

ON THE NATURE OF THINGS: ESSAYS
New Ideas and Directions in Botany

Finding the seasons in tree ring stable isotope ratios

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The annual diameter increments of tree rings provide insight into past climate influences on tree growth, wildfire intervals, insect outbreaks, and the history of human civilizations (Stahle and Dean, 2011). Most recently, decadal-to-millennial tree-ring records have been used as foundational paleoclimate proxies in assessments and reports from the United Nations Intergovernmental Panel on Climate Change (IPCC). An inherent assumption in the use of tree rings to reconstruct tree–climate interactions is that the activities of stem cambial cells and their derivatives are modulated in response to environmental resource limitations and climate variations and that the modulated responses are predictable. These relations have been recognized since at least the 16th century. For example, Leonardo da Vinci wrote: “The rings around the branches of the sawn trees show the number of years and which were formed in the wetter or drier years by their greater or lesser thickness” (translated generally from the *Trattato della pittura*; Manzi, 1817, p. 396, see Sarton, 1954). Over the subsequent four centuries, anecdotal observations on the correlations between climate and tree rings gradually coalesced into the discipline of dendrochronology, with the modern form of the discipline emerging in the early 20th century under the leadership of A. E. Douglass at the University of Arizona. Here, we highlight some recent discoveries in one part of dendrochronology, known as dendroclimatology, which have allowed us to extract details about seasonal climate variations during past decades and their influences on forest ecophysiology. With this capability, we are on the cusp of accessing even greater insights about the nature of past climate cycles, their influences on forest productivity and the feedbacks within forest–climate interactions that will determine future climate regimes on Earth.

In past dendroclimatological studies, the focus has most often been on inter-annual variation in climate. Recent studies have revealed even greater resolution in the anatomical structure and chemical composition of tree rings, such that we can now recognize the patterns and causes of intra-annual (seasonal) dynamics in tree–climate interactions (Leavitt et al., 2011; Cuny and Rathgeber, 2016; Rinne et al., 2015). The knowledge that specific anatomical zones within individual rings reflect seasonal variation in climate is not new. In past studies, seasonal variation in cambial activity has been inferred through the recognition of earlywood and latewood tissues, which are assumed to reflect growth during the earlier and later parts of the growing season, respectively (Meko and Baisan, 2001; Pallardy, 2008). What is new, however, is the use of various techniques to cut or ablate thin sections of cellulose from across individual rings and molecular techniques to infer kinetic patterns in tree physiological processes (Loader et al., 2017). These finely resolved serial sections allow us to assess tree–climate interactions at scales of days to weeks, and we can identify seasonal patterns in the use of environmental resources and climate space, such as those defined by precipitation and temperature and nutrient resources stored in the trees (Rinne et al., 2015). In essence, we can much more accurately “find the seasons in tree rings”.

We have been studying the stable isotope ratios of selected compounds (¹³CO₂/¹²CO₂ and ²H¹HO/¹H₂O or H₂¹⁸O/H₂¹⁶O) that are incorporated into tree-ring cellulose in *Pinus ponderosa* trees distributed across the geographic domain defined by the North American monsoon climate system (Szejner et al., 2016). When pine needles assimilate atmospheric CO₂ through photosynthesis or lose H₂O through transpiration, isotopic ratios of the elements

that compose these compounds are altered through the process of isotopic discrimination—i.e., the heavier and lighter isotope forms of compounds (known as isotopomers) are distinguished through biochemical and physical processes (Gessler et al., 2014). To align the seasonal determinants of isotope discrimination with specific components of a tree ring, we sample sections from the earliest earlywood (EW1), later earlywood (EW2), false latewood (FLW, embedded within the earlywood), summer wood (SW, the earlywood that follows the FLW) and latewood (LW) (Fig. 1), thus, providing five seasonal fractions for each annual ring.

