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REVIEW

Defining cambial activity: the limitations of indirect indicators and the need for direct cellular markers

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Abstract

The vascular cambium is a key lateral meristem responsible for secondary growth in woody plants, producing secondary xylem and phloem. Understanding its activity is crucial for studies on plant phenology, carbon sequestration, and environmental responses. However, defining the precise period of cambial activity remains challenging due to reliance on indirect indicators, such as cambial zone width or the presence of undifferentiated cells adjacent to the cambium. These parameters often misrepresent the true timing of cambial cell division, conflating it with subsequent differentiation processes. This study critically examines the limitations of indirect indicators and advocates for a more precise definition of cambial activity strictly as the process of cellular division. Direct cellular markers, such as mitotic figures, phragmoplasts, and newly formed tangential walls, provide more accurate assessment of cambial activity. By distinguishing cell division from differentiation, we can refine growth periodicity analyses and improve our understanding of environmental influences on cambial function. We review the structure and activity of the vascular cambium, demonstrating how indirect indicators can misrepresent cambial activity dynamics and lead to errors in determining its onset, duration, and cessation. By integrating direct cellular markers, we propose a more accurate methodology for assessing cambial activity, improving phenological studies and providing a clearer framework for evaluating plant responses to climatic variability.

Keywords Cambium · Seasonality · Phenology · Secondary growth · Cell production · Cell differentiation

Introduction

The vascular cambium is the lateral meristem that produces secondary xylem and phloem, driving the thickening of stems and roots. This process strengthens plant structure, improves water and nutrient transport and storage (Baas et al. 2004; Evert 2006), and contributes to atmospheric carbon sequestration (Babst et al. 2014; Cuny et al. 2015). As a key factor in plant development and global carbon cycling (Barford et al. 2001; Spicer and Groover 2010; Doughty et al. 2015), understanding the timing and duration of cambial activity is crucial in phenological studies (Wilcox 1962; Bosio et al. 2016). Accurately defining its active period provides insights into how environmental factors regulate growth cycles, resource allocation, and long-term ecological adaptations. Given its role in seasonal responses, precise assessments of cambial activity are essential for understanding plant reactions to climate variability and environmental pressures. In this context, numerous studies have sought to clarify the environmental control of cambial activity/xylogenesis, aiming to improve the prediction of growth responses to environmental change. Such efforts have increasingly focused on modeling cambial dynamics, wood production, and carbon sequestration under ongoing climate change scenarios (e.g., Castagneri et al. 2017; Begum et al. 2018; Van Camp et al. 2018; Cabon et al. 2020; Chen et al. 2022;).

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Despite its importance, accurately defining the onset, duration, and cessation of cambial activity remains challenging, as different researches have used different parameters. Many studies rely on indirect parameters—such as cell enlargement or the number of cell layers in the cambial zone—to infer the periods of active cell division in the cambium. However, these indirect indicators reflect processes occurring downstream of cellular division, often conflating the activity of cambial initials with subsequent stages of cell differentiation and maturation. Such confusion can distort the true timing and duration of the cambial activity period, potentially resulting in misleading conclusions about the dynamics of wood formation and the external factors modulating cambial phenology.

To address these limitations, it is essential to recognize cambial activity strictly as a process of cellular division. Direct cellular markers, including the presence of phragmoplasts (Fig. 1a–b, f), mitotic figures (Fig. 1a, c–d), and a very thin newly formed tangential wall (Fig. 1f), offer the most accurate approach for identifying an active cambium. These features allow for precise differentiation between the phases of cell division and subsequent differentiation processes, which, in the secondary xylem, are typically well-defined and include cell expansion, secondary wall deposition, and lignification. In contrast, the differentiation of

