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Review

A Conceptual Framework for Winter Dormancy in Deciduous Trees

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Abstract: The perennial life strategy of temperate trees relies on establishing a dormant stage during winter to survive unfavorable conditions. To overcome this dormant stage, trees require cool (i.e., chilling) temperatures as an environmental cue. Numerous approaches have tried to decipher the physiology of dormancy, but these efforts have usually remained relatively narrowly focused on particular regulatory or metabolic processes, recently integrated and linked by transcriptomic studies. This work aimed to synthesize existing knowledge on dormancy into a general conceptual framework to enhance dormancy comprehension. The proposed conceptual framework covers four physiological processes involved in dormancy progression: (i) transport at both whole-plant and cellular level, (ii) phytohormone dynamics, (iii) genetic and epigenetic regulation, and (iv) dynamics of nonstructural carbohydrates. We merged the regulatory levels into a seasonal framework integrating the environmental signals (i.e., temperature and photoperiod) that trigger each dormancy phase.

Keywords: phenology; chilling; cell-to-cell communication; genetics; carbohydrates; phytohormones; vascular transport; *DAM* genes

1. Introduction

In the context of climate change, winter dormancy in temperate forest and fruit trees is increasingly attracting the attention of both scientists and growers. Warming winters can cause striking and unpredictable effects on deciduous fruit tree orchards by advancing or delaying phenological stages such as flowering, fruit ripening, or leaf senescence. Along the same lines, climate change is causing shifts in the suitability of orchard locations for particular species and cultivars, leading growers to modify the array of fruit tree species and varieties that are cultivated in some of the most important growing regions [1–3]. The development of tools and strategies for adapting orchards to future climate conditions helps to ensure the viability of temperate fruit production. However, despite more than two centuries of research, a comprehensive understanding that integrates the physiological basis of dormancy at the whole-plant and cellular level remains elusive.

Scientists have studied tree dormancy since at least the beginning of the 19th century. In 1801, Sir Thomas Knight discovered that temperate woody perennials require exposure to cold winter temperatures to resume growth in spring [4]. By the middle of the 20th century, the basis of our current understanding of dormancy had been established [5–7]. During the same period, experiments were conducted with numerous bioactive compounds to replace the effect of chilling [6], and some observations on the effects of warm winter temperatures on dormancy release led to one of the first temperature-based models, the Chilling Hours model [8]. Already in 1920, Frederick V. Coville introduced an experimental methodology (which is still used today) to study dormancy by exposing seedlings, potted trees, or shoots to a sequence of different temperature regimes, already hypothesizing

that low temperatures trigger dormancy release by inducing the degradation of starch into soluble sugars [9].

In the second half of the 20th century, many researchers focused on phenology modeling, relating temperature to phenological events, thus identifying the most effective temperature range to overcome dormancy [10]. A number of temperature-based mathematical models emerged to estimate the chilling requirement (CR) of several species and cultivars aiming to forecast bloom dates. Together with the Chilling Hours model [8], the chill models that are currently most widely used are the Utah model [11] published in 1974 and the Dynamic model [12–14], which appeared in 1987. Compared to the other common models, the Dynamic model is based on the most sophisticated biological concept, assuming that chill accumulation is mediated by a thermally labile precursor [1]. However, like all earlier models, even the Dynamic model uses only temperature data as input.

Also in the year 1987, Lang et al. [15] proposed a new terminology for dormancy research, distinguishing between para-, endo-, and eco-dormancy phases. According to their definition, para-dormancy refers to growth suppression that is imposed on particular organs by other tree structures (e.g., apical dominance) due to the production and/or action of inhibitory molecules. During endo-dormancy, growth is not possible even under suitable temperature conditions, as buds have not received sufficient exposure to chilling. Eco-dormancy refers to the subsequent period, in which buds on trees only need sufficient exposure to warm temperatures to resume growth [15]. Although some shortcomings have been pointed out in the nomenclature by Lang et al. [16], it is the most widely used terminology for the dormancy of temperate fruit trees.

