Review

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Coordination of spring vascular and organ phenology in deciduous angiosperms growing in seasonally cold climates

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Summary

In seasonally cold climates, many woody plants tolerate chilling and freezing temperatures by ceasing growth, shedding leaves and entering dormancy. At the same time, transport within these plants often decreases as the vascular system exhibits reduced functionality. As spring growth requires water and nutrients, we ask the question: how much does bud, leaf and flower development depend on the vasculature in spring? In this review, we present what is known about leaf, flower and vascular phenology to sort out this question. In early stages of bud development, buds rely on internal resources and do not appear to require vascular support. The situation changes during organ expansion, after leaves and flowers reconnect to the stem vascular system. However, there are major gaps in our understanding of the timing of vascular development, especially regarding the phloem, as well as the synchronization among leaves, flowers, stem and root vasculature. We believe these gaps are mainly the outcome of research completed in silo and urge future work to take a more integrative approach. We highlight current challenges and propose future directions to make rapid progress on this important topic in upcoming years.

I. Introduction

Phenology plays a critical role at many different levels of biological organization. At the ecosystem level, it impacts agroecosystem processes such as productivity, water and energy exchanges (Cleland *et al.*, 2007) and thus feedbacks of the biosphere to the atmosphere (Richardson *et al.*, 2013). At the species level, phenology impacts species performance and distributions (Chuine, 2010; Gaüzère *et al.*, 2020) along with interspecific interactions and food webs (Renner & Zohner, 2018). At the population and individual levels, phenology mediates the impact of biotic and abiotic conditions on a plant's growth, survival and reproduction

(Forrest & Miller-Rushing, 2010). And finally, within an individual, the timing of changes in organ function impacts patterns of resource allocation and growth. Despite the importance of phenology at all of these scales, we are only beginning to understand the physiological basis of large changes in plant growth like leaf out and flowering, and we have a limited understanding of physiological and developmental constraints on phenology (Duputié *et al.*, 2015; Fahey, 2016), a topic that is becoming more important in the light of growing concern about the impact of climate change on ecosystems (Richardson *et al.*, 2013; Renner & Zohner, 2018). To make reliable projections for the future about phenology and the range of processes it influences, it is necessary to determine the internal and external factors that control and regulate plant phenology.

In seasonally cold environments, plants go through successive periods of dormancy in winter and resumption of growth in the spring, which is often marked by bud break (see Box 1 for a glossary of terms used in this review). Growth resumption requires remobilization of resources (water, nutrients) and transport of signaling molecules (hormones and proteins) regulating development between sources (soil, roots, stems, branches) and sinks (growing buds, leaves, flowers, fruits), when nutrient demands exceed locally available storage. This often necessitates that the plant vascular system (xylem and phloem) be at least partly functional at the beginning and optimally functional at the peak of growth. However, in seasonally cold environments, the vascular system often does not remain fully functional throughout winter. Indeed, phloem conduits (sieve tubes) and meristematic cells are susceptible to freezing damage like other living cells (Cavender-Bares, 2005), and xylem conduits (vessels) are prone to embolism triggered by freezing (Cochard & Tyree, 1990; Sperry & Sullivan, 1992; Fig. 1). Therefore, in many plant species, the vascular system exhibits reduced function in the winter and transport has to be reactivated in the spring through the production of new xylem and phloem conduits, maturation of immature conduits and/or repair of the existing vascular system, all of which require water and/or nutrients.

Because of the presumed functional dependency of developing new organs on the vascular system, some authors have suggested that there might be a relationship between leaf phenology (the timing of leaf out) and wood anatomy that is driven by differences in the timing of vascular reactivation in the spring (Lechowicz, 1984; Wang *et al.*, 1992). However, most studies in the last 20 yr have focused either on bud, leaf and flower phenology or on vascular system development and its regulation by internal and external drivers; it is less common for studies to link multiple organs and/or physiological processes (except see Suzuki *et al.*, 1996; Kudo *et al.*, 2015; Gričar *et al.*, 2017; Lavrič *et al.*, 2017; Savage, 2019). Thus, today many questions remain regarding whether there is a strong relationship between whole-organ phenology (bud, leaf and flower phenology) and that of the vascular system in the spring. There is no consensus on how and when the bud transport system reconnects to the stem vascular system and whether this reconnection takes place before or after dormancy break or whether one precludes the other. It is also uncertain how much leaf and floral bud development is dependent on the stem and root vascular system and how the reactivation and development of the two overlap and interplay.

Box 1 Glossary.

Anthesis – when a flower exhibits pollen release and/or stigma receptivity.
Apoplast – area external to plasma membranes including space inside open vessels in the xylem.
Bud break – when leaf or floral tissue is first visible emerging from the bud.
Callose – polysaccharide that builds up along plasmodesmata and sieve plates, seasonally and during stress and certain developmental stages.
Diffuse-porous wood – wood type with uniform vessel size throughout the year.
Ecodormancy – state during which development and metabolism are inhibited by external factors (e.g. temperature, photoperiod).
Endodormancy – state during which development and metabolism are inhibited by internal factors (e.g. circadian clock, hormonal controls).
Paradormancy – state during which development and metabolism are inhibited by competition for nutrients and water used by strong sinks (e.g. leaves, fruits).
Phenophases – stage during annual development, for example anthesis or bud break.
Leaf out – when leaf blade is reflexed and the whole leaf and petiole are visible.
Leaf expansion – when leaves are not full-sized and are expanding.
Leaf maturation – when leaves reach final size.
Phloem – vascular tissue that transports carbohydrates and signals from carbon sources to sinks.
Plasmodesmata – cell wall channels containing a strand of endoplasmic reticulum connecting the symplast of two adjacent cells.
Ring-porous wood – wood type with two vessel size classes: wide vessels formed in the spring and small vessels formed later in the year.
Semi-ring porous wood – wood type with a gradual transition from wide early vessels to narrow late vessels.
Sieve element – phloem transport cells with reduced cellular components that form sieve tubes, also called sieve tube elements.
Symplast – space inside the plasma membrane where water and low-molecular-weight solutes can freely diffuse.
Vessel elements – xylem transport cells without a plasma membrane at maturity that form vessels.
Xylem – vascular tissue that transports water and nutrients from the roots to transpiring organs including leaves and flowers.

In this review, we clarify the relationships between development of buds, leaves, and flowers and changes in the vascular system. We restrict the review to broadleaf deciduous angiosperms living in seasonally cold environments because these species experience a large flush of new growth in the early spring when the vascular system is potentially the most compromised. We break down the transport pathway into three pieces, all which have to be functional to meet high resource demands of growing organs: the bud, stem and root vasculature. We treat these three pieces separately because there are limited data on how physiological changes are coordinated among them. However, we draw parallels and connections between them when possible. We aim to answer the following questions: are bud endodormancy break and bud break dependent on the vascular

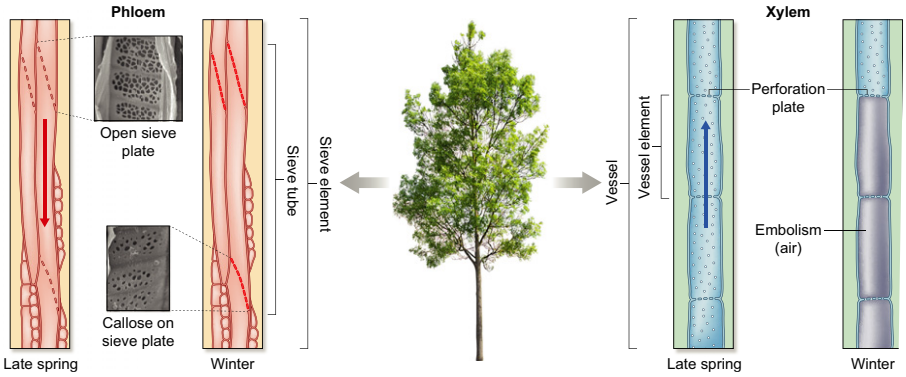


Fig. 1 Seasonal changes in xylem and phloem function. In the phloem, a sugar-rich sap is transported in conduits (sieve tubes) made of stacked cells (sieve elements) connected by porous plates (sieve plates with sieve pores). Because these cells have an intact plasma membrane at maturity that is continuous within the tube, transport occurs within the symplast (red arrow). During the growing season, sap flow in the phloem is driven by a positive pressure gradient between carbon sources and sinks in the tree. However, in the winter, transport is reduced because of increased sap viscosity, decreased sink and source activity, and occlusion of sieve pores with callose (red dashed line). In the xylem, water and nutrients are transported in conduits (vessels) composed of stacked cells (vessel elements) that are often completely continuous with each other because of open perforation plates. Because vessels do not have an intact plasma membrane at maturity, transport occurs in the apoplast and is driven by a negative water potential gradient from the roots to the canopy (blue arrow). In the winter, xylem sap can freeze, causing the formation of bubbles. These bubbles can expand and cause embolisms, decreasing transport capacity in the xylem after thawing. Images are based on the anatomy of *Populus tremuloides*.

system; are there differences between leaves and flowers in this dependency on the vascular system; and what is the variability in the synchronization between organs and vascular system development among species?

