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Seasonal Cambial Activity and Formation of Secondary Phloem and Xylem in White Oaks (*Quercus alba* L.)

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Abstract: (1) Background: the cambium has seasonal activity, forming earlywood and early phloem with relatively wide conducting cells, which will function during the most favorable season, and latewood and late phloem with narrower conducting cells, which typically function during the less favorable season. However, few studies have focused on when these two contrasting tissue types are formed in relation to climatic conditions. (2) Methods: the senior author of this paper made weekly collections for an entire year of four specimens per collection back in the 1960s, using traditional anatomical methods to study in detail what the cambium was producing progressively. (3) Results: annual growth rings are evident in both secondary xylem and secondary phloem. The cambium resumes activity in early April, with simultaneous formation of wood and secondary phloem. Both latewood and late phloem production are initiated in early June, the peak of the favorable season. The cambium ends its activity in early August. Phloem growth rings are marked by radially narrow sieve elements interspersed among a band of axial parenchyma with dark contents. Most specimens produce only one fiber band per season. This feature may be used as an indirect phloem growth ring marker. Wood growth rings are marked by very wide vessels and thick-walled, radially narrow fibers. (4) Conclusions: growth rings are evident in both secondary xylem and secondary phloem. The trees produce their latewood and late phloem long before the beginning of autumn, indicating that they prepare ahead of the selective regime, a phenomenon most likely dependent on the photoperiod. Living sieve elements are present yearlong.

Keywords: bark; seasonality of cambial activity; phloem growth rings



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1. Introduction

The vascular cambium responds to environmental cues, switching on and off according to external favorable and unfavorable conditions [1–5]. This phenomenon commonly results in the formation of growth rings, with initial and terminal markers in both secondary xylem and secondary phloem in species across all latitudes and altitudes [1–6]. Most commonly, growth rings are annual when a single annual seasonal-climatic regime occurs, such as the alternation of hot summers and cold winters in temperate zones, or a dry and wet season in the tropics [4–6]. However, in some other cases, more than one or no growth rings may be produced annually [7,8]. A classic example of supra-annual growth rings are the trees in Amazonian *igapós*, which are subject to an annual dry and wet season in addition to an

annual flooded and non-flooded season, therefore forming two growth rings per year [7,8]. While it is well known that the cambium is either active or inactive according to the season, more information is needed to understand the periodic, seasonal formation of the products of the cambium, i.e., wood and secondary phloem; whether they are formed in synchrony or asynchrony; when earlywood/early phloem and latewood/late phloem are formed; and which cell types are produced in each period of the year, giving rise to their final tissue arrangement. Previous studies have indicated that in some cases the onset of secondary xylem and secondary phloem formation is practically synchronized [9,10], while in other cases phloem formation precedes that of xylem [11–14] or xylem formation precedes that of phloem [15–17], in both cases by as much as a month. Evert [18], who led most of these studies, summarized the results in a table (page 345), with seven species exhibiting synchronicity and four with phloem formation preceding xylem formation. A number of studies indicate that in temperate regions, the entire growth ring is completely formed by early August when the cambium is no longer active, and the last cells are completing their differentiation [9–17]. This is important because latewood, also known as autumn wood in various countries [5], is generally interpreted as an adaptation against the risk of embolism caused by the first frosts, since narrow vessels and tracheids were shown to be more resistant to embolism [1,19,20]. From the literature, it appears that latewood formation precedes the first frosts (selective regime), suggesting that the trees prepare for autumn and early winter before frost occurs.

Our study is the first of an intended series to rescue historic collections from the senior author, undertaken by himself back in the 1960s and which were still unpublished. These collections aimed to conduct a detailed study of cambial activity, xylem, and phloem formation and can contribute to perfecting current knowledge on how the cambium works across different seasons in Northern temperate areas. Within this same area, other deciduous [10,12,14] and perennial trees [13] have also been studied, which allow us to directly compare results and extrapolate our data to understand how different species react to the exact same environmental inputs. These species were all collected on the premises of the University of Wisconsin in Madison. Our results can serve as basis for further studies in forest dynamics, dendrochronology, and cambial activity in some of the most common North American temperate tree species and can help pave the way to studies in less common species, other plant habits (shrubs and lianas), and trees in tropical areas. The first species explored in this series is the white oak (*Quercus alba* L.), a long-living, deciduous tree broadly distributed in Central and Eastern North America, whose resistant wood is economically important. Here, we compile previously published data on its wood and bark anatomy [17,18] and show for the first time which cell types are progressively produced across entire growing seasons. Oaks (*Quercus* spp., Fagaceae) are among the most common genera from the Northern hemisphere temperate zones and tropical high-altitude mountains [21–23], being either deciduous or evergreen. White oak is a deciduous oak and is notable for its conspicuous ring-porous woods, with very wide vessels in the earlywood and extremely narrow vessels and/or tracheids in the latewood [24].

2. Materials and Methods

2.1. Area of Study and Climate

The collection of material used in this study began in January 1961 and ended in August 1962, from trees growing in the University of Wisconsin Arboretum in Madison, Wisconsin (USA), latitude 43°2'34.3356" N, longitude 89°25'27.5556", elevation 262 m.

Climate diagrams were based on data extracted from Climate at a Glance on the National Centers for Environmental Information, National Oceanic and Atmospheric Administration (NOAA) website [25]. The climate in Madison, Wisconsin, according to the Köppen–Geiger climate classification system, is a Dfa: a typical Northern-continental climate characterized by extremely seasonal temperatures, with the coldest months below freezing, contrasted to wet, warm, bright summers (Figure 1). The difference in daylight and temperature are the most marked, whereas precipitation is more evenly distributed

(Figure 1). In 1961, September was the month with the highest precipitation, and July 1962 was the wettest month (Figure 1).

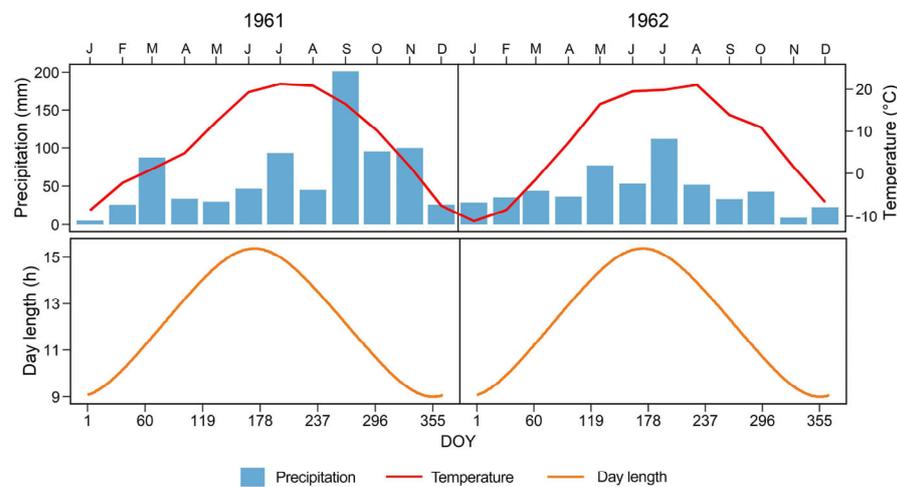


Figure 1. Climate diagram for Madison, Wisconsin, between 1961 and 1962.