Especially since the beginning of the 21st century, advances in dormancy research have been achieved by several scientific disciplines. Studies on the genetics of dormancy resulted in the discovery of a group of genes that regulate seasonal rest, i.e., *DORMANCY ASSOCIATED MADS-box* (*DAM*) genes in peach (*Prunus persica*) [17,18]. The application of techniques such as genomics, proteomics, and metabolomics in dormancy research resulted in numerous analyses that revealed a period of activity at the cellular level compared to the previous conception of null activity during this stage [19,20]. Transcriptomic analyses have managed to link gene expression profiles to hormonal, metabolic, and physiological changes during dormancy progression [21,22]. Today, innovative research approaches promise to close some of the remaining knowledge gaps. Epigenetic mechanisms that modify gene expression patterns without any changes to the DNA sequence appear to be involved in mediating dormancy release in several temperate fruit tree species [23]. Reactive oxygen species (ROS), which are associated with cellular stress, have been proposed as key molecules integrating the environmental cues and metabolic processes that regulate plant growth and development [24]. Recent years have thus produced great advances in dormancy research, which has come a long way since the discovery of the need for chilling more than two centuries ago. However, dormancy research is still lacking a comprehensive framework that integrates all these knowledge gains.

The current literature offers numerous reviews summarizing previous work on certain aspects of dormancy. Among these works are reviews focusing on molecular biology [25,26] and transcriptomics [27], experimental approaches that contributed to dormancy knowledge [28], ROS activity [24], terminology [16], self-organization of the shoot apical meristem [29], *DAM* genes [30], epigenetic regulation [23], and the impacts of climate change on fruit and nut tree phenology [3,31]. In the present work, we attempt to offer a holistic review of winter dormancy in temperate woody perennials, which we integrate into a conceptual framework aiming to provide a foundation and guidance for further research in this field.

For an integrative understanding of dormancy, attempting to achieve a reasonable balance between disciplinary depth and a multi-disciplinary perspective, we describe biological and physiological processes, in floral and vegetative buds, according to the distinct dormancy phases and the environmental stimuli that are involved. The manuscript is divided into two main parts to facilitate the interpretation of the conceptual framework. First, we describe the background regarding dormancy phases and the phenological stages of the tree (Figure 1). We also describe how environmental signals

such as photoperiod and temperature might trigger phase transitions (Figure 2). Second, we group the physiological processes linked to dormancy into four main topics, which we contextualize and frame according to the main dormancy phases (Figure 3).

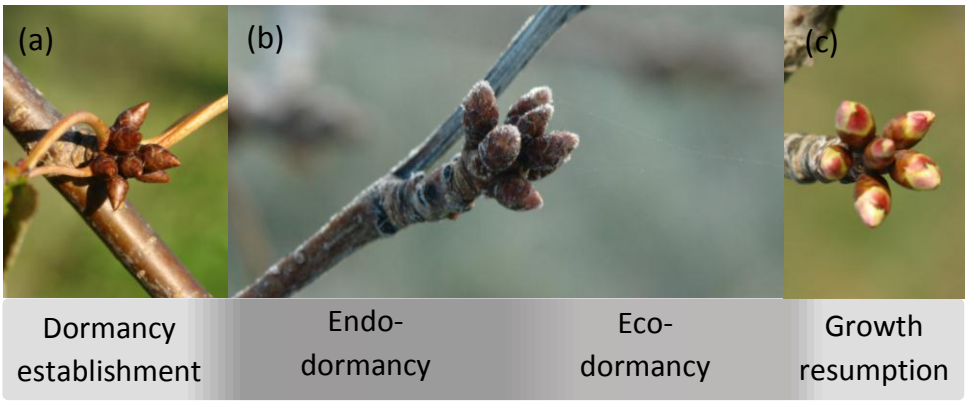

Figure 1. Phenological appearance of sweet cherry buds throughout winter dormancy: (**a**) dormancy establishment (BBCH 93, beginning of leaf fall), (**b**) endo- and eco- dormancy (BBCH 00 for dormant vegetative and BBCH 50 for dormant flower bud), and (**c**) growth resumption after eco-dormancy release (BBCH 01 for beginning of vegetative bud swelling and BBCH 51 for inflorescence buds swelling).

