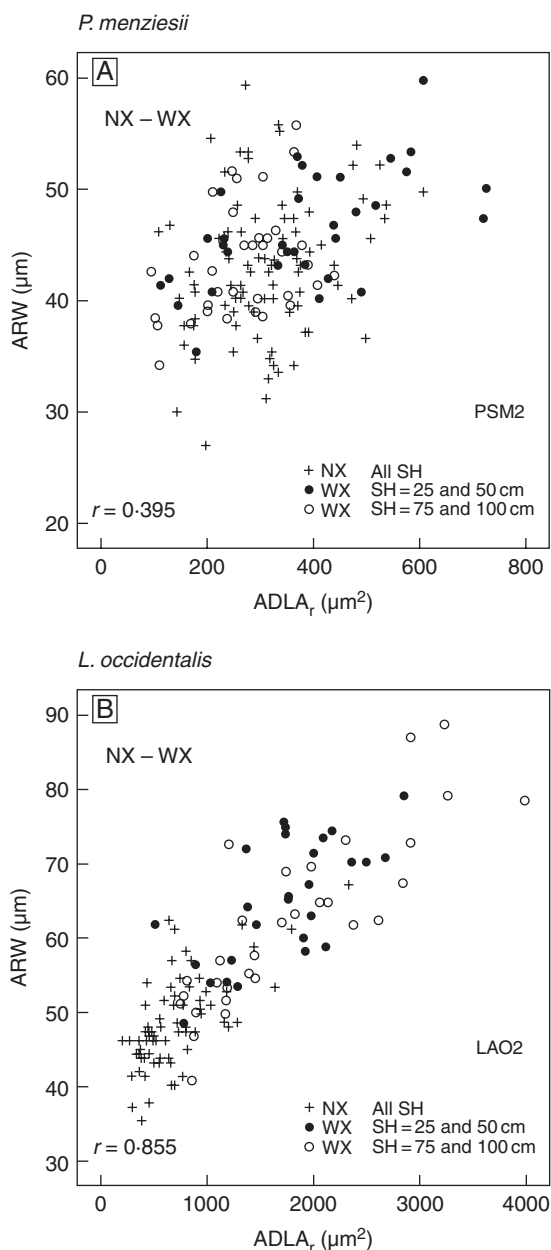
FIG. 3. Changes in average duct lumen area of radial resin ducts (ADLA$_r$) and in average ray width (ARW) of their associated fusiform rays, presented for (A) one tree of *P. menziesii* and (B) one tree of *L. occidentalis*. Discriminant analysis plots allow comparison between normal xylem (NX) and wound xylem (WX). Section heights (SH) were paired in WX and considered all together in NX.

is consistent with the theoretical description of RD formation from epithelial cells (Kuroda, 1986). In more detail, *P. menziesii* axial RDs were narrower in the first year after injury (Fig. 4A), and the magnitude of this change increased with stem height. In contrast, *L. occidentalis* axial RDs were wider in the second year after injury (Fig. 4B). Radial RDs were of equivalent size in *P. menziesii*, but were wider in *L. occidentalis* (Fig. 4C). Similarly, injury inflicted on trees with an increment borer decreased axial RD size in *P. menziesii* and increased it in *L. occidentalis* (Hudgins *et al.*, 2003*a*). It is likely that mechanical wounding of either origin

raised auxin and ethylene concentrations in the injured area (Aloni and Zimmermann, 1984; Imaseki, 1985). These phytohormones are believed to have caused the differentiation of narrower tracheids and larger rays in fire-scarred *P. menziesii* and *L. occidentalis* (Arbellay *et al.*, 2014).

We suggest that the narrower axial RDs in wound xylem of *P. menziesii* are the result of rapid conduit differentiation due to high auxin concentration, as is theorized in the case of reduced size tracheids and vessels (Aloni and Zimmermann, 1983, 1984; Lovisolo *et al.*, 2002; Anfodillo *et al.*, 2012). This decrease in RD size was most obvious in the first year after injury, presumably because the level of auxin was higher. The magnitude of this change increased with stem height, as the fire scar locally obstructed the basipetal flow of auxin and raised the auxin concentration, with decreasing importance from the top to the bottom of the scar (Aloni and Zimmermann, 1983, 1984). Moreover, cambial reactivation in *Pseudotsuga* occurs on the xylem side first, as opposed to on the phloem side in *Larix* (Larson, 1994). The prompt start of xylogenesis in *Pseudotsuga* ensures that traumatic resinosis in *P. menziesii* occurred at a high auxin level. Vice versa, the wider axial RDs in wound xylem of *L. occidentalis* might reflect slower conduit differentiation due to lower auxin concentration, as the start of xylogenesis is delayed in *Larix* compared with *Pseudotsuga*. This increase in RD size was most obvious in the second year after injury, presumably because the level of auxin was even lower.

