

Variation in wood anatomical structure of Douglas-fir defoliated by the western spruce budworm: a case study in the coastal-transitional zone of British Columbia, Canada

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Received: 26 May 2014/Revised: 21 August 2014/Accepted: 27 August 2014
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Abstract

Key message An outbreak of the western spruce budworm temporarily modifies cellular wood anatomy of stem wood in natural and mature Douglas-fir stands impacting wood quality properties.

Abstract Western spruce budworm (*Choristoneura occidentalis* Freeman) is a widespread and destructive defoliator of commercially important coniferous forests in western North America. In British Columbia, Canada, Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] is the most important and widely distributed host. Permanent sample plots were established at a number of locations in southern interior at the beginning of a severe western spruce budworm outbreak in the 1970s. Two of the sites were sampled in 2012 to determine whether modifications had occurred in the anatomical characteristics of stem wood formed during outbreak years. We determined that

rings formed during the western spruce budworm 1976–1980 outbreak had a significantly lower proportion of latewood, reduced mean cell wall thickness and smaller radial cell diameters. While the cellular characteristics of the earlywood remained fairly constant, significant reductions in lumen area occurred in 1978 and 1979 at each site. Our study shows that western spruce budworm outbreaks not only reduce annual radial growth, but also temporarily modify cellular characteristics in latewood cells, which has implications for wood density and quality in Douglas-fir.

Keywords Cellular anatomy · Tracheids · Dendroecology · Douglas-fir · Western spruce budworm · Defoliation event

Communicated by Y. Sano.

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Introduction

Western spruce budworm (*Choristoneura occidentalis* Freeman; WSB) is the most widespread and destructive defoliator of coniferous forests in western North America (Wickman et al. 1992; Mason et al. 1997). Populations of WSB periodically intensify due to increased reproduction and survival, resulting in outbreaks that typically last for several years over large geographical areas (Alfaro et al. 2014). WSB defoliation severely reduces the radial growth of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) host-trees (Furniss and Carolin 1977; Fellin and Dewey 1982), with sustained outbreaks resulting in stem defects, mortality in understory trees, and regeneration delays due to feeding of staminate flowers and developing cones (Alfaro et al. 1982; Fellin and Dewey 1982; Alfaro and Maclauchlan 1992; Maclauchlan et al. 2006).

In the last two decades WSB outbreaks within the Douglas-fir forests of the interior of British Columbia (BC), Canada, have increased in severity, frequency and spatial distribution (Maclauchlan et al. 2006) in response to ongoing climate change (Dale et al. 2001; Logan et al. 2003; Volney and Hirsch 2005; Battisti 2008). Given that Douglas-fir is a widely distributed and commercially important species in this region, its increased vulnerability to WSB outbreaks (Walker and Sydenysmith 2008) is having immediate economic and social consequences for forestry dependent communities (Spittlehouse 2008; Johnston et al. 2010). These issues are exacerbated by the recent catastrophic outbreak of mountain pine beetle (*Dendrotonus ponderosae* Hopkins) in this region that resulted in the cumulative mortality of over 710 million m³ of *Pinus* trees (British Columbia Ministry of Forests, Lands and Natural Resource Operations 2012). Given the long-term loss of this timber resource, forest managers have refocused their attention on minimizing the impact of WSB outbreaks on Douglas-fir forests through application of the biological agent *Bacillus thuringiensis* var. *kurstaki* (Buxton and Maclauchlan 2001–2012). While the expectation is that a reduction in the incidence of outbreaks would enhance wood formation by minimizing growth suppression, stem defects and mortality, there has been an absence of anatomical research focused on evaluating the cellular-level impact of WSB on Douglas-fir growth. Given the increasing importance of Douglas-fir to the forest industry, and the uncertain impact of climate change on disturbances arising from WSB outbreaks, it is crucial to acquire a fundamental understanding of the cellular-level changes in anatomical structure resulting from defoliation during WSB outbreaks.

The goal of this study was to evaluate the anatomical characteristics of Douglas-fir trees impacted during an outbreak of the WSB by examining inter-annual changes in xylem characteristics in stem wood formed during an outbreak, relative to wood formed during non-outbreak years. The findings of the research will contribute to our understanding the effects of WSB defoliation on wood density, wood quality properties, and the resilience and plasticity of Douglas-fir.

Study sites

In the 1970s a number of permanent sample plots (PSPs) were established in southwestern BC during a WSB outbreak to study the effects of defoliation on stand structure, tree growth, volume, and tree form. Two PSPs located in the Fraser River Canyon, approximately 200 km northeast of Vancouver, BC, were selected for this study: East Anderson (49°46′34″N, 121°21′05″W) and Gilt Creek

(49°42′05″N, 121°22′35″W). Both PSPs are located on steep slopes (25° and 34°, respectively) between 660 and 700 m and have similar south-facing aspects (Fig. 1).