II. Winter and spring phenology: transition from dormancy to growth

1. Endodormancy and winter physiology: the shutdown of transport pathways

In most temperate tree species, leaf and sometimes flower buds are formed during the summer of the previous growing season and remain dormant throughout the winter. Dormancy probably evolved to tolerate periodic adverse growing conditions in extratropical climates. In the winter, temperature and light conditions are not optimal for growth and subzero temperatures can lead to freezing and desiccation damage (Neuner, 2014). Therefore, dormancy is often marked by a decrease in metabolic function and transport (Rinne & van der Schoot, 2003; Rinne & van der Schoot, 2004). There are three successive stages of dormancy called paradormancy, endodormancy and ecodormancy (Lang *et al.*, 1987; Anderson *et al.*, 2010). Both para- and endodormancy correspond to physiological states during which general development and metabolism are inhibited by internal factors (e.g. hormones, transcriptional factors). While paradormancy is mainly a result of the dominance effect of active sinks of nutrients and water, such as functional leaves and growing fruits (e.g. apical dominance), endodormancy is under complex internal regulation pathways that are not yet fully elucidated (Allona *et al.*, 2008; Campoy *et al.*, 2011; van der Schoot *et al.*, 2014). Ecodormancy is regulated by extrinsic factors (e.g. temperature and photoperiod) and is the last period of dormancy in the spring.

There are four potential pathways of exchange between buds, the stem and the root system: two nonvascular (the symplast and apoplast in and around cells) and two vascular (the phloem and xylem). During endodormancy, all these pathways probably have reduced function in the bud (Fig. 2). First, callose disrupts the symplastic pathway in all cells including sieve elements. Callose also decreases the concentrations of oxygen, water, nutrients and other molecules by blocking the plasmodesmata of all meristematic cells, and thus prevents or decreases metabolic and physiological activities in these cells (for a review see Rinne & van der Schoot, 2003). Second, water transport in the apoplast can be limited by small intracellular spaces (Quamme *et al.*, 1995). Third, movement of water between the apoplast and symplast is minimized as a result of low aquaporin expression (Yooyongwech *et al.*, 2008). Lastly, water transport in the xylem is disrupted at the junction between the bud and the twig, which minimizes any exchange through the vascular tissue (Xie *et al.*, 2018). It is not clear whether this disruption is a result of immature xylem cells in the junction, as Xie *et al.* (2018) suggested, or of other types of obstruction including modifications to cell walls (Flinn & Ashworth, 1994; Jones *et al.*, 2000), occlusion with a tannin-like substance (Goodwin, 1967) and/or embolism. In general, the release of endodormancy seems to be related to the opening of some pathways, but which pathways and when each pathway becomes functional remain unclear.

As in the buds, transport in the vascular tissue (xylem and phloem) throughout the plant exhibits reduced activity in the autumn, winter and following spring. In the phloem, sap flow is reduced because of low metabolic activity and growth in sinks, and a build-up of sap viscosity (for a review see Cavender-Bares, 2005). In many plants during endodormancy, sieve elements show a heavy deposit of callose on sieve plates and lateral pores and many sieve tubes collapse in the trunk, stems and twigs (Evert, 1990; Aloni & Peterson, 1997). As a result, the amount of sap transported in sieve

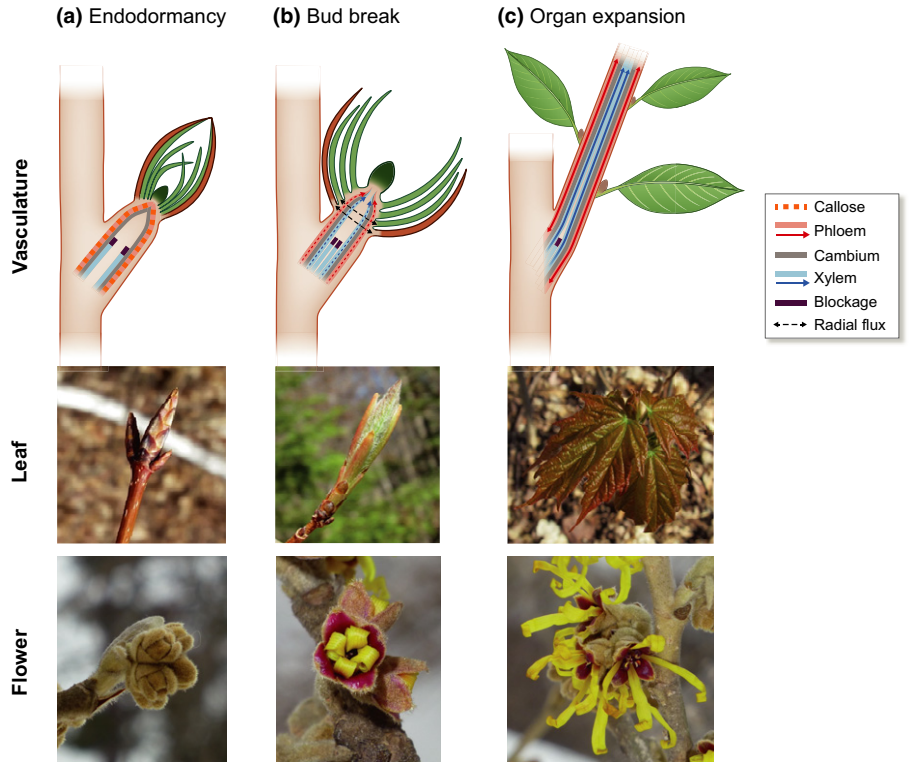


Fig. 2 Vascular, leaf and flower phenology during the winter and spring. (a) When leaf and flower buds are endodormant, vascular transport into the bud is greatly reduced in the phloem (in orange) by callose build-up (red boxes) and in the xylem (in blue) by blockage of vessels by embolism, cell wall modification and/or build-up of tannin-like substances at the junction with bud (black boxes). At this stage, the cambium (in gray) remains inactive. The vasculature of a leaf bud is imaged with brown bud scales (brown), green immature leaves and a darker green apical meristem. (b) At bud break, transport may resume in the stem xylem (dashed blue arrows) and phloem (dashed red arrows), but the exact nature of this transport is unclear. Essiamah & Eschrich (1986) suggested that in the early stages of development, bud cells can rely on a remobilization of water and nutrients within the bud through radial fluxes (black dashed arrows). Other studies have proposed that the xylem is a major contributor to carbon transport and water backflow occurs in the phloem (Tixier *et al*., 2017). However, more research is needed to understand vascular transport at this stage. (c) When organs begin expanding and maturing, transport capacity in the xylem (blue arrows) and phloem (red arrows) increases. The xylem becomes the major transport pathway for moving water into the organ and the phloem either delivers or removes sugars depending on whether the organ is a carbon source or sink. Leaf and flower phenology photographs are from *Acer saccharinum* and *Hamamelis mollis*, respectively.

elements during the winter and early spring in the stem decreases (Aloni *et al*., 1991; Aloni & Peterson, 1997). However, there are large differences between species in the amount of callose that accumulates on sieve plates and the functional status of mature sieve tubes during the winter (Evert, 2006). Assessment of callose accumulation can also be influenced by sampling procedure, making it hard to generalize across studies (Montwé *et al*., 2019).