Furthermore, these contrasting results between *P. menziesii* and *L. occidentalis* might also stem from species-specific sensitivity to wound-induced ethylene, as suggested by Arbellay *et al.* (2014) to explain the different amount of ray tissue among *Pinaceae* following fire injury. Ethylene promotes RD formation as well as ray enlargement (Barker, 1979; Yamamoto and Kozlowski, 1987; Yamamoto *et al.*, 1987; Fahn, 1988; Lev-Yadun and Aloni, 1992). Fusiform rays associated with radial RDs were indeed larger in wound xylem of both species whether they accommodated RDs of equivalent or enlarged size as in *P. menziesii* and *L. occidentalis*, respectively. Several studies have contributed to establishing methyl jasmonate as the signalling agent responsible for ethylene biosynthesis and the induction of defence responses in conifers (Franceschi *et al.*, 2002; Martin *et al.*, 2002; Hudgins *et al.*, 2003*a*, 2004; Hudgins and Franceschi, 2004).

Changes in RD density

Axial RDs are usually less numerous than radial RDs in *Pseudostuga* and *Larix* (Larson, 1994), which was no longer verified in *P. menziesii* and *L. occidentalis* following fire injury. While radial RD density did not change significantly, axial RD density increased in wound xylem of both species due to the formation of tangential rows of traumatic RDs (Fig. 4A). The production of numerous traumatic RDs in wood and bark tissues along with the activation of polyphenolic parenchyma cells in the bark are characteristic induced defence responses of *P. menziesii* and *L. occidentalis* (Hudgins *et al.*, 2003*a*, 2004; Hudgins and Franceschi, 2004). Moreover, the trees of this study were injured by fire after cessation of cambial growth. Traumatic resinosis was initiated in the following growing season and was most manifest in the first and second years after injury, concurring with observations in *Larix decidua*,

TABLE 3. *Changes in the size of radial resin ducts (ADLA$_r$) and fusiform rays (ARH, ARW) between normal xylem (NX) and wound xylem (WX) for the four trees analysed*

		SH = 25 cm		SH = 50 cm		SH = 75 cm		SH = 100 cm		All SH	
		P-value	Δ (%)	*P*-value	Δ (%)	*P*-value	Δ (%)	*P*-value	Δ (%)	*P*-value	Δ (%)
PSM1											
NX – WX	ADLA$_r$	0·826	+3	0·393	−10	0·671	−4	**0·034**	**−27**	0·058	−11
	ARH	0·653	+3	0·906	−1	**<0·001**	**+25**	0·417	+5	**0·002**	**+9**
	ARW	**<0·001**	**+14**	0·326	+3	**<0·001**	**+17**	**0·009**	**+10**	**<0·001**	**+13**
PSM2											
NX – WX	ADLA$_r$	0·969	+1	**0·010**	**+43**	**0·015**	**−22**	0·744	+3	0·329	+7
	ARH	**0·036**	**+20**	**0·007**	**+17**	**<0·001**	**+31**	0·198	−8	**<0·001**	**+14**
	ARW	0·177	−6	0·112	+5	0·868	−1	**<0·001**	**+19**	**0·034**	**+4**
LAO1											
NX – WX	ADLA$_r$	**<0·001**	**+192**	**<0·001**	**+104**	**<0·001**	**+186**	**<0·001**	**+109**	**<0·001**	**+162**
	ARH	**0·002**	**+28**	**0·003**	**+34**	**0·037**	**+17**	**0·040**	**+15**	**<0·001**	**+23**
	ARW	**<0·001**	**+26**	**<0·001**	**+25**	**<0·001**	**+36**	**0·001**	**+18**	**<0·001**	**+26**
LAO2											
NX – WX	ADLA$_r$	**<0·001**	**+124**	**<0·001**	**+177**	**<0·001**	**+157**	**<0·001**	**+171**	**<0·001**	**+214**
	ARH	0·133	+15	0·786	+3	0·179	+8	0·272	+10	0·080	+8
	ARW	**<0·001**	**+25**	**<0·001**	**+56**	**<0·001**	**+40**	**<0·001**	**+28**	**<0·001**	**+38**