The two PSPs are found in a region that is climatically transitional between the BC coast and the interior characterized by warm-dry summers and moist-cool winters with moderate snowfall (Green and Klinka 1994). Interpolated climate data (Climate BC, v4.71, Wang et al. 2006, 2012) indicate the mean annual temperature is 6.3° C, with spring and summer temperatures averaging 12 and 23 °C, respectively. Annual precipitation totals in this setting average 1,587 mm, 72 % of which falls in the autumn and winter seasons. Dry summers in the study area historically gave rise to stand replacing fires, resulting in even-aged Douglas-fir forests with minor components of western hemlock (*Tsuga heterophylla* (Raf.) Sarg) and western red cedar (*Thuja plicata* Donn ex D. Don). Both PSPs are characterized by poorly developed shrub and herb layers in the understory, but have a well-developed moss layer, dominated by step moss (*Hylocomium splendens* (Hedw.) Br. et Sch.), and red-stemmed feather moss (*Pleurozium schreberi* (Brid.) Mitt.) (Green and Klinka 1994).

The East Anderson and Gilt Creek PSPs were established in 1976, at the beginning of a WSB outbreak that lasted from 1976 to 1980. At both sites the original sampling strategy was designed to incorporate 10 plots that transected the forest type (i.e., forest polygon with homogenous structure and composition) with inter-plot spacing of 80 m (Fig. 1). Initial stand structure was determined using a variable radius prism with a basal area factor of five (m²/ha). Each plot tree was measured for diameter at breast height (cm), total tree height (m), and stem features such as top-kill (i.e., dieback of the apical portion of the tree top due to severe defoliation). Annual visual defoliation data were collected from 1976 to 1980 at East Anderson and from 1977 to 1979 at Gilt Creek from the top, middle and bottom thirds of the crown. In 1982 these parameters were re-measured, by which time the WSB outbreak had collapsed and defoliation levels were zero.

Methodology

Field procedures

The East Anderson and Gilt Creek PSPs were revisited in August 2012, 36 years after their establishment. To study the effect of defoliation on tree growth, two 5.2 mm increment cores were collected from 25 trees in each PSP at ground and breast height, 0.3 and 1.3 m, respectively. To study the effects of defoliation on wood anatomy, a single 12 mm core was collected at breast height core from the same trees.