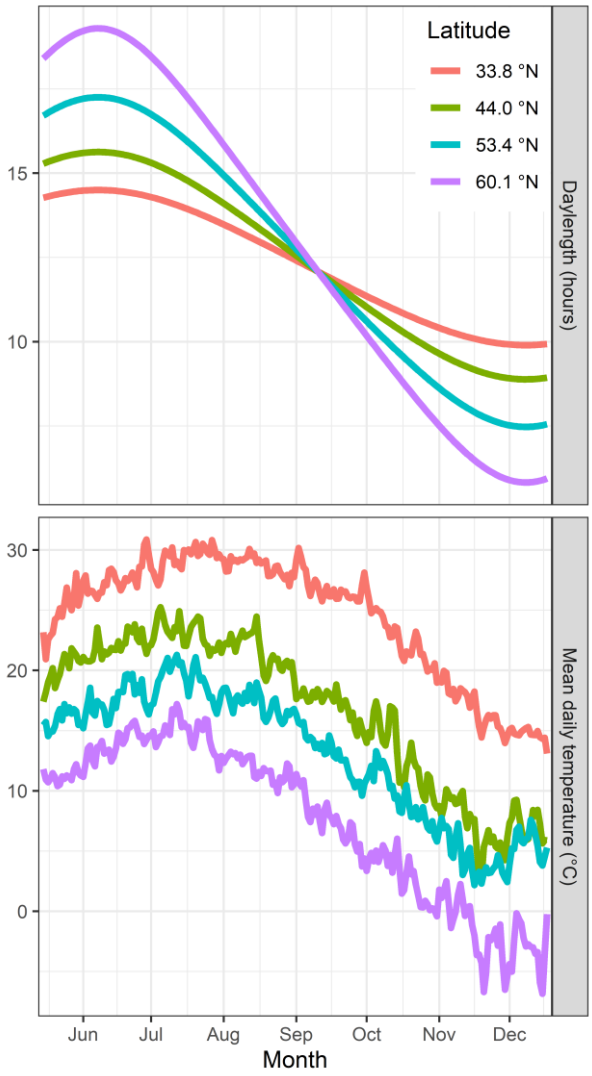

Figure 2. Effect of latitude (colored lines) on two environmental responses (daylength and daily mean temperature) in four northern-hemisphere locations. Data were acquired from the weather stations at Zarzis in Mellita, Tunisia (33.8° N, 10.7° E, 1 meters above sea level, m.a.s.l.); Montauban in Montauban, France (44.0° N, 1.3° E, 107 m.a.s.l.); Neuwiedenthal in Altes Land, Germany (53.4° N, 9.9° E, 3 m.a.s.l.); and Gustavfors in Bengtsfors, Sweden (60.1° N, 13.8° E, 188 m.a.s.l.). In the top panel, we show daylengths for each day of the year computed with equations by Spencer [32] and Almorox et al. [33] through a function in the chillR package [34]. The bottom panel shows mean daily temperatures based on daily minimum and maximum records of a six-year period (2012 to 2017). We downloaded these records from the Global Summary of the Day (GSOD) database from the National Climatic Data Centre (NCDC) of the United States National Oceanic and Atmospheric Administration (NOAA).