2.2. Trees Sampled

Only fully grown, adult trees that appeared healthy and vigorous were sampled, avoiding trees growing in shaded areas. These trees had been growing for a long time in the University of Wisconsin Arboretum, therefore their exact age was not available. Samples were taken each week except from October through February, when collections were made monthly. Typically, samples were taken from four different trees each time; on occasion a second sample was taken from a previously sampled tree, but in this case making sure it was from an area of the trunk distant from the previous collection, to avoid wound healing processes having an effect on the seasonal interpretation of cambial activity. Pieces containing wood, cambium, and bark were taken at DBH from the main trunk by sawing into the trunk above and below the portions desired, and then removing them with the aid of a hammer and chisel. The samples were fixed immediately in either Craff III [26] or FAA [26] and aspirated shortly later in the lab. The samples were softened for 48 h in a solution of 96% ethanol and hydrofluoric acid (HF) within a fume hood and subsequently embedded in celloidin [27]. Serial transverse, radial, and tangential sections 15 μm thick were made with a sliding microtome, double-stained in lacmoid and Foster's tannic acid-ferric chloride [28] and mounted in Harleco synthetic resin.

2.3. Anatomical Criteria and Equipment Used

The terms used in this article are those defined by the IAWA Committees for wood and bark [29]. Sieve-tube elements were considered functional or living when their companion cells were alive, nucleated, and the sieve elements had contents. Completely differentiated sieve tubes are devoid of nucleus and turgid [29], while phloem fibers and sclereids are fully differentiated when lignified; secondary wall deposition in them has ended. Completely differentiated secondary xylem elements are those that are fully expanded and with lignified, secondary walls.

Samples were analyzed under a light microscope (Axioscop 40 Zeiss, Jena, Germany) equipped with an AxioCAM MRC and Axiovision software.

3. Results

3.1. General Description of the Wood and Bark

3.1.1. Wood Anatomy of *Quercus alba*

The wood of *Quercus alba* is ring-porous (Figure 2a). The growth rings are delimited by wide vessels (initial markers) of the earlywood and the thick-walled, radially flattened

fibers (terminal markers) in the latewood (Figure 2a). The size of the growth rings can greatly vary. For the most vigorous years, annual growth rings can reach up to 2 mm or more, while for less vigorous years, growth rings can be as short as 120 μm and be composed of only earlywood. The wide vessels ($154 \pm 28 \mu\text{m}$ in diameter) of the earlywood are tangentially arranged at the beginning of the growth ring (Figure 2a). The narrow vessels (Figure 2a,d) ($15 \pm 3 \mu\text{m}$ in diameter) and vascular tracheids of the latewood are solitary or in radial multiples of 2–4, in dendritic arrangement (Figure 2a). The vessels of both earlywood and latewood have simple perforation plates (Figure 2c); intervessel pits are alternate. Vessel-ray pits have greatly reduced borders with apparently simple, rounded, and vertical borders. Tyloses are common in earlywood vessels (Figure 2a,c). Vasicentric tracheids are present around the wide vessels (Figure 2b). Fibers have simple to minutely bordered pits. The fibers have thin-to-thick walls. Diffuse-in-aggregates axial parenchyma form more or less discontinuous tangential bands (Figure 2a,d) and are vasicentric in the earlywood (Figure 2a). Each parenchyma strand consists of 6–8 cells (Figure 2e). Rays are uniseriate (Figure 2e), except for the aggregate rays, which are multiseriate, over 10 cells in width (Figure 2d). All ray cells are procumbent (Figure 2c).

3.1.2. Bark Anatomy of *Quercus alba*

The bark of *Quercus alba* consists of several yearly increments of secondary phloem (6–12 in the collections used in this study) recognized indirectly by the number of fiber bands, which in this species is typically one per year, and a rhytidome consisting of several periderms and with dead phloem between them (Figure 3a). A complete increment of phloem spans the end of its formation from 150 to 200 μm in length. The cortex and pericyclic fibers are typically sloughed when multiple periderms have already been formed (Figure 3a). True phloem fibers, associated with typical prismatic crystal-containing cells (Figure 3b), form a stratified structure, with discontinuous tangential bands 3–5 cells in width and interrupted largely by the rays (Figure 3a,b). During dormancy, radially narrow sieve elements, interspersed among a band of axial parenchyma cells with dark contents, occur outside the cambial zone (Figure 4a). These sieve elements represent the last of the late phloem produced in the fall. They remain functional throughout the next growth cycle. These are the smallest sieve elements in the secondary phloem of *Quercus alba* ($97 \pm 32 \mu\text{m}^2$ of area, $11 \pm 2 \mu\text{m}$ in diameter). The remainder are considerably larger ($311 \pm 68 \mu\text{m}^2$ of area, $20 \pm 2 \mu\text{m}$ in diameter) (Figure 4a). The other sieve elements are solitary or in multiples of 2–3 (Figure 3b), with one or two companion cell strands lying in corners of the sieve elements.

End walls of sieve elements are mostly inclined (Figure 3d), with compound sieve plates (Figure 3e) of 2–8 sieve areas per sieve plate (Figure 3e). Only rarely are simple sieve plates present on one end of a sieve element. Axial parenchyma cells occur diffuse to diffuse-in-aggregates or in radial rows (Figure 3b). Crystal-containing cells border the fiber bands (Figure 3b,e). The dark contents, probably tannins, in axial and ray parenchyma cells become increasingly more abundant in the nonconducting phloem (Figure 3b). Axial parenchyma strands commonly consist of 5–8 cells (Figure 3d). The course of rays is slightly undulated (Figure 3a,b), especially where sieve elements have collapsed. Rays are uniseriate (Figure 3d), except for aggregate rays (Figure 3c). The latter may be over 10 cells in width (Figure 3c). Uniseriate rays do not sclerify when in contact with the fiber bands (Figure 3b). Aggregate rays are greatly sclerified, even in the conducting phloem (Figure 3a). Some axial parenchyma cells greatly enlarge in the nonconducting phloem and sclerify, forming large sclereid clusters (Figure 3a). Such clusters are associated with large crystal-containing cells. In summary, the nonconducting phloem is marked by dilatation of parenchyma cells, collapse of the sieve elements, formation of sclereid clusters, and an increased accumulation of presumably tannins in the axial and ray parenchyma (Figure 3a). A rhytidome is present, with sequent periderms reticulated (Figure 3a). The first periderm arises in the cortex, while the subsequent ones arise within nonconducting phloem. Lenticels were not observed.

