Tree Physiology 00, 1–13
doi:10.1093/treephys/tpab045

Research paper

Bimodal cambial activity and false-ring formation in conifers under a monsoon climate

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Received July 30, 2020; accepted March 30, 2021; handling Editor Roberto Tognetti

Tracking wood formation in semiarid regions during the seasonal march of precipitation extremes has two important applications. It can provide (i) insight into the adaptive capacities of trees to drought and (ii) a basis for a richer interpretation of tree-ring data, assisting in a deeper understanding of past and current climate. In the southwestern USA, the anatomical signature of seasonally bimodal precipitation is the 'false ring'—a band of latewood-like cells in the earlywood. These occur when a particularly deep drought during the early growing season ends abruptly with timely, mid-growing season monsoonal rains. Such conditions presented in southern Arizona in 2014, enabling us to explore false-ring formation in ponderosa pine (*Pinus ponderosa Lawson and C. Lawson*) and Douglas-fir (*Pseudotsuga menziesii Mirb. Franco*) in mixed-conifer forest at 2573 m above sea level. We ask: what were the cell-by-cell timings and durations in the phases of wood cell development in 2014? How do these seasonal patterns relate to strongly fluctuating environmental conditions during the growing season? We took weekly microcores from March through November from six ponderosa pine and seven Douglas-fir trees at a well-instrumented flux tower site. Thin sections were prepared, and we counted cells in cambial, expansion, cell wall thickening and mature phases. For ponderosa pine trees forming a false ring, the first impact of intensifying seasonal drought was seen in the enlarging phase and then, almost a month later, in cambial activity. In this species, recovery from drought was associated with recovery first in cambial activity, followed by cell enlargement. This timing raised the possibility that cell division may be affected by atmospheric moisture increases before soil recharge. In both species, the last false-ring cells matured during the summer rainy season. Bimodal cambial activity coincident with moisture availability was observed in both species, whether or not they formed a false ring. This deeper knowledge of the precise timing of both developmental and environmental events should help define mechanistic connections among these factors in creating bimodal growth patterns.

Keywords: Arizona, Douglas-fir, false ring, IADF, inter-annual density fluctuation, North American Monsoon, *Pinus ponderosa*, ponderosa pine, *Pseudotsuga menziesii*.

Introduction

In semiarid regions, water limitation exerts a strong control over tree growth with consequences for forest productivity, biogeochemical cycles and ecosystem services. The capacity of trees to adapt to fluctuating moisture availability is central to mediating their vulnerability to and recovery from drought. Climate change, however, may be pushing the limits of their capacity to adapt (Allen et al. 2010, Williams et al. 2010). Identifying growth responses of trees to extremes in moisture conditions is needed to better understand the limits of wood formation in buffering climate variability and the mechanisms of drought-induced mortality (Friend et al. 2019, Kannenberg et al. 2020).

Wood formation, or xylogenesis, responds to drought at a cellular level. As cells differentiate, recent and current environmental conditions over time scales of days to months matter (Cuny et al. 2014). The conifer wood cell, or tracheid, is first formed in the cambium—a sheath of meristematic tissue, sometimes only a few cells thick, which covers the entire tree. Once formed through cell division, tracheid development proceeds in three phases: cell enlargement, cell wall thickening and programmed cell death (maturation; Rathgeber et al. 2016). Upon maturation, cells fulfill their two primary functions: structural support and conduits for the transport of water from soil to crown. While water availability impacts all phases of development, cell production and cell enlargement are considered the most sensitive (Hsiao 1973). As the site of cell production, the cambium is the fundamental driver of wood formation, controlling the number of cells formed during the growing season. Drought can truncate the period of cambial activity, resulting in a reduced number of cells formed (de Luis et al. 2011, Eilmann et al. 2011, Ren et al. 2015, Vieira et al. 2014). After a cell is produced, it will expand to its final size during the enlarging phase. Cell expansion is regulated by factors including cell wall extensibility, osmotic potential and turgor pressure (Cosgrove 1993, Hsiao et al. 1976). Both theory and modeling studies have highlighted the fundamental role of turgor pressure in cell growth (Steppe et al. 2006, 2015). When water pressure internal to the cell exceeds external pressures, cell expansion occurs (Lockhart 1965). Given the central role of turgor pressure in cell growth, soil moisture is a key driver of xylogenesis (Peters et al. 2020). Wood anatomy and experimental studies have both shown a strong association between low soil moisture and the formation of radially narrow cells (Carvalho et al. 2015, Rossi et al. 2009). The role of turgor pressure in the enlarging phase is likely more direct compared with its role in cambial activity, where chemical signaling also plays an important role in cell division rates (Vaganov et al. 2011). Thus, during a developing drought as well as drought recovery, the response of cambial activity will lag cell growth (Abe et al. 2003, Abe and Nakai 1999, Hsiao 1973). Careful consideration of the dynamics of both cambial activity and cell enlargement under conditions of fluctuating water availability is warranted given their critical complementary roles in wood production and function.

Regions with bimodal precipitation are a natural laboratory to examine the adaptive capacities of wood formation to extreme seasonal fluctuations in water availability. To date, the majority of our knowledge about bimodal xylogenesis in mature conifers derives from studies of trees growing in the Mediterranean region (see reviews in Battipaglia et al. 2016, De Micco et al. 2016*b*). Here, intraseasonal fluctuations in water availability leave an anatomical imprint widely referred to as an intra-annual density fluctuation (IADF)—either a band of latewood-like cells embedded in the earlywood (radially wide cells with thin cell walls) or a band of earlywood-like trees embedded in the latewood (radially narrow cells with thick cell walls). Few studies have aimed to identify the specific environmental triggers and subsequent responses of cell enlargement (De Micco et al. 2016*a*). Instead, based on the inextricable link between cambial activity and radial growth, bimodal xylogenesis and IADF formation has primarily been examined through the lens of cambial activity. During spring, declining cambial activity occurs alongside decreasing soil moisture (Camarero et al. 2010, Vieira et al. 2015). Under prolonged seasonal drought, cambial activity will be greatly reduced, only to be reactivated by the return of favorable moisture conditions. In this region, xylogenesis studies have shown that cambial reactivation is spurred by the arrival of precipitation during the late summer or early autumn (Camarero et al. 2010, Vieira et al. 2015, 2017). However, while the associations between environmental conditions and cambial activity are generally recognized, the specific triggers for intra-seasonal changes in cell production have yet to be identified. For instance, late season precipitation does not consistently result in cambial reactivation (Campelo et al. 2018, Vieira et al. 2017, 2020). Vieira et al. (2020) posit that the timing of drought-terminating precipitation is critical—if it comes too late, the cambium will not respond with any additional cell division for that growing season.