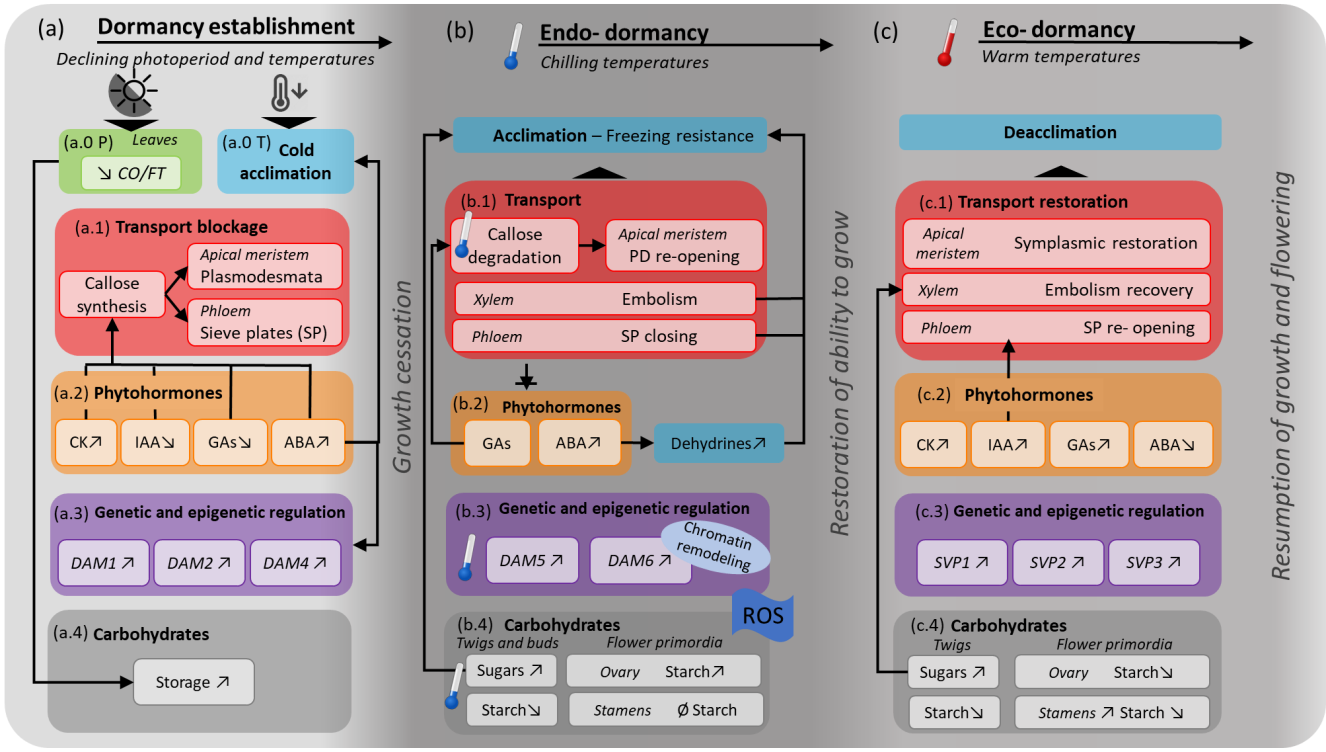

Figure 3. Conceptual framework of winter dormancy in deciduous trees. The dormancy framework (gray background) indicates three main phases: (**a**) dormancy establishment (light gray), (**b**) endo-dormancy (dark gray), and (**c**) eco-dormancy (medium gray). For each phase (**a**–**c**), the dormancy-related physiological processes are represented by colored shapes and numbers (0 to 4). These processes are (0) the regulatory module *CONSTANS (CO) /FLOWERING LOCUS T (FT)* (green square), (1) the transport at both whole-plant (phloem and xylem) and meristematic/cellular (plasmodesmata—PD) level (red squares), (2) phytohormone dynamics (cytokinins—CK, auxins—IAA, gibberellins—GAs, and abscisic acid—ABA) (orange squares), (3) genetic and epigenetic regulation of the *DORMANCY-ASSOCIATED MADS-box (DAM)* genes and the *SHORT VEGETATIVE PHASE (SVP)* genes (purple squares), and (4) carbohydrate dynamics (gray squares). Within each colored square, the light squares indicate the concrete substance or process (plain text) and where it occurs (italics). ↗ indicates rising levels, ↘ decreasing levels, and Ø indicates absence. The shaded sun refers to declining photoperiod whereas the black thermometer stands for declining temperatures. The blue and red thermometers indicate that cold or warm temperatures, respectively, are directly associated with a given process. Black arrows establish a causal relationship between processes.

2. A Seasonal Framework of Dormancy

Winter dormancy in temperate woody perennials is one of the phases of the plants' seasonal cycle. It is characterized by the temporary suspension of visible growth [35]. In temperate climate regions, trees normally grow and develop during the climatically most favorable seasons, spring and summer. In late autumn, trees establish a dormant state in order to survive unfavorable winter temperatures (Figure 1) [36]. Acknowledging that winter dormancy is likely a continuous process, we divided dormancy into three commonly distinguished phases to facilitate comprehensive understanding. It is worth noting that we did not consider para-dormancy (e.g., the dormancy imposed in formed buds during summer) but focused on essential stages of winter dormancy. These phases are (a) "dormancy induction" (Figure 1a); (b) "endo-dormancy" (also called "true dormancy" or "rest") (Figure 1b); and (c) "eco-dormancy" (also called "climatic dormancy" or "quiescence") (Figure 1b). Winter dormancy induction is associated with leaf fall, whereas the second phase (endo-dormancy) is characterized by meristems being unable to grow even under favorable conditions [15,24]. The third phase (eco-dormancy) is controlled by external factors. It is characterized by buds progressively becoming more responsive to favorable growth conditions, although they are still acclimated and resistant to winter cold [15,16,24]. Finally, eco-dormancy release and growth resumption are associated with the observation of the first phenological changes that lead to bud burst and bloom in response to favorable temperature conditions (Figure 1c).

