Research paper

Functional xylem anatomy of aspen exhibits greater change due to insect defoliation than to drought

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The study of tree rings can reveal long-term records of a tree's response to the environment. This dendroecological approach, when supplemented with finer-scale observations of the xylem anatomy, can provide novel information about a tree's year-to-year anatomical and hydraulic adjustments. Here we use this method in aspen (*Populus tremuloides* Michx.) to identify xylem response to drought and insect defoliation. Surprisingly, we found that precipitation influenced vessel diameter mostly in the trees' youth, while this correlation was less pronounced at maturity. This is likely due to a reduction in stress the stand experiences as it ages, and reflects an ability to mediate drought stress as trees mature. Defoliation events caused consistent and profound changes in fiber anatomy likely leading to reduced structural support to vessels. We therefore expect that in years of defoliation trees may be vulnerable to drought-induced cavitation when leaf area recovers. This study highlights how the inclusion of cellular level measurements in tree ring studies provides additional information on how stress events may alter tree functioning through alterations in structure.

Keywords: aspen, drought, insect defoliation, tree rings, white rings, xylem transport.

Introduction

The variation in tree ring width is widely used to study tree growth response to changing environmental conditions; sensitivity to the environment can be observed especially in areas where tree growth is limited by precipitation or temperature (Fritts 1976, Speer 2010). However, there is also variation within the cellular structure of tree rings. Tree rings are composed of cells belonging to the water-transporting xylem tissue: cells with a direct link to tree physiology. Analyses of xylem structure along a time series have been less widely studied than tree ring width, but remain an important source of information about year-to-year physiological adjustments to the environment (Fonti et al. 2010).

With the global rise of tree dieback due to climate change-induced drought (e.g., Allen et al. 2010, Venturas et al. 2016, Adams et al. 2017), understanding the anatomical structure of the xylem could provide additional insight into this high-interest topic of tree death (McDowell et al. 2008). Drought may lead to narrower vessels, and this will correspond with lower transport efficiency (Plavcová and Hacke 2012, Hacke et al. 2017), and xylem pit membranes can become more porous (and leaky to air), resulting in increased vulnerability to drought-induced cavitation (Hillabrand et al. 2016). Additional aspects of cellular xylem anatomy may also be altered by drought and should be explored further. Although most studies of functional xylem anatomy have focused on vessel size, the structure of fibers can also influence water transport. Increased fiber wall area was positively correlated with cavitation resistance in at least one study (Jacobsen et al. 2005), suggesting a mechanical role for fibers in cavitation resistance. Similarly, inter-vessel wall thickness also remains an important contributor to the integrity of the vessel structure (Hacke et al. 2001).

Here we asked how the xylem anatomy of trembling aspen (*Populus tremuloides* Michx.) is altered by stress events like drought and insect defoliation. Aspen is a promising study system, because its ecophysiology and water relations have been extensively studied (e.g., Galvez et al. 2011, Schreiber et al. 2011, Anderegg et al. 2013, Schreiber et al. 2015), and because it is the most widespread tree species in North America (Peterson and Peterson 1992). Aspen stands have experienced massive dieback as a result of severe drought (Worrall et al. 2008, Michaelian et al. 2011), but xylem anatomy has not been studied in this context and over longer time scales, i.e., decades.

Increases in drought due to climate change and insect outbreaks may interact to promote tree mortality (Anderegg et al. 2015). In western Canada, a major defoliator of trembling aspen is the forest tent caterpillar (Hogg et al. 2005). Defoliation by the caterpillar often first occurs in early spring before the leaves are fully expanded; as a result, these defoliation events lead to the formation of distinctive 'white rings' with reduced width and density (Hogg et al. 2002, Sutton and Tardif 2005). Forest tent caterpillar outbreaks in aspen stands may last successive years (Cooke and Roland 2000, 2007), leading to the formation of multiple adjacent white rings. Though the fiber wall thickness appears to be influenced by defoliation events (Sutton and Tardif 2005), the impact of defoliation on the xylem anatomy remains understudied. It is also unknown whether defoliation events can alter xylem structure in the following year, i.e., whether there is a lag effect.

Besides stress-induced changes in xylem anatomy, the cellular dimensions of xylem are known to change radially, from pith to bark, i.e., there is a trend for an increase in conduit size with age (Lachenbruch et al. 2011). As a tree grows larger, it experiences different micro-environments and mechanical demands. Wood produced in the early years of a tree's life is generally characterized by large gradients of change in anatomy. In the growth rings of later years, this change becomes more gradual (Lachenbruch et al. 2011). Disturbance events such as drought and insect defoliation could therefore impact the structure of the wood to different degrees based on the age of the tree at the time of disturbance.