Bimodal precipitation is a prominent climatic feature of other regions besides the Mediterranean. In the southwestern USA (hereafter the Southwest), winter precipitation arises from cyclonic Pacific frontal storms (Sheppard et al. 2002) and summer precipitation from the North American Monsoon (NAM). The NAM is an atmospheric pattern that delivers moisture to the region primarily during July and August (Adams and Comrie 1997, Higgins et al. 1999). The impact of this bimodal precipitation regime on the process of cell formation has been little studied; however, its imprint on tree-ring anatomy in the Southwest has long been recognized. Namely, severe drought during the spring followed by a timely monsoon was found to be associated with a band of latewood-like followed by earlywood-like cells (Douglass 1919). Early tree-ring scientists referred to this feature as a 'false ring' (Douglass 1919, Schulman 1938) and we maintain this nomenclature in the present study. Even though the false ring is integrally linked to bimodal precipitation, only recently has it been used to shed light upon the multicentennial dynamics of seasonal precipitation in this region (Griffin et al. 2011, 2013). A better understanding of false-ring xylogenesis would certainly enhance our interpretation of the tree-ring record. Thus far, however, only two xylogenesis studies have been conducted in this region (Budelsky 1969, Ziaco et al. 2018). Between these, only the more recent study specifically examined cells in the cambial and enlarging phases. Moreover, this study appears to suggest important differences in bimodal growth in the

southwestern USA compared with the Mediterranean region (Ziaco et al. 2018). They observed, at the beginning of the growing season, the onset of cambial activity coincided with increases in humidity even as soil conditions remained very dry. This novel finding is particularly intriguing, because it suggests that atmospheric moisture may, under some conditions, be the primary driver of xylogenesis. Additionally, in contrast to Mediterranean xylogenesis studies, Ziaco et al. (2018) observed that false-ring formation was not associated with bimodal cambial activity. A greater mechanistic understanding of false-ring formation in the southwestern USA would thus serve a dual purpose of providing a deeper reading of the tree-ring archives and lending insight into potential vulnerabilities of these forests to climate change.

In the present study, our main goal was to identify potential mechanistic links between tree growth and environmental conditions by comparing cell phenology to high-resolution biophysical data. Knowledge of the precise timing of both developmental and environmental events should help define mechanistic connections during the mid-season decline in cambial activity and its subsequent acceleration resulting in a bimodal growth pattern. In this exploratory study, we focus on events in a single year, 2014, in which a notably dry fore-summer (May and June) was followed by a sharp onset of the summer monsoon (early July). We focused on identifying cell phenology—the timing of cell entry into each differentiation phase for two ecologically and dendrochronologically important species: ponderosa pine (*Pinus ponderosa* Lawson and C. Lawson) and Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco). We then compared cell phenology with high-resolution, site-specific biophysical data that are associated with the availability of moisture to developing xylem, including soil moisture, vapor pressure deficit (VPD), rainfall events and sapflow. Within this biophysical framework, we assume that soil moisture, in its overarching regulation of turgor pressure, will be a primary driver in xylogenesis. Consequently, we hypothesize that the gradual decline of soil moisture during spring and early summer, followed by a sudden recharge of soil moisture due to the monsoon, will be associated with bimodal cambial activity and the formation of a false ring. As the 2014 growing season unfolds, we expect that:

(i) Drying soil during the spring and early summer will negatively impact the more sensitive cell enlarging phase and then cambial activity.
(ii) The radially narrow cells of the false ring will be mature by the time the monsoon arrives.
(iii) Recovery from seasonal drought will be spurred by the arrival of monsoon precipitation and will have a positive effect on both cell enlargement and cambial activity.
(iv) Bimodal cambial activity will be linked to false-ring formation.

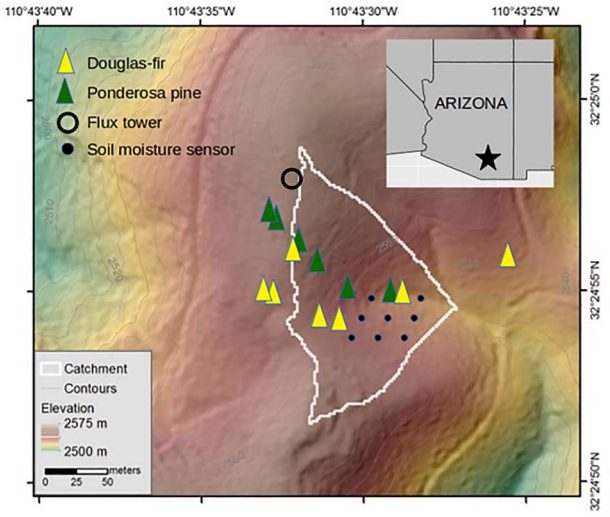

Figure 1. Site map. Study area is in the Santa Catalina Mountains of southeastern Arizona. Locations of the sampled trees and environmental data collection sites are shown.

Materials and methods

Study site and trees

The study site is located in a Critical Zone Observatory in the Santa Catalina Mountains (https://czo-archive.criticalzone.org/catalina-jemez/) north of Tucson, in southern AZ (32° 25′ 00″N, 110° 43′ 31″W, elevation 2573 m above sea level; Figure 1; Chorover et al. 2011, Knowles et al. 2020). The bimodal precipitation pattern typical of this region is comprised of winter (October through March) and summer (July through September) rainy seasons. May and June consistently have very low precipitation, which is the same period when temperatures are increasing toward annual maxima in July (Figure 2B). Year 2014 was notable for the extremely low precipitation during the winter months (Figure 2A). For the same time period, mean monthly temperatures were also markedly higher than normal. Summer rains were timely and above average in July, but below average in August (Figure 2A).

The forest stand is second-growth, likely dating from the 1930s. It is a mixed-conifer forest, composed of *P. menziesii*, *P. ponderosa* and *Pinus strobiformis* (Knowles et al. 2020, Potts et al. 2017). We selected dominant or co-dominant, healthy trees with similar growth during the most recent 5–10 years. We targeted seven Douglas-fir (average diameter at breast height = 52 cm) and six ponderosa pine (average diameter at breast height = 46 cm; Figure 1). Local weather data from 2014 were collected onsite at the flux tower (Ameriflux site ID—US-MtB). Site climate was characterized using PRISM data for the period spanning 1980 through 2014 (PRISM Climate Group 2015). Within the footprint of this tower, we measured sap flow on the north side of three ponderosa pine individuals using the thermal dissipation probe method (Granier 1985, 1987). Data

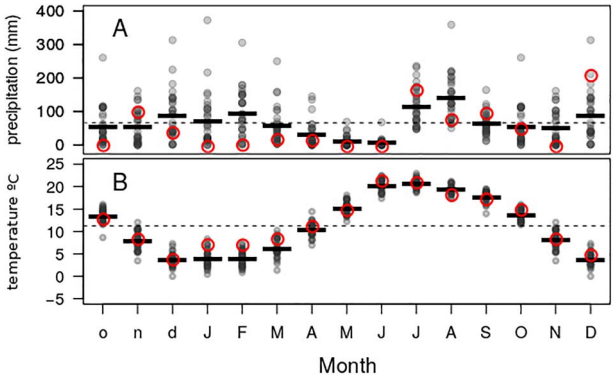

Figure 2. Site climate. Year 2014 monthly data at the field site (red circles) are shown in relation to: monthly data from 1985 to 2014 (gray dots), average monthly climate (black bars) and average annual climate (dashed lines). Data are from PRISM, interpolated to our field site. (A) Precipitation. (B) Temperature.