Transport is reduced in the xylem, similar to the phloem, but as a result of different processes. In the autumn, transpirational area decreases in deciduous species, which leads to a drop in the driving gradient for xylem transport. At the end of the growing season, when water in the xylem freezes, dissolved gases come out of solution, forming bubbles. If these bubbles expand and form embolisms, they can lead to reduced hydraulic conductivity (for a review see Cochard, 2006). As a result, in plants that experience a large amount of embolism in the winter, xylem function often remains limited until the formation of new vessels from the cambial initials during xylogenesis (Evert, 2006). The main exceptions are some species that exhibit refilling, for example, through positive xylem root pressure in the spring (Hacke & Sauter, 1996; Hao *et al*., 2013). With the xylem, it is also important to note that local sources of water in twigs and stems can support early growth (Kudo *et al*., 2015), but high rates of water movement can only occur after xylem function is restored throughout the plant, allowing for a continuous pathway for water movement from the soil.

The majority of research on vascular phenology is focused on stem material either in the main trunk or in the branches and twigs. Like leaves, some fine roots in cold climates can be short-lived and senesce in the autumn and winter (Hendrick & Pregitzer, 1993). However, root phenology can be asynchronous in different parts of the root system depending on root depth and size (Germon *et al*., 2020). In general, little is known about root vascular overwintering, but research suggests that roots do not undergo true dormancy like buds and stem cambium and that roots are able to grow at soil temperature as low as 5°C (Radville *et al*. 2016). Although callose is produced in roots in response to various stressors (chemical stress, pathogen agent infection) (Kortekamp *et al*., 1997), we found no studies that looked at callose build-up in root phloem in autumn and winter, leaving uncertainty about the degree of interconnectedness that remains in the roots throughout the year.

2. Resource use during organ development

After endodormancy release, there is an increase in the nutrient and water requirements of buds as organs resume development and buds begin to expand. Before budburst, water and biomass in the bud can increase up to four hundred-fold (Savage, 2019) and bud respiration spikes (Hatch & Walker, 1969; Young *et al.*, 1987). This increase in respiration often coincides with organ development, which resumes in many species after the end of endodormancy. For example, in *Actinidia deliciosa* (kiwifruit), bud respiration increases 3–6 wk before budburst, which is close to the time when flowers start forming in the bud (McPherson *et al.*, 1997). After budburst, resource use continues to increase as leaves and flowers require additional nutrients to support expansion and higher rates of respiration and water loss.

Although some of the initial water and nutrients required for supporting developing buds can come from stored reserves in branches and the stem, at some point, resource requirements exceed the supply capacity of nonvascular apoplastic and symplastic pathways. In deciduous species, it is clear that by the time of optimal cambial growth and leaf out, leaves need to be connected to a functional vascular system because of their large water and nutrient requirements (Savidge, 2001; Lacointe *et al.*, 2004; Lavrič *et al.*, 2017). The same is likely to be true for flowers, which can use 20–60% of the daily water required by leaves (Roddy *et al.*, 2016; Liu *et al.*, 2017) and may require extra carbon and nutrients to support attractants and rewards, including nectar (De la Barrera & Nobel, 2004). However, the importance of different transport pathways probably depends on the organ, its stage of development and the phenology of the vascular system. This is one of the reasons why it is important to understand the interplay between organ and vascular phenology. Changes in water and nutrient use need to be matched by an increase in vascular function at the appropriate time or spring growth could be limited. For this reason, we need to look at the timing of vascular reactivation in multiple parts of the plant to try to understand how they are connected to each other and how the vascular system and leaf/flower phenology impact each other (Fig. 3).

It is important to note that not all carbon used for organ growth needs to come from outside the bud, leaf or flower. Some plants have green buds that can fix carbon before budburst, but typically their total assimilation does not offset respiration until after budburst (Landhausser, 2011). Because young, expanding leaves have higher rates of respiration and lower rates of photosynthesis than fully expanded leaves (Jurik, 1986; Reich *et al.*, 1991), they are often heterotrophic. Once they are large enough, they transition to be carbon sources. Direct measurement using labeled carbon isotopes and estimates based on models suggest that between 44% and 68% of all the carbon in leaves comes from stored reserves, the remaining carbon coming directly from assimilation (Dyckmans *et al.*, 2000; Barbaroux *et al.*, 2003). During early leaf expansion, most stored carbon accessed for new growth comes from a branch close to the developing leaves but not from the bud itself (Tixier *et al.*, 2017; Furze *et al.*, 2019).

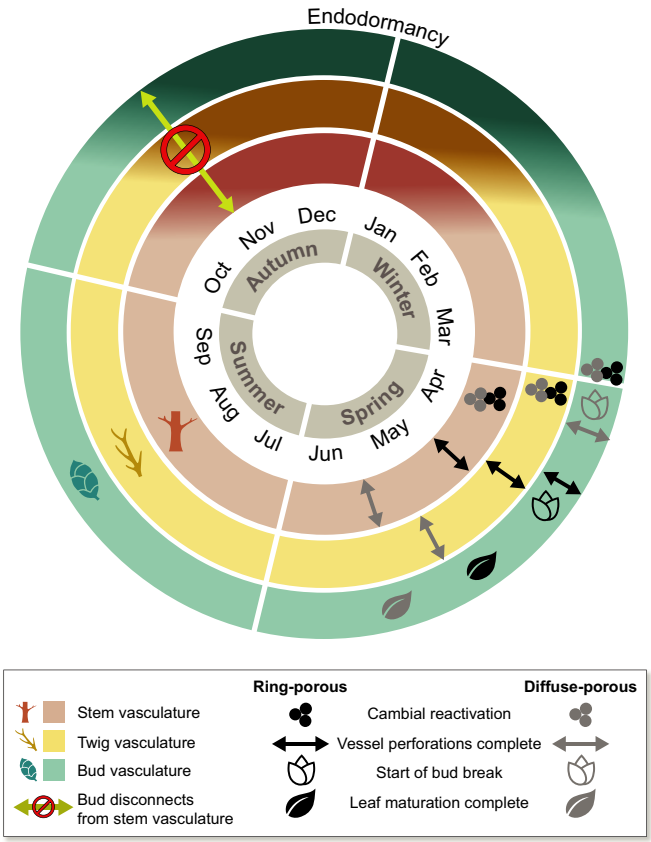


Fig. 3 Comparison of stem, twig and bud phenology in diffuse-porous (gray symbols) and ring-porous (black symbols) species broken down spatially from the bud to the main stem (colored concentric circles) based on what is currently known for a handful of species. The color in the circles is darkened when the organ is in endodormancy. The phenology of the cambium is very poorly known in buds. It has been shown that in diffuse and semi-diffuse porous species, bud cambial reactivation either starts or is completed shortly before bud break, and we assume it is similar in ring-porous species in this figure, although it is not known. In all species, the cambium in twigs and stems is often reactivated approximately at the same time, but the start of vessel function (marked by vessel perforation completion) occurs later in the stems than in the twigs. Major differences between diffuse- and ring-porous species include earlier bud break and leaf out in diffuse porous species (Panchen *et al.*, 2014), and earlier leaf maturation and reactivation of stem and twig cambium in ring-porous species (Lavrič *et al.*, 2017). However, in ring-porous species, xylem transport from the roots in newly formed vessels (after perforations are open) is only possible during leaf expansion but not at bud break as previously thought (Kitin & Funada, 2016). This late timing of new vessel activation does not preclude transport through older conduits or other apoplastic pathways. The calendar is based on the northern hemisphere.