The radial extent of change varies by species, and though this pattern is found in both hardwoods and softwoods, anatomical changes due to age are understudied in diffuse-porous wood (Lachenbruch et al. 2011). In *Populus* species, research in this area has mainly focused on wood density and fiber length, important commercial traits (Koubaa et al.1998, DeBell et al. 2002). Analyses of hydraulic traits such as vessel diameter would provide an additional insight into how trembling aspen respond to their environment and stress events over time.

For this study, aspen trees were chosen from Ministik forest, a site in central Alberta where aspen trees have experienced periods of both drought and defoliation over their lifetimes. This provided an opportunity to observe potential changes in tree ring anatomy with environmental stress and provide a comprehensive examination of aspen xylem anatomy over time. Our objectives were to (i) determine how drought and defoliation events influence the structure and inferred function of the xylem anatomy and (ii) to determine how xylem anatomy changes due to age and whether age alters the response to drought and defoliation.

Materials and methods

Tree core sampling and cross-dating

Tree cores were collected from an even-aged stand of trembling aspen that originated around 1926 in Ministik, Alberta, Canada (53° 16′ 40.8″ N, 112° 54′ 46.8″ W). Two increment cores were taken at 1.3 m height from 20 aspen trees along with measurements of diameter at breast height. The cores were dried and sanded with progressively finer sand paper. Cores were visually cross-dated using a binocular microscope (Fritts 1976). Tree-ring width (*rw*) was measured to a precision of 0.01 mm using a LINTAB measuring device (Rinntech, Heidelberg, Germany). The program COFECHA was used to check the quality and correct dating of the series (Holmes 1983).

Identification of drought and defoliation events

Years of drought were identified using the Climate Moisture Index (CMI), a measure derived from the difference between annual precipitation and annual potential evapotranspiration (Hogg 1994, Hogg et al. 2013), calculated yearly over successive 12-month periods ending on 31 July (Hogg et al. 2005). With this index, four separate years were identified as having values below −15 cm and were classified as 'drought years' for this study: 1949, 1950, 2002 and 2009. Five years of severe defoliation, primarily by forest tent caterpillar, were identified by the repeated presence of 'white rings' (Hogg et al. 2002) in the chronology: 1960, 1987, 1988, 1994 and 1995.

Thin section preparation and wood anatomical variables

To measure wood anatomical characteristics spanning the years 1946–1963 (Age Group 1) and 1984–2012 (Age Group 2), thin sections were produced to represent each year by four to six individual trees. The lengths of these two Age Groups were determined by beginning 3 years prior to the first stress event and ending at 3 years post last year of stress. Transverse thin sections were prepared using a sliding microtome (Leica SM2400, Leica, Wetzlar, Germany). The tissue was stabilized with a starch-based non-Newtonian solution to fill the cells and keep the structure rigid during the cut (Schneider and Gärtner 2013). Afterwards, the sections were stained with a combination dye of safranin and astrablue to indicate lignification (Gärtner and Schweingruber 2013).

Prepared slides were imaged using a light microscope (Leica DM3000) with a mounted camera. The resulting images were stitched together to create larger sections containing adjacent tree rings. Images were analyzed with ImagePro Premier (Media Cybernetics, Rockville, MD, USA) for wood anatomical variables.

The average diameter (dv) was taken from all vessels across an entire ring between 3 and 4 xylem rays. From this, the hydraulically weighted mean vessel diameter (dh) (Sperry et al. 1994) was calculated per ring as:

$$dh = \frac{\sum dv^5}{\sum dv^4} \qquad (1)$$

Xylem vessel contact fraction (cf) was measured as the total length of contact between vessels per the total perimeter of all vessels (Wheeler et al. 2005) across an entire ring for the same vessels on which the diameter measurements were made. Using xylem rays as an outline surrounding measured vessels, vessel lumen fraction (lf) was measured as the total vessel lumen area per total xylem area. A greater vessel lumen fraction corresponds with a higher percentage of xylem cross-sectional area dedicated to water transport.

All sections containing rings representing defoliation years were also analyzed for the fiber lumen fraction. The fiber lumen fraction was calculated by selecting two sections of fibers from the middle of each ring and determining the ratio of total fiber lumen area to total fiber lumen and wall area. Higher values of fiber lumen fraction correspond with larger fibers and/or thinner fiber walls.

Additionally, sections in Age Group 2 were analyzed for vessel roundness and inter-vessel double wall thickness (t); the lower quality of the sections in Age Group 1 did not allow for accurate measurements of t. With t, the thickness to span ratio tb was calculated as $(t/b)^2$ to estimate vessel resistance to implosion (Hacke et al. 2001), with b represented by dh. Within each ring, only vessel pairs within $\pm 5\ \mu$m of the calculated dh were chosen for these measurements.

Vessel roundness was calculated by ImagePro Premier with an area to perimeter ratio:

$$\text{roundness} = \frac{4\pi \text{Area}}{\text{Perimeter}^2} \qquad (2)$$

where a value of 1 represents the ratio of a perfect circle. Values further from 1 are less circular because of protrusions from the ideal shape: outward protrusions give a value greater than 1, and inward protrusions give a value less than 1.