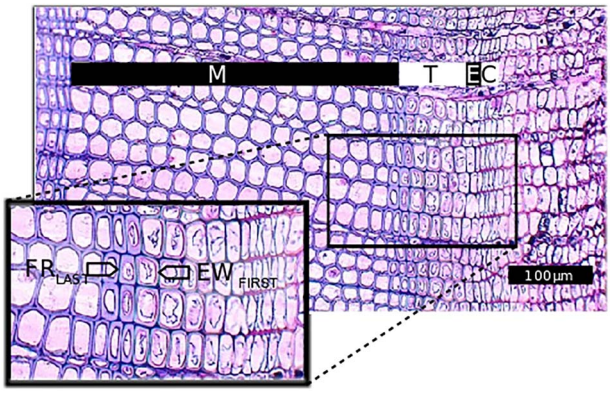

Figure 3. Thin-section (6 µm) image of a microcore collected on 5 September 2014 from ponderosa pine (PP224). Matured cells (M) are solid blue in color with no visible cell contents; cells in the cell wall thickening phase (T) show a blue outer and violet inner wall; cells in the enlarging phase (E) are dark blue with thin cell walls and cells in the cambial zone (C) are very narrow with flimsy cell walls. The 'signature' cells—FR_{LAST} and EW_{FIRST}—are shown in the inset.

were logged at 30-min resolution using an upper heated probe and lower reference probe (TDP-30, Dynamax Inc., Houston, TX, USA) implanted in the sapwood of the tree approximately 40-mm apart. Further details on the calculations and calibrations of sap flux sensors at this site are described by Yang et al. (2020). Soil moisture data were collected downslope of the tower. Soil moisture sensors (5TE, METER Group, Pullman, WA, USA) at 10-cm depth were located on the north-facing slope and configured in a regular grid pattern with a 25-m spacing (Figure 1, Murphy et al. 2020).

Sample collection and preparation

Microcores were collected using a Trephor™ punch tool on a weekly basis from the north side of the tree (Rossi et al. 2006*b*). According to established methods, spacing between microcores was at least 5 cm to minimize the potential effects of injury response (Deslauriers et al. 2003). Microcores were placed in a solution of 50% ethanol in 1.5-ml Eppendorf tubes and stored at about 5 °C until they were embedded in paraffin and processed following Rossi et al. (2006*a*). Transverse thin sections 6- to 8-µm thick were cut using a Thermo Scientific Microm HM355S automatic rotary microtome. Thin sections were mounted on a slide and stained with cresyl violet acetate (0.16% in water; Rossi et al. 2006*a*). Slides were then observed using an Olympus BX43 microscope at ×200 to ×400 magnification.

On each stained slide, three cell files were selected to evenly represent the sample (Deslauriers et al. 2003, Rossi et al. 2006*a*). Counts were made, in each of the three files, of the number of cells in each phase represented by their respective zones: cambium, enlarging, cell wall thickening and mature. Cell walls in the cambial and enlarging phases are comprised only of primary cell wall, which stains pink with cresyl violet. Enlarging cells have evident diameter increments compared with cambial cells. The cell wall thickening phase was identified by birefringence of the cellulose microfibrils of the secondary cell walls in polarized light. The end of cell wall development occurs when lignification is complete. Lignin stains blue in cresyl violet and mature cells were identifiable by their solid blue color.

False-ring timing: identification of signature cells

The abrupt difference in cell size between the last formed cell of the false ring (FR_{LAST}) and the first earlywood-like cell formed after the false ring (EW_{FIRST}) provides anatomical markers that can be observed on thin sections (Figure 3). For these two cells, we noted the timings of three differentiation events: (i) the maturation of FR_{LAST}; (ii) enlarging phase onset of EW_{FIRST}; and (iii) maturation of EW_{FIRST}. To identify the timing of FR_{LAST} maturation, we identified the last date when FR_{LAST} exhibited a pinkish color within the inner cell walls—that is when lignification was still incomplete (Figure 3). Entrance into the mature phase was assigned to the time between that date and the next sampling date. To identify the timing of enlarging phase onset of EW_{FIRST}, we identified the date when we first observed cells with a larger radial diameter than the last formed cells of the false ring, indicating the timing, albeit delayed, of EW_{FIRST} entry into the enlarging phase. Lastly, to identify the timing of EW_{FIRST} maturation, we identified the date when EW_{FIRST} appeared solid blue, indicating entrance into the mature phase. For each tree forming a false ring, each differentiation event was ascribed a window comprising the period between the previous sampling date and the date of observation. Then, the beginning and end of differentiation event windows were averaged among trees in each species.

Modeling the timing of cell differentiation

For each tree, cell count data in the enlarging, cell wall thickening and mature phases were first normalized using cell counts of the prior year's growth (Rossi et al. 2003). In each species, we

divided the trees into two groups: trees that did form a false ring (FR) and trees that did not (noFR). An FR was noted when the radial diameter of EW_{FIRST} was visually assessed to be at least 1.5 times that of FR_{LAST}. Cell count curves for enlarging, cell wall thickening and mature phases were compiled by group. Each cell count curve was then modeled using the Generalized Additive Model function in the mgcv package (Wood 2017) of R statistical software (R Development Core Team 2016).