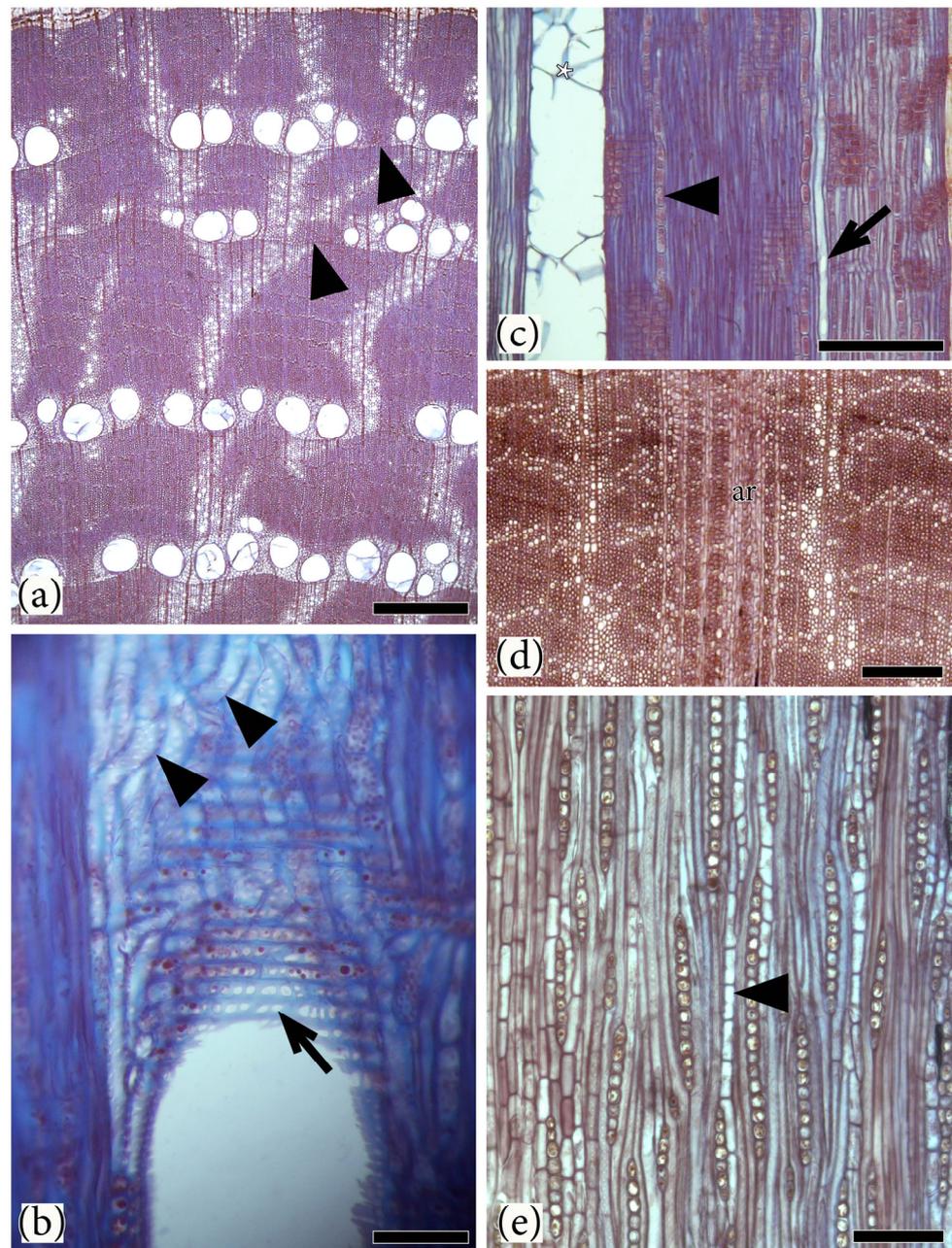


Figure 2. General aspect of the wood of *Quercus alba*. (a) Transverse section. Growth rings distinct, marked by ring-porosity, axial parenchyma associated with the vessels, and thick-walled, radially flattened fibers (arrowheads). Earlywood with very wide, solitary vessels, latewood with narrow vessels in a dendritic arrangement associated with axial parenchyma. Paratracheal parenchyma vasicentric, apotracheal parenchyma diffuse-in-aggregates forming narrow bands (Collected 4 November 1961). (b) Radial section. Sinuous vasicentric tracheids present around earlywood vessels (arrowheads). Vessel-ray pits with much reduced borders to apparently simple, rounded (arrow) (Collected 9 September 1961). (c) Radial section. Earlywood vessel with tyloses (asterisk). Rays homocellular with procumbent cells, axial parenchyma form narrow bands (arrowhead). Perforation plate (arrow) in a latewood vessel (Collected 21 October 1961). (d) Transverse section. Latewood showing axial parenchyma diffuse-in-aggregates, narrow tracheary elements, and aggregate rays (ar) (Collected 4 November 1961). (e) Tangential section. Note axial parenchyma strand with eight cells (arrowhead), uniseriate rays, and libriform fibers (Collected 4 November 1962). Scale bars: (a) = 400 μm , (b) = 50 μm , (c,d) = 200 μm , (e) = 100 μm .