ADLA$_r$, average duct lumen area of radial resin ducts; ARH, average ray height; ARW, average ray width; SH, section height above the ground surface. One-way ANOVA. Significant results appear in bold.
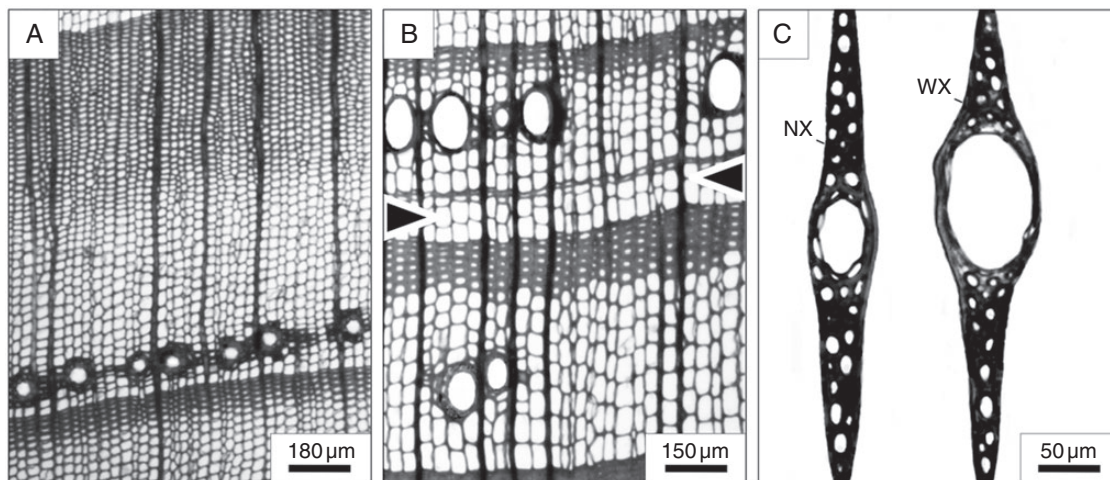

FIG. 4. Resin ducts (RDs) in fire-scarred *P. menziesii* and *L. occidentalis*. (A) Following fire injury, axial RDs in wound xylem of both species were aligned in one or more tangential rows of traumatic RDs, as in *P. menziesii* where traumatic RDs were narrower than normal RDs in the first post-fire ring. (B) In *L. occidentalis*, traumatic RDs were sometimes locally absent in the first post-fire ring, designated by black arrows, and were wider than normal RDs in the second post-fire ring. (C) Fusiform rays were larger in wound xylem of both species, but only in *L. occidentalis* did radial RDs increase in size between normal xylem (NX) and wound xylem (WX).

Picea abies and *Abies alba* trees injured by mass-movement processes during dormancy (Bollschweiler *et al.*, 2008; Stoffel and Hitz, 2008; Schneuwly *et al.*, 2009*a*, *b*). The reaction faded over time, with the density of RDs already reduced by about 50 % in the second year after injury in both species, and often disappeared completely as early as in the fourth year after injury.

Ecophysiological significance of traumatic resinosis

Pseudotsuga and *Larix* synthesize relatively little resin constitutively compared with *Pinus* (Lewinsohn *et al.*, 1991*b*). They must rely on further constitutive defences against pests and pathogens, such as lignified cells (Wainhouse *et al.*, 1997) and calcium oxalate crystals (Hudgins *et al.*, 2003*b*). More

importantly, they deploy a highly inducible defence strategy in which the formation of traumatic RDs is crucial (Hudgins *et al.*, 2003*a*, 2004; Hudgins and Franceschi, 2004). Both species produced numerous traumatic RDs in the axial duct system in an attempt to compartmentalize decay after wounding (Tippett and Shigo, 1981; Oven and Torelli, 1999). Resin can act as a mechanical barrier when its volatile terpenes evaporate and leave a hardened plug in the ducts that prevents the entry of water and invaders (Hillis, 1987; Pearce, 1996). Traumatic resin, in particular, might act as a chemical barrier, as it appears to be more toxic or fungistatic than constitutive resin (Solheim, 1991; Nagy *et al.*, 2000). Interestingly, *L. occidentalis* produced wider RDs in both the axial and radial duct system, thereby increasing resin biosynthesis and accumulation within the whole tree. It

could be argued that *L. occidentalis* allocates more energy to defence than *P. menziesii*, which only produced RDs of equivalent or reduced size.

This study demonstrates that fire injury altered RD expression in *P. menziesii* and *L. occidentalis* and, more importantly, highlights traumatic resinosis as a species-specific response.

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