III. Reactivation of bud vascular system

1. Ecodormancy: the reactivation of transport pathways

The last stage of bud development, ecodormancy, corresponds to a physiological state during which general development and metabolism are inhibited by external factors (e.g. temperature, photoperiod, water). Leaf and flower growth during ecodormancy leads to buds opening, and to the organs (e.g. flowers or leaves)

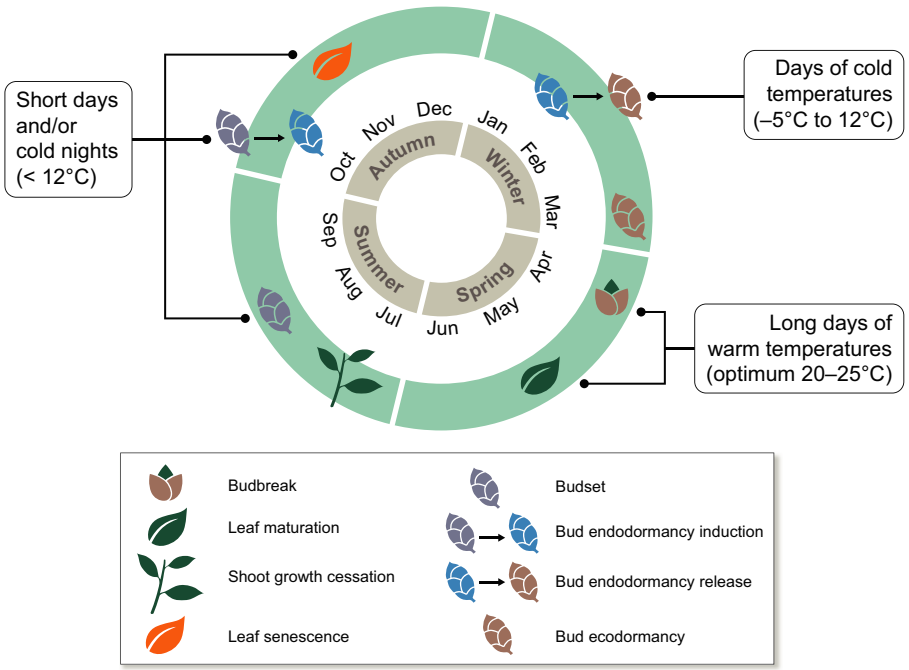
gradually expanding and maturing. The different cues driving endodormancy and ecodormancy are presented in Box 2. Until recently, it was not clear how much of the early stages of ecodormancy necessitated water, nutrients, and hormones from the stem and roots. As long as the sink remains weak, that is, a few weeks after endodormancy release, the symplast and the apoplast in the bud might support the bud's needs (Xie *et al.*, 2018). Nonetheless, later on in ecodormancy, when the sink has increased substantially, both vascular and nonvascular pathways need to be functional in the stem, root and bud, if there is going to be an exchange between all of these organs. Because tracking carbon, nutrients and water in developing buds is challenging, the origin of resources mobilized to sustain bud, leaf and flower growth is not very well identified. Some studies suggest that soluble sugars in the cambial zone (area including initial and undifferentiated derivative cells) might be translocated radially in xylem ray parenchyma and serve partly as an energy source for the resumption of cell division and partly for attracting water osmotically (Essiamah & Eschrich, 1986).

2. Leaf bud vascular reactivation

So far, we know how the vascular system in the bud differentiates but we know very little about the timing, especially relative to the timing of restoration of the vascular system in the stem and the rest of the plant. Procambium is always initiated at the base of the strands of residual meristem when buds are initiated (Garrison, 1955). In some species it forms concomitantly with the first leaf, but it can also form before the first leaf primordia (Garrison, 1955; Goffinet & Larson, 1981). In general, the timing of the reactivation of the procambium and of the differentiation of phloem and xylem cells in the bud is not known in most species. The differentiation of bud's procambial cells into phloem cells occurs acropetally and

Box 2 Environmental cues of bud development.

Some external factors play a role in endodormancy induction and breakdown, including cold night temperatures and/or short photoperiods (induction cue) (Anderson *et al.*, 2010; Basler & Körner, 2012), and number of cold or cool days (Arora *et al.*, 2003; Horvath *et al.*, 2003; Campoy *et al.*, 2011), also called chilling temperatures (breakdown cue). Note that chilling temperatures can be as high as 12°C in some species (Malagi *et al.*, 2015) and potentially as low as −5°C in others (Jones *et al.*, 2012). During ecodormancy, bud development is mainly dependent on temperature, and also photoperiod in some species (Chuine *et al.*, 2013; Zohner *et al.*, 2016): the higher the temperature and the longer the photoperiod, the quicker the development. In particular, a long photoperiod has been shown to compensate for a lack of chilling temperatures during winter in some species (Caffarra *et al.*, 2011; Laube *et al.*, 2014; Zohner *et al.*, 2016). Cues of growth cessation and subsequent events (i.e. bud set, endodormancy induction, leaf senescence) are less well known. Depending on the species, cold night temperature and/or short photoperiod are suspected to play a role, in addition to water stress for leaf senescence (for a review see Delpierre *et al.*, 2016). Some studies also suggest that drought during summer may hasten growth cessation and bud set, leading to a longer bud development before endodormancy induction, which translates into an earlier budburst in spring (Sanz-Perez & Castro-Diez, 2010; Marchand *et al.*, 2020). In the image in this box, environmental cues are linked to bud phenophases. The calendar is based on the northern hemisphere.



generally continuously (Esau, 1943) and begins before production of xylem cells (Avery, 1933; Swidrak *et al.*, 2014). Phloem translocation is possible when leaves are only 5% expanded (Larson & Gordon, 1969; Isebrands & Larson, 1973). The first xylem cells mature in or near a leaf and then differentiate basipetally in the axis and acropetally in the leaf (Esau, 1953). In *Populus*, vessel differentiation begins in the bud close to budburst and continues as leaves begin to emerge (Goffinet & Larson, 1982). However, in some species, only the smallest areoles and vein endings continue to mature after the leaves are exposed (Lersten, 1965), and in others hydraulic conductance between the stem and the buds increases 1 month before bud break (Bonhomme *et al.*, 2010).

One important study on leaf bud vasculature concerned *Vitis vinifera*, grapevine (Xie *et al.*, 2018). This study showed that buds formed during summer had differentiated xylem at their base and were connected to the stem's xylem. In the winter, dye could not be pulled into the bud xylem, indicating that the pathway was obstructed/altered or the transport gradient reduced. In this species, new xylem conduits begin to differentiate in the spring coincidentally with bud swelling. However, water uptake by the buds takes place before vascular reconnection of the bud to the stem and therefore bud break does not rely on exogenous water and nutrients.

3. Flower bud vascular reactivation

In contrast to leaf buds, more is known about floral buds, especially in cultivated species including *Prunus* species (apricot and peach) and *Forsythia*. These species flower early in the spring and have separate flower and leaf buds. Studies have shown that in these species during endodormancy the vascular system of the bud is undifferentiated or not completely differentiated, and the vascular tissue progressively differentiates during endo- and ecodormancy (Ashworth, 1984; Aloni, 1987; Ashworth *et al.*, 1992; Bartolini & Giorgelli, 1994). At the beginning of floral bud differentiation, only elongated procambial cells are present (Ashworth, 1984), and xylem conduits differentiate while floral whorls develop (Faust *et al.*, 1995). Vessels develop from the base of the bud axis up to the floral primordia, reaching the rudimentary sepals and petals, then the anther filaments, and finally the pistil (Andreini *et al.*, 2012). Interestingly, it has been shown that endodormancy break occurs when xylem conduits are present in up to three-quarters of the bud axis in apricot varieties (Bartolini & Giorgelli, 1994), especially in those requiring low chilling, while this stage of xylem development is reached before endodormancy break in varieties requiring high chilling (Viti *et al.*, 2013). It has also been shown that xylem vessel elements do not form a continuous pathway with the bud primordium until the preflowering stage (Ashworth, 1984). In peach, xylem elements in the bud are not fully functional until a few days before bud break, suggesting that nutrient uptake by the primordia at this time does not strongly depend on the vascular system (Bondada *et al.*, 2005).