For each species, we modeled the timing of cell development of only those trees forming an FR. Computing the timing and duration of cell differentiation on a cell-by-cell basis is based on cumulative cell counts (Cuny et al. 2013, Rossi et al. 2003). To this end, the numbers of cells in each phase—enlarging (E), cell wall thickening (T) and mature (M)—at each sampling date were summed to yield three cumulative curves: (i) ETM ($n_{ETM} = n_E + n_T + n_M$); (ii) TM ($n_{TM} = n_T + n_M$); and (iii) M ($n_M = n_M$). In addition to the M-curve based on cell counts alone (M), we developed an adjusted M-curve (M_{adj}) based on observed timings (see Supplemental Methods available as Supplementary data at *Tree Physiology* online). For estimating cell phenology, we used the M-curve adjusted for observations (M_{adj}). The timing of entry into the enlarging, cell wall thickening and mature phases, i.e., the cell phenologies, was derived analytically from the ETM_{adj}, TM_{adj} and M_{adj} cumulative curves, respectively (Cuny et al. 2013). Upon estimating the onset of the enlarging, cell wall thickening and mature phases for each cell, the duration in the enlarging and cell wall thickening phases were computed as the difference in entry dates between enlarging and cell wall thickening phases then cell wall thickening and mature phases, respectively (Cuny et al. 2013). For cambial cell counts, we conducted an additional analysis to characterize cambial activity patterns in the false-ring group of ponderosa pine. We computed the rate of cell production using the cumulative curve: CETM ($n_{CETM} = n_C + n_E + n_T + n_M$), where the rate for cell formation was the difference in number of cells between time, t_t and t_{t-1}, at daily time steps (Cuny et al. 2013).

Results

2014 biophysical conditions

Micrometeorological data from the flux tower at the site (Figure 1) show daily and weekly patterns for 2014 (Figure 4). Daily precipitation data show few rain events during the late winter and spring of 2014 (Figure 4A). A robust summer monsoon rainy season began with a 5 July event that yielded over 10 mm of precipitation. Periodic precipitation events occurred through mid-September after which dry conditions prevailed through the end of November. Surface soil moisture levels were highly responsive to individual rain events. Minimum volumetric soil moisture was about 2% just before the onset of the summer rains (Figure 4A). Increases in temperature

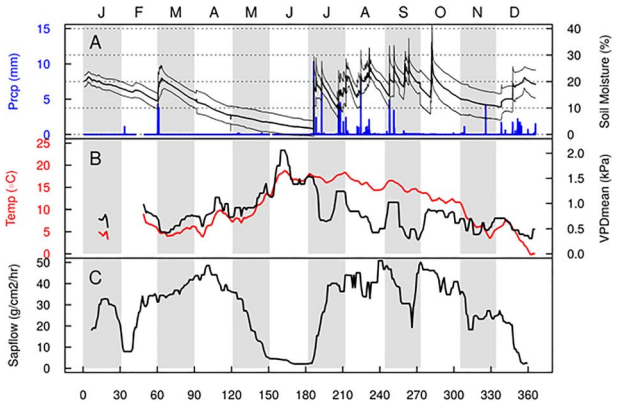

Figure 4. Year 2014 local environmental data. (A) Hourly precipitation data (blue bars) collected from the top of the flux tower (31 m). Hourly volumetric soil moisture (solid line; ±1 standard deviation shown as thin black line) collected from nine soil sensors in the lower basin at 10-cm depth, configured in a regular grid pattern with 25-m grid cells (Figure 1). (B) Smoothed 13-day daily mean VPD (black) and smoothed 13-day daily temperature (red). Both variables plotted on the center of the 13-day window. (C) Smoothed 13-day sapflow velocity during day (6 a.m. to 6 p.m.) from three ponderosa pines, plotted on window center.

and VPD occurred from March through early June (Figure 4B). From this point, VPD decreased abruptly from about 2 to 1.5 kPa, where it stabilized until the onset of the monsoon at the beginning of July when it rapidly decreased to ~0.5 kPa. Temperatures remained relatively constant at ~17 °C in June and July and slowly decreased to about 10 °C from the beginning of August through the end of October. Temperature remained high during the summer months. Sapflow data for ponderosa pine show two periods of sapflow activity separated by negligible sapflow during June (Figure 4C). The first peak in transpiration occurs around mid-April. Sapflow declines rapidly during May to minimum rates in June. Sapflow responds rapidly to the first summer rain event and recovers to early season rates within a couple of weeks.

Phenology of tree-ring formation

In 2014, three out of six ponderosa pines formed an FR, whereas five out of seven Douglas-fir formed an FR. Trees forming an FR tended to be slightly larger in diameter, ~8 cm. Ring widths for 2014, and the mean ring width for the 10 years 2005–14 were smaller for two noFR ponderosa pine than in the three FR of the same species. No such clear difference was found between the two noFR *P. menziesii* and the five FR trees of the same species. For both ponderosa pine and Douglas-fir, the onset of the growing season, defined by the timing of the first cell to enter the enlarging phase, occurred in late March/early April (Figure 5B and F). The end of radial growth, indicated when there are no longer any cells in the expansion phase, showed different timings both within and between species. In ponderosa pine, FR trees tended to have a slightly extended period of radial growth compared with noFR trees, ending the

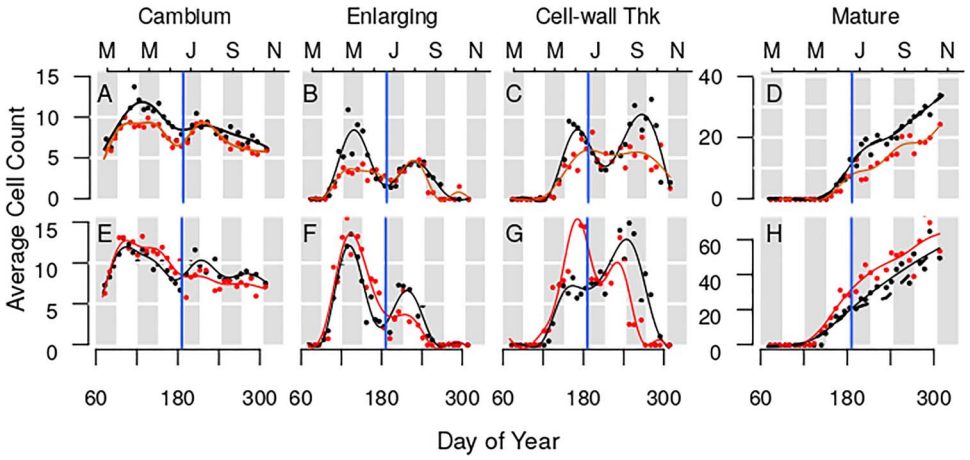

Figure 5. Modeled cell counts for ponderosa pine (top row) and Douglas-fir (bottom row) trees grouped by false ring formation (black = yes; red = no). Cell counts in the cambium, enlarging, cell wall thickening and mature phases for ponderosa pine (A–D) and Douglas-fir (E–H). For trees that formed a false ring (black), the adjusted mature cell counts are shown by a dashed line. Vertical blue line shows date of first summer rain event (5 July; day of year 186).

enlarging phase in early October compared with mid-September (Figure 5B). In Douglas-fir, all trees regardless of FR formation, had a similar timing for the end of the enlarging phase—mid-September (Figure 5F). The end of the cell wall thickening phase marks the time when carbon assimilation has ended for the growing season and the tree ring is fully mature. The end of the cell wall thickening phase was not observed in two ponderosa pine (PP215 and PP216; see Figure S1 available as Supplementary data at *Tree Physiology* online) by the time of the last sampling date (4 November 2014). For both species, tree-ring maturation for FR trees occurred around late October/early November. For ponderosa pine, noFR trees matured at about the same time as FR trees (Figure 5C), whereas for Douglas-fir, noFR trees matured earlier than FR trees (Figure 5G).