Regulation of phase transitions during the dormancy process by environmental conditions such as photoperiod and temperature is highly species-specific. The chilling requirement, for example, differs greatly between species, with clearly distinguishable high- and low-chill species.

2.1. Dormancy Induction

When temperate trees shift from a fully active stage in summer to a dormant phase during autumn, buds set, growth is suspended, and leaves lose their green color and drop (Figure 1a). Temperature and photoperiod have been found to contribute to dormancy induction in many species [25]. Photoperiod is a reliable indicator of the time of year, and it decreases progressively after summer solstice. The intensity of this change varies according to latitude. For example, although the photoperiod shortens by nearly 10 h in the northern regions of the temperate zone, only a reduction by 4 h occurs in the south of the northern hemisphere (Figure 2). Temperature decreases after the reduction of the photoperiod as a consequence of the diminished radiation energy input (Figure 2). However, temperatures are also influenced by geographical location and climate. Winter temperatures are highly variable across temperate fruit production areas, ranging from several degrees below 0 °C in cold temperate climates to temperatures above 15 °C in Mediterranean regions. Similarly, photoperiod during winter ranges from 4–8 h of light at northern latitudes to 10–12 h in southern locations. Although photoperiod is highly predictable in any location, temperature can greatly vary across seasons due to year-to-year variation in weather.

The relative importance of temperature and photoperiod in the establishment of dormancy depends on the species and cultivar [37]. Early studies reported that critically short photoperiods trigger the cessation of growth and the onset of dormancy in forestry species such as yellow poplar or *Pinus* sp. (reviewed in [25]). Other species showed an interaction between photoperiod and temperature. For instance, short days with high temperatures at the end of summer can induce growth cessation and deep dormancy in many species, but they can delay the onset of dormancy in others, such as the temperate woody model plant *Populus* sp. [38,39]. Fruit tree species belonging to the Rosaceae family have been found to be non-sensitive to changes in photoperiod and to require mainly low nighttime temperatures to initiate dormancy [26,40–43]. Determining the exact moment of dormancy onset is difficult, as this state is progressively established. In temperate climate areas, where autumn is relatively cold, natural leaf fall can be a point of reference, whereas in other regions buds may already be dormant, when trees still carry foliage [44].

Bud set implies the initiation of scale leaves that protect the embryonic shoot. This process occurs at the end of summer and in early autumn. By the time dormancy establishes, the flower primordia appear to present different levels of development according to species [37,45]. In *Populus* sp., the gynoecium (stigma, style, ovary, and ovules) and the stamens (filament and anthers with sporocytes in each locule) present a more advanced developmental stage compared to *Prunus* spp. In species such as apricot (*Prunus armeniaca*) [46,47], sour cherry (*Prunus pseudocerasus*) [48], and sweet cherry (*Prunus avium*) [49], verticiles are distinguishable, but ovules do not show any sign of growth inside the ovary. Finally, in *Malus* spp. flower development during dormancy is suspended at an even earlier stage. In this group of species, the gynoecium and stamens are not distinguishable during dormancy initiation [50].

2.2. Endo-Dormancy

Unlike the phase of dormancy induction, endo-dormancy is controlled by plant-endogenous factors. This state is characterized by the inability to grow, even under favorable environmental conditions. Exposure to chilling is required for a certain period to overcome this state of rest and recover the buds' responsiveness to growth-promoting environmental signals. This inhibitory mechanism prevents bud burst during temporary spells of mild temperatures during the cold season [36].

Trees acclimate to low temperatures during dormancy, becoming able to survive harsh freezing temperatures, which would cause severe damage or even the death of the tree if they occurred during any other phenological stage (Figure 1b) [51]. The sensitive parts of the tree, such as the reproductive and vegetative meristems, remain protected inside the buds. Flower primordia present all verticiles differentiated and clearly distinguishable (petals, sepals, anthers, and pistil) [49].

Low temperature has been shown to be the main environmental signal that regulates dormancy release. Nevertheless, whether photoperiod is also involved in controlling this transition is still a matter of debate. Because only leaves contain photoreceptors, the absence of these organs during dormancy may imply that photoperiod can only have a weak effect, or no effect at all, on dormancy progression. Although biological evidence on how buds perceive the light signal is scarce, some studies indicate that photoperiod influences bud burst [52–54].