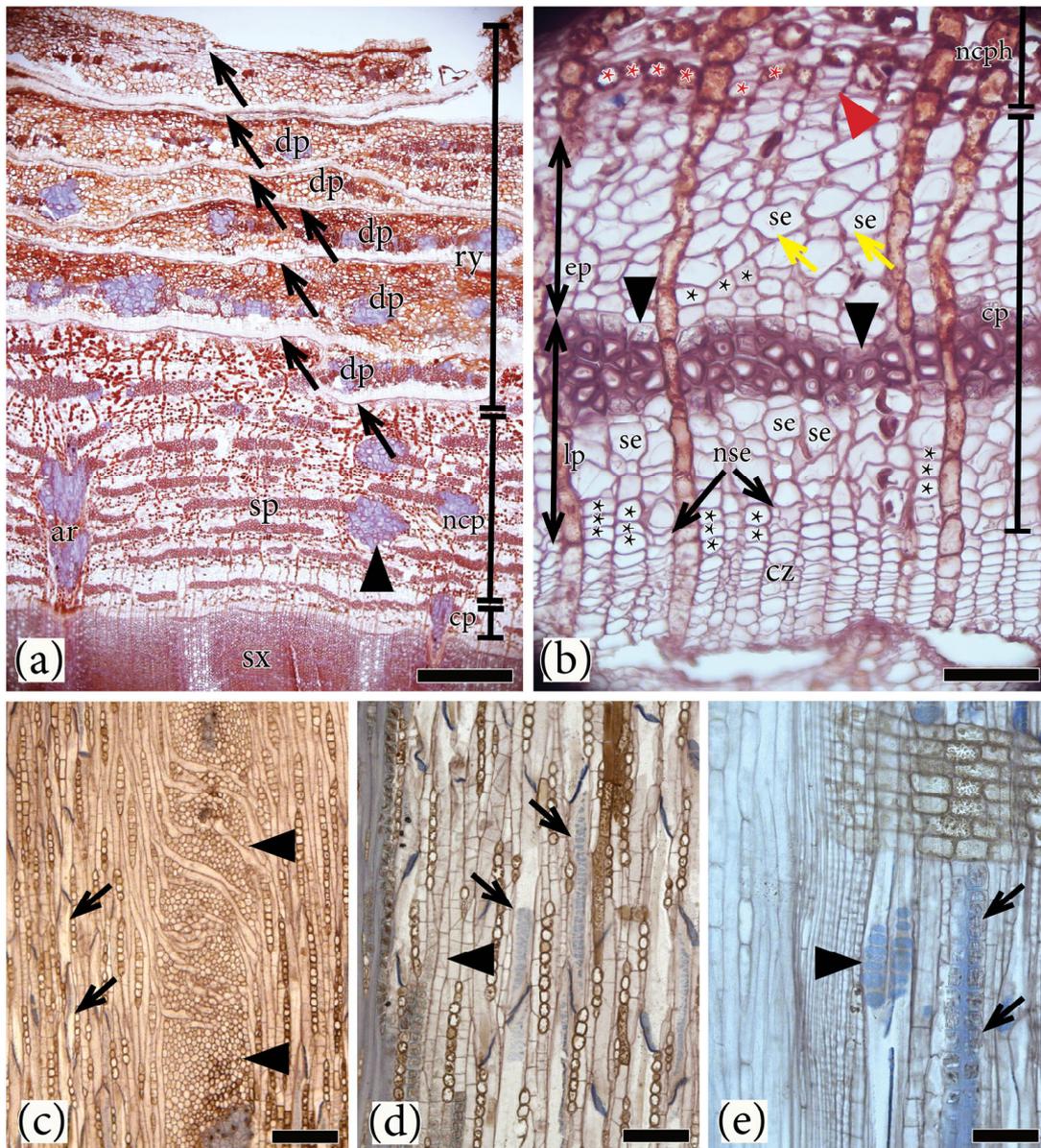


Figure 3. General aspect of the bark of *Quercus alba*. (a) Transverse section showing from below to above some secondary xylem (sx) latewood, stratified secondary phloem (sp), marked by alternating bands of phloem fibers, sclerified aggregate rays (ar), conducting phloem (cp), large sclereid clusters (arrowhead) in the nonconducting phloem (ncp), and rhytidome (ry). Rhytidome is formed by sequent periderms (arrows) and portions of dead secondary phloem (dp) (Collected 4 November 1961). (b) Transverse section. Detail of the conducting phloem (cp), which corresponds to one year's increment, showing narrow late phloem sieve elements (nse, bottom arrows), axial parenchyma cells (asterisks), those with crystals bordering the fiber band (arrowheads), and early phloem sieve elements (yellow top arrows). Last year's late phloem sieve elements (red arrowhead) and a narrow band of axial parenchyma delimit the growth ring. Most of the parenchyma band is in nonconducting phloem (ncph) (Collected 5 July 1962). (c) Tangential section showing aggregate rays (arrowheads), uniseriate rays, and sieve elements (arrows) (Collected 26 July 1962). (d) Tangential section showing sieve elements with inclined sieve plates and conspicuous lateral sieve areas (arrows), axial parenchyma cells (arrowhead) and uniseriate rays (Collected 26 July 1962). (e) Radial section, showing compound sieve plates (arrowhead), homocellular rays with procumbent cells and fibers surrounded by crystal-containing cells (arrows) (Collected 26 July 1962). Additional acronym: cz = cambial zone. Scale bars: (a) = 400 μm , (b) = 50 μm , (c) = 200 μm , (d,e) = 100 μm .

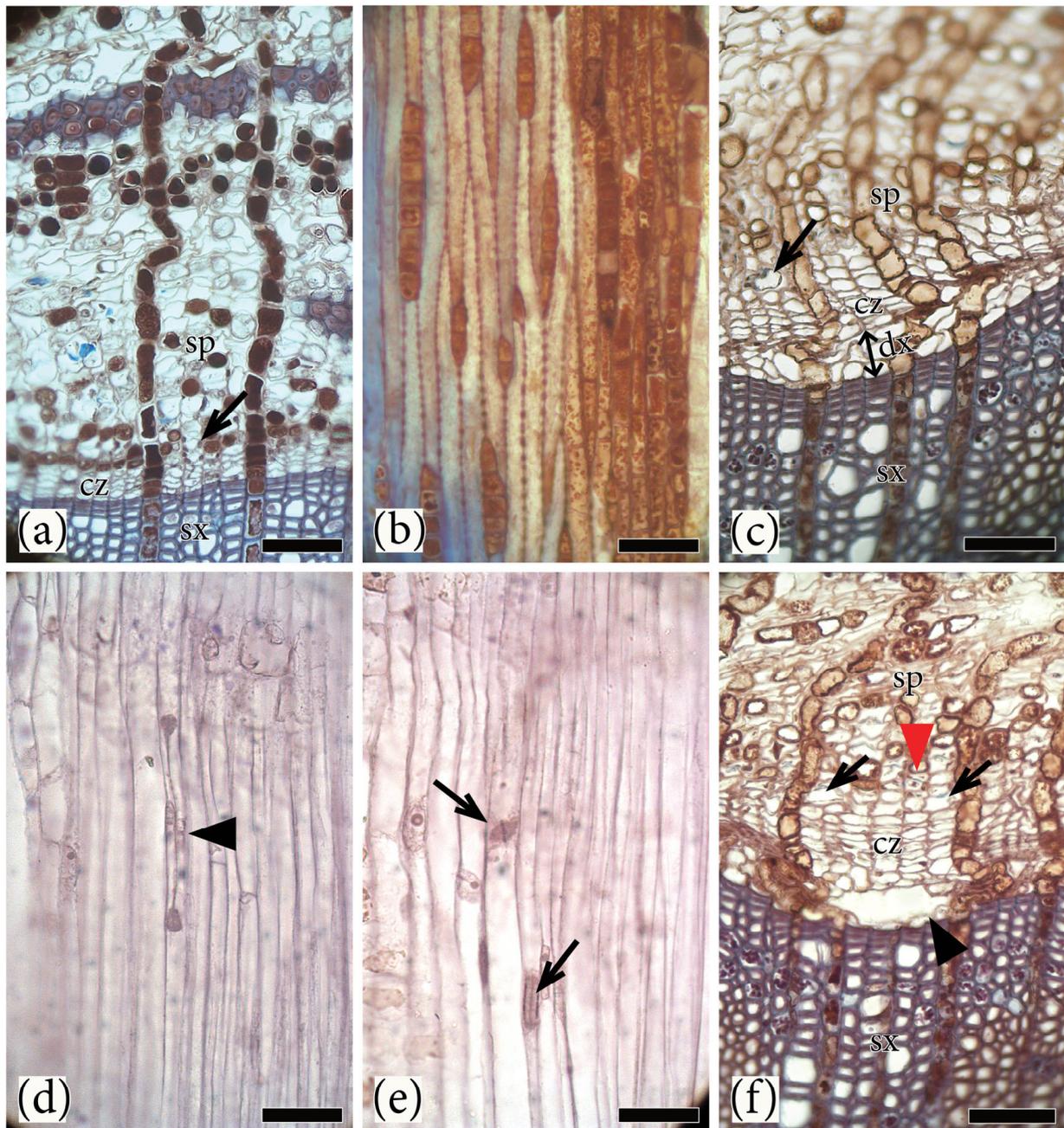


Figure 4. (a) Transverse section. At this stage, the cambium is dormant and consists of 5–6 layers of fusiform cells. Mature late phloem sieve elements (arrow) overwinter on the outer margin of the cambial zone (cz). Their dead counterparts from the previous year have collapsed and can no longer be identified. Both axial and ray parenchyma cells contain dark contents, probably tannins (Collected 2 December 1961). (b) Tangential section of dormant cambium. The deeply depressed primary pit-fields of the cell walls give these walls a distinctive beaded appearance (Collected 21 October 1961). (c) Transverse section. The now active cambium (cz) is adding cells to the secondary phloem (sp) and secondary xylem (sx). Arrows points to an early phloem sieve element (Collected 27 April 1962). (d,e) Radial sections of active cambial zone showing phragmoplast (arrowhead) in (d) and mitotic figures (arrows) in (e) (Both collected 27 April 1962). (f) Transverse section. Whereas an early xylem vessel is just beginning to expand (black arrowhead), some new sieve elements are already mature (arrows). The red arrowhead is pointing to a late phloem sieve element that overwintered. It is still living (Collected 27 April 1962). Additional acronyms: dx = differentiating xylem, sp = secondary phloem. Scale bars: (a–c,f) = 50 μm , (d,e) = 20 μm .