Stable carbon isotope analysis

Additional cores were chosen for analysis of carbon isotope discrimination. In total, each year in the analysis was represented by four individual trees in the same Age Groups as used in the anatomical analyses. Entire rings were separated using a scalpel for the same years of interest as chosen for the thin sections. As some rings were very small, we did not extract cellulose; whole wood was used to ensure there was enough material for the analysis. Samples were weighed, cut into slivers and sent to be analyzed at the University of British Columbia Stable Isotope Laboratory (http://isotopes.forestry.ubc.ca/) for the isotope ratio $^{13}C/^{12}C$, expressed as $\delta^{13}C$ in parts per thousand (‰) relative to the standard Vienna PeeDee Belemnite. These values ($\delta^{13}C_p$) were used to calculate the physiological discrimination of ^{13}C by the tree ($\Delta^{13}C$), accounting for changes in the atmospheric ratio ($\delta^{13}C_a$) over time (McCarroll and Loader 2004):

$$\Delta^{13}C = \frac{\delta^{13}C_a - \delta^{13}C_p}{1 - \delta^{13}C_p/1000} \qquad (3)$$

Statistical analysis

Data analysis was completed using R statistical software (R Development Core Team 2016). Significant changes in mean values of $\delta^{13}C$, fiber lumen fraction, and vessel roundness were assessed with analysis of variance (ANOVA) performed using linear mixed models with wood Age Group and tree core id as random effects to assess these changes independent of age.

Time series of tree ring width and wood anatomical data were detrended using a 10-year cubic smoothing spline with a 50% frequency cut-off to remove age trends. First-order autocorrelation was non-significant in the detrended series. This detrending procedure was applied to series in both Age Group 1 and Age Group 2, and carried out using the *dplR* package (Bunn 2008).

After detrending, correlation matrices of Pearson's r were calculated using the standardized data to assess the relationships between wood anatomical variables, as well as anatomical variables and climate. Years of defoliation were removed from the calculation of correlations between wood anatomical variables and climate since changes in wood anatomy due to defoliation events were not climate-driven.

Annual and seasonal climate data for Ministik, AB was generated through interpolation with the BioSIM software tool, available at ftp://ftp.cfl.forestry.ca/regniere/software/BioSIM/ (Régnière et al. 2016). Annual variables such as mean annual temperature (T_aj) and total annual precipitation (P_aj), as well as the CMI and additional Soil Moisture Index (SMI) values (Hogg et al., 2013) were calculated from the previous 1 August to 31 July. Total growing season precipitation (P_gs) included the months of May through September. A mean of the last 4 years CMI (CMI_4y) was also included based on strong correlations with ring width (Hogg et al. 2008), as well as a mean of the last 2 years SMI (SMI_2y).

For further investigation of correlations between wood anatomical variables, standard major axis regression was used to model the relationship. This analysis was used because the

wood anatomical variables could not be considered to be independent of one another (Warton et al. 2006).

Results

The chronology of aspen ring width showed considerable variation year to year from 1933 to 2014. Years with CMI values lower than −15 cm, considered as severe drought, showed narrow ring width, but not as narrow as years of defoliation (Figure 1).

Stable carbon isotope analysis of the tree rings showed interannual variation in $\Delta^{13}C$ values and generally supported the choice of years of severe drought as previously determined with the CMI (Figure 2a). Expectedly, mean drought year $\Delta^{13}C$ was significantly lower than those of defoliation or control, meaning that drought years were significantly more enriched in ^{13}C. Mean $\Delta^{13}C$ for the defoliation years was significantly higher than control (Figure 2b).

Pearson's r correlations between wood anatomical variables in the separate wood age groups showed similar results (Figure 3a and b). However, correlations between wood anatomical variables and climate generally showed a stronger relationship in the Age Group 1 than in the Age Group 2, including rw, dv and dh (Figure 3c and d; see Tables S1–S4 available as Supplementary Data at Tree Physiology Online for P-values). A strong positive relationship between vessel diameter (dv and dh) and precipitation variables was found in Age Group 1, but was less pronounced for Group 2. The relationship between vessel diameter and growing season precipitation (P_aj) was even stronger than the relationship between ring width and P_aj (Figure 4), though both these relationships were not found in Age Group 2. Age Group 1 ring width and vessel diameter was also found to correlate strongly with the current year CMI and

SMI, while the Age Group 2 ring width was more strongly correlated with the mean of the last 4 years for the CMI (CMI_4y) and 2 years for the SMI (SMI_2y). The relationship between $\Delta^{13}C$ data and climate was significantly positive for all climate variables in Age Group 2 except T_aj, while in Age Group 1, $\Delta^{13}C$ was only significantly correlated with CMI_4y.