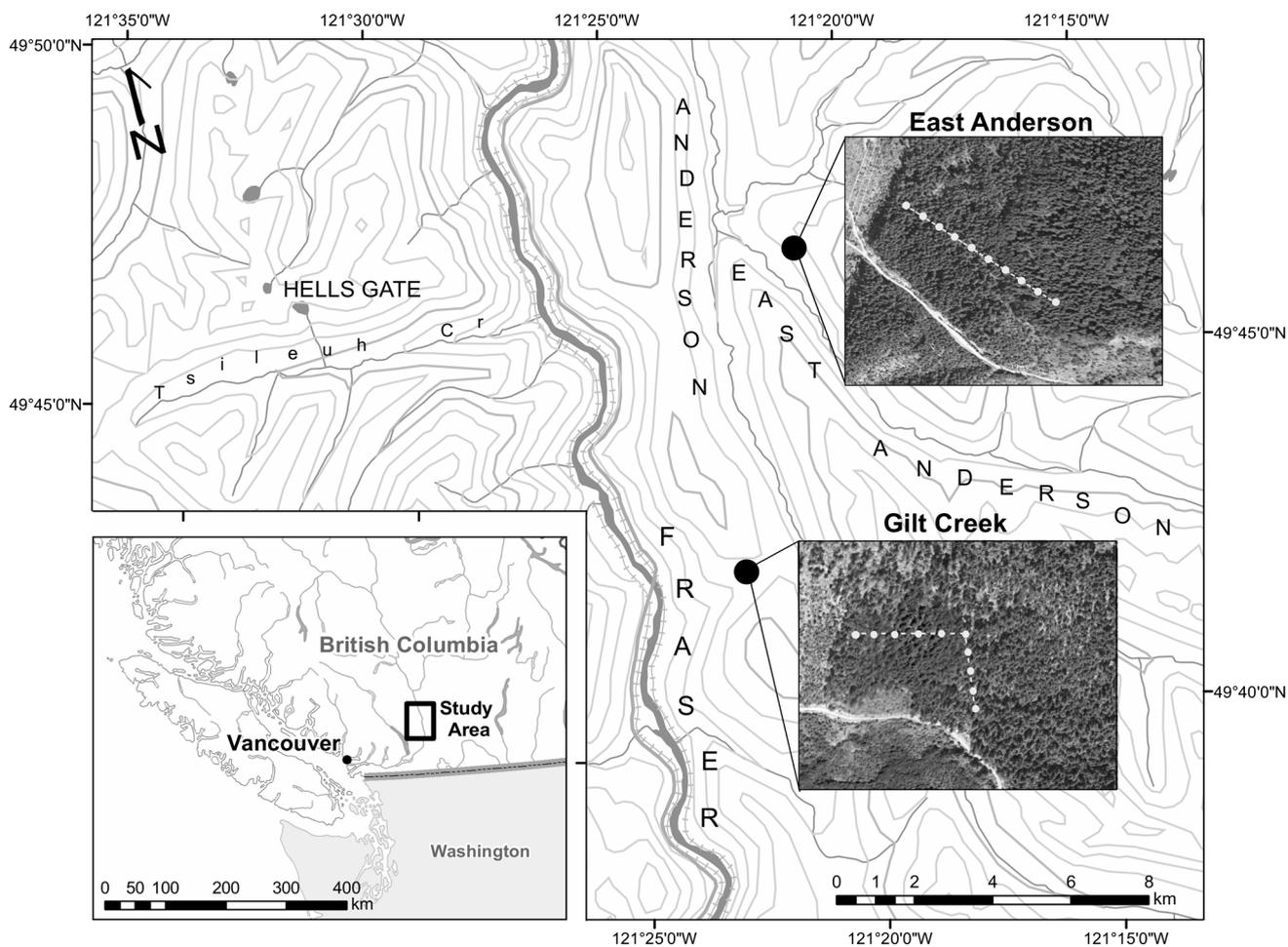


Fig. 1 Location of 2 permanent sample plots, East Anderson and Gilt Creek, used to measure impacts of western spruce budworm on cellular anatomy of stem wood in Douglas-fir British Columbia,

Canada. Inset maps of 2003 orthophoto images showing the single plots (*white dots*) with an inter-plot spacing of 80 m

Laboratory

Tree-ring chronologies were developed using standard dendrochronological methodologies (Stokes and Smiley 1968). Polished tree rings were measured with WinDendro (2009d, Regents Instruments Inc. 2009), and subsequently visually cross-dated and verified using COFECHA (Holmes 1986) to ensure exact calendar years were assigned to each tree ring. Individual tree-ring series were standardized using a negative exponential curve to remove biological growth trends (Cook et al. 2007), and stand-level chronologies were developed by computed a bi-weight robust mean that minimizes the effect of outliers and produces a dimensionless stationary index with a defined mean of 1.0 (Cook and Kairiukstis 1990).

For anatomical measurements, the 12 mm cores were cut into 4–5 cm segments and the portion spanning the years 1973–1988 identified for further analysis. Core segments were split radially and micro sections were cut on

the cross-sectional plane using a GSL1 microtome (Gärtner et al. 2014) equipped with removable NT-cutter blades. Micro sections were cut to a thickness of 15 μm using a non-Newtonian fluid (Schneider and Gärtner 2013), which greatly reduced preparation problems related to the separation of secondary cell walls and cell distortion, especially in the earlywood portion of the rings. Micro sections were double stained with cellulose and lignin specific dyes (Astrablue and Safranin), dehydrated with alcohol, rinsed in xylol and embedded in Canada balsam (Gärtner and Schweingruber 2013). Permanent slides were photographed with a digital camera system (Colorview IIIu camera mounted on an Olympus BX41 microscope) at 40 \times magnification.

Analysis

Microscopic images were measured using WinCell (2004a, Regents Instruments Inc, 2004). Twelve annual rings were

measured (1974–1986) along 10–14 radial files per ring on each sample, with the following parameters recorded: proportion of the radial file comprised of earlywood/latewood (%), cell lumen area (μm^2), cell wall thickness (μm), and radial cell diameter (μm). Every cell along each radial file was measured, and cellular parameters were averaged into a single radial file value for earlywood and latewood.

All statistical analyses were performed using the software R, version 3.0.2 (R Core Team 2013). The non-parametric Kruskal–Wallis (Quinn and Keough 2002) was computed to test for differences in wood anatomical properties between individual defoliated years (1976–1980), post-defoliation years (1981–1983) and non-defoliated periods 1974–1975 and 1984–1986 ($p < 0.05$). After a significant Kruskal–Wallis test result, the multiple comparison test *kruskalmc* from the R library *pgirmess* was applied to determine which groups were different from one another (Giraudoux 2013). Individual years had to be significantly different from both non-defoliated periods to reject the null hypothesis, and only differences between individual years and the non-defoliated periods were evaluated. Details of the Kruskal–Wallis test and the outcome of multiple comparisons were summarized by the anatomical parameter tested: proportion of latewood tracheids (%); lumen area (μm^2); secondary cell wall thickness (μm); and, radial cell diameter (μm). We averaged annual radial file data into a single ring value for each year and each micro section to assist in visual interpretation. For visualization, a smoothing line based on a robust locally weighed regression (Cleveland 1979) was added to scatter-plots of anatomical variables (Wickham and Chang 2013) to highlight the original data points, and the standard error of the smoothed surface.

Results

Defoliation history

Defoliation at East Anderson in 1976 averaged 50 % of the crown area (no data for Gilt Creek), and peaked at both

sites in 1977, when an average 65 % (East Anderson) and 60 % (Gilt Creek) of the crowns were defoliated. After 1977, average defoliation decreased at East Anderson to 60 % (1978), 40 % (1979) and 20 % (1980), while at Gilt Creek average defoliation stayed relatively constant at 55 % in 1978 and 1979 (no data for 1980) (Fig. 2).