Three of five noFR trees, two ponderosa pine (PP215 and PP218, see Figure S1 available as Supplementary data at *Tree Physiology* online) and one Douglas-fir (DF228, see Figure S2 available as Supplementary data at *Tree Physiology* online) had shorter growing seasons than FR trees. For DF228 and PP218, radial growth started about the same time as FR trees but cell maturation, and therefore tree ring formation, was completed earlier—around the beginning of September. For PP215, radial growth started later than FR trees—around the end of May, but tree-ring formation was complete about the same time as FR trees.

Seasonal patterns of cell count data

Bimodal cell counts were observed in the cambial zone for both species. In Douglas-fir the bimodal pattern was observed in FR trees but not in noFR trees (Figure 5E). However, in ponderosa pine, this pattern was only observed in cambial cell counts among noFR trees (Figure 5A). The rate of cell production, as derived from cumulative cell counts

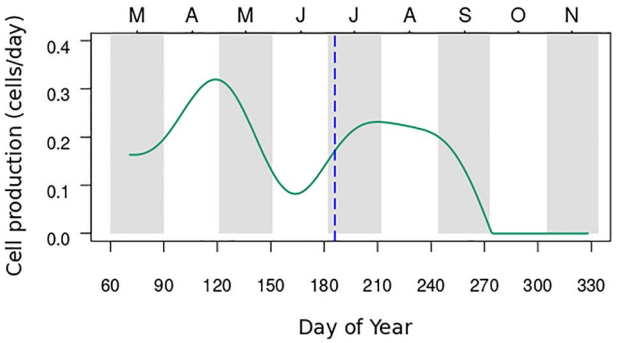

Figure 6. Rate of cell production for ponderosa pine forming a false ring. Dashed blue line shows timing of the first summer rains (5 July; day of year 186).

($n_{CETM} = n_C + n_E + n_T + n_M$), however, showed a bimodal pattern of cambial activity for ponderosa pine FR trees with peak spring activity at the end of April and peak summer activity spanning from the end of July through August (Figure 6). Minimum cambial activity levels, when cell production rates were estimated to be <0.1 cells day_{-1}, occurred in mid-June before the onset of the monsoon.

Both species showed bimodal patterns of counts for cells in the enlarging (Figure 5B and F) and cell wall thickening zones (Figure 5C and G). In ponderosa pine, the bimodal pattern appeared to be stronger among FR trees compared with noFR trees. This, however, may be an artifact of averaging. On a tree-by-tree basis, the enlarging and cell wall thickening phases, in fact, showed a strong bimodal pattern, but the timing of peak numbers was not synchronous among trees (see Figures S1 and S2 available as Supplementary data at *Tree Physiology* online).

Two curves are shown for the number of cells in the mature phase: one where counts were adjusted based on observed

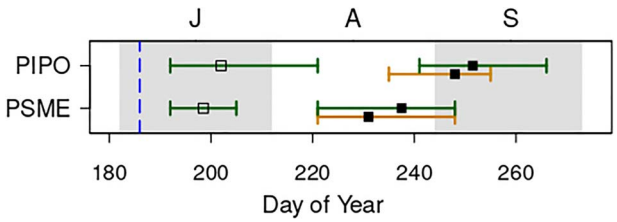

Figure 7. Observed timings for beginning of enlarging phase (open squares show median date) for EW_{FIRST} (green) and end of cell wall thickening phase (solid squares show median date) for EW_{FIRST} (green) and FR_{LAST} (yellow). Ranges (horizontal lines) show earliest and latest date for observed timing. Top row is ponderosa pine (PIPO); bottom row is Douglas-fir (PSME).

timings (M_{adj}; see Supplemental Methods available as Supplementary data at *Tree Physiology* online) and one where counts were not adjusted (M; Figure 5D and H). For ponderosa pine, there was a negligible difference between the two curves. Cells began entering the mature phase around the end of May. They did so at relatively high rates until about mid-July, when the rate of entry into the mature phase decreased appreciably. Around the end of August rates increased again and remained stable until the end of the growing season in November.

Among Douglas-fir, adjusted and unadjusted curves for cell counts in the mature phase are different. Whereas the unadjusted curve shows a constant rate of cells entering the mature phase over the course of the growing season, the adjusted curve shows a decrease in number of cells entering the mature phase during July and August. These different curve forms resulted in relatively large differences in the estimated timings of cell phenologies for Douglas-fir (see Figure S3 available as Supplementary data at *Tree Physiology* online).

Timing of false-ring signature cell development

In ponderosa pine and Douglas-fir, EW_{FIRST} was in the early stages of enlarging within 1–2 weeks after the first summer rain event (5 July 2015, day of year 186; Figure 7). Likewise, for each species, both signature cells matured within a similar time frame, with FR_{LAST} maturing on average almost a week earlier than EW_{FIRST}. However, in ponderosa pine, the timing of maturation for EW_{FIRST} and FR_{LAST} occurred ~2 weeks later than Douglas-fir—around early September (Figure 7).

Modeled cell phenologies for false-ring trees

Between the two species, Douglas-fir FR trees produced more cells during the 2014 growing season than ponderosa pine FR trees (Figure 8A and C). This appears to primarily be the result of more cells produced during the first half of the growing season—up to the onset of the summer rains (Figure 8A and C). The total time for cell maturation in ponderosa pine peaked at the beginning and end of the growing season (Figure 8B), whereas for Douglas-fir, it was highest during the middle of the growing season (Figure 8D). In ponderosa pine, the enlarging and cell wall thickening phases showed a repeating pattern for the first and second halves of the tree ring. For about the first 20 cells, time spent in the enlarging phase gradually decreased, whereas duration in the cell wall thickening phase increased (Figure 8B). In the first half of the tree ring, minimum time spent in the enlarging phase occurs in the cell formed just before the first summer rain event (cell rank 20). After cell 20, there is an abrupt increase in time spent in the enlarging phase, then time spent in the enlarging phase again decreases gradually until the last cell formed (Figure 8B). A mirror image of this pattern was observed for the cell wall thickening phase (Figure 8B). In Douglas-fir, similar to ponderosa pine, minimum durations for the enlarging phase were observed for cells formed in the middle and end of the tree ring (Figure 8D). The mid-season minimum, however, occurs several cells prior to the first summer rain. There are other differences in the pattern for the enlarging phase over the course of the growing season. For most of the first half of the tree ring, time spent in the enlarging phase shows gradual but small increases. During this period, average time in the enlarging phase is about three and a half weeks. Then, there is an abrupt decrease, from ~3 to 1 week, over a sequence of four cells (Figure 8D). For cells formed after the first summer rain, there is first (cells 30 through 39) a gradual increase in time spent in the enlarging phase up to a maximum of just under 3 weeks (Figure 8D). Then, in cells 40 through 48, enlarging phase duration decreases to ~5 days for the last cell formed (Figure 8D).