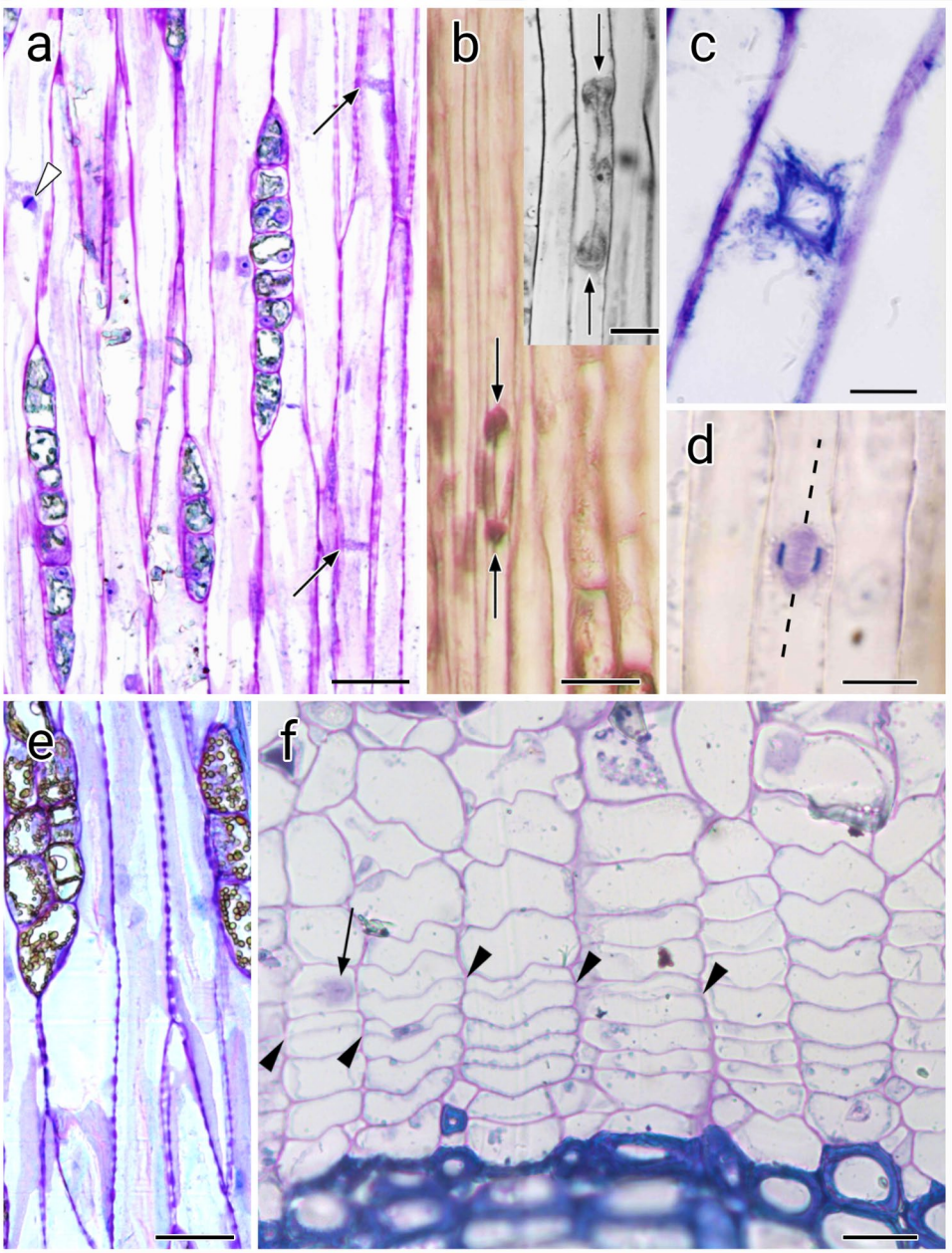

Fig. 1 Cambial activity in different species. **a**–**d** Cytokinesis in the vascular cambium. **a** Phragmoplasts (arrows) in the longitudinal tangential section of *Kielmeyera grandiflora* (Calophyllaceae). Note the thin, smooth radial walls of fusiform initials and the absence of prominent primary pit fields, as well as a mitotic figure (white arrowhead), and phragmoplasts (arrows), as seen in longitudinal tangential section. **b** Phragmoplasts (arrows) in a longitudinal radial section of *Quercus alba* (Fagaceae). Inset: Higher magnification view in *Citharexylum myrianthum* (Verbenaceae). **c**, **d** Mitotic figures in early stages of division in the longitudinal radial section of *Citharexylum myrianthum* (Verbenaceae). **d** The cell plate is in the early stage of formation (dashed line), and daughter nuclei appear in blue. **e** Dormant cambium with fusiform cells showing a beaded appearance of radial walls in the longitudinal tangential section of K. grandiflora (Calophyllaceae). **f** Recently formed periclinal walls (arrowheads) and a phragmoplast (arrow) in a cross section of *C. myrianthum* (Verbenaceae). **a** and **e** from Bosio et al. 2016; **b** inset from Angyalossy et al. 2020. Scale bars: **a** 50 µm; **b** 20 µm (inset: 10 µm); **c** 4 µm; **d** 8 µm; **e** 50 µm; **f** 25 µm