Dendrochronology

The tree-ring data indicates that tree establishment occurred in the early 1900s at East Anderson and in the 1890s at Gilt Creek (Table 1; Fig. 3). The standard deviation around establishment dates was greater for Gilt Creek indicating that the age structure was more variable at this site (Table 1). Tree-ring series at each site strongly cross-date, as indicated by the inter-series *r* statistic that was well above the value of 0.328 ($p < 0.01$) (Table 1). Standardized tree-ring indices, truncated to the average establishment year, show inter-annual variability around the reference mean of 1.0 at both PSPs (Fig. 3a).

The 1970s outbreak is evident in the tree ring series as a pronounced period of growth suppression starting in 1976 and ending in 1983 (Fig. 3a), a duration that corresponds to the typical tree-ring signature of WSB outbreaks (Alfaro et al. 1982). Individual (i.e., non-averaged) raw ring growth (mm) increment value for the analysis window 1974–1986 demonstrated high inter-tree variability in growth during the 1970s outbreak at East Anderson (Fig. 3b) and Gilt Creek (Fig. 3c), as well as the strong growth suppression in response to budworm feeding. Annual growth rates at East Anderson were 1.2 mm pre- and post-outbreak, decreasing to a low of 0.2 mm during defoliation years (Fig. 3b). At Gilt Creek pre- and post-outbreak growth rates were slightly lower at 1.0 mm, and decreased to around 0.1 mm during the defoliation period (Fig. 3c). At both PSPs the lowest growth increment occurred in 1979, 2 years after the maximum crown defoliation in 1977 (Figs. 2, 3). Annual growth increment and inter-tree variability, as evidenced by outliers, was higher at East Anderson than at Gilt Creek (Fig. 3b, c).

Fig. 2 Average crown defoliation (%) (ND = no data) during the 1970s outbreak at East Anderson and Gilt Creek permanent sample plots. Boxes represent the interquartile range and median, whiskers indicate the variability outside the upper and lower quartiles, and outliers are plotted as individual points

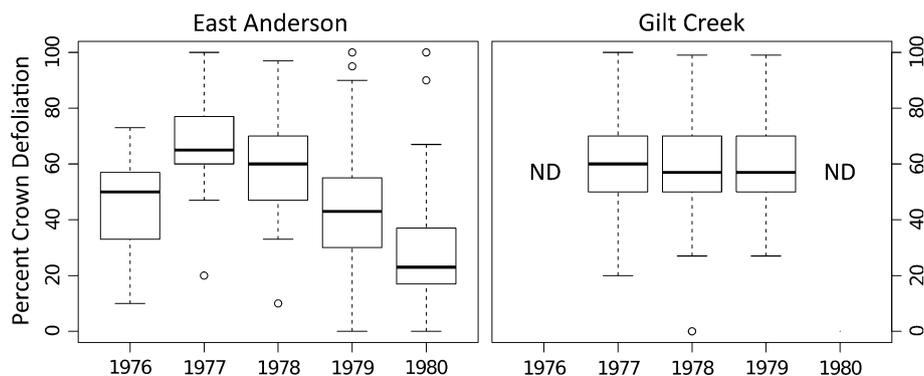


Table 1 Properties of tree-ring data from permanent sample plots used to measure impacts of western spruce budworm on wood cell anatomy of Douglas-fir, British Columbia, Canada

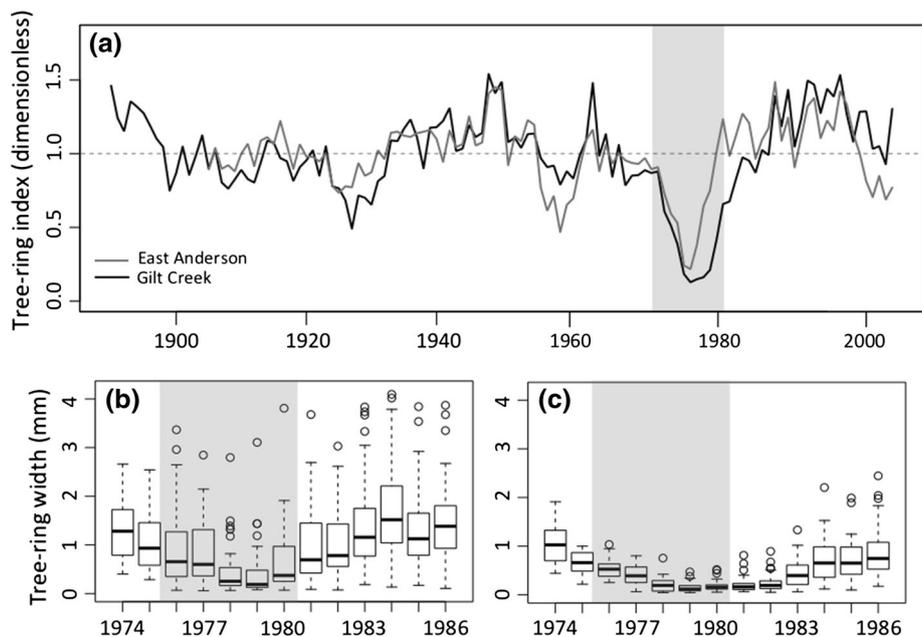
Stand name	Length of master tree ring series	Mean year of tree establishment ^a (\pm SD)	Inter-series (r)	Number of dated series
East Anderson	1892–2011	1904 (\pm 15)	0.57	47
Gilt Creek	1800–2011	1890 (\pm 43)	0.59	38