Discussion

The capacity of trees to adjust to environmental fluctuations is a critical factor in determining tree vulnerability to environmental change. Our goal for this study was to improve inferences of environmental causality over the course of the growing season by comparing more precise timings of the phases of cell differentiation with high-resolution, local environmental fluctuations. Our results suggest that ponderosa pine and Douglas-fir growing in the southwestern USA can exhibit high capacities to respond to evolving moisture conditions over the course of the growing season due to sensitivities of cell production and cell growth.

Response of wood formation to developing drought

Our findings indicate that, in ponderosa pine, the enlarging phase, specifically its duration, was more sensitive than cambial activity during a developing drought. Early in the growing season, the rate of cell production increased steeply, whereas the time spent in the enlarging phase gradually decreased with each newly formed cell. The synchronous patterns of decreasing amounts of time in the enlarging phase and declining soil moisture suggests a direct impact of water limitations where declining soil moisture may have led to increasingly

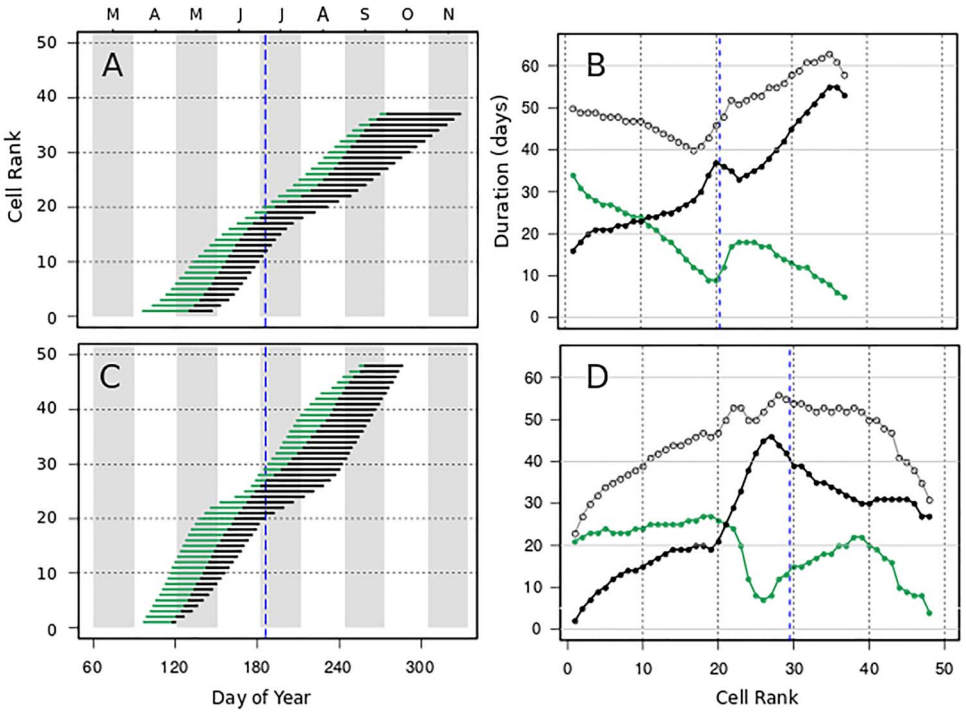

Figure 8. Estimated timing and duration of the enlarging and cell wall thickening phases for ponderosa pine (top row) and Douglas-fir (bottom row). First plots in each row (A, C) show the timing and duration of the enlarging (green bars) and cell wall thickening (black bars) phases. Dashed blue line in all plots shows timing of the first summer rains (5 July; day of year 186). Second plots (B, D) are the duration of enlarging (green circles), cell wall thickening (black circles) phases by cell rank and total maturation (enlarging + cell wall thickening; empty circles) for each cell formed. In plots (B) and (D), the first summer rain event (dashed blue line) is shown relative to cell rank, namely the first cell formed after the first rain event.

shorter periods that cells could maintain turgor pressure above threshold (Lockhart 1965). Similar patterns of decreasing time in the enlarging phase have been found and associated with decreasing cell size for trees growing in both semiarid (Vieira et al. 2020) and temperate (Cuny et al. 2013, 2014) regions. Certainly, we observed the smallest cell sizes, i.e., the false ring, associated with the shortest time spent in the enlarging phase during the final stages of early summer drought. In the absence of cell measurements, however, we do not know whether the gradual decrease in time spent in the enlarging phase is reflected by a gradual decrease in cell size.

Initial impacts of declining soil moisture have a direct impact on cell expansion (Abe and Nakai 1999). However, the gradual development of drought, as would occur in the field, would also invoke physiological changes that could, in turn, mediate this fundamental relationship. In ponderosa pine, sapflow data indicate increasing levels of stomatal regulation over the course of the developing drought. Daytime sapflow rates began decreasing early in the growing season (early April) when volumetric soil moisture was ~10% in the upper layers. This soil moisture threshold has been observed to be associated with decreases in sapflow and canopy conductance in other studies in the Southwest (Liu and Biondi 2020). In our study, we cannot be sure whether the observed decrease in sapflow indicates stomatal regulation or a transient decrease in evaporation demand. If, however, the onset of stomatal regulation occurred in early April, the timing is intriguing: around the same time that the first cell formed entered into the enlarging phase. In other words, soil moisture depletion was stemmed as trees began to grow radially. Further research is necessary to verify whether there is, in fact, a coordinated response of stomatal regulation and the onset of radial growth, and whether it acts as a mechanism to extend soil water availability to growing cells. It seems plausible that this type of adaptation would benefit trees growing in semiarid regions.