secondary phloem involves distinct cellular modifications, such as the development of sieve elements with sieve areas or sieve plates and degeneration of organelles, the formation of companion or Strasburger cells, and the thickening or sclerification of fibers or sclereids (Esau 1977; Evert 2006). By focusing on these direct indicators, researchers can minimize misinterpreting cambial activity with downstream developmental events, thus improving the accuracy of phenological assessments, enhancing the interpretative power of phenological studies in woody plants and enabling more precise evaluations of environmental modulation of growth. It is important to note that, on the other hand, cambial dormancy is characterized by the absence of these direct markers, as well as by the distinct morphological features of dormant cells, such as thickened radial walls and prominent primary pit-fields, which give the walls a beaded appearance, observable in longitudinal tangential sections (Evert 1963; Tucker and Evert 1969; Fig. 1e). These characteristics provide a clear contrast to the active phase, in which cells exhibit thin, smooth walls, further supporting accurate temporal assessments of cambial dynamics.

To support this approach, we first review the structure and activity of the vascular cambium, emphasizing how a more restrictive definition—as a single layer of cambial initials—enhances clarity about its role in plant growth. This precise definition helps to distinguish true cambial activity, which is limited to cellular division, from the subsequent process of cellular differentiation. Next, we address the main limitations of indirect parameters commonly employed to assess cambial activity, critically examining evidence from studies that have thoroughly analyzed the cambial phenology and the differentiation of its products. Specifically, we focus on two widely used indicators—the presence of undifferentiated cells adjacent to the cambium and the count of cell layers in the cambial zone. Finally, we will discuss how an integrated approach, incorporating direct cellular markers, can improve the accuracy of phenological assessments of cambial activity, thereby advancing our understanding of how environmental factors influence plant growth dynamics.

Vascular cambium: structure and activity

Structurally, the vascular cambium is composed of a single layer of meristematic cells known as **cambial initials** (Sanio 1873; Berlyn 1982; Shi et al. 2019; Smetana et al. 2019). Cambial initials are categorized into two types: fusiform initials, which are elongated cells responsible for forming axial elements of both secondary xylem and phloem, and ray initials, which are nearly isodiametric cells that form the phloem and xylem rays.

Cambial initials undergo two primary types of division, both essential for the maintenance and function of the cambium. In **periclinal divisions**, where the plane of division is parallel to the plant's surface, a cambial initial produces two daughter cells: one retains the identity of a cambial initial, sustaining the cambium's ability to continue dividing, while the other becomes a derivative cell. Depending on its position relative to the cambium, the derivative cell differentiates into a **phloem mother cell**, which will undergo additional divisions to produce secondary phloem, if located toward the exterior of the plant organ, or a **xylem mother cell**, which will further divide to give rise to secondary xylem, if positioned toward its interior. Additionally, anticlinal divisions, which occur perpendicular to the plant's surface, increase the number of cambial initials, ensuring the continuity of the cambium as the organ's circumference expands with growth (Lachaud et al. 1999; Evert 2006).

Notably, only the cambial initial has the potential to produce both secondary xylem and secondary phloem (Shi et al. 2019; Smetana et al. 2019). Though morphologically indistinguishable from the cambial initial and capable of undergoing multiple divisions, the mother cell differs in that it is committed to forming only one type of secondary vascular tissue, xylem or phloem. A phloem mother cell will continue dividing before differentiating into functional phloem elements, while a xylem mother cell will divide and give rise to secondary xylem. Although distinguishing initial cells from their immediate derivatives can be challenging, it is nevertheless crucial to make this distinction to correctly understand cambial activity.