Between wood anatomical variables, a significant negative correlation was found between ring width and vessel lumen fraction (Figure 3). In both Age Groups, defoliation rings were characterized by small width and increased vessel lumen fraction (Figure 5). Drought years did not exhibit this characterization as strongly. Another significant negative correlation was found between vessel diameter and contact fraction, indicating that tree rings with smaller vessels also had vessels in greater contact with another. Drought and defoliation years did not appear to be characterized by either extreme (Figure 6).

No anatomical variables strongly characterized drought rings by having significantly different means in drought years. By contrast, defoliation rings were characterized by having increased fiber lumen fraction and decreased vessel roundness (Figure 7). Fiber lumen fraction was significantly greater in the years that experienced defoliation as compared with the year immediately prior to or post a 1–2 year defoliation event (Figure 7a). Fiber lumen fraction measurements were taken as close to the middle of each ring as possible and high fiber lumen fraction seemed to reflect both thinner walls and larger fiber lumens (Figure 7b). No lag effect was seen; following defoliation, mean fiber lumen fraction returned to a value equal to the year prior the defoliation. Additionally, the vessels within the defoliation years were significantly less round as compared with vessels in the years prior to and post a defoliation event (Figure 7c). Vessels exhibited wavy margins in defoliation years (Figure 7d), indicating a weakened structure. The combination dye of safranin and astrablue also
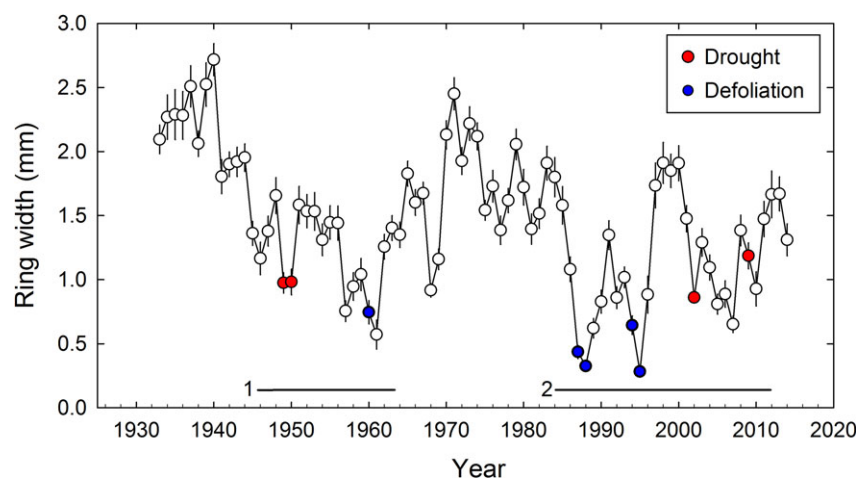