Cambial activity has long been presumed to be less sensitive to a developing drought than cell enlargement (Hsiao 1973, Hsiao et al. 1976). The lagged response of cambial activity to declining soil moisture appears to support this assertion. At the beginning of the growing season, cambial activity rates in ponderosa pine showed steep increases while soil moisture was decreasing. It was not until the beginning of May—almost 2 months after the onset of cambial activity, when cambial activity in trees forming false rings began to slow down (Figure 6). The link between soil moisture and cambial activity is believed to be indirect whereby cambial activity begins to decrease as a result of accumulated physiological adjustments (Abe et al. 2003). In our study, we observed decreases in the enlarging phase around the beginning of April. Shortly thereafter, sapflow rates also decreased. Then, in early May, when cambial activity

began to decrease, there was an increase in the rate of sapflow decline (Figure 4C). The coincident timings and patterns of sapflow rate and cambial activity again point to potentially important associations between stomatal regulation and the phases of xylogenesis, in this case, cell production. Future studies that include multi-year assessments of the environmental drivers of stomatal regulation and cambial activity could prove useful in elucidating the direct and indirect biophysical controls of tree growth during drought.

Transition from dry to wet conditions: false-ring formation

Like a 'true' ring, the false ring is defined by the juxtaposition of a radially narrow and radially wide tracheid. Unexpectedly, for both ponderosa pine and Douglas-fir, observed timings of cell differentiation revealed that the last cell of the false ring (FR_{LAST}) matured during the summer rainy season, entering the mature phase around the beginning of September. This is in contrast to Budelsky (1969), who reported that for ponderosa pine, the false ring was already formed—cells matured by the onset of the summer rains. While close in proximity (~2 km from our site), site conditions in this earlier xylogenesis study were more xeric—a steeper, rockier slope with a south-facing exposure. Moreover, during Budelsky's study, the monsoon arrived ~2 weeks later than average. In combination, these two factors may have intensified drought conditions and advanced the timing of maturation in false-rings cells by truncating the time spent in the cell wall thickening phase. Environmentally induced truncation of cell wall thickening has been previously observed when low temperatures at the end of the growing season terminate this phase for the last-formed cells (Cuny and Rathgeber 2016, Tardif et al. 2020). This environmental sensitivity in false-ring formation may provide information with which to evaluate seasonal drought severity and/or the timing of the monsoon over multi-centennial scales using wood anatomy. Assuming that the rate of cell wall thickening is constant, if a false ring matures before monsoon onset, its last-formed cells will have thinner cell walls than a false ring that completes its maturation during the monsoon season. While we did not measure cell dimensions, Budelsky (1969), who observed the false ring to mature before the onset of the monsoon, found cell wall thickness among cells comprising the false ring to be more similar to earlywood than latewood cells.

Our finding that the last cell of the false ring matured during the monsoon also appears to contradict prior studies that have used isotope analyses to infer timing of false-ring formation. Enriched carbon isotopes indicate conditions of high evaporative demand, i.e., hot and dry (Leavitt 2002). Thus, when cellulose derived from false rings contains enriched carbon isotopes, this has been interpreted to mean that false-ring cells matured under conditions of drought (De Micco et al. 2007). A recent isotope study presents findings that suggest otherwise. Belmecheri et al. (2018) analyzed both carbon and oxygen isotopes in subdivided tree rings with a false ring in ponderosa pine within 2 km of our site. They found that the false ring had isotopic signatures that pointed to maturation during humid conditions (Belmecheri et al. 2018; their Figure 4). Carbon isotopes in the false ring did not show the same levels of enrichment as observed in wood formed during seasonal drought (May–June). Rather, false rings exhibited enrichment levels intermediate to the highly enriched wood formed during seasonal drought (i.e., May–June) and the minimally enriched wood formed during the rainy season (July–August). Meanwhile, oxygen isotopes in the false ring were more closely aligned to oxygen isotopes from wood formed under humid, compared with dry conditions. A possible reduction in carbon turnover may also have led to higher oxygen exchange during cellulose synthesis (Song et al. 2014, Szejner et al. 2020).

The anatomical emergence of the false ring is finalized with the formation of the first earlywood-like cell after the false ring (EW_{FIRST}). In this study of events in 2014, EW_{FIRST} first appeared in individual trees of both species ~1 week after the first summer rains. This timing represents a point early in the enlarging phase, not the onset (see Materials and methods, subsection False-ring timing: identification of signature cells). Thus, it is possible that, for some trees, cell expansion occurred within days after initial increases in soil moisture. On the other hand, we also observed the first cell formed after the false ring to appear as late as early August and late July for ponderosa pine and Douglas-fir, respectively. While anecdotal, these findings indicate high variability among trees in capacity to respond to renewed moisture availability.

Cell maturation of EW_{FIRST} occurred around the beginning of September, at the end of the monsoon. That EW_{FIRST} differentiation occurs during the summer rainy season is an unsurprising but important finding that is consistent with an earlier nearby xylogenesis study (Budelsky 1969). Moreover, it provides a biological basis for assumptions made in subdividing tree rings for the reconstruction of winter and summer precipitation in the NAM region (e.g., Griffin et al. 2011).

Transition from dry to wet conditions: cambial reactivation

Understanding recovery from drought provides the greatest insight into the capacity of trees to adapt to changing climate. In the enlarging phase, recovery manifests in abrupt changes in cell size and has important implications for tree-ring morphology. For cambial activity, recovery manifests in renewed cell production rates and has implications for radial tree growth. We expected that, based on previous findings, tree growth recovery would first manifest in the cell enlarging phase followed by cambial activity (Abe et al. 2003). Surprisingly, we found that for ponderosa pine trees forming a false ring, cambial activity preceded recovery in the cell enlarging phase. Even more surprisingly, increased cambial activity preceded the first rain event of the 2014 monsoon. This timing of cambial reactivation suggests

that contrary to our current understanding, soil moisture may not be the sole environmental driver of xylogenesis recovery from drought (Battipaglia et al. 2016, Peters et al. 2020). In the southwestern USA, the development of the monsoon begins with increases in air humidity as upper-level winds shift (Douglas et al. 1993, Higgins et al. 1999). Then, within weeks, the first precipitation event occurs. Given that increases in air humidity are a reasonably reliable signal for imminent precipitation in the NAM system, it is not surprising that trees growing in the region may be sensitive to this atmospheric shift. If the increase in cell production rates is indeed triggered by increases in air humidity, it would mean that when the monsoon arrives, one or more cells can quickly move into the enlarging phase to take advantage of renewed soil moisture conditions. This finding aligns with a recent xylogenesis study conducted in the southwestern USA where Ziaco et al. (2018) found that the onset of cambial activity was associated with increases in air humidity prior to the monsoons. Clearly, more work is needed to verify this association and, if corroborated, investigate the underlying mechanism.