Scientists and growers currently use temperature-based models to quantify the exposure to effective cold temperatures that is needed to overcome endo-dormancy. Such models are also applied to predict bloom dates in several fruit tree species. Despite their extensive use to characterize the biological processes involved in tree dormancy, chill models are still fairly rough mathematical approximations. Currently available chill models do not include any known biological or physiological parameters [10]. Among these models, the Chilling Hours model is probably the most widely used in horticulture, as it is fairly easy to apply. This model considers any temperature between 0 and 7.2 °C (45 Fahrenheit) equally effective for endo-dormancy release. Despite extensive citations that the work of J.H. Weinberger has received as a reference for this model, its actual origin remains unclear. In 1950, J.H. Weinberger cited an oral presentation in 1932, given by the renowned phytopathologist Lee Milo Hutchins, as the source of this model [8], but it seems doubtful that this model was developed through rigorous scientific procedures. The next major innovation in chill modeling was the Utah model, published in 1974, which established a weight function for different temperature intervals, implying differences between these intervals regarding their effectiveness in contributing to endo-dormancy release [11]. In 1987, the Dynamic model emerged as the first process-based dormancy model. This model relied on the assumption that low temperatures stimulate the production of a chill precursor compound (not yet identified) that then needs to be converted into a permanent "chill portion" (quantifying the duration of effective chilling temperature) by a process that is most effective at moderate temperatures [12–14]. In this regard, among currently available chill models, the Dynamic model is based on the most sophisticated concept. Forestry studies on phenology prediction resulted in interesting advances, offering alternatives to the traditional chill–heat models currently used in agricultural sciences. These models account for the relationships between photoperiod and temperature [55,56], or even allow the

quantification of an interaction of physiological and environmental factors in addition to considering them separately [57].

2.3. Eco-Dormancy

Once trees have experienced a certain level of chill, and endo-dormancy has been overcome, buds recover their capacity to grow. However, they do not immediately resume growth. The external appearance of the tree does not change, but remains at the phenological stage "BBCH 50" (reproductive bud) or "BBCH 00" (vegetative buds). These stages describe the dormant state (Figure 1b) [58]. During this period, when the tree is acclimated (freezing tolerant) but not deeply dormant, growth is simply prevented by unsuitable environmental conditions. Therefore, this phase is referred to as eco-dormancy [15], although the term quiescence has also been proposed [16].

Also during this dormancy phase, temperature appears to be the main environmental driver. Mild temperatures may lead to cold de-acclimation triggering the resumption of growth [59]. A number of studies have highlighted that in this phase buds require heat exposure in order to reach subsequent phenological stages. To quantify the necessary heat, researchers have developed mathematical models that correlate temperatures with the transitions from a given phenological stage to the next one. The Growing Degree Hours model (GDH) [60] quantifies temperatures that are suitable for growth by using parameters for base, optimum, and critical temperature thresholds for growth. This model was initially developed to predict phenological stages in annual plants, but it was later applied, in combination with chill models, to predict bloom dates in trees, even though trees do not show any visible sign of activity during eco-dormancy [61].

The lack of a phenological trait to distinguish between endo- and eco-dormancy complicates the use of heat models. The first visible sign of growth occurs after a certain amount of heat has been accumulated by the bud. Therefore, the use of a model developed for clearly defined stages may be inaccurate [62]. Nonetheless, this transition point can be experimentally determined by evaluating bud growth under controlled conditions in growth chambers after different periods of chilling exposure [63,64]. To some extent, a long or intense period of heat can compensate for a lack of chill. Conversely, long or intense exposure to chill can compensate for a lack of heat [65,66].

First apparent phenological changes occur at the end of winter or in early spring, depending on the species and cultivar (Figure 1c). Trees progressively de-acclimate to cold and become more sensitive to freezing temperatures before bud burst. Changes inside the buds precede external changes at bud burst [49,58]. Important processes for sexual reproduction, such as anther meiosis and pollen formation or pistil growth, concomitantly occur inside the buds at this stage. Unlike in evergreen species, in deciduous trees all these processes rely on stored reserves from the previous growing season [67]. Moreover, in histerant species, in which the reproductive buds (i.e., flowers) resume growth before the vegetative buds (i.e., leaves), storage becomes a critical prerequisite for a successful start of the season. During this period, as in the previous stage, warm temperatures again promote growth progression. This allows the use of heat models such as growing degree days (GDD) or growing degree hours (GDH).