Figure 1. Chronology of aspen ring width. Points in red indicate years with Climate Moisture Index (CMI) values lower than −15 cm, indicating a severe drought. Points in blue indicate years with 'white rings,' a sign of defoliation. Horizontal black bars indicate the lengths of the series which were chosen for further wood anatomical measurements: 1946–63 (Age Group 1) and 1984–2012 (Age Group 2). Error bars indicate standard error of the mean ($n = 40$).
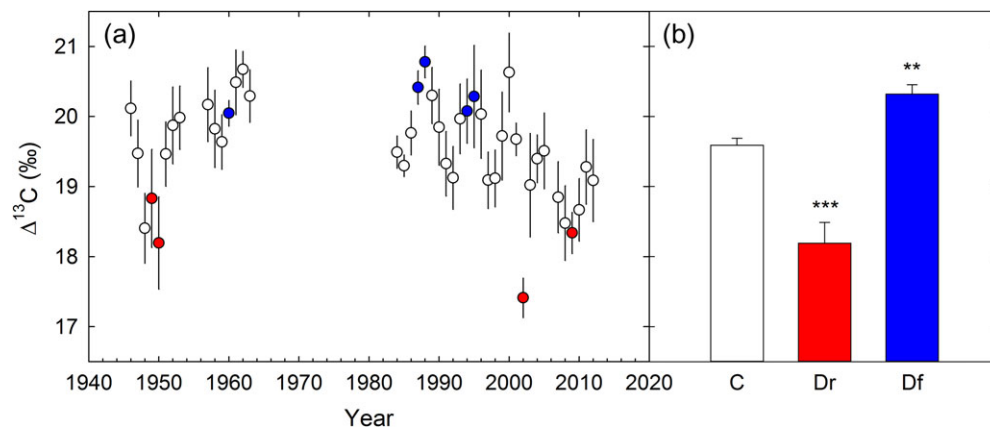
Figure 2. (a) Chronology of $\Delta^{13}C$ values for both Age Groups, $n = 4$. Points in red indicate drought, and points in blue indicate defoliation years. (b) The mean $\Delta^{13}C$ values for each group are presented with an ANOVA test of significance comparing drought with control values and defoliation with control. Error bars indicate standard error of the mean and stars indicate significant differences at a $P < 0.001$*** and $P < 0.01$**; C = control, Dr = drought, Df = defoliation.
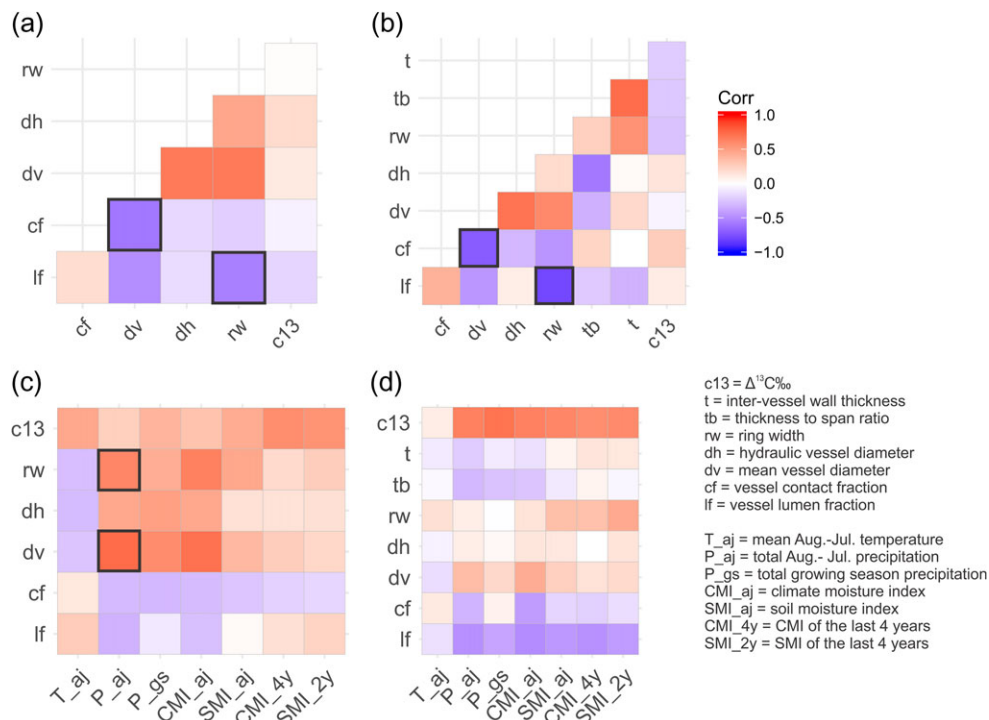

Figure 3. Pearson's r correlations of wood anatomical variables with other wood anatomical variables in Age Group 1 (a) and Age Group 2 (b). Pearson's r correlations of wood anatomical variables and climate variables for Age Group 1 (c) and Age Group 2 (d), in which years of defoliation were removed from the analyses. Wood anatomy correlations with climate were generally stronger with Age Group 1: here, mean vessel diameter (dv) showed a strong positive relationship with annual and seasonal precipitation variables—a slightly stronger relationship than ring width (rw) and precipitation. For rw, only the rings used for anatomical measurements were included in these analyses. Resulting P-values for these correlations can be found in Tables S1–S4 available as Supplementary Data at *Tree Physiology* Online. Black outlines indicate wood anatomical relationships that are explored in more depth in Figures 4–7. Due to time constraints and section quality, t and tb were only measured for Age Group 2.

suggested that white rings were less lignified by the darker staining (see Figure 7b).

Discussion

Our first objective was to determine wood structural changes induced by drought and defoliation. We found that these two types of stress events have different impacts on xylem anatomy. This may be expected from the ^{13}C data, which showed contrasting influences of these events on tree water relations in the year of their occurrence. The enrichment of drought years in ^{13}C, and the reduced ^{13}C in defoliation years is a proxy indicator of stomatal behavior; stomatal closure in drought years results in
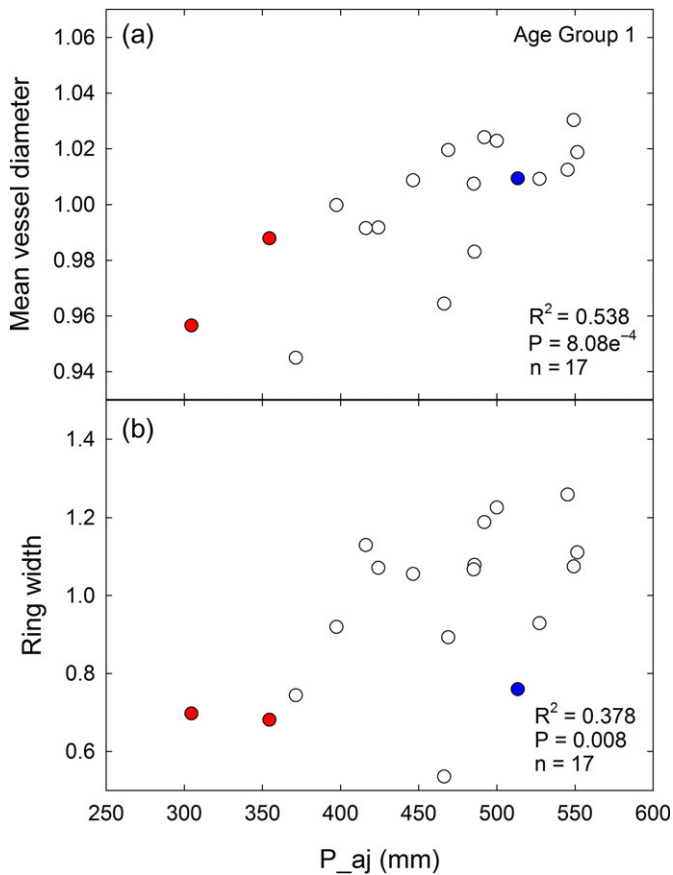
Figure 4. A significant correlation was found between the standardized mean vessel diameter values and August to July precipitation (P_aj) in Age Group 1 (a). A still significant, though weaker, correlation was found for the standardized ring width values and P_aj (b). Red points indicate drought and blue points indicate years of defoliation. Defoliation years were removed from the analysis.