^a Mean year of establishment based on cross-dated cores collected at stump height (0.3 m) with standard deviation in brackets

Wood Anatomy

Twenty high quality micro sections were measured over a 12 year period from 1974 to 1986 from each PSP (e.g., Fig. 4). Table 2 summarizes the Kruskal–Wallis test and multiple comparisons for each anatomical parameter tested. The proportion of latewood decreased in both PSPs during the outbreak period (Fig. 5); at East Anderson this effect lasted from 1977 to 1979 with a 20 % reduction in the proportion of latewood (Table 2). At Gilt Creek there was a 13 % reduction in latewood in 1979 and 1981 (Table 2). For the other cellular parameters three main trends were evident: (1) independent of the parameter analyzed reductions in area or size were observed during defoliation years as compared to the years with no defoliation; (2) reductions in cell wall thickness and radial cell diameter in the latewood tracheids were the most acute and prolonged of any of the parameters analyzed; and, (3) the largest reductions occurred in 1979 (Fig. 5).

Fig. 3 **a** Tree ring index series truncated to average year of tree establishment, dashed line indicated chronology mean (1.0). **b** East Anderson raw tree-ring growth (mm) during analysis window, 1974–1986. **c** Gilt Creek raw tree-ring growth (mm) during analysis window, 1974–1986. Boxes represent the interquartile range and median, whiskers indicate the variability outside the upper and lower quartiles, and outliers are plotted as individual points. Gray shading highlights the western spruce budworm outbreak period from 1976 to 1980



At East Anderson earlywood lumen area and radial cell diameter decreased between 5 and 8 % in 1978 and 1979 compared to undefoliated years; in the latewood tracheids cell wall thickness decreased between 11 and 25 % from 1977 to 1980, and radial cell diameter decreased between 8 and 22 % from 1977 to 1980 compared to undefoliated years (Table 2; Fig. 5). At Gilt Creek earlywood lumen area decreased by 10 and 12 % in 1978 and 1979, respectively, compared to undefoliated years (Table 2); in the latewood tracheids lumen area decreased by 13 % in 1978 and 1979, cell wall thickness decreased between 18 and 22 % from 1977 to 1982, and radial cell diameter was 12–22 % smaller from 1977 to 1982 compared undefoliated years (Table 2; Fig. 5).

Discussion

We examined cellular variations in the wood anatomy of Douglas-fir trees arising from severe WSB defoliation at two sites during the late-1970s. We are unaware of other studies that have examined the anatomical characteristics of Douglas-fir in relation to defoliation by the WSB, either in artificial defoliation experiments or naturally occurring outbreaks in mature forest stands. We hypothesized that in addition to radial growth suppression (Kulman 1971; Alfaro et al. 1982) that anatomical parameters such as proportion of latewood, lumen area, cell wall thickness, and/or radial cell diameter would change in the stem wood during an outbreak of the WSB.

In this study, reductions in radial increment during the defoliation period were 80 % at East Anderson and 83 % at

Fig. 4 Example of a composite micro section for analysis window (1974–1986) at East Anderson permanent sample plot

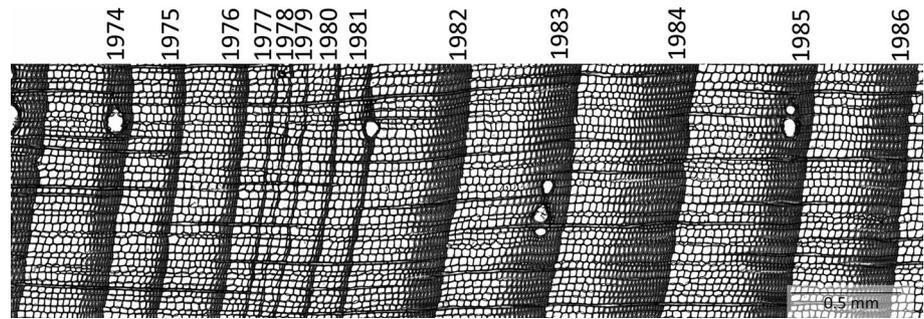


Table 2 Kruskal–Wallis test for each anatomical parameter: proportion of latewood, lumen area, cell wall thickness, and radial cell diameter for early- and latewood tracheids