During the peak of cambial activity in woody plants, cell divisions may occur so rapidly that older derivative cells are still meristematic while new cells are being produced by the initials. This dynamic can result in the formation of a relatively wide band of undifferentiated cells, comprising cambial initials, phloem mother cells and xylem mother cells that are still dividing. This band of cells is commonly referred to as the cambial zone (Esau 1977).

Following the division of the mother cell, the resulting newly formed daughter cells undergo a series of differentiation processes. In the xylem, these include cell expansion, secondary wall deposition, and lignification, while in the phloem, they involve sieve element development, the formation of companion or Strasburger cells, and the thickening or sclerification of fibers or sclereids (Esau 1977; Evert 2006). In tracheary elements, the differentiation process culminates in programmed cell death, contrasting sharply with the ongoing mitotic divisions that define the cambium itself. Therefore, cambial activity—strictly defined as cellular division— must be clearly distinguished from differentiation, which begins as soon as mother cells are formed. Together, these two distinct yet complementary processes drive secondary growth, the radial expansion that underlies the development of woody plants.

Indirect indicators of cambial activity and their limitations

Direct observation of cambial cell division can be challenging. Consequently, indirect indicators such as the presence of undifferentiated cells adjacent to the cambium or the number of cell layers in the cambial zone are frequently employed (León-Gómez and Monroy-Ata 2005; Rahman et al. 2020; Buajan et al. 2023; Silva et al. 2023; Silvestro et al. 2024). However, this approach may result in either underestimating or overestimating the cambium's active period, as cell differentiation may occur independently of cambial division.

Differentiating cells next to the cambium

Using the presence of undifferentiated or differentiating cells adjacent to the cambium as an indicator of cambial activity can lead to misinterpretations of the timing, duration, and extent of cambial activity.

Undifferentiated cells overwintering next to a dormant cambium: in many species, undifferentiated cells can overwinter next to the cambium throughout dormancy, which can be misleading if interpreted as evidence of ongoing cambial activity. Several studies have reported overwintering undifferentiated cells on the phloem side of the cambium (Evert 1960, 1962, 1963; Alfieri and Evert 1968, 1973; Angyalossy et al. 2020), whereas others have found them on the xylem side (Imagawa and Ishida 1972; Barnett 1992; Frankenstein et al. 2005; Marcati et al. 2006). Additionally, some studies have noted the presence of undifferentiated cells on both the xylem and phloem sides of the dormant cambium (Murmanis 1971). Misinterpreting these overwintering cells as newly formed derivatives of cambial division can lead to errors in determining the precise timing of cambial reactivation, potentially skewing phenological and growth-related analyses.

Cells next to the cambium can initiate differentiation prior to the reactivation of the cambium: undifferentiated cells next to the cambium may begin to differentiate—either by growing or dividing—prior to the actual reactivation of the cambium. In many species, for instance, phloem differentiation starts one to two weeks before the onset of cambial activity, as seen in *Pinus banksiana* Lamb., *P. resinosa* Ait., *P. strobus* L., *Larix laricina* (Du Roi) K. Koch, *Picea mariana* (Mill.) BSP., and *Abies balsamea* (L.) Mill. (Pinaceae; Alfieri and Evert 1968, 1973; Murmanis 1971). Similarly, Barnett (1992) observed that in *Aesculus hippocastanum* L. (Sapindaceae), phloem differentiation preceded cambial activity by two weeks, while in *Fraxinus excelsior* (Oleaceae) xylem cell differentiation began three weeks before the first cambial divisions (Frankenstein et al. 2005). This early differentiation, occurring before any actual cambial division, can be misinterpreted as evidence of cambial reactivation, leading to an overestimation of the cambial active period.