3.2. Seasonal Cycle of the Cambium, Wood, and Secondary Phloem Development

The cambium of *Quercus alba* is dormant from late August to early April. During this period, the cambial zone ranges from 5–8 layers of fusiform cells (Figure 4a). In tangential sections, the primary pit-fields in the radial walls of these cells have a distinctive beaded appearance (Figure 4b). During dormancy, the only living sieve elements found in the bark are the radially narrow sieve elements found next to the cambial zone. These narrow sieve elements persist alive, and presumably functional, throughout the new growing season. They cease to function in the fall.

The cambium resumes its activity in early April. The first indication of cambial activity is a small radial enlargement of the cambial cells (Figure 4c), followed by cell divisions, detectable by the presence of phragmoplasts (Figure 4d) and mitotic figures (Figure 4e). The number of cells in the cambial zone remains more or less constant year round, with 5–8 layers of fusiform cells. Some of the first divisions are found next to the narrow, overwintering sieve elements (Figure 4f). With the resumption of cambial activity, these sieve elements are carried outward with the new season's early phloem and represent the first functional sieve elements of the new year. By the end of April, all layers of cells in the cambial zone at one site or another are dividing, most frequently in median cells of the cambial zone (Figure 4c). At that time, 1–3 rows of new sieve elements are differentiating, as evidenced by the presence of dispersed P-protein and callose at the developing sieve plates. At the same time, vessel elements are beginning to expand (Figure 4f).

In early May, the first expanded vessels undergo secondary wall deposition (Figure 5a). By that time, 2–3 rows of new sieve elements are mature (Figure 5b), and new rows of sieve elements are differentiating. By the end of May, 5–7 rows of new sieve elements are mature (Figure 5c), and 1–2 rows of new sieve elements are differentiating (Figure 5c).

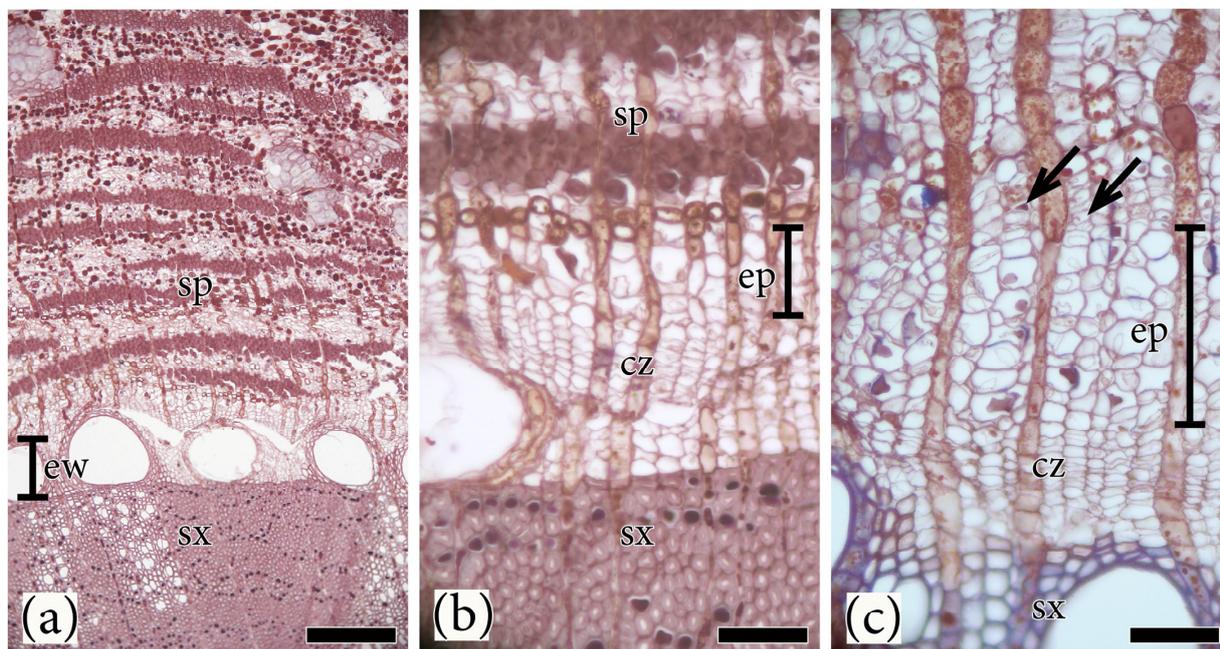


Figure 5. Cont.