Tracheid Type	Cell parameter	Kruskal–Wallis (<i>p</i> value)	Difference between year and undefoliated periods (1974–75 and 1984–86)									
			1976	1977	1978	1979	1980	1981	1982	1983		
East Anderson												
Earlywood	Lumen area (μm^2)	$p = 3.6^{-06}$			*	**						
	Cell wall thickness (μm)	$p < 0.001$										
	Radial cell diameter (μm)	$p < 0.001$			*	**						
Latewood	Proportion of latewood (%)	$p < 0.001$		**	**	**						
	Lumen Area (μm^2)	$p = 1.1^{-09}$										
	Cell wall thickness (μm)	$p < 0.001$		**	**	**	**					
Radial cell diameter (μm)	$p < 0.001$		**	**	**	**						
Gilt Creek												
Earlywood	Lumen area (μm^2)	$p = 4.6^{-07}$			**	*						
	Cell wall thickness (μm)	$p = 5.3^{-13}$										
	Radial cell diameter (μm)	$p < 0.001$										
Latewood	Proportion of latewood (%)	$p < 0.001$					**		**			
	Lumen area (μm^2)	$p < 0.001$			*	*						
	Cell wall thickness (μm)	$p < 0.001$			**	**	**	**	**	**	**	**
	Radial cell diameter (μm)	$p < 0.001$		**	**	**	**	**	**	**	**	**

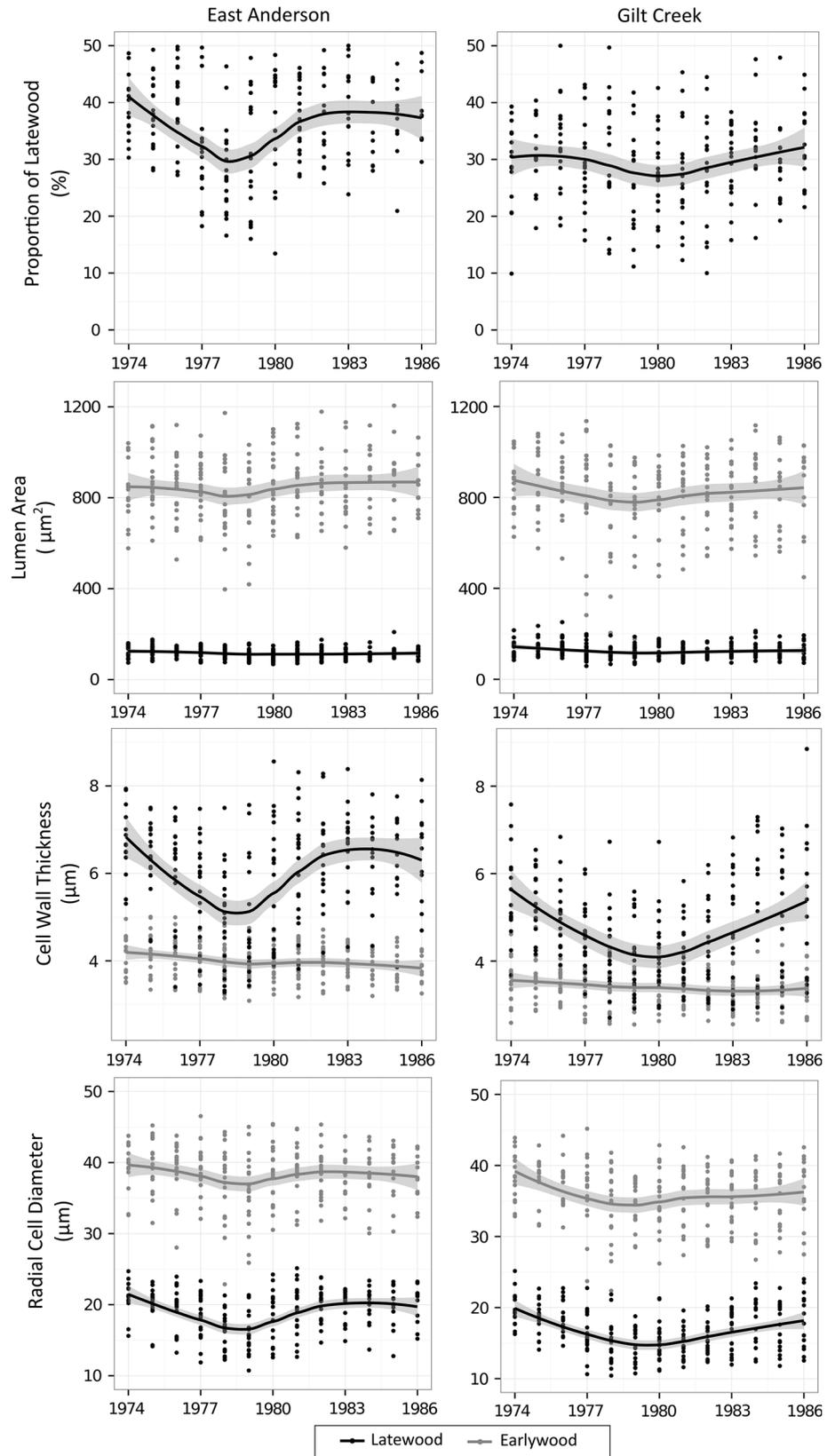
Multiple comparisons determined where significant differences occurred between tracheids produced during defoliation years and undefoliated years (1974–1975 and 1984–1986). Significant differences are denoted by * $p < 0.5$ level and ** $p < 0.01$ level. An individual year had to be significantly different from both sets of undefoliated years to reject the null hypothesis, and differences between individual years were not considered at permanent sample plots East Anderson and Gilt Creek