increased fixation of ^{13}C (McCarroll and Loader 2004), while the ^{13}C values of defoliation years likely reflect more favorable tree water potentials due to reduced leaf area for transpiration. Additionally, direct measures have shown increased stomatal conductance in residual leaves following partial defoliation in aspen (Hart et al. 2000).

We begin our discussion with drought and will then consider the impact of insect defoliation. In Age Group 1, mean vessel diameter was strongly correlated with growing season precipitation. In fact, vessel diameters were more sensitive to precipitation than ring width was. Surprisingly, however, this sensitivity differed between tree rings formed in youth and at maturity. Therefore, when considering both Age Groups, vessel diameter could not serve as a defining characteristic of drought years.

Subsequently, our finding was that correlations between precipitation variables and wood anatomical variables (rw, dv, dh) were generally stronger in Age Group 1 than Age Group 2 (Figure 3). We suggest that when the trees in the aspen stand are young, they are more growth-limited by water stress than they are at maturity. Competition for soil water may be a factor; young aspen stands can carry more leaf area than mature stands (Pinno et al. 2001), leading to more water lost through transpiration in young stands. Older trees may also mediate stress by using larger amounts of stored water (Phillips et al. 2003, Scholz et al. 2011) and by having larger or deeper roots. Additionally, older trees may mediate stress through a greater stomatal sensitivity to the environment as indicated by the significant Δ^{13}C correlations with climate variables in Age Group 2. The ability to mediate water stress would then provide an explanation for
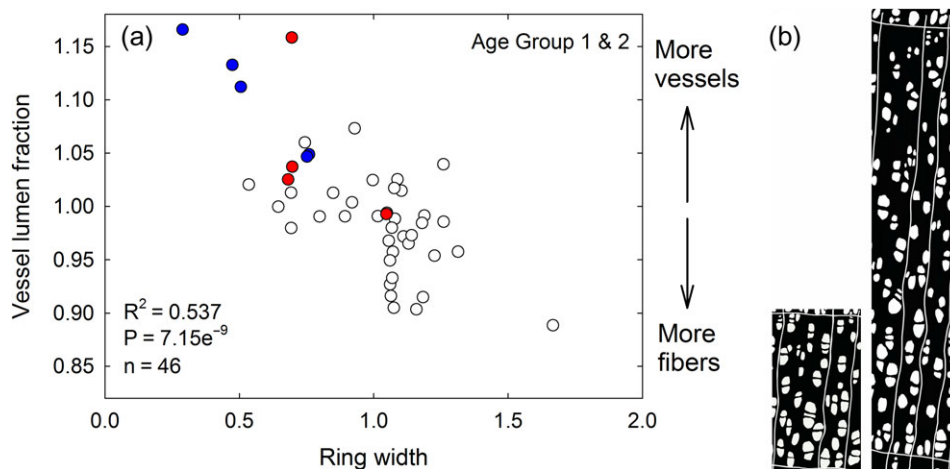

Figure 5. Ring width vs vessel lumen fraction (standardized values) for both Age Groups (a). Vessel lumen fraction represents the proportion of vessel lumen area per xylem area across the entire ring. Within both groups, the defoliation years are characterized by small ring width and high vessel lumen fraction. Red points indicate years of drought and blue points indicate years of defoliation. The semi-ring porous nature of aspen tree rings is illustrated: larger rings tend to have less vessel lumen area, as the trees produce smaller vessels and a higher proportion of fiber area in the latewood (b).