3. Physiological Processes that Regulate Dormancy

Dormancy as a whole is the result of complex interactions between numerous physiological processes that occur in different parts of the tree, such as buds, twigs, meristems, and vascular tissues. We divide these processes into four main themes: transport, phytohormones, genetic and epigenetic regulation, and carbohydrates.

3.1. Transport

During dormancy, the transport of water and solutes stops both at the whole-plant level and at the cellular level within the meristems. In higher plants, the function of long-distance transport is fulfilled by the vascular system, which is composed of xylem and phloem. The xylem transports

water and solutes from roots to aboveground structures. This transport depends on the build-up of negative pressure generated by water evaporating from the leaves. In the spring, the xylem facilitates sufficient water supply to allow flower and leaf expansion [68]. The phloem is responsible for maintaining connectivity between the various plant organs. It supports the long-distance transport of photosynthates and provides a signaling pathway [69]. In contrast to the passive transport mechanism within the xylem, transport within the phloem requires active symplastic transport through cellular membranes. In the shoot apical meristem, plasmodesmata allow cell-to-cell transport of molecules such as ions, hormones, minerals, amino acids, sugars, proteins, transcription factors, and various classes of RNA. Thus, plasmodesmata located in the sieve plates of the xylem as well as in the meristems play a major role in the orchestration of plant growth and development [50].

3.2. Phytohormones

Plant hormones are short- and long-distance signaling compounds acting in very low concentrations. They are major regulators of plant growth and development and mediate responses to biotic and abiotic stressors. Phytohormones also work as the internal drivers that regulate phenology according to environmental and seasonal variability [68]. Several phytohormones such as abscisic acid, gibberellic acid, auxin, cytokinins, and possibly jasmonic acid are implicated in direct or indirect regulation of different phase transitions during bud dormancy [59].

3.3. Genetic and Epigenetic Dormancy Regulation

In numerous fruit tree species regulation of bud dormancy has been shown to involve the action of *DORMANCY-ASSOCIATED MADS-box (DAM)* genes. This group of genes was identified and related to dormancy thanks to the *evergrowing* (*evg*) peach mutant, which presented a continuous growth behavior throughout all seasons of the year [17,18,70,71]. *DAM* genes are phylogenetically related to *SHORT VEGETATIVE PHASE* (*SVP)* genes in *Arabidopsis thaliana*. [72]. Subsequent work on several temperate tree species has consolidated the role of this gene family in regulation of growth–dormancy cycles, relating their expression patterns to each dormancy phase [73,74]. In *Populus*, the model tree, *FLOWERING LOCUS T* (*FT*) and other *FT*-like genes have been associated with the regulation of bud dormancy [60]. These genes regulate flower development and flowering in the model plant *Arabidopsis thaliana* and act as a main component in long-distance signaling. Recently, transcriptomic studies have shown global changes in expression patterns of genes associated with a broad range of structural and physiological functions during dormancy progression [22].

Epigenetic regulation has been postulated to be involved in environmental and molecular control of bud dormancy in perennials [26,75]. Epigenetic modifications to DNA and the proteins surrounding and packaging DNA (histones) alter gene expression patterns (active versus inactive genes) but do not affect the DNA sequence—a change in phenotype without a change in genotype. Changes in the expression of histone and DNA modification genes, as well as in the levels and patterns of DNA methylation and histone acetylation, suggest a role for chromatin remodeling in coordinating global changes in gene expression during growth–dormancy cycles [76,77].

3.4. Carbohydrates

Nonstructural carbohydrate dynamics have often been assigned critical roles in tree dormancy. Trees exhibit strong fluctuations between dormancy stages in the rates at which soluble sugars (i.e., glucose, fructose, and sorbitol, among others) and starch are synthesized and degraded. Nonstructural carbohydrates have three main functions: energy storage, transport and supply, and osmotic regulation and signaling. These main functions clearly vary within and between seasons.

Metabolic pathways in almost all cells produce reactive oxygen species. Plants take advantage of these metabolic products to work as signaling compounds that mediate plant responses to abiotic and biotic stresses and fulfill important functions in plant growth and development. Reactive oxygen species interact with plant phytohormonal networks and can affect basic cellular processes, such as the

cell cycle (the ordered sequence of events for cell division: size increase, DNA duplication, preparation to divide, and division or mitosis) [78]. The importance of reactive oxygen species for dormancy regulation has recently been reviewed [24].