Our initial studies in southern Arizona revealed a unique and puzzling pattern. Based on theory (see Saurer et al., 1997), the lowest ratios of the heavier to lighter stable isotopes ($^{13}\text{C}/^{12}\text{C}$ and $^{18}\text{O}/^{16}\text{O}$) were predicted to occur in the earlywood (EW1 and EW2), which is produced during spring. We hypothesized that with cooler springtime temperatures, higher relative humidities, and ample supply of snowmelt water, the production of photosynthate to support cambial activity would occur at a lower leaf-to-atmosphere water vapor pressure difference (VPD) and higher steady-state ratios of intercellular to ambient CO_2 concentration (c_i/c_a), both of which favor discrimination leading to lighter isotope ratios in tree-ring cellulose. These conditions are predicated on the assumption that during favorable seasonal moisture regimes, a cooler more humid atmosphere is associated with reduced transpiration rates at any given stomatal conductance (g_s), and a lower intrinsic water-use efficiency ($\text{iWUE} = A/g_s$, where A is net CO_2 assimilation rate). In contrast, the highest $^{13}\text{C}/^{12}\text{C}$ and $^{18}\text{O}/^{16}\text{O}$ ratios (and highest iWUE) were predicted to be found in the FLW band. The small cells and high density of the FLW band are known to be produced during the early-summer, hyper-arid

phase, presumably in response to seasonal drought (Leavitt et al., 2011). The lower g_s and c_i/c_a ratios that are often observed during drought should favor discrimination leading to heavier isotope ratios in tree-ring cellulose. With the start of monsoon rains, in mid-summer, we hypothesized that the increased availability of water would be reflected in a return to lighter isotope ratios in the cellulose of SW and LW.

In contrast to these hypothesized patterns, however, we observed that the EW1 and EW2 fractions contained the heaviest cellulose isotope ratios, reflecting the highest seasonal iWUE and highest seasonal VPDs, presumably due to the use of photosynthate that was produced during periods with limited soil moisture, a dry atmosphere, and warm temperatures (S. Belmecheri and P. Szejner, unpublished data). The isotope ratios of the FLW band were isotopically among the lightest of the entire annual ring, presumably reflecting the use of photosynthate produced in a climate with wetter soil and more humid atmospheric conditions. The SW and LW cells contained isotope ratios that were heavier than in the FLW, but lighter than in the EW1 and EW2 bands. What could cause the apparent paradoxes between the predicted and observed patterns?

Insight came when Kiyomi Morino, Ed Wright, and Malcolm Hughes, of the Laboratory of Tree Ring Research in Tucson, took a closer look at the phenology of cambial processes. Their observations confirmed the formation of EW cells during cooler springtime weather. However, tracheids mature during the subsequent weeks that extend well into the hot, early-summer drought. Thus, while the large-diameter cell size of EW reflects springtime production, the stable isotope composition of mature EW reflects hotter, drier summer weather. The fact that the phases of xylogenesis occur