Compared with fruit trees, less is known about vascular phenology of other flowering species and there is reason to expect variation among species based on floral and bud morphology. Flowers that bloom early in the growing season are often preformed in their buds and require fewer resources than those that flower later in the year (Savage, 2019). These species often reach anthesis before leaves are formed (precocious flowering), leading to noticeable differences in the coordination of bud development with stem vascular phenology. In a classic study, Gill (1933) found that flowers in species with naked catkins do not increase in biomass before anthesis and do not reactivate the vascular system in their catkins in the spring. Meanwhile, species with covered catkins exhibit vascular differentiation when catkins open, at which time phloem cells begin to differentiate basipetally and eventually reconnect with the stem vascular system. Whether the xylem in floral buds connects to the stem in all species remains unclear (Gill, 1933) and some have argued that flowers are sometimes phloem-hydrated, but evidence for this remains sparse (Roddy *et al.*, 2016; Savage *et al.*, 2016). In general, we expect that flowers that experience anthesis later in the season and flowers that share a bud with shoot primordia are more similar in their vascular phenology to leaf buds, but more work is needed to clearly document the relationship between floral morphology, phenology and vascular development.

4. Connecting to the stem vasculature

An important part of dormancy in many species appears to be a disconnection between the bud and stem vascular system, specifically with the xylem (Xie *et al.*, 2018). This disconnection is thought to be important in reducing both freezing damage and desiccation. In cold climates, ice often forms in the apoplast and, once nucleated, can propagate through the xylem (Neuner, 2014). As ice forms, water is drawn into the apoplast, desiccating neighboring cells and tissues. Therefore, a bud that is disconnected from the stem has a lower chance of experiencing freezing and desiccation damage in the winter, which might allow plants that delay connecting to the stem xylem to have better performance in early spring. This pattern is best demonstrated by work on freezing tolerance in flowers. In a study on six *Prunus* species, Ashworth (1984) demonstrated that in species that can supercool there is a barrier that prevents ice from spreading into the bud before vessel maturation in the pedicel. Once vessels are functional, these flowers lose their ability to protect themselves from ice and show visible damage in response to freezing temperatures. As a result, the timing of organ reconnection to the stem can be important in reducing freezing risk to developing floral buds.

There are three critical elements that need to be understood when considering how and when the bud vascular system connects to the stem. First, transport in the phloem requires that both bud and stem sieve tubes are free from callose. So far, no study has investigated whether callose degradation occurs simultaneously in the bud and the stem sieve elements. Second, we know very little about the connection between bud and stem xylem and when it is functional (and not just present) because only a few studies have investigated connectivity to the stem by tracking dye (Essiamah & Eschrich, 1986; Xie *et al.*, 2018) or ice spread in conduits (Ashworth *et al.*, 1989). It is also possible that there are obstructions to xylem transport even after the vascular connections are present.

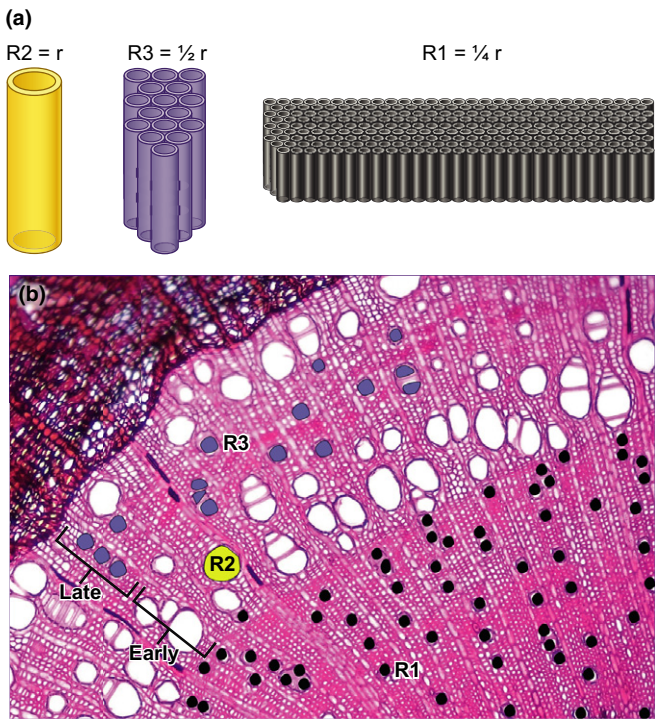


Fig. 4 Large conduits are responsible for the majority of transport in the xylem and phloem because hydraulic conductivity scales with radius (*r*) to the fourth power. (a) One large conduit (yellow) has the same conductivity as 16 conduits of half the radius (purple), and 256 conduits of one-quarter the radius (black). As a result, hydraulic conductivity is highly influenced by changes in conduit radius and seasonal occlusion of conduits (embolism in xylem and callose occlusion in the phloem). (b) In ring-porous species, conduit radius often changes from early in the season (early) to late in the season (late) and between years (R1, first year of growth; R2, second year; R3, third year). In this image, late-wood vessels are half the radius of early-wood vessels in R2, and R1 has vessels a quarter of the size of these late-wood vessels (only 60 in the image). In this type of species, larger vessels might become embolized in the winter, which would greatly decrease xylem hydraulic conductivity, until the cambium produces new vessels in the spring (as seen in R3). Therefore, in the spring, it is important to know not just the number but also the size of the conduits to judge differences among species in transport capacity.

For example, in *Solanum tuberosum* (potato), xylem vessels of the stem are filled with tannin-like substance a short distance below the bud during endodormancy, and after endodormancy break, buds have both old obstructed xylem and new functional xylem at the junction with the stem (Goodwin, 1967). These tannin-like structures might be a supplementary security to prevent transport of water and nutrients in the buds before endodormancy breaking. Third, it is not clear what transport pathways are required at different stages of dormancy and organ development. No study has, to our knowledge, investigated the potential role of other tissues such as pith and cortex in conducting water and nutrients during ecodormancy and bud break when the vascular system is not yet fully functional. There is also uncertainty about the role of the xylem and phloem in early organ development because both parts of the vascular system can transport sugar, water and nutrients at this time of year (Tixier *et al.*, 2017; Savage, 2019).

Box 3 Role of auxin in vasculature development.

Dormancy and auxin transport

In general, auxin is synthesized in the shoot apex and moves basipetally through the twig (Thimann & Skoog, 1933). During bud ecodormancy, auxin is probably produced locally by meristematic cells and subsequently leaves. Low production of auxin might be associated with mitotic activity in dormant buds and may activate the cambium and phloem in trees with auxin-sensitive vascular tissue (Aloni & Peterson, 1997). Auxin is also involved in upregulation of callose breakdown, which is accelerated by leaf development (Aloni & Peterson, 1997; Prislan *et al*., 2013). On bud break, auxin transport capacity can increase 10-fold in a single day (Schrader *et al*., 2003). At the end of the growing season, auxin transport capacity progressively decreases and ceases at dormancy induction (Schrader *et al*., 2003). Auxin synthesis is partially governed by environmental cues (Schrader *et al*., 2003): short photoperiod seems to play a role in auxin transport capacity at dormancy induction.

Sensitivity to auxin

Organ development and phenology are tied to auxin sensitivity. For example, phloem, which is very sensitive to auxin, matures before xylem, which is less sensitive (Aloni, 2013). However, sensitivity to auxin varies seasonally, including when the cambial meristem switches from auxin-sensitive to -insensitive, a process that may have a role in maintaining endodormancy (Little & Bonga, 1974). Differences between ring- and diffuse-porous species may also be tied to their auxin sensitivity, with ring-porous species being more sensitive to auxin than diffuse-porous species. This difference could explain why ring-porous species reactivate their cambium before leaf out, when auxin concentrations increase (Aloni, 2013). The higher sensitivity of ring-porous species may explain why callose disappears almost completely before bud break in these species but not in diffuse-porous species (Aloni *et al*., 1991).