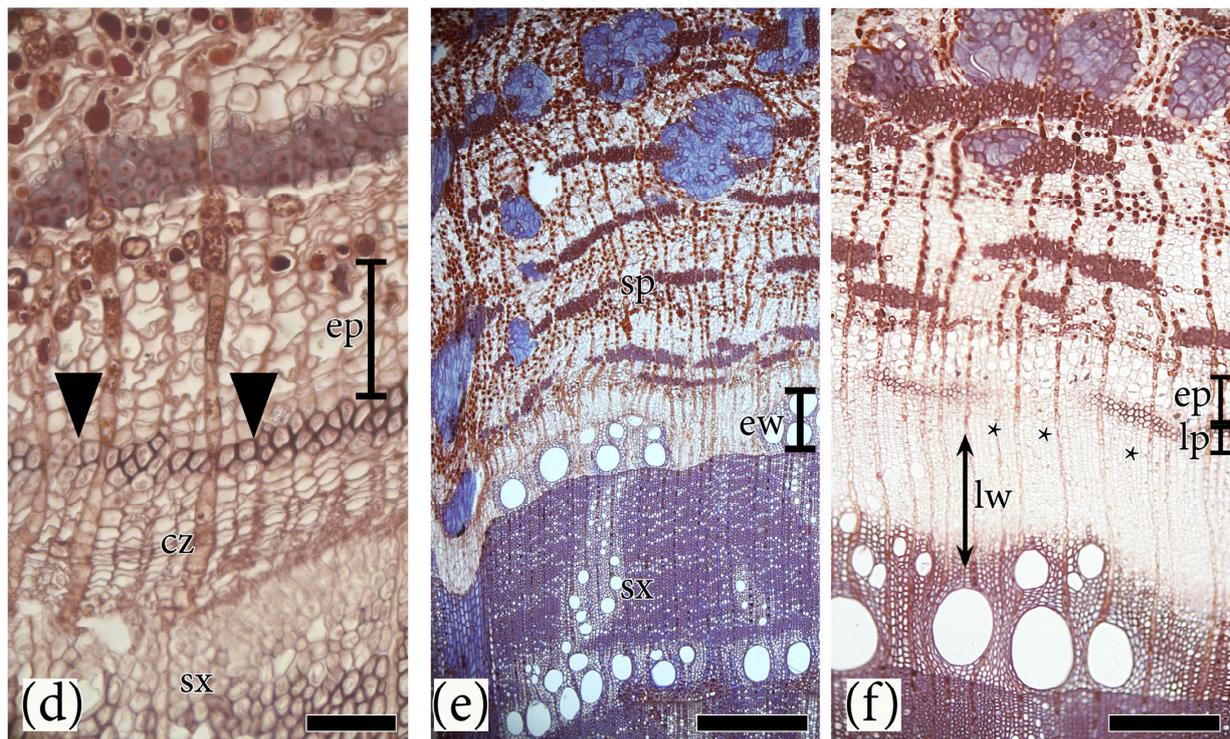


Figure 5. All transverse sections. (a,b) The initiation of earlywood (ew) differentiation and early phloem (ep) differentiation occurs simultaneously. (b) Early phloem (ep) with 2–3 layers of mature sieve elements (Both (a,b) collected 5 May 1962). (c) Early phloem (ep) now with 5–7 layers of mature sieve elements. Arrows point to narrow late phloem sieve elements that overwintered. They are still living (Collected 23 May 1962). (d) Here a developing fiber band (arrowheads) marks the beginning of late phloem (lp) development. Latewood formation has also begun on the xylem side (sx) of the cambial zone (cz) of some specimens (Collected 7 June 1962). (e) Earlywood (ew) development well along (Collected 7 June 1962). (f) Latewood (lw) formation well along. The cambium (asterisks) is actively forming derivatives to both sides (Collected 13 June 1962). Additional acronym: sp = secondary phloem. Scale bars: (a,f) = 200 μm , (b–d) = 50 μm , (e) = 400 μm .

In early June, a new fiber band begins to differentiate (Figure 5d), marking the onset of late phloem development. When present, the tangential bands of fibers may be used to delimit roughly yearly increments of growth (growth rings), as they separate early phloem from late phloem. However, in some individuals a fiber band may fail to form each year; in others, more than one may form in a given year (Figure 6e). Returning to the xylem side of the cambium in early June, the last earlywood vessels are fully expanded (Figure 5e) and latewood elements are beginning to develop. By the end of June, all earlywood is mature and latewood formation is well advanced (Figure 5f).

In early July, approximately 5–6 rows of early phloem and 4–5 rows of late phloem sieve elements are mature, the early sieve elements being somewhat wider than those of the late phloem (Figure 6a,d). Sclerification of the aggregate rays occurs in both the present and previous years' phloem increments (Figure 6b). Scleroid clusters, which are derived from axial parenchyma cells (Figure 6b), occur only in nonconducting phloem, where they may become very extensive (Figure 6c). By late July, both xylem and phloem differentiation are nearing completion (Figure 6b,c). The last sieve elements to be accounted for are the radially narrow ones that overwinter next to the cambial zone. As noted previously, with the reactivation of the cambium, these sieve elements are carried outward by the new year's phloem increment. There, these sieve elements remain in a functional state until cessation of function begins with them in early October. Cessation of function then spreads from them toward their counterparts next to the cambial zone. Similarly to their counterparts, these sieve elements will function for a second season.

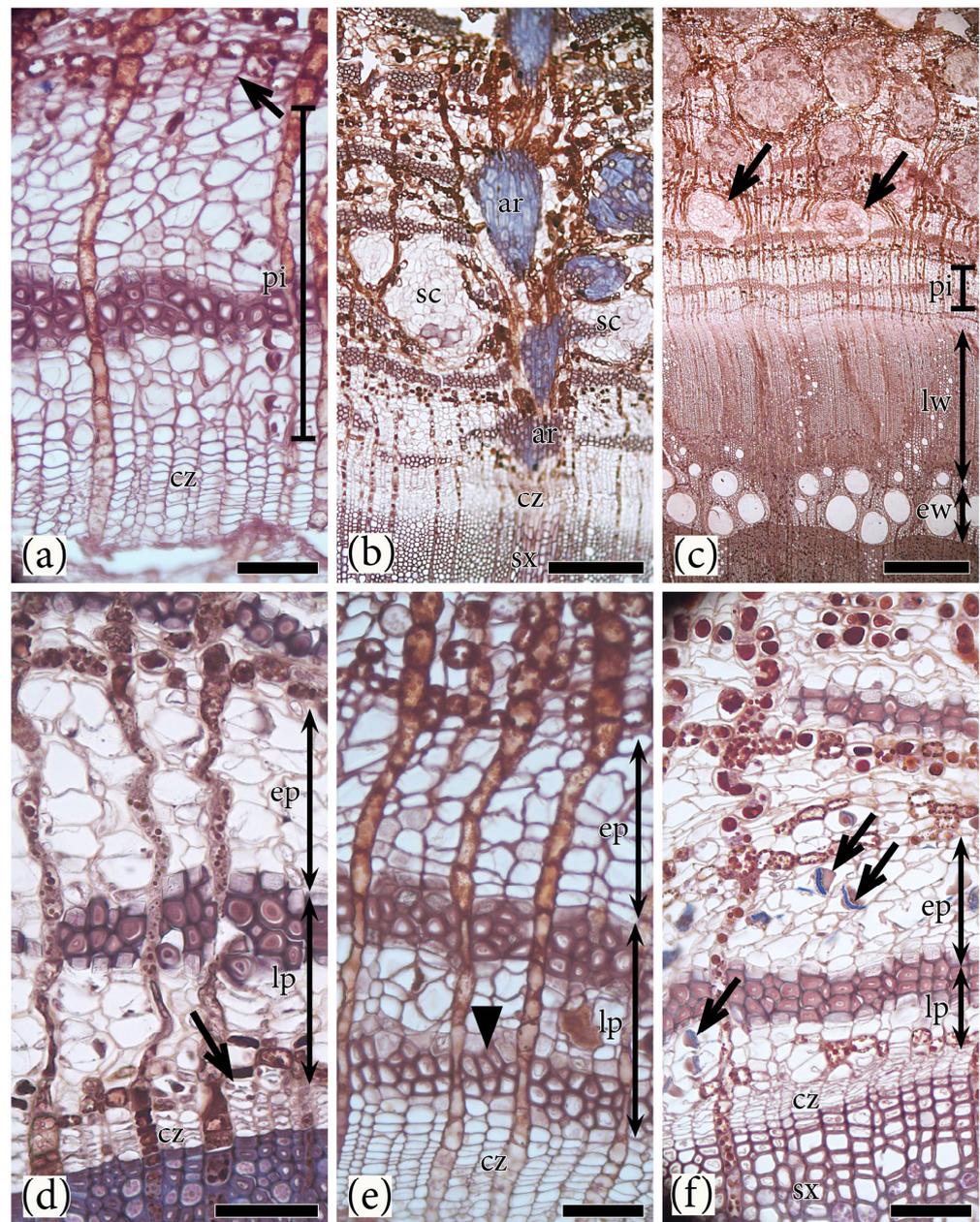


Figure 6. All transverse sections. (a) Almost all of the 1962 phloem increment (pi) has been formed. Radially narrow sieve elements of the previous year's phloem increment (1961) are still turgid (arrow). Practically the entire 1962 phloem increment has formed (Collected 5 July 1962). (b) Clusters of sclereids (sc) have developed from axial parenchyma cells in nonconducting phloem and the aggregate ray (ar) has sclerified (Collected 12 July 1962). (c) Current xylem and phloem increments are nearing completion. Numerous sclereid clusters (arrows) are present in nonconducting phloem (Collected 21 July 1962). (d) Radially narrow terminal sieve elements (arrow) next to the cambial zone (cz). These sieve elements mark the end of the 1962 phloem increment (Collected 22 September 1962). (e) Some phloem increments contain two fiber bands. Both bands shown here are still depositing secondary walls, most notably the band (arrowhead) nearest the cambial zone (cz) (Collected 5 July 1962). (f) Apparently definitive callose (blue stain, arrows) has been deposited on the sieve plates of some sieve elements in both early phloem (ep) and late phloem (lp). (Collected 2 August 1962). Additional acronyms: ep = early phloem, ew = earlywood, lp = late phloem, lw = latewood, sx = secondary xylem. Scale bars: (a,d–f) = 50 μm , (b) = 200 μm , (c) = 400 μm .