Bimodal cambial activity and false-ring formation

False-ring formation, or IADFs, has generally been assumed to be an outcome of bimodal cambial activity (De Micco et al. 2016*a*, De Micco et al. 2019). Our findings support this association for both ponderosa pine (Figure 8) and Douglas-fir (Figure 5E). This is in contrast to a recent xylogenesis study of ponderosa pine in the southwestern USA where only a single peak was observed (Ziaco et al. 2018). In that study, cambial activity was estimated based on cell counts in the cambial zone. In fact, we found a similar unimodal pattern of number of cells in the cambial zone (Figure 5A). However, when we derived rates of cell production from cumulative cell counts—tallies of cells in all phases of development–a pattern of bimodal cell production emerged (Figure 6). We argue that this is a more accurate representation of cambial activity, because there is an implicit recognition that the number of cells in any one phase is the result of both the rate of entry into and exit from that phase. This approach takes into account the inextricable link between the phases of cell differentiation. Moreover, estimating cambial activity using cumulative cell counts may be especially important in detecting bimodal activity when there are only small increases in rates of cell production and when this occurs around the same time that there is an increase in rate of entry into the enlarging phase from the cambium due to a sudden improvement in environmental conditions.

Unexpectedly, in ponderosa pine, we observed bimodal cambial activity not only among trees forming a false ring but also among trees not forming a false ring. This novel finding—bimodal cambial activity in the absence of false-ring formation—is intriguing and suggests a more complex response of xylogenesis to fluctuating moisture conditions than has thus far been identified. One reason why this pattern has not previously been confirmed (or denied) is that xylogenesis data are typically not grouped by false-ring/IADF formation. While some bimodal xylogenesis studies have identified which trees did and did not form a false ring/IADF (e.g., De Micco et al. 2016*b*, Vieira et al. 2020, Ziaco et al. 2018), we are unaware of any study that tabulated cell counts separately for trees forming and not forming a false ring/IADF. An important implication of this finding is that under certain conditions cambial activity is not a proxy for cell enlargement. In other words, the presence of bimodal cambial activity is not an implicit indication of false-ring/IADF formation. The seemingly incongruous occurrence of bimodal cambial activity without the formation of a false ring/IADF may be the result of compensatory adjustments in xylogenesis subprocesses. Experimental manipulations of water availability revealed adjustments made in the enlarging phase for both saplings (Balducci et al. 2016) and mature trees (Vieira et al. 2020). As water availability was reduced, cell division rates decreased but the time spent in the enlargement phase increased. As a result, cell size was maintained despite low water availability. If this was the mechanism operating in our study, an emergent question is why compensation was invoked in some trees but not others. Future studies should focus on a more detailed examination of how differences in vigor and/or the physiological responses among trees could play a role.

Conclusions and future directions

Estimating the timing of cell entry into the different phases of differentiation is critical for advancing our knowledge about when, how rapidly and by what mechanisms environmental conditions affect wood formation. Our efforts to reconcile the timing of environmental triggers and cell phenology at our study site in 2014 revealed the following:

(i) As we expected, the first xylogenetic response to the developing drought occurred in the enlarging phase, not in cambial activity, at least in ponderosa pine.

(ii) The last cell of the false ring (FR_{LAST}) did not mature before the onset of the monsoon in either species studied in 2014. This finding departs from earlier reports and has implications for the relationship between radial growth and carbon fixation.

(iii) In the recovery from within-season drought, at least for ponderosa pine trees forming a false ring, cambial activity recovery preceded cell enlargement. Our findings further suggest that the trigger for cambial recovery was not increases in soil moisture but rather increases in atmospheric moisture. This raises the interesting possibility that atmospheric moisture content may play a distinct role in determining the xylogenetic recovery from mid-season drought in monsoon climates.

(iv) Cambial activity was seasonally bimodal but was not strictly associated with false-ring formation. In ponderosa pine,

trees that did not form a false ring also showed bimodal cambial activity.

These results, taken together, are generally consistent with our hypothesis that the gradual decline of soil moisture during spring and early summer, followed by a sudden recharge of soil moisture due to the monsoon, will result in bimodal cambial activity. To this, however, we add that increases in atmospheric moisture may spur cambial reactivation before soil recharge by precipitation. Our findings were also consistent with our expectation that bimodal cambial activity would be linked to the formation of a false ring. However, we also found that bimodal cambial activity occurred in the absence of false-ring formation.

Although the study site was well instrumented as a result of the location of a flux tower, local micrometeorological measurements, sapflow and of a Critical Zone Observatory, the work reported here would have benefited from fuller information on the individual health, vigor and access to soil moisture of each of the trees sampled. This is particularly the case because of the complex topography and geology of the site. Ideally, it would have been possible to sample and analyze a larger number of trees. One way to moderate these limitations would be to extend the study of these same trees for further years. Another would be to extend a roughly latitudinal transect to other well-instrumented sites extending from the strongly monsoon-affected location reported here to regions outside major monsoon influence. Both these approaches, although still descriptive, would provide opportunities to observe the reaction of xylogenesis to differing seasonal climates, in particular differing seasonal patterns of precipitation. This in turn could inform the design of experimental modifications of soil moisture to simulate early monsoon arrival prior to VPD decline and its consequences for the detailed trajectory of xylogenesis. By focusing on the effects on tracheid development of the sharp transition from deepening drought to dramatic relief over a week or two in early July 2014, light was cast on the sequence of environmental effects on the mechanisms of xylogenesis.

Data and materials availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Supplementary Data

Supplementary data for this article are available at *Tree Physiology* online.

Acknowledgments

We are grateful for inspiration and guidance from Drs S. Rossi, A. Deslauriers and H. Morin of the University of Quebec at Chicoutimi. Drs Paul Szejner of UNAM, Mexico City and Ellis Margolis of the USGS made many helpful suggestions on an earlier version of the manuscript. Thanks are due to Mabel Alsina, Sarah Gritis, Abby Gritis, Jackie Hannifan, Jennifer Salazar, Johnathon Somers, Paul Torregrossa and Mary Mathilde Westermann for field and laboratory work. Finally, we thank the Communicating Editor and two anonymous reviewers who provided comments and suggestions that improved the manuscript.

Funding

Funding for this study was provided by National Science Foundation (AGS-1303740) P2C2 Program to M.K.H. Funding for Mt Bigelow operation and maintenance was provided by National Science Foundation (EAR-1331408) to the Catalina-Jemez Critical Zone Observatory. Local and micrometeorological data were managed and collected by R.L.M.

Conflict of interest

None declared.

Authors' contributions

M.K.H. and P.M.B. initiated the project, part of which is reported here; K.M. and M.K.H. developed the 2014 'natural experiment' reported here; K.M. supervised and conducted the field, laboratory and data analysis; G.A.B.-G. and R.L.M. provided access to the Ameriflux site on Mt Bigelow and made site environmental data available; K.M., with help from M.K.H., wrote this manuscript.

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