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

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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., 1991a). 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; Martin-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) 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 Larix 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.

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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., 2009b). 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>
<thead>
<tr>
<th>Age (years)</th>
<th>SH = 25 cm</th>
<th>SH = 50 cm</th>
<th>SH = 75 cm</th>
<th>SH = 100 cm</th>
</tr>
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<tr>
<td>P. menziesii</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PSM1</td>
<td>97</td>
<td>68</td>
<td>64</td>
<td>60</td>
</tr>
<tr>
<td>PSM2</td>
<td>47</td>
<td>57</td>
<td>52</td>
<td>51</td>
</tr>
<tr>
<td>L. occidentalis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAO1</td>
<td>108</td>
<td>44</td>
<td>39</td>
<td>42</td>
</tr>
<tr>
<td>LAO2</td>
<td>124</td>
<td>68</td>
<td>64</td>
<td>62</td>
</tr>
</tbody>
</table>

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.
One-way analysis of variance (ANOVA) was used to determine differences in RD traits associated with normal and wound xylem. Results were similar in the two rings analyzed.

### RESULTS

#### Axial RDs in L. occidentalis

The ADLAr values pre-fire were 3062 and $3388 \, \mu \text{m}^2$ in LAO1 and LAO2, respectively. Results differ between the two rings of wound xylem. ADLAr decreased significantly in ring 1, by 20% in LAO1 (LAO2 only showed that reaction at 75 cm to 100 cm above the ground surface). This narrowing of RDs increased with stem height (Fig. 2A, B).

#### Radial RDs and fusiform rays in P. menziesii

The ADLAr values pre-fire were $360 \, \mu \text{m}^2$ and $315 \, \mu \text{m}^2$ in PSM1 and PSM2, respectively. The ARH and ARW values pre-fire were respectively $264 \, \mu \text{m}$ and $274 \, \mu \text{m}$ in PSM1 and PSM2. The size and density of RDs did not change significantly in wound xylem compared with normal xylem (Fig. 2A, B). The density of RDs decreased by 44% from ring 1 to ring 2.
Radial RDs and fusiform rays in *L. occidentalis*

The ADLA<sub>r</sub> values pre-fire were 583 and 705 μm<sup>2</sup> 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<sub>r</sub> 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
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., 2003a, 2004; Hudgens and Franceschi, 2004).

**Changes in RD density**

Axial RDs are usually less numerous than radial RDs in Pseudotsuga 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., 2003a, 2004; Hudgens 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 (ADLAr) and fusiform rays (ARH, ARW) between normal xylem (NX) and wound xylem (WX) for the four trees analysed

<table>
<thead>
<tr>
<th></th>
<th>SH = 25 cm</th>
<th>SH = 50 cm</th>
<th>SH = 75 cm</th>
<th>SH = 100 cm</th>
<th>All SH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P-value</td>
<td>Δ (%)</td>
<td>P-value</td>
<td>Δ (%)</td>
<td>P-value</td>
</tr>
<tr>
<td>PS M1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NX – WX</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADLAr</td>
<td>0.826</td>
<td>+3</td>
<td>0.393</td>
<td>–10</td>
<td>0.671</td>
</tr>
<tr>
<td>ARH</td>
<td>0.653</td>
<td>+3</td>
<td>0.906</td>
<td>–1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ARW</td>
<td>&lt;0.001</td>
<td>+14</td>
<td>0.326</td>
<td>+3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PS M2</td>
<td></td>
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<td></td>
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<td>NX – WX</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADLAr</td>
<td>0.969</td>
<td>+1</td>
<td>0.010</td>
<td>+43</td>
<td>0.015</td>
</tr>
<tr>
<td>ARH</td>
<td>0.036</td>
<td>+20</td>
<td>0.007</td>
<td>+17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ARW</td>
<td>0.177</td>
<td>–6</td>
<td>0.112</td>
<td>+5</td>
<td>0.368</td>
</tr>
<tr>
<td>LA O1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>NX – WX</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADLAr</td>
<td>&lt;0.001</td>
<td>+192</td>
<td>&lt;0.001</td>
<td>+104</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ARH</td>
<td>0.002</td>
<td>+28</td>
<td>0.003</td>
<td>+34</td>
<td>0.037</td>
</tr>
<tr>
<td>ARW</td>
<td>&lt;0.001</td>
<td>+26</td>
<td>&lt;0.001</td>
<td>+25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LA O2</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADLAr</td>
<td>&lt;0.001</td>
<td>+124</td>
<td>&lt;0.001</td>
<td>+177</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ARH</td>
<td>0.133</td>
<td>+15</td>
<td>0.786</td>
<td>+3</td>
<td>0.179</td>
</tr>
<tr>
<td>ARW</td>
<td>&lt;0.001</td>
<td>+25</td>
<td>&lt;0.001</td>
<td>+56</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

ADLAr, 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.

Picea abies and Abies alba trees injured by mass-movement processes during dormancy (Boilschweiler et al., 2008; Stoffel and Hitz, 2008; Schneuwly et al., 2009a, 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., 1991b). 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., 2003b). More importantly, they deploy a highly inducible defence strategy in which the formation of traumatic RDs is crucial (Hudgins et al., 2003a, 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 defense 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.

**ACKNOWLEDGEMENTS**

We thank the Lolo National Forest, Missoula Ranger District for their co-operation during the planning and sampling phase of this study. This work was supported in part by US Forest Service Research and Development and National Fire Plan Research funds. We are grateful for the assistance of Josh Farella, David K. Wright and especially Ian R. Hyp (all US Forest Service Rocky Mountain Research Station) for sampling and preparing the cross-sections.

**LITERATURE CITED**