IV. Reactivation of stem and root phloem

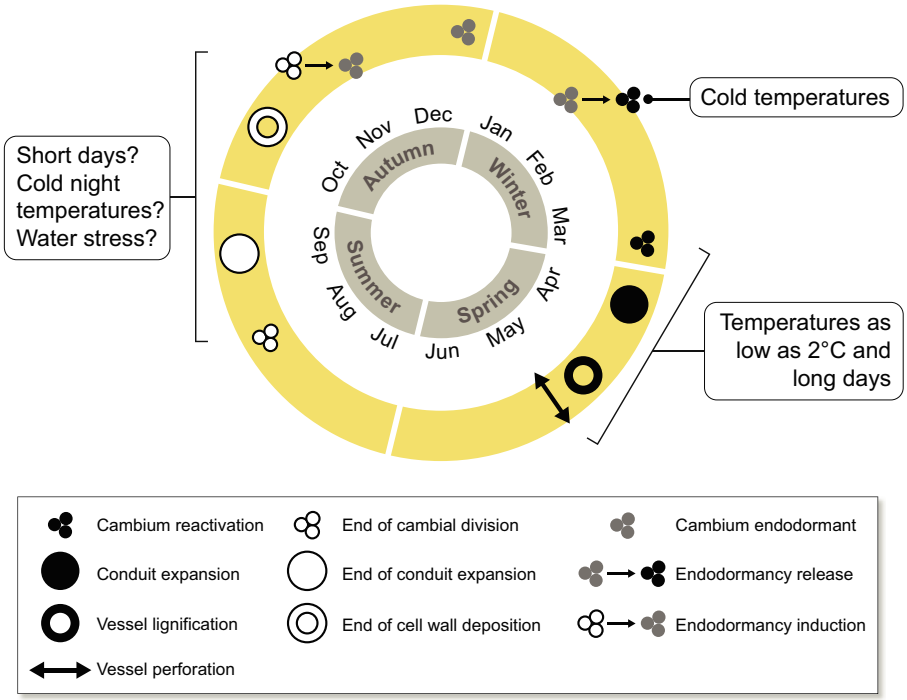
1. Increasing transport in the spring

In the spring, stem phloem resumes function before the xylem in many species (Fromm, 2013) but the exact timing of when the phloem begins transport is species-specific and may depend on the method of phloem overwintering/reactivation (Aloni & Peterson, 1997; Savage, 2020). In general, there are three ways that plants resume carbon transport in the spring. First, there are species that appear dependent on the formation of new sieve tubes because all their older conduits become occluded by callose and/or collapse during the winter (Evert, 2006). Second, there are species that reactivate their sieve tubes in the spring by dissolving dormancy callose enzymatically and thus reusing sieve tubes during consecutive years. In these species, phloem sieve tubes can resume function before callose has completely disappeared (Aloni & Peterson, 1997). Lastly, there are species that rely on mature or immature sieve tubes and/or initials that were formed by cambial division during the previous year at least in the early part of spring (Davis & Evert, 1970; Evert, 2006). Because of the challenge of

Box 4 Environmental cues of vasculature development.

Stem vasculature

Cambial initials divide and produce undifferentiated phloem cells outwards and undifferentiated xylem cells inwards. Sieve elements then differentiate by losing their nucleus and most of their organelles, and become functional after perforating their sieve plates. Xylem conduits (e.g. vessels) differentiate, expand and then exhibit secondary cell wall deposition, wall lignification and wall perforation. Studies on conifer species suggest that temperature is a major determinant of stem cambial activity, with warmer temperatures in spring stimulating cambial cell division and growth (Gricar *et al*., 2014; Swidrak *et al*., 2014), and lower temperatures in summer downregulating cambial activity as well as drought stress (Swidrak *et al*., 2014). However, environmental cues of cambial activity are not as well known as cues of bud development (for a review see Delpierre *et al*., 2016). In the image in this box, environmental cues are linked to vascular phenology. The calendar is based on the northern hemisphere.



Root vasculature

The mechanisms regulating cambial activity and differentiation are often presumed to be similar in stems and roots. However, environmental drivers of root vascular phenology have not been investigated in broadleaf deciduous species. In conifers, Thibeault-Martel *et al*. (2008) found that xylem formation was synchronous in stem and root except when air and soil temperature rise were not synchronous (e.g. soil temperature remaining near zero while air temperature rose above 6°C). It is possible that the determinants of root vascular phenology might be similar to root primary growth, that is, soil temperature and humidity, nutrient, plant growth regulators and carbon allocation (see Radville *et al*., 2016 for review). Root growth phenology is also dependent on leaf and stem growth phenology, which are impacted by air temperature, day length and light intensity (Radville *et al*., 2016). Abramoff & Finzi (2015) suggested that root growth occurs across species, on average, 25 d later than stem growth, probably because roots cannot grow when shoots are consuming the majority of photoassimilates.

distinguishing cells adjacent to and in the cambial zone with certain types of microscopy, there are discrepancies in the literature about the nature of overwintering immature cells in many species and whether they are differentiated or not (Artschwager, 1950; Evert, 1963; Davis & Evert, 1968). There are also a few clear cases of species that primarily overwinter open sieve tubes, such as in *Acer negundo* and *Salix* spp. (Tucker & Evert, 1969; Lawton, 1976). Research in *Salix* suggests that sieve tubes in some species might remain functional all winter and can exhibit sap flow at temperatures as low as −4°C and −13°C (Weatherley & Watson, 1969; Fisher, 1983). Other species appear only to overwinter sieve tubes formed late in the season in the late phloem (Prislan *et al*., 2018). Overall, there is limited information on whether patterns of phloem reactivation are consistent throughout the plant and most of the work has been done on stem tissue.

Leaf out and xylem formation (xylogenesis) are costly processes that probably require resources transported in the phloem. For this reason, it is not surprising that there are always open sieve tubes in the stem before either of these important spring events take place. Most of the phloem in the stem is made before or during the period of rapid leaf expansion (Lavrič *et al*., 2017; Prislan *et al*., 2018). For example, in *Quercus pubescens*, 40% of annual phloem growth in

the stem is produced before leaf out, and at this time of year, growth in the phloem can exceed growth in the xylem (Gričar *et al.*, 2017). Branch phloem often reactivates before phloem in the main stem, but it is important to note that this should only influence which carbohydrate sources can be accessed at different times of the year. The phloem does not need to be reactivated throughout the whole plant to be operational. It can function as long as there are local carbon sources like nonstructural carbohydrates, which are in ample supply and easily accessible in branches (Tixier *et al.*, 2017; Furze *et al.*, 2019).

2. Phloem reactivation and leaf phenology

The apparent role of phloem in facilitating new growth has led some to hypothesize that there should be a relationship between phloem reactivation and leaf phenology (Aloni & Peterson, 1997; Savage, 2020). There is some evidence that this may be the case because the earliest plants to leaf out appear to overwinter functional sieve elements (Tucker & Evert, 1969; Lawton, 1976) and callose in the phloem has been shown to be dissolved before budburst or leaf out in some ring-porous species (Aloni & Peterson, 1997). Unfortunately, assessment of broader patterns across species is limited by a paucity of data on phloem phenology and transport capacity (Savage, 2020). This is apparent when examining species that overwinter functional sieve tubes produced later in the year. These species still leaf out late, despite having mature sieve elements early in the spring (Tucker, 1968; Prislan *et al.*, 2018). However, a large difference between these species and other species that overwinter open sieve tubes (Tucker & Evert, 1969; Lawton, 1976) is that these species only overwinter late phloem, which should have a lower transport capacity than early phloem because of the narrow size of the conduits. Therefore, it is possible that these species do not have a high enough transport capacity in their phloem in the early spring to support a developing canopy (Fig. 4). In the end, without understanding how much phloem is needed to support leaves and whether there is a relationship between phloem anatomy and transport capacity, it is not possible to determine how phloem phenology might relate to resource use and organ phenology.

The relationship between leaf and phloem phenology is further complicated by the role of auxin in callose breakdown in sieve tubes. The length of time between callose breakdown and cambial division can vary from less than a week to a month depending on the species. In some species, callose breakdown can begin before bud break as in *Quercus robur*, while in other species such as *V. vinifera* and *Magnolia kobus*, callose removal occurs when at least one leaf is almost fully expanded (Davis & Evert, 1970; Aloni & Peterson, 1997). This difference in the timing of callose removal is thought to be related to species' sensitivity to auxin and might be associated with wood type, with ring-porous species showing a higher sensitivity to auxin (see Box 3). The same pattern of callose removal is observed when leaves are removed and substituted with auxin (Aloni *et al.*, 1991; Aloni & Peterson, 1997). One implication of this research is that leaf phenology (because it impacts auxin concentrations) could directly influence phloem phenology and the timing of reactivation of sieve tubes in the spring.