Xylem differentiation lags behind cambial reactivation: using xylem differentiation as an indicator of cambial activity may lead to an underestimation of its onset. In many species, xylem differentiation lags behind cambial reactivation by one to two weeks or more. For instance, delays of one to two weeks have been observed in *A. balsamea* (Pinaceae; Alfieri and Evert 1973), *Robinia pseudoacacia* L. (Fabaceae; Derr & Evert 1967), and *Vitis riparia* Michx. (Vitaceae; Davis and Evert 1970), while in other species, such as *Pinus banksiana*, *P. resinosa*, *P. strobus*, *Larix laricina*, *Picea mariana* (Pinaceae; Alfieri and Evert 1968, 1973), *Acer negundo* L. (Sapindaceae; Tucker and Evert 1969), and *Parthenocissus inserta* (Kerner) Fritsch (Vitaceae; Davis and Evert 1970), the delay extends to over a month. In some cases, this lag may extend up to a month and a half, as in *Malus domestica* (Suckow) Borkh. (Rosaceae; Evert 1963) and *Populus tremuloides* Michx. (Salicaceae; Davis and Evert 1968). These delays in xylem differentiation highlight a significant limitation of using it as a sole marker for cambial activity, as it may fail to accurately capture the true period of cambial division, especially in species with pronounced delays in xylem formation.

Additionally, the persistence of cellular differentiation after the cessation of cambial division can further complicate the assessment of cambial activity duration. For example, in *Pinus banksiana*, *P. resinosa*, and *P. strobus* both xylem and phloem production ceased around one month before differentiation was complete (Alfieri and Evert 1968). Similarly, in *Robinia pseudoacacia* (Fabaceae), cambial division ended nearly two months before xylem and phloem differentiation concluded (Farrar and Evert 1997). In tropical species, such as *Aphananthe monoica* (Hemsl.) J.-F.Leroy (Cannabaceae) and *Pleuranthodendron lindenii* (Turcz.) Sleumer (Salicaceae), cambial activity ceased approximately two months before differentiation was complete (Yañez-Espinosa et al. 2006). This lag between cambial division and the completion of differentiation can lead to an overestimation of cambial activity duration, misrepresenting the true period of active cell division.

Thus, using the presence of differentiating cells adjacent to the cambium as an indicator of cambial activity may be misleading. Due to the asynchronous timing of division and differentiation processes, this approach may inaccurately extend or shorten the apparent period of cambial activity, leading to misinterpretations of growth dynamics and the environmental factors modulating cambial function.

Number of cell layers in the cambial zone

The number of cell layers in the cambial zone (CZ) is often used as an indirect indicator of cambial activity. That is because, as previously noted, during the peak of cambial activity, cell divisions may proceed at such a rapid pace that older derivative cells remain meristematic while new cells are being produced by the initials. This dynamic can lead to the increase of the number of CZ cell layers (Esau 1977). However, numerous studies highlight the limitations of this approach, as many species show either a delayed increase in the number of CZ cell layers relative to cambium reactivation or minimal differences in the number of CZ cell layers between dormant and active stages (Fig. 2), making it an unreliable indicator of cambial activation.

For instance, the number of CZ layers increased only after two weeks and most commonly one month following the onset of cambial activity, coinciding with the initiation of xylem differentiation in several Pinaceae species, including *Pinus banksiana*, *P. resinosa*, *P. strobus*, *Abies balsamea*, *Larix laricina*, and *Picea mariana* (Alfieri and Evert 1968, 1973; Murmanis 1971). A similar delay was observed in *Robinia pseudoacacia*, where an increase in CZ layers was noted approximately one month after cambial reactivation (Farrar and Evert 1997). These results indicate that cambial activity begins before the increase in CZ layers, preceding the initiation of xylem differentiation. This highlights a temporal separation between the early stages of cell division and the visible manifestations of cambial zone expansion and cell differentiation, further demonstrating the limitation of using CZ layer counts as a reliable indicator of cambial reactivation.

Other studies have similarly reported little to no variation in the number of CZ cell layers between dormant and active states. Minimal variation or stable CZ cell layer counts, regardless of the cambial state, was found in distantly related taxa, both in tropical species—such as *Cordia trichotoma* (Vell.) Steud. (Cordiaceae; Amano 2002), *Paubrasilia echinata* (Lam.) Gagnon, H.C.Lima & G.P.Lewis (Fabaceae; Amano 2007), *Cordiera concolor* (Cham.) Kuntze (Rubiaceae; Lara and Marcati 2016; Lara et al. 2017), *Cedrela fissilis* Vell. (Meliaceae), and *Citharexylum myrianthum* Cham. (Verbenaceae; Angyalossy et al. 2020)—and temperate species such as *Quercus alba* L. (Fagaceae; Pace et al. 2023; Fig. 2). Earlier research by Srivastava (1966) and Murmanis (1977) had also documented negligible differences in