why the relationship in Age Group 2 for ring width is stronger for an average of multiple past years CMI and SMI, than the concurrent year.
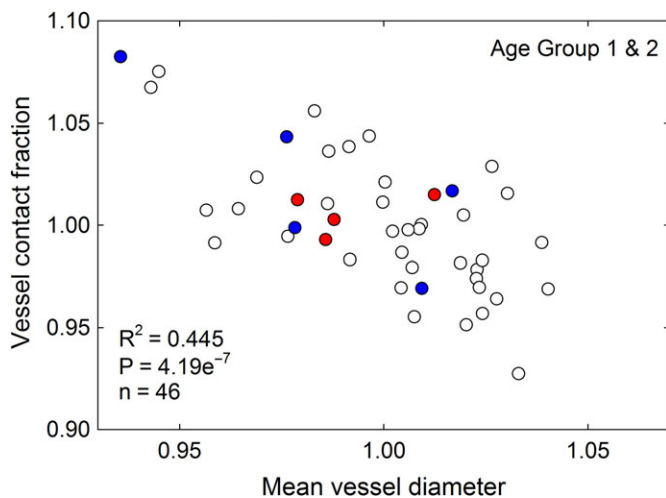

Figure 6. Mean vessel diameter vs vessel contact fraction (standardized values) for both wood Age Groups. Across both groups, tree rings with smaller vessels tend to also have vessels in greater contact with each other. Red points indicate years of drought, and blue points indicate years of defoliation.

Compared with drought, insect defoliation caused more consistent and profound changes in xylem anatomy. The 'white rings', that were formed during years of defoliation had significantly increased fiber lumen fraction (less cell walls per cross-sectional area) and decreased vessel roundness in both Age Groups. Interestingly, defoliation primarily altered fiber properties, and observed changes in vessel anatomy may be partially explicable in the light of their support by the altered fiber matrix surrounding the vessels. Increased fiber lumen fraction in the xylem of defoliation years seemed to be due to both thinner fiber walls and larger fiber lumens. This finding contrasts slightly with the observations of Sutton and Tardif (2005), who found thinner fiber walls, but also smaller fiber lumens in white rings of aspen. It may be that the size of the lumen area is more variable depending on environmental conditions of the time, i.e., precipitation, while the fiber wall thickening is more directly impacted by the loss of photosynthates in the early spring. Regardless, the fiber cellular dimensions recovered in the following year; there was no lag effect.

Though the inter-vessel wall thicknesses were not found to be significantly thinner in defoliation years, the vessel walls were found to be 'wavy' and less circular. This is likely due to the thinner-walled fibers being unable to keep the xylem
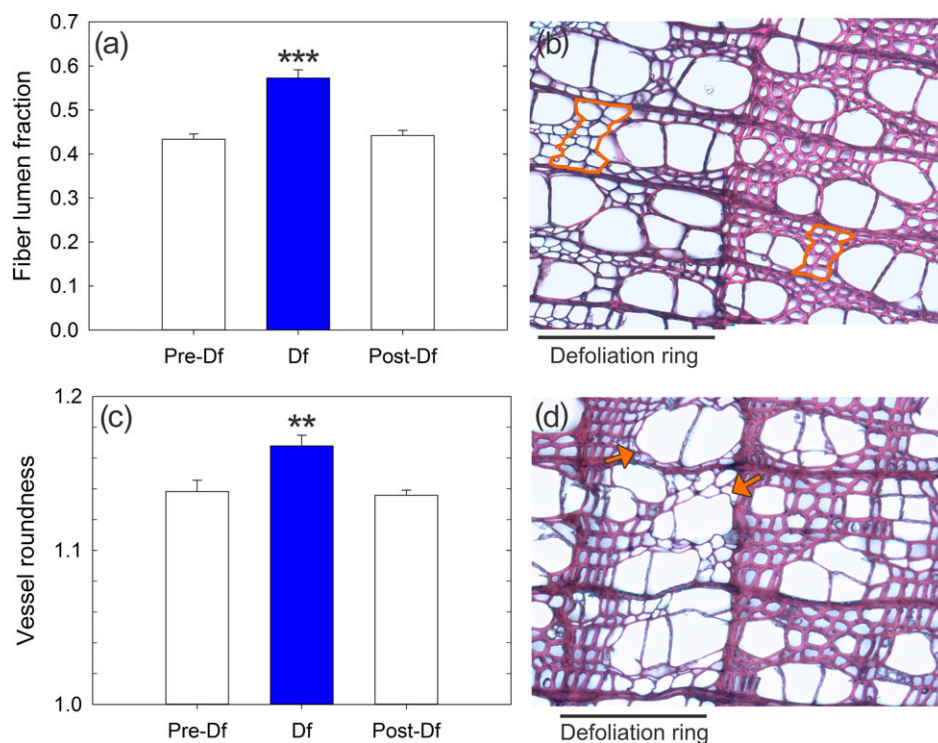