V. Reactivation of stem and root xylem

1. Cambial activity and xylogenesis

Stem cambial cells undergo changes between endodormancy and active growth including cell wall thickening by extension of the microfibrilar scaffold and increased amount of xyloglucan, and helically arranged microtubules (Lachaud *et al.*, 1999). Environmental cues affecting cambial activity and vasculature development are not as well known as those of bud development (see Box 4). The timing of cambial activity onset and ending in the different parts of the plant is still controversial. While some authors have proposed that cambial activity should reactivate first close to buds and propagate basipetally to the roots, some studies on conifers have found no clear pattern and direction (for a review see Thibeault-Martel *et al.*, 2008). However, Aloni (2013) showed that cytokinin and strigolactones, which are both primarily produced in the roots and then transported to the stems, regulate cambial activity and promote vascular differentiation. This new finding suggests that root cambial activity might be independent of exogenous auxin regulation.

In both diffuse-porous and ring-porous species, vessel differentiation occurs later in the stems than in the twigs (Ladefoged, 1952), while cambium in the twigs is often reactivated at approximately the same time as in the stem (Fig. 3; Takahashi *et al.*, 2013). In ring-porous species, cambial reactivation usually occurs almost simultaneously throughout the whole tree (simultaneous reactivation), while in diffuse-porous species the cambium is usually reactivated first at the base of buds and then proceeds basipetally (Lachaud *et al.*, 1999; Kudo *et al.*, 2015; Gričar *et al.*, 2017). However, these patterns are not ubiquitous (Sass-Klaassen *et al.*, 2011) and both types of reactivation (simultaneous and basipetal) can occur in all species (Begum *et al.*, 2007) and within individual plants (Lachaud *et al.*, 1999).

2. Cambial activity and leaf phenology

When comparing cambial phenology with leaf phenology, additional differences become apparent between ring- and diffuse-porous species (Fig. 3; for a review see Kitin & Funada, 2016). In general, ring-porous species reactivate their twig and stem cambium several weeks before bud break (Imagawa & Ishida, 1972; Atkinson & Denne, 1988; Frankenstein *et al.,* 2005; Lavrič *et al.,* 2017; but see Fromm, 2013), flush leaves, on average, later than diffuse-porous species (Panchen *et al.*, 2014), and exhibit vessel lignification in twigs and stems before or at the same time as leaf out and subsequent expansion (Suzuki *et al.*, 1996; Takahashi *et al.*, 2013; Takahashi *et al.*, 2015; Gričar *et al.*, 2017). By contrast, diffuse-porous species often reactivate their twig cambium concurrently with or after budburst (Aloni & Peterson, 1997), flush leaves earlier and exhibit vessel lignification in the twigs and stems at the same time as or after leaf out and expansion (Suzuki *et al.*, 1996; Prislan *et al.*, 2013; Takahashi *et al.*, 2013). Recently, Kitin & Funada (2016) highlighted that while the first vessel elements in upper parts of the stem are fully functional at bud break in ring-porous species, the opening of perforation plates was completed

later in the lower stem but nevertheless before leaf maturation. Therefore, contrary to what was previously thought, the current-year early-wood vessel elements in the stem of ring-porous species probably become functional during bud break and leaf expansion and not before bud break. Water and nutrients are thus first supplied to the developing buds and leaves either by narrower late-wood vessels, which can remain functional for several years (Umebayashi *et al.*, 2008), or new vessels that use more local water sources in branches.

Several hypotheses have been proposed to explain differences in cambial phenology between diffuse-porous and ring-porous species. One hypothesis focuses on competition for carbohydrates remobilized from the reserves between the cambium and buds (Bonhomme *et al.*, 2010). Another hypothesis argues that the greater length of vessels in ring-porous species (up to 18 m vs *c.* 1 m in diffuse-porous) would take longer to build up than shorter vessels in diffuse-porous species (Aloni, 1987). A third hypothesis ties this difference to whether or not species produce leaves continuously during a few months (like many diffuse-porous species) or over a shorter period (like many ring-porous species), which would require a higher flux of resources earlier in the season (Aloni & Peterson, 1997). However, in the end, there are limited data to support any of these hypotheses.

3. Vessel diameter and leaf-out time

A plant's susceptibility to freezing-induced embolism is tied to wood anatomy because large bubbles are more likely to expand and form embolisms than smaller bubbles, and wider vessels can accommodate larger bubbles than can narrow vessels (Ewers, 1985; Sperry & Sullivan, 1992). As a result, plants with large vessels such as those with ring-porous wood lose greater function in the winter than those with small vessels, such as plants with diffuse-porous wood (Fig. 4; Sperry & Sullivan, 1992; Hacke & Sauter, 1996). A major implication of this pattern is that ring-porous species often cannot reuse their large, early-wood vessels the following year unless they are refilled (for a review see Cochard, 2006). Because large vessels provide most of the wood transport capacity in ring-porous species, these species often rely on xylogenesis for the resumption of water transport in the spring (Fig. 4). Meanwhile, species with smaller vessels (diffuse-porous species) can resume flow as soon as the conditions are favorable (Sperry & Sullivan, 1992; Kudo *et al.*, 2015; Jacobsen *et al.*, 2018).

Because of this known association between vessel diameter and freezing-induced embolism, it has been proposed that vessel size is a good predictor of leaf phenology (Zimmermann, 1983; Lechowicz, 1984). Lechowicz (1984) was one of the first to show a positive relationship between vessel diameter and leaf-out time across species (i.e. that species with smaller vessels flush leaves earlier than those with larger vessels). However, it was not until a subsequent study by Wang *et al.* (1992) that the link between hydraulic conductivity and timing of leaf out was examined directly. Since these early studies, there has been limited work examining the relationship between wood anatomy and leaf phenology, with the exception of recent macroecology studies which found that wood porosity is a good indicator of leaf-out time and its plasticity (Panchen *et al.*, 2014; Fahey, 2016).

On a functional level, a relationship between leaf phenology and vessel diameter is frequently explained in terms of the water requirements of a developing canopy (Zimmermann, 1983; Lechowicz, 1984). For species that are vulnerable to freezing-induced embolism (i.e. wider vessels and/or ring-porous wood), access to water would be limited in the spring because of low wood conductivity. Meanwhile for species that maintain xylem function during the winter (i.e. narrower vessels and/or diffuse-porous wood), there would be no hydraulic limitation on leaf expansion. Although this rationale is frequently cited to explain the potential relationship between vessel diameter and leaf-out time, it is not the only explanation for this pattern. It is also possible that this relationship is driven directly by the susceptibility of large vessels to freezing-induced embolism. Selection would favor later initiation of xylogenesis in species with larger vessels because of the higher risk of embolism. If ring-porous species require the production of new vessels before leaf out, they would be unlikely to leaf out early in the spring. A third possible explanation for the relationship between leaf phenology and vessel size is that it is a side-effect of the role of auxin in vessel maturation and its connection to leaf development (see Box 3; Aloni, 2013).

VI. Future directions: an integrated phenological approach

Most studies on vascular phenology focus on one part of the vascular system or a handful of species, phenophases or stages of development, because of the intense sampling required for this type of work. As a result, we lack important information about how organ and vascular phenology are coordinated and the physiological basis of this coordination. Additionally, a substantial part of what we know about this topic is based on work done over 20 yr ago, and there have been significant improvements in our sample preparation and imaging techniques since that time. Here, we lay out four ways that future studies could help to advance our understanding of the timing and physiology of organ reactivation and growth. This list is not comprehensive but highlights major gaps in our knowledge that exist today.