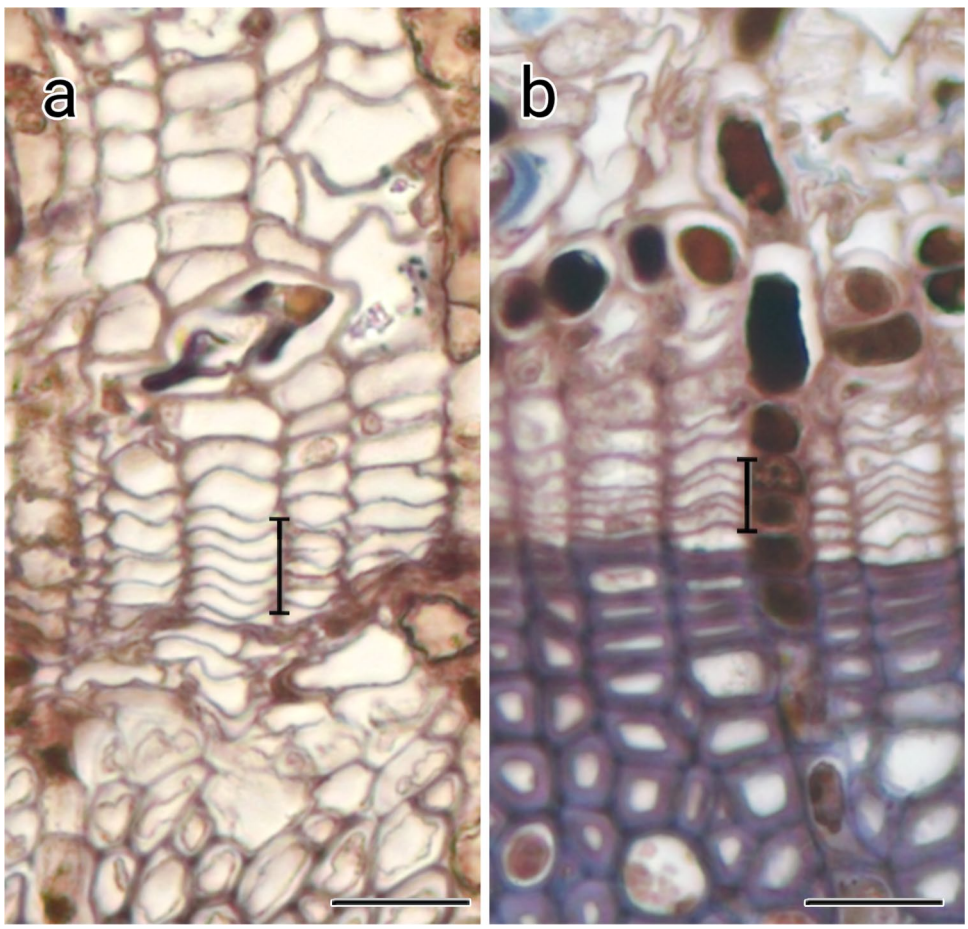

Fig. 2 Cambial zone (CZ) cell layers in active **a** and dormant **b** states in *Quercus alba* (Fagaceae), showing minimal variation in CZ cell layer counts. Scale bars: **a**, **b** 50 µm

the number of CZ cell layers between dormant and active phases.

This lack of variation in CZ layer counts suggests that they may not reliably reflect cambial activity in many species. Since increases in CZ cell layers can lag significantly behind the onset of cambial reactivation, relying on this parameter alone may lead to an underestimation of the active growth period. Moreover, the timing of CZ expansion often coincides with xylem differentiation rather than the initiation of cambial activity itself. This suggests that CZ cell layer counts may reflect processes influencing xylem formation, rather than cambial division alone.

Overall, these findings demonstrate that using CZ cell layer counts as a proxy for cambial activity is problematic due to the asynchronous relationship between layer expansion and cambial reactivation. Similar to the use of differentiating cells near the cambium, this approach can distort the perceived timing of cambial activity, highlighting the need for complementary or alternative markers that provide a more accurate assessment of cambial dynamics and the timing of active growth phases.