4. Discussion

4.1. Latewood and Late Phloem Are Produced While Environmental Conditions Are Still Favorable

The cambium of *Quercus alba* growing in Madison, Wisconsin, is active for about four months, activating in early April, when the photoperiod is around 12 h/day and increasing daily, and mean temperatures are well past those of around the time of the last frost date (Figure 7).

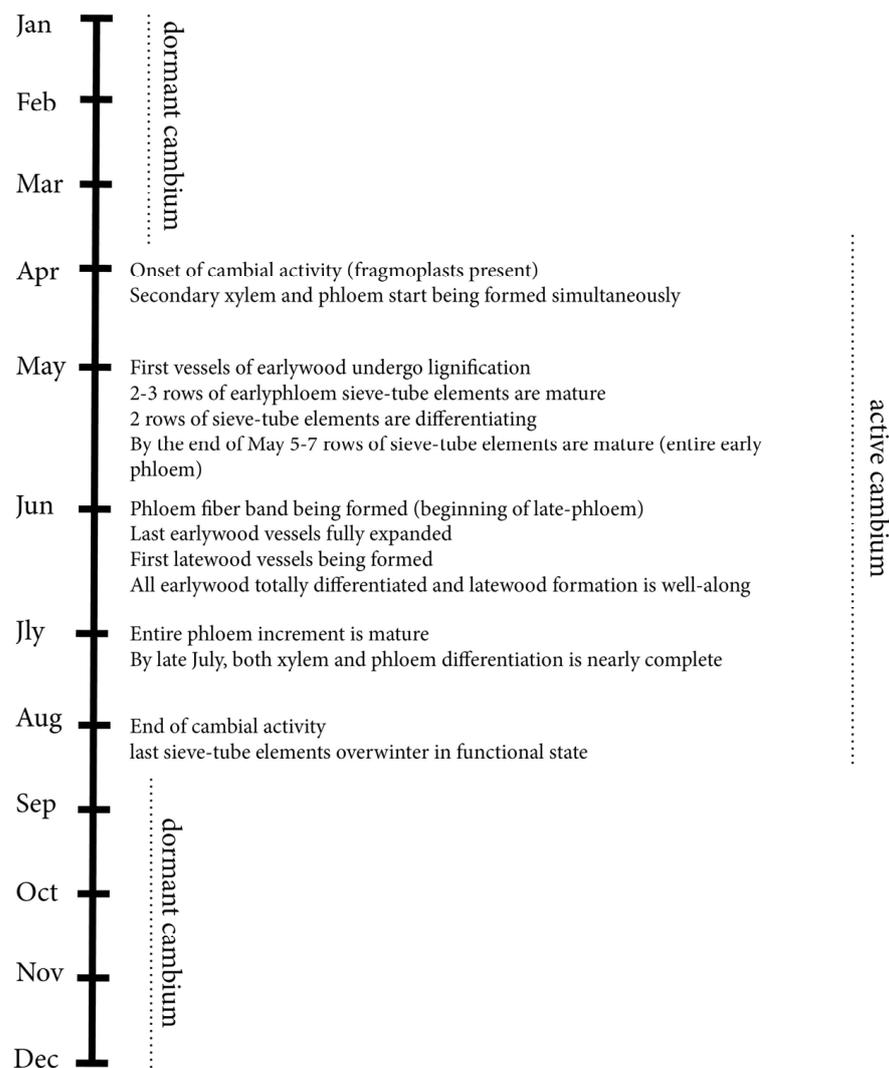


Figure 7. Summary of the events happening across the four months in which the cambium of *Quercus alba* is active.

We considered only the presence of mitotic figures and phragmoplasts as reliable indicators of cambial activity. Increasingly, more studies have shown that the number of cells in the cambial zone, or cells differentiating next to it, are not reliable indicators of cambial activity [30–32]. Moreover, not all species present fluctuating numbers of cells in the cambial zone, while in others undifferentiated cells overwinter next to the cambium [11,32–35]. Cambial activity in *Quercus alba* stops in early August, when daylength is still quite high (above 13 h/day), although already declining, and mean temperatures are as high as in the previous two months. Water availability fluctuates throughout the year, similarly. Latewood and late phloem production begin in early June and end in mid-August, when external conditions are the most favorable. This phenomenon is not unique to *Quercus alba*. It has been documented in all temperate species studied thus far, both angiosperms and conifers, trees and lianas [9–14,36,37]. Therefore, it is not the low temperatures or

water deficit that trigger the formation of narrower latewood and late phloem cells but most likely a matter of photoperiodism, something likely regulated hormonally [38]. The photoperiod has been shown to be the main trigger in previous studies of seasonal cambial activity [30,39].

Ring-porous and semi-ring-porous species have long been recognized as prime examples of climatic adaptation [1–3,5]. During the favorable season, wide vessels allow for maximum conductivity, while during the unfavorable season, narrow vessels maintain conductivity, being safer [1–3]. This has been supported by physiological studies that show that wide vessels are more vulnerable to embolism triggered by low temperatures and frost, explaining the selective pressure to latewoods with narrower vessels and tracheids, which are safer against embolism [40–43]. Nonetheless, ring porosity and semi-ring porosity are not excluded from the tropics. In the semi-deciduous tree *Cedrela fissilis* Vell. (Meliaceae) growing in Brazil, the narrow vessels are produced during the favorable season in early March (Southern-hemisphere summer), when water is at a maximum in the soil [44]. Another even more curious case was registered for the liana *Dalbergia frutescens* (Vell.) Britton (Leguminosae), where on the same stem and at the same time of the year, one side of the stem was producing wide vessels while the opposite side was producing small vessels of the latewood [45]. As the stem of lianas is commonly inclined, the authors suggested auxin imbalance in the stem [45]. We do not know how frequently something like this occurs in trees, since periodical collections of an entire transverse section are not feasible with them. This clearly evidences that both for plants growing in the temperate zone and in the tropics, formation of latewood (and late phloem) occurs prior to the selective regime. While narrow vessels have been accepted as safer against embolism caused by frost [40–43], the same is not true for embolism caused by drought [41,46], posing a problem. For decades, based on comparative wood anatomical studies, especially those championed by Carlquist [1,47,48], researchers have proposed that narrow vessels would be safer against drought embolism [49,50], such as the embolisms caused by frost. However, nowadays there is a heated debate among experimental wood anatomists, where one group's experiments seem to corroborate the hypothesis that narrow vessels are safer against drought embolism [49,50], while another's vehemently dispute that conclusion, demonstrating by their experiments that the decisive character promoting safety against drought embolism is the thickness of intervessel pit membranes [46]. The narrow vessels of tropical ring-porous species were suggested to occur as a response to lack of water by the latter authors, impairing the full expansion of the cells [46]. However, similarly to temperate species, tropical species also form the latewood long before the selective regime, contradicting this idea and suggesting a possible selective importance to narrow vessels in the tropical dry seasons as well. This scenario merits additional tests [48].