Gilt Creek (Fig. 3), corresponding to an average of 60 % defoliation of the crown (Fig. 2). Growth suppression was immediate at each site (Fig. 3) and persisted for approximately 8 years. Radial growth has been shown to be progressively reduced with every consecutive year of defoliation, in a cause–effect relationship that usually has a 1–2-year lag (Alfaro et al. 1982). This lag was apparent in the patterns of radial tree growth at both sites (Fig. 3), where the maximum growth reduction (1979) did not occur until 2 years after maximum crown defoliation (1977). This 2-year lag was also evident in the anatomical variables tested.

Significant inter-annual changes in xylem characteristics, principally in latewood tracheids, were detected. When compared to non-outbreak years these changes

include decreases in proportion of latewood, cell wall thickness and radial cell diameter during WSB defoliation (Table 2; Fig. 5). In contrast, very few sustained changes were detected in earlywood tracheids (Table 2, Fig. 5). Latewood proportion in growth rings is the main driver of overall wood density (Zobel and Van Buijtenen 1989), which from a timber perspective is the most important feature of Douglas-fir (Renninger et al. 2006). In Douglas-fir, latewood proportion has been shown to decrease after thinning and fertilization treatments (Brix and Mitchell 1980) and exposure to air pollution (de Kort 1993, 1991). In slow growing Douglas-fir infected with Swiss needle cast (*Phaeocryptopus gaeumannii* (Rohde) Petrak), a disease that reduces the leaf area of infected individuals, trees produced higher proportions of latewood and had better

Fig. 5 Scatterplots (fit with a locally weighed regression line) of anatomical parameters: proportion of tree-ring comprised of earlywood and latewood (*top*), lumen area (*top-middle*), cell-wall-thickness (*bottom-middle*), and radial cell diameter (*bottom*) for earlywood (*gray*) and latewood tracheids (*black*) at East Anderson (*left*) and Gilt Creek (*right*) permanent sample plots



strength characteristics (Johnson et al. 2003). In this study we found that during WSB defoliation latewood proportion was significantly lower at both sites (Table 2; Fig. 5) suggesting that slower radial growth did not necessarily lead to denser wood. However, the degree of latewood reduction required to meaningful impact wood density is unclear and was not one of the goals of this study.

We suggest that the changes in latewood tracheid properties identified in this study are related to the timing of latewood formation, as well as processes such as cell wall thickening, and budworm phenology and feeding behavior. In Douglas-fir, vegetative bud burst occurs in mid-May (Owens 1968) but can occur as early as mid-April in coastal regions (Brix and Mitchell 1980). Early-stage WSB larvae emerge several days to weeks in advance of bud suitability (i.e., bud burst) (Nealis 2012) and then feed on buds and new needles until early July, after which they pupate (Furniss and Carolin 1977). By this time earlywood formation is complete (Brix and Mitchell 1980; Vargas-Hernandez and Adams 1994) and latewood transition and formation is underway (Li and Adams 1994; Vargas-Hernandez and Adams 1994; Grotta et al. 2005). Consequently latewood formation, including cell wall thickening, occurs when the tree has undergone its maximum defoliation in a given year and foliar resources are the lowest.

The extent to which tree growth, and by association cellular properties, are a function of carbon availability has important implications for the understanding and predicting tree and forest responses to environmental change (Sala et al. 2012). The removal of current-year shoots near the beginning of the growth period stimulates starch mobilization to increase net photosynthetic rate of the remaining foliage (Reich et al. 1993; Lavigne et al. 2001). In artificially defoliated young red pine (*Pinus resinosa* Ait.) photosynthetic rate and leaf conductance increased due to altered root: needle balance, leading to a shift in allocation to new needles rather than the roots, followed by a temporary increase in photosynthesis. This compensatory response has a net energy cost and carbohydrate reserves were lower in defoliated individuals (Reich et al. 1993). Foliage replacement requires carbon reserves for the production of new needles at a considerable energy cost for tree, and cumulative loss of current-year foliage results in gradually consumed and depleted carbohydrate reserves (Reich et al. 1993). In Douglas-fir stands infected with Swiss needle cast, retention of non-structural carbohydrates in the crown has a greater priority than exporting photosynthates required for diameter growth in the trunk ensuring crown growth was favored (Saffell et al. 2014). Plants allocate carbohydrates to different sinks, and foliage production takes priority over stem growth to ensure that there is an adequate amount of resources available for

developing foliage (Piene and Little 1990; Kaitaniemi et al. 1999; Polák et al. 2006). Thus, carbon storage may be an active sink, where trees actively regulate storage at the expense of short-term growth to optimize growth and survival in the long term (Sala et al. 2012).