Direct indicators of cambial activity—phragmoplasts, mitotic figures, newly formed cells walls

It is unsurprising that many researchers rely on indirect methods to infer cambial activity. While identifying thin walls of recently divided cells is relatively straightforward, spotting mitotic figures and phragmoplasts presents significant practical challenges and demands greater technical expertise. In this section, we provide practical guidance for locating phragmoplasts and mitotic figures in vascular cambia.

Sample preparation is a critical step, as inadequate fixation or embedding can easily compromise the preservation of delicate mitotic figures. To ensure optimal penetration of fixative and embedding medium, samples should ideally measure between 3 and 5 mm in length, width, and thickness. Smaller samples help minimize the risk of uneven fixation and resin infiltration, which are common causes of structural distortion or loss. The choice of fixative is also critical. We recommend using fixatives that preserve nuclear and chromatin detail with good contrast, such as Craf III (10% formalin, 0.3% chromic acid, 0.2% acetic acid; Sass 1958; Kraus and Arduin 1997). Immediate vacuum infiltration greatly enhances fixative penetration, and should continue for 24–48 h at 4 °C to ensure proper preservation of cellular details.

Following fixation, samples should be dehydrated through an ascending ethanol series (30%, 50%, 70%, 90%, and 100%) and infiltrated with methyl methacrylate following the manufacturer's protocol. This embedding medium supports the production of thin, high-quality sections and preserves cellular detail exceptionally well. After polymerization, excess resin should be trimmed from the sample block using a sharp blade to facilitate stable positioning during sectioning. Sections should be cut with a thickness of 3–6 µm using a rotary microtome. Thinner sections improve light transmission and resolution, which is essential for detecting subtle cytological features. We strongly recommend preparing both tangential and radial sections. Radial sections are particularly effective for observing periclinal divisions, as these occur parallel to the radial axis of the cambium.

Once slides are prepared, begin observations using a 20 × or 40 × objective. A systematic examination of multiple sections is necessary, as mitotic events are infrequent and may not appear uniformly throughout the cambium. The phragmoplast, which forms between the daughter nuclei during cytokinesis, exhibits different visual characteristics depending on the section plane. In tangential sections, the cambium is located between differentiating xylem and phloem and is identifiable by elongated, fusiform cells with thin primary walls. In this view, the phragmoplast appears as a grid-like structure marking the site of new cell wall formation, with denser staining at the ends where microtubules concentrate (Fig. 1a). In radial sections, it spans the space between two daughter nuclei, bisecting the initial cell (Fig. 1b). In transverse sections, the phragmoplast appears more circular or oval, located centrally within the dividing cell, again showing denser microtubules presence at the margins (Fig. 1f).

Mitotic figures are even less frequently encountered but can be reliably identified when preparation is meticulous. In our experience, metaphase and anaphase are the most easily recognized stages of mitosis in vascular cambia (Fig. 1a, c, d). During metaphase, chromosomes are highly condensed and aligned along the equatorial plane of the cell, forming a conspicuous dark-staining metaphase plate. In anaphase, sister chromatids are pulled toward opposite poles, forming a distinctive, symmetrical arrangement that is readily observable under light microscope (Evert 2006). These phases are relatively easier to identify due to the compact nature and orderly distribution of chromosomes at these stages.

An additional diagnostic feature is the newly formed tangential cell wall, which typically appears thinner than the original cell wall and forms an angular junction with its radial cell wall (initial or mother cell). The outline of the original (mother) cell can often remain discernible by its rounded shape, aiding in the recognition of recently divided cell (Fig. 1f).

The detection of phragmoplasts and mitotic figures remains a technically demanding task, subject to variability in tissue condition, fixation quality, and the inherent rarity of cell divisions at the moment of sampling. Therefore, while

direct markers provide unequivocal evidence of cambial activity, these practical challenges must be acknowledged and carefully addressed in any anatomical study of cambial dynamics.