4.2. Synchronicity in the Formation of Cambial Products and Growth Rings

Previous seasonal studies indicate a correlation between porosity type and the relative time during which either phloem or xylem begins to be produced by the cambium [9–14]. Diffuse-porous species typically exhibit the initiation of phloem formation before that of xylem, commonly by a month or a month and a half, which is the case of apple (*Malus domestica* (Suckow) Borkh., Rosaceae) [11], poplar (*Populus tremuloides* Michx., Salicaceae) [12], and maple (*Acer negundo* L., Sapindaceae) [14]. On the other hand, in semi-ring and ring-porous species, such as pecan (*Carya illinoensis* (Wangenh.) K. Koch, Juglandaceae) and black locust (*Robinia pseudoacacia* L., Leguminosae), the production of xylem and phloem begin simultaneously [9], or nearly so [10]. The initiation of secondary xylem production before that of phloem has, to the best of our knowledge, been reported only for species growing in semi-arid or tropical regions, such as for guayule (*Parthenium argentatum* A. Gray, Asteraceae) from the Chihuahuan desert [15], *Mimusops elengi* Wight (Leguminosae), from tropical India [16], and the liana *Dalbergia frutescens* (Leguminosae), from southeastern Brazil [45]. We found *Quercus alba* to start producing both xylem and phloem simultaneously, corroborating the studies with temperate ring-porous species [9,10]. In

addition, *Quercus alba* behaves similarly to *Quercus robur* L. and *Quercus pyrenaica* Willd. growing in Galicia (Spain), with no overwintering undifferentiated xylem elements [51].

In *Quercus alba*, both wood and secondary phloem form growth rings. With regards to the wood, this is a well-known phenomenon for deciduous oaks, where the earlywood is marked by wide, solitary vessels and the latewood by dendritically arranged vessels and tracheids, in addition to thick-walled, radially narrow fibers [52–55]. For the phloem, our study found less-distinct growth rings, marked by somewhat wider sieve elements in the early phloem and narrower sieve elements in the late phloem, and also terminal, narrow sieve elements, commonly arranged in short radial rows, associated with a narrow band of axial parenchyma with abundant tannins. Since generally a single fiber band is produced every year, the fiber bands may be taken as an indirect growth marker. The direct growth markers are the radially flattened sieve elements and the narrow parenchymatic band. The narrow parenchymatic band is very conspicuous and, in addition, has been used to identify growth rings in the Turkish species *Quercus cerris* L. var. *cerris* [56]. The formation of a single fiber band per season was also recorded in the sessile oak (*Quercus petraea*) growing in Slovenia, and the authors were also able to estimate the yearly phloem increments by counting them [57]. For the Mediterranean holm oak (*Quercus ilex* L.), seasonal studies showed the formation of three fiber bands per year [58]. In our study, however, sometimes two fiber bands or none were formed in certain specimens, making the fiber bands' growth marker less reliable. The same situation was recorded for *Cedrela fissilis* (Meliaceae) [32], where generally a single fiber band was formed every year, but not always. For *Robinia pseudoacacia* multiple fiber bands were produced annually, but the first one was always thicker, allowing therefore for the recognition of growth rings across the entire bark, even in the nonconducting phloem [10].

4.3. Sieve Elements' Longevity, Phloem and Wood Anatomical Features

As a conductive tissue, the secondary phloem of *Quercus alba* is short-lived [Figures 4a and 6f]. With the exception of the narrow sieve elements that overwinter next to the cambial zone, by October–November all other sieve elements produced during the growing season will be devoid of contents, except for definitive callose. This same growth pattern has been recorded for other *Quercus* species [57,58]. Thus, before cambial activity resumes in the spring, the only functional sieve elements in the bark are the narrow ones that overwintered. These same sieve elements are eventually carried outward by the new year's phloem increment. They will be the first sieve elements to become nonfunctional in the fall, as cessation of function spreads toward their overwintering counterparts near the cambial zone.

Overwintering sieve elements are common both in angiosperms and gymnosperms, and were shown experimentally to start conducting even before cambial reactivation [59,60]. In addition to *Quercus*, mature overwintering sieve elements were also recorded in *Ulmus* [61] and *Acer* [14]. In other plants, immature sieve elements may overwinter next to the cambial zone [62]. Short-lived sieve tubes functioning for just one season seems to be the most common pattern [18,63], although in a few cases the sieve tubes may be conductive for at least two growing seasons both in the temperate (e.g., poplar and grape [12,36]) and tropical regions [32], and more rarely even up to five or 10 years (*Tilia americana* L. and *T. cordata* Mill. [33,64]).

In terms of wood and bark anatomy, *Quercus alba* exhibits the typical features of deciduous, temperate white oaks, with wood marked by ring-porosity; wide earlywood solitary vessels surrounded by vasicentric tracheids; development of tyloses by the end of the growing season; latewood vessels in dendritic arrangement, associated with vascular tracheids; apotracheal axial parenchyma diffuse to diffuse-in-aggregates forming lines; paratracheal axial parenchyma vasicentric, quite abundant in the earlywood; a combination of uniseriate and aggregate homocellular rays; and simple vessel-ray pits [52–54]. Oak woods can be very homogeneous, making their distinction a long-standing problem for wood anatomists [65,66]. The presence of vasicentric tracheids around solitary vessels

is a common pattern and thought to provide a bypass mechanism against embolism by vulnerable, wide vessels [67–70]. Experimental studies support a correlation between the percentage of vasicentric tracheids and vulnerability curves in *Eucalyptus* [71]. Aggregate rays are well known for the entire order Fagales, being especially common in oaks, where they can be uniseriate or multiseriate aggregate rays [52,53,65], being present both in wood and secondary phloem. In secondary phloem they very quickly undergo sclerification, forming large sclereid clusters already in the conducting phloem [58,72,73]. The secondary phloem of *Quercus alba* is also typical for the genus, with stratified phloem marked by the alternation of fiber bands and non-fibrous tissues (axial parenchyma and sieve tubes), and with the formation of large sclereid clusters in the nonconducting phloem, rays of two distinct sizes, and crystals in chambered axial parenchyma cells [52,56,58,72–76]. The crystal-containing cells are associated with the fiber bands. Only the part of the parenchyma in contact with the fibers becomes lignified. A similar development has been reported for many different species [63].

5. Conclusions

This work shows that white oaks produce growth rings both in wood and secondary phloem. Phloem growth rings are delimited by radially narrow sieve elements interspersed with bands of parenchyma cells with dark contents as direct markers, and fiber bands as indirect markers. Both latewood and late phloem are produced during the summer, long before the beginning of autumn and lower temperatures, suggesting that the trees “anticipate” the selective regime, and the main seasonal trigger may be the photoperiod. Sieve elements are short-lived, being active for just one growing season, except for the radially narrow sieve elements, which overwinter next to the cambial zone and remain functional throughout the next growing season.

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