While the growth response of latewood tracheids was similar at each site (Table 2; Fig. 5), they were most pronounced and prolonged at Gilt Creek where the defoliation pressures were highest. Crown defoliation at Gilt Creek averaged 60 % from 1977 to 1979 (Fig. 2). More intense budworm feeding extended the lag effect at Gilt Creek, where the growth suppression phase (Fig. 3) and reductions in cell wall thickness and radial cell diameter (Fig. 5) persisted through the outbreak phase and into non-outbreak years (1981 through 1982) (Table 2). This prolonged response was not evident at East Anderson where defoliation levels steadily decreased after 1977 to around 20 % by 1980 (Fig. 2), suggesting that defoliation intensity is a particularly important feature to consider when evaluating tree response to herbivory (Kozłowski 1969; Kulman 1971; Alfaro et al. 1985; Krause and Raffa 1996). The modifications to latewood tracheid properties during a WSB outbreak, especially when prolonged, may impact the drought tolerance of Douglas-fir as latewood has been shown to have a greater water storage capacity than earlywood (Domec and Gartner 2002). Thus, trees with higher wood density with thicker cell walls and smaller lumen diameters are expected to be more resistant to drought (Martinez-Meier et al. 2008; Dalla-Salda et al. 2009).

Rossi et al. (2009) conducted a study where spruce budworm (*Choristoneura fumiferana* Clemens) defoliation was simulated by removing two-thirds of the current-year needles in balsam fir (*Abies balsamea* (L.) Mill.) seedlings. This removal had no effect on either cambial activity or xylem differentiation in the stem, or the anatomy of the xylem produced. Significant changes in xylem anatomy in the stem wood (Filion and Cournoyer 1995; Liang et al. 1997) was, however, detected during the study of a mature eastern larch (*Larix laricina* (Du Roi) K. Koch) forest in subarctic Quebec during a larch sawfly (*Pristiphora ericsonii* (Htg.)) defoliation event. Filion and Cournoyer (1995) found that a light ring formed in the first year of the outbreak, and in subsequent years the tracheid size decreased abruptly in the most severely impacted trees. Liang et al. (1997) compared light rings resulting from sawfly defoliation and climate-limiting conditions. They demonstrated that average latewood cell wall thickness was reduced in insect-generated light rings, and smaller still in climate-induced light rings, while cell wall thickness in earlywood tracheids remained constant.

In natural outbreak conditions insect defoliation varies in intensity according both to the severity of the attack and to the vigour of the tree at the time, consequently tree

response to defoliation is highly variable and light rings due to defoliation are more variable than those produced by climatic conditions (Liang et al. 1997). In experimental conditions trees (seedlings or juveniles) are growing in controlled environments and receiving the same defoliation treatments (Wodzicki 2001; Rossi et al. 2009). However, mature trees undergoing natural defoliation sustain different feeding pressures, and have different physiological status, and vigour. The complexity of host plant reaction to insect defoliation can be important in contributing to the variability of cell structure patterns among different years and different trees (Liang et al. 1997). This pattern was readily apparent in this study where cell structure patterns were highly variable between earlywood and latewood tracheids, and between non-outbreak and outbreak years from tree to tree (Fig. 5).

Conclusions

In this study we focused attention on the anatomical impact of a single WSB outbreak in Douglas-fir stem wood collected at breast height. We demonstrated that this outbreak resulted in statistically significant annual reductions in the proportion of latewood, cell wall thickness and radial cell diameter. As environmental factors affect whole plants not just stem wood (Gartner et al. 2002), future research should examine the anatomical impact of multiple WSB outbreaks in samples collected at different heights on the tree bole, branches and roots. This approach would provide additional insight into the cumulative effects of outbreak disturbances on wood structure and would enhance our understanding of how different parts of a tree respond to these disturbances. Further, sampling on the tangential plane could provide insights into the response of other anatomical structures, such as ray parenchyma cells in nutrient storage during disturbances events.

Author contribution statement J. A. developed the study and hypothesis, conducted field, laboratory work, and statistical testing, primarily wrote the manuscript and produced tables and figures. A. B. supported statistical analyses. R. A. assisted in re-locating original permanent sample plots, provided the permanent plot dataset, contributed to the Study Area and Methods sections, and reviewed edited the manuscript. D. S. assisted in hypothesis development, contributed to the Introduction, Methods and Results sections and reviewed and edited the manuscript. H. G. contributed to hypothesis development, methodological development, supported wood anatomy and micro sectioning methods and interpretation, and contributed to the entire manuscript.

Acknowledgments This study was made possible through funding from the Natural Science and Engineering Research Council (Axelson and Smith) and Michael Smith Foreign Supplement Award (Axelson) and the Pacific Institute for Climate Solutions (Axelson and Smith). We are grateful for assistance provided in the field by Peter Sprague,

and at WSL Birmensdorf from Fritz Schweingruber and Loïc Schneider.

Conflict of interest The authors declare that they have no conflict of interest.

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