Emerging technologies for the assessment of cambial activity

Recent advances in molecular biology and imaging techniques offer promising tools to overcome the challenges associated with studying vascular cambium dynamics. Expression analyses conducted across the seasonal cycle of cambial activity have revealed a wide array of candidate genes that may serve as precise molecular markers for the onset and cessation of cambial activity (Qiu et al. 2013; Chen et al. 2021; Hu et al. 2021; Du et al. 2024). Studies in *Populus* and *Cunninghamia lanceolata* have identified consistent upregulation of cyclin-dependent kinases (CDKA, CDKB), cyclins (CYCA/B), and histone H4 transcripts during reactivation phases, reflecting their roles in regulating cell cycle progression and chromatin replication in meristematic tissues (Espinosa-Ruiz et al. 2004; Chen et al. 2021).

These molecular markers expression can be detected through quantitative real-time PCR (qRT-PCR), in situ hybridization, or immunolocalization, and in model species, their expression patterns can be tracked in vivo by fusing their promoters to reporter genes such as GUS or GFP. Because they are closely tied to core components of the cell cycle, these markers offer a more temporally resolved and mechanistically grounded alternative to genes involved in downstream processes like cell wall thickening and cell expansion, which may persist after cambial division has ceased.

Although live imaging of cambial cells by confocal microscopy, which has been successfully applied to visualize stem cell dynamics in primary meristems in vivo, is not yet feasible due to the cambium's location deep within secondary tissues (Wybouw et al. 2024), technical advancements such as computational cannula microscopy may overcome this obstacle in the near future (Guo et al. 2023; Menon et al. 2024). Nevertheless, combining techniques such as confocal laser scanning microscopy, Raman spectroscopy, lineage tracing, and tissue-specific gene manipulation may provide similar resolution also for studies investigating the seasonality of cambial activity (Siligato et al. 2016; Wu et al. 2016; Decaestecker et al.2019; Shi et al. 2019; Smetana et al. 2019; Pérez-de-Lis et al.2024). Despite current limitations, including technical complexity, high cost, and reduced applicability in the field—especially in tropical tree species with low population densities and remote distributions—these emerging technologies hold great promise. As costs decline and collaborative genomic and transcriptomic databases expand, such tools are expected to become increasingly accessible for ecological studies across a wider range of species and environments.

Together with anatomical markers, molecular and imaging approaches can transform the study of cambial activity. By enabling precise tracking of cell division in situ and across time, they provide an important foundation for improving phenological models and deepening our understanding of how woody plants respond to climatic and environmental variability.

Concluding remarks

Direct indicators of cellular division, such as mitotic figures, phragmoplasts, and very thin newly formed tangential walls, are crucial for conclusively identifying cambial reactivation. However, most studies on cambial phenology rely on indirect parameters related to xylem differentiation, such as the presence of undifferentiated cells near the cambium or changes in the number of cell layers in the cambial zone, as proxies for cambial activity. Given the frequent asynchrony between the onset of cambial division and these subsequent differentiation processes, exclusive reliance on indirect indicators can obscure key signals of true cambial activity (*i.e.*, cellular division). As a result, such studies may primarily capture xylem phenology—xylogenesis—rather than providing an accurate representation of cambial reactivation dynamics.

While these studies have made significant contributions to our understanding of secondary growth and carbon dynamics, especially in the context of climate change and increasing pressures on forest ecosystems, there remains an opportunity to refine their precision. By improving the accuracy of phenological studies to more clearly distinguish between the phases of secondary growth—cell division, phloem and xylem production, the timing intervals between xylem and phloem production, and the stages of cell differentiation (including cell growth, secondary wall formation, lignification, sieve areas/plate development, organelles degeneration, formation of companion or Strasburger cells)—we can enhance our understanding of how climatic factors influence each stage of secondary growth.

A more detailed understanding of the phenology of cambial activity and its controlling factors in tropical species remains a key gap in our current understanding. In addition to the challenges presented here, the low population density of many tropical tree species—due to the region's high biodiversity—combined with the often-remote locations of study sites, makes it difficult, if not impossible, to conduct frequent sampling. As a result, studies using direct parameters in tropical taxa are often based on relatively long intervals between sampling events, which limits the

temporal resolution needed to precisely track the dynamics of cambial activity and the differentiation of secondary xylem and phloem. Given the ongoing impacts of climate change, developing a more comprehensive understanding of the environmental drivers of cambial activity in the most biodiverse region of the planet is not only a pressing scientific challenge but also a key to understanding the resilience of tropical ecosystems.

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Declarations

Conflict of interests The authors have no conflicts of interest to declare.

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