1. Providing temporal and spatial context

Whenever possible, it is best to increase the temporal resolution of a study by monitoring multiple phenophases and/or increasing sampling frequency. Phenological transitions are continuous and phenophases that are clear in one species may not be present in another. As a result, it is easier to compare studies that have more than one benchmark. For example, relatively simple changes like noting multiple organ phenophases (e.g. Puchalka *et al.*, 2017), or determining when new vessels are first lignified in addition to when they are fully expanded (e.g. Suzuki *et al.*, 1996; Čufar *et al.*, 2008; Kudo *et al.*, 2015; Puchalka *et al.*, 2017), could greatly increase the impact of a study. Another way to increase temporal resolution is to change the sampling frequency. With frequency, the key is understanding how quickly the changes you want to observe

happen. Many studies select sampling intervals of 1–2 wk, which can capture large-scale differences in leaf and vascular phenology but may miss differences in the timing of events that occur over shorter periods of time. Whenever possible, we recommend conducting a pilot study to determine the appropriate sampling frequency.

In addition to time, space, in terms of sampling location along a main plant axis, and tree height can impact the interpretation of vascular phenology data (Lachaud *et al.*, 1999; Seiwa, 1999; Osada & Hiura, 2019). Only when sampling location is taken into account can comparisons be made between individual plants and among species. This fact is nicely highlighted in a recent study examining differences in the vascular phenology of ring- and diffuse-porous species (Takahashi *et al.*, 2013). However, this study, similarly to many others, relies on broad classifications like twig and stem instead of providing quantitative data on plant height and sampling location. We suggest that future work be made more comparable to other studies by describing sampling location and recording the height of the sample (or distance of the sample from the tip of the branch).

2. Measuring and inferring vascular function

A central challenge to understanding the relationship between organ and vascular phenology is determining when the vascular system is functional and quantifying transport capacity. Because documenting transport typically requires dyes, sap flow sensors, magnetic resonance imaging or isotopes, and these methods can be complicated, require specialized equipment or have limited use in some species (Windt *et al.*, 2006; De Schepper *et al.*, 2013; Savage *et al.*, 2013; Lavrič *et al.*, 2017), most work on vascular phenology relies on anatomical data to estimate transport function. This approach requires information on the size and number of conduits (Fig. 4) and a key awareness of the assumptions made about the structure–function relationships in the xylem and phloem. For example, there are currently several benchmarks used to determine when vessels are 'functional'. Three of the most common stages are the initiation of vessel expansion, secondary cell wall deposition and wall lignification. Unfortunately, all of these stages precede vessel maturation and the initiation of xylem transport. Jacobsen *et al.* (2018) found that dye was not transported in the xylem until weeks after the vessels became fully expanded in *Q. robur*. There are also issues with the common method used to determine when lignification is initiated (safranin dye) because this stain is not specific to lignin and will stain other structures inside cells.

To advance our understanding of vascular phenology, we need to know when vascular function resumes and when there are significant changes in transport seasonally. Ideally, we need to quantify changes in hydraulic capacity or sap flow in the xylem and phloem in the spring and summer. However, when this is not possible, we need simple tests to determine transport functionality in buds and stems, and/or good anatomical benchmarks that relate to functional changes. One trait in the xylem that appears to be a good indicator of function is the disintegration/opening of perforation plates separating vessel elements. This step appears to be the final stage in vessel maturation and coincides with when dye can move in xylem (Kudo *et al.*, 2015). Unfortunately, in the phloem, there are not simple benchmarks for determining when sieve tubes become functional and only limited research linking seasonal structural changes to sap flow. However, there are current efforts to measure sap flow *in situ* (Windt *et al.*, 2006; De Schepper *et al.*, 2013; Savage *et al.*, 2013; Knox & Oparka, 2018) along with promising preliminary data on cell wall changes that may relate to phloem function (Ray & Savage, 2020). There are also an increasing number of studies aimed at understanding symplastic and apoplastic pathways in plant tissue using dyes (e.g. Werner *et al.*, 2011) that could be applied to developing buds and roots. Expansion of this type of research could close the gap between our understanding of phloem structure and function and advance our understanding of seasonal changes in the vascular system.

3. Investigate gene and transcripts regulating phenology

An exciting new frontier in understanding the physiological basis of organ development is research that examines seasonal changes in gene expression and how this relates to phenology. One promising new approach is the use of transcriptomics, which has allowed recent advances in our understanding of molecular-genetic pathways involved in environmental regulation of plant development and cell activity in model organisms. In particular, studies have investigated how the expression of genes involved in cell activity varied with environmental conditions using high-throughput phenotyping techniques that can be deployed in the field (e.g. Nagano *et al.*, 2012; Satake *et al.*, 2013; Kudoh, 2016). So far, these studies have not been employed to thoroughly examine changes in cambial activity, although some studies have investigated the transcriptome of the cambium. Schrader (2004), for example, has shown that the complexity of the transcriptome was significantly reduced during endodormancy. Although cell division stops at this time, cells maintain a very low amount of activity, as suggested by the presence of cell cycle transcription factors. Future work examining the state of the transcriptome in buds, the cambium and vascular tissue as buds transition from dormancy induction to bud break would improve our understanding of the timing and sequence of physiological changes that occur during bud development.

4. Whole-plant or multiple organ approach

Many of the questions we asked in this paper can only be answered by research that includes both parts of the vascular system and/or multiple organs (e.g. Prislan *et al.*, 2013; Gričar *et al.*, 2017; Prislan *et al.*, 2018). So far, phenology studies have mostly focused on single organs. While leaf and flower phenology have received considerable attention, fruit, wood and root phenology have received much less, and very few studies have integrated the phenology of the different organs at the scale of an individual tree (Delpierre *et al.*, 2016). Examining what is happening in the buds, terminal shoots, stems and roots at the same time is the only way to understand how tightly they are connected and whether we should integrate these dependencies in predictive phenology models and tree functioning models. Similarly, research that examines both

xylem and phloem phenology is necessary for us to understand the interplay between these two critical transport pathways. Research that spans multiple organs and tissue types could have a significant impact on our understanding of spring phenology and help us understand the impact of climate change on phenology. In particular, this type of research could allow us to determine whether climate change might lead to a desynchronization of organ phenology within a plant, a topic that has been largely unexplored.

VII. Conclusions

When water and nutrient requirements for growth exceed the local supply, buds need to draw on resources from the stem vasculature, but access to resources could be limited by reduced vascular transport in the spring. Therefore, the key to understanding resource delivery to developing organs is determining how organ and vascular phenology relate to each other and the implications of seasonal changes in vascular physiology on growth and freezing tolerance.

Although the break of endodormancy is associated with an increase in cell connectivity in buds, there is limited evidence that the vascular system is needed in the early stages of ecodormancy. However, reconnection to the stem must occur before a certain amount of growth is reached, in order to sustain flower and/or leaf development. For this reason, after bud break and once flowers and leaves begin to expand, there is more evidence that organs are supported by the vascular system. As a result, the timing of phenophases such as leaf out and floral anthesis may be more limited by vascular phenology than by dormancy release. This pattern is consistent with research documenting a relationship between xylem anatomy/phenology and leaf out (Lechowicz, 1984; Panchen *et al.*, 2014), and hypotheses about how phloem physiology might be linked to leaf phenology (Savage, 2020). The overarching idea behind these relationships is that plants with xylem and phloem conduits that remain functional or quickly resume function before cambial division in the spring may be able to support earlier growth. In the xylem, this is tied to a plant's susceptibility to freezing-induced embolism and the amount of hydraulic conductance that is lost in the winter. For example, diffuse-porous species that have more functional vessels in the spring may be able to leaf out before species with higher levels of embolism such as ring-porous species.

With growing interest in how phenology is changing in response to climate change, it is important that we understand how plant physiology might impact the plasticity of organ phenology (Fahey, 2016) and vice versa (Aloni *et al.*, 1991; Aloni & Peterson, 1997). Therefore, further research addressing key gaps in our knowledge about bud phenology, phloem phenology and the interplay of organ and vascular phenology is critical to understanding how plants will respond to climate change (Delpierre *et al.*, 2016). In this review, we provide suggestions on future directions and highlight the type of data needed to understand the interplay between vascular and organ phenology. We focused on deciduous species in cold climates, but this is just a starting point. Vascular phenology is important to a wide variety of species, including evergreens and deciduous species in other seasonal environments. We will not know the extent of the impact that resource allocation and vascular physiology has on plant phenology until we have a more comprehensive perspective on vascular phenology and a more integrated view of plant phenology.

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