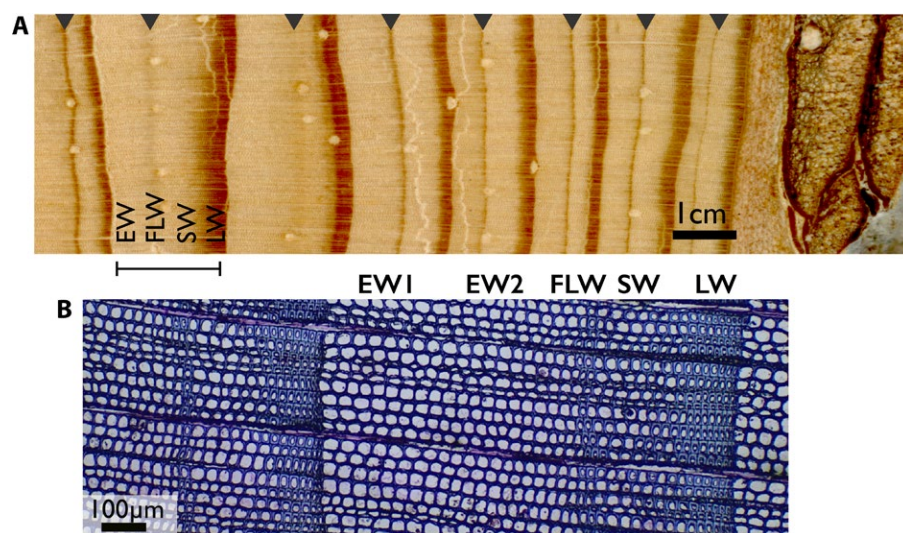


FIGURE 1. (A) Photograph of tree rings from a *Pinus ponderosa* tree growing in the Santa Catalina mountains (Arizona). The arrows at the top of the image show the locations of the false latewood (FLW) bands, which are typically produced during the hyperarid period in early summer, but which mature during the subsequent monsoon period. The typical anatomical subdivisions of an annual ring (indicated by a horizontal bar) are shown and labelled as: earlywood (EW) located before the FLW, summer wood (SW) and latewood (LW) both located after the FLW. (Image credit: S. Belmecheri, University of Arizona, unpublished). (B) Magnified thin section (6 μm thick, stained with creels violet) showing seasonal designations and anatomical details for the earliest earlywood (EW1), latest earlywood (EW2), FLW, SW, and LW for one annual ring. From the cellular structure, it is evident that EW1, EW2, and SW cells have wider-diameter lumens than in FLW and LW cells. (Image credit: K. Morino, University of Arizona, unpublished).

across many weeks is well established (Skene 1969), to the point of being “textbook knowledge” (Pallardy 2008). However, refinement of that knowledge to align specific phases of xylogenesis to the seasonal timing of photosynthate production, and its reflection in the stable isotope record of cellulose, has only recently emerged. In one recent high-profile report, Cuny et al. (2015) reported that lags between annual increases in stem girth and woody biomass average 1 month in many global forests. This discovery called into question the use of tree-ring diameter measurements to indicate seasonal patterns in woody biomass production in analyses and models of the global carbon cycle. In the past, limited awareness of how the seasonal phenology of xylogenesis lines up with specific radial components of annual rings has been noted as contributing to “fuzziness” in interpretations of isotope-environment correlations (Leavitt et al., 2011). As a result, seasonal analyses of tree-ring isotope ratios have focused on the earliest EW or latest LW as the most reliable markers of seasonal climate (e.g., Leavitt et al., 2011; Rinne et al., 2015). With a quantitative understanding of seasonal lags during xylogenesis, we can achieve more accurate assessments of seasonality across the entire ring.

Having resolved the mystery of the gap between anatomy and isotope composition, we are now in a position to address several aspects of tree–climate interactions. In ponderosa pine, for example, which is widely distributed across the western United States, we can begin to dissect the independent influences of winter versus summer precipitation and seasonal droughts on forest tree growth and water use. We can also assess the potential for long-lasting effects due to extreme seasonal climates (e.g., the multi-year legacies of extreme droughts). Going forward, one of our more salient needs will be to expand these perspectives to include additional species. Broadleaf, deciduous trees, for example, often utilize stored carbohydrate resources to support construction of the earliest EW and latest LW when photosynthetic production is seasonally constrained. The dependence on stored photosynthate for seasonal wood production in deciduous trees can obscure efforts to extract seasonal insight on tree–climate stable isotope connections (Helle and Schleser, 2004). The clearest opportunities at the moment for elucidating isotope–climate relations through serial tree-ring analyses appear to lie with needle-leaf, evergreen conifers, especially those in the Pinaceae (Barbour et al., 2002; Leavitt et al., 2011; Rinne et al., 2015).

Tree rings continue to inspire curiosity about how some of the oldest organisms on the planet accommodate and record their surrounding environment. There is growing recognition that the annual rings of trees contain considerably more information about their environment than what is determined from diameter increments alone. As molecular analytical techniques improve, it is likely that we will be able to deconstruct the glucose units that compose cellulose and extract even more insight about the seasonality of woody biomass production (Augusti and Schleucher, 2007). Recently, the resolution of isotope ratios in specific thin sections of tree rings has improved with the use of laser ablation techniques coupled to in-line combustion and mass spectrometry. Now, we are able to assess isotope ratios of samples in the diameter range of 40–100 μm (Rinne et al., 2015; Loader et al., 2017). The seasonal resolution capabilities of such systems are currently limited by the sample size required for isotope ratio resolution. As the analytical capabilities for assessing isotope ratios in extremely small samples improves, the seasonal resolution capabilities of tree-ring analysis will likely also improve.

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