Figure 7. Bar graph showing the mean fiber lumen fraction for rings in the year immediately preceding, during and post a 1 or 2 year defoliation event; bars represent the mean of all tree rings per all relevant years (Pre-Df = 18, Df = 30, Post-Df = 18) (a). Cross section of two adjacent tree rings; circles in orange exemplify how fiber lumen fraction was measured (b). Vessel roundness in the year immediately preceding, during, and post a 1 or 2 year defoliation, measured for rings in Age Group 2 (Pre-Df = 10, Df = 20, Post-Df = 10). A value of 1 indicates a perfect circle while values higher than 1 are less circular (c). Cross section of two adjacent tree rings with visibly less round vessels in the years of defoliation, as indicated by arrow (d). Error bars indicate standard error of the mean. Stars indicate significant differences at a $P < 0.001$*** and $P < 0.01$**; Df = defoliation.

structure rigid, which allowed the vessel walls to be pulled inward due to the negative hydrostatic pressure within the vessels (Jacobsen et al. 2005). Consequently, we might expect that vessels in white rings are more susceptible to cavitation as the compromised structure may create nucleation points for air through micro-fractures in the cell wall or stretching of pit membranes (Jacobsen et al. 2005). White rings may also weaken the wood structurally and functionally, if cell walls are reduced in lignin content (Kitin et al. 2010, Voelker et al. 2011), and may provide pathways for the spread of pathogens, similar to frost damage (Diamandis and Koukos 1992). In addition, despite the recovery of the xylem structure in the year following defoliation, loss of stored carbon from the tree may be a long-term risk factor for tree dysfunction. In mature aspen, loss of root tissue carbon reserves from defoliation can take up to 2 years to recover (Landhäusser and Lieffers 2012). In the year of defoliation, the reduced leaf area and reduced total transpiration are not likely to generate very low water potentials and may mitigate the risk of cavitation. However, there is potential for delayed effects. Trees may be at an increased risk for cavitation if drought occurs in the year following defoliation. An early season drought, before the current year's xylem is fully functional, may be especially detrimental to twigs containing only wood formed in a defoliation year. We conclude that the changes caused by insect defoliation may have a range of negative effects on tree performance in future years.

The anatomy and the extent of fiber production were also highly variable across years. This was observed in the correlation between ring width and vessel lumen fraction, which inversely describes the percentage of area occupied by fibers. Although vessels were produced throughout the growing season as is typical in diffuse porous growth, vessels comprised a larger cross-sectional area in earlywood than in latewood. In other words, earlywood had a higher vessel lumen area than latewood. It appears that the increased vessel lumen area of smaller rings is a result of early truncation of growth in years of unfavorable conditions, reducing the addition of fiber area to the latewood. This correlation characterized defoliation years as small rings with high vessel lumen fraction, likely because defoliation causes early cessation of radial growth (Jones et al. 2004).

The fact that fiber properties appeared to be more sensitive to defoliation events than vessel properties may indicate that trees prioritized the need for maintaining adequate water transport (at least in the short term) over structural support during these years. As already mentioned though, transport and structural support go hand in hand, hence alterations in fiber properties are likely to have a negative effect on transport, at least when water potentials become negative enough to trigger cavitation – presumably when full leaf area recovers.

The maintenance of adequate water transport may also be influenced by the arrangement of vessels. The grouping of vessels, expressed as contact fraction, exhibited the strongest correlation with mean vessel diameter: rings with smaller vessels had vessels that were in greater contact with each other. Similar patterns are found between mean vessel area and vessel frequency in growth rings of other species (Leal et al. 2004, Martínez-Sancho et al. 2017, Noyer et al. 2017). Greater contact between vessels is an anatomical character with conflicting attributes. It can offer redundancy in the hydraulic pathway (Carlquist 1984), but it also may increase the vulnerability of vessels to embolism by providing more surface area for air-seeding (Loepfe et al. 2007). It is possible that the benefits of increased contact are maximized when vessels are small, and the risk of embolism is low.

Conclusions

Although vessel diameters were sensitive to precipitation in Age Group 1, this was not the case in Age Group 2. We suggest that older trees were more buffered from the impacts of drought through a larger root system, increased stomatal sensitivity and reduced stand leaf area as compared with their youth. White rings produced by defoliation had a weaker fiber matrix than control or drought rings in both Age Groups. Also, likely due to the weakened fiber structure, white rings had vessels with reduced circularity, implying that these vessels would be more susceptible to cavitation. Fortunately, the year of defoliation is less likely to generate strongly negative water potentials that would promote cavitation. Surprisingly, the fiber properties in growth rings were more influenced by stress than were the vessels. Perhaps this is because vessel development, with its direct tie to water transport, is more conserved. These measurements increase our knowledge about annual adjustments in xylem cellular anatomy in aspen, and associated physiological implications that could not be seen from chronologies of ring width alone.

Supplementary Data

Supplementary Data for this article are available at *Tree Physiology* Online.

Conflict of interest

None declared.

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