The regulatory and metabolic processes during dormancy that we described above have mostly been studied independently, and thus we try to interrelate them to construct a single story. The following sections integrate these physiological processes during the commonly distinguished dormancy phases: dormancy induction, endo-dormancy, and eco-dormancy.

4. Physiological Processes during Dormancy Induction

Temperate woody perennials establish dormancy (Figure 3a) in response to shortening photoperiod (process a.0 P in Figure 3) and decreasing temperature (process a.0 T in Figure 3) in autumn. Photoperiodic induction of bud dormancy has been described in *Populus*, the model tree (process a.0 P in Figure 3). Phytochromes in plant leaves have been shown to act as detectors of changes in the levels of red and far-red light, which trigger downregulation of the regulatory module *CONSTANS (CO)/FLOWERING LOCUS T (FT)* [75]. In response to short days, the CO protein loses stability, which leads to decreased *FT* expression and down-regulation of gibberellin synthesis, establishing physiological conditions that allow bud formation [26,75] (process a.0 P in Figure 3). During bud formation, FT moves through the phloem towards the apex, where it initiates the formation of scale leaves and embryonic shoots [79]. However, the role of *CO/FT* has to be considered with extreme caution in temperate fruit trees, where its function has not been demonstrated, and the importance of low temperatures in this species suggest the influence of other mechanisms.

Moderately low temperatures during autumn promote dormancy establishment and cold acclimation, which is the development of cold hardiness, long before very low temperatures occur (process a.0 T in Figure 3). In contrast with photoperiod, temperature is perceived by the whole plant and affects multiple cellular components. Membrane fluidity and membrane-bound proteins act as temperature sensing systems, as falling temperatures slow down movements of proteins and lipids in membranes and make them more rigid. As a consequence, rigidification of membranes leads to activation of calcium channels in the membranes, causing an influx of Ca^{2+} into cells. The cytoskeleton may also participate in temperature perception processes, as dropping temperatures can lead to cytoskeleton disassembly by depolymerization of microtubules and microfilaments [80]. Temperature strongly affects plant metabolism, influencing enzymatic activities and thereby changing catalytic rates. Enzymes and structural proteins may unfold in response to a temperature drop, which entails loss of structural and enzymatic functionality and shifts in metabolite pools and redox status [81]. Much of the data cited is from the model plant *Arabidopsis thaliana*, but wherever possible, we also present evidence from other plants [51,80]. We are not aware of any studies that have produced direct evidence for woody perennials on how these mechanisms regulate dormancy establishment in autumn.

Dormancy establishment is largely driven by callose deposition in the plasmodesmata, which block cell-to-cell communication in the shoot apical meristem, as shown in hybrid aspen trees [82,83] (process a.1 in Figure 3). Phytohormones regulate this process (process a.2 in Figure 3), which is promoted by increasing levels of abscisic acid (ABA) [84,85]. In *Populus,* decreasing levels of gibberellins (GAs) also play a role [24,70,71], whereas this does not seem to be the case in grapevine [86]. ABA, a growth inhibitor and storage promoter, has been described as a key hormonal regulator in the initiation and progression of dormancy, mediating environmental signals such as short-day conditions. Apart from the key role of ABA in interrupting intercellular communication, abscisic acid also induces suspension of the cell cycle through inhibition of DNA replication at the G1 stage [87]. ABA has been proposed as an integral component in the *DAM* signaling pathway (process a.3 in Figure 3), and it has also been reported to affect *DAM* expression via a feedback mechanism [88,89]. ABA is antagonized by the growth promoter gibberellic acid (GA), which shows a decline during dormancy establishment. The ABA/GA ratio was found to be correlated with the depth of dormancy in sweet cherry [90].

In parallel to processes that block communication in the shoot apical meristem, transport at plant level is progressively limited as dormancy establishes (process a.1 in Figure 3). Callose plugs in the sieve plates interrupt sap flow through the phloem in response to the relative cytokinin (CK) vs. auxin (indole-3-acetic acid, IAA) content. CK stimulates callose production towards the end of the growing season, when IAA concentrations decline (process a.3 in Figure 3) [91]. Xylem transport also stops, because leaf senescence and leaf fall cause a progressive reduction in transpiration, which together with freeze-thaw cycles (mainly in temperate cold climates) may result in the presence of air bubbles and later cavitation [92,93].

The expression of *DAM* genes is associated with growth cessation and dormancy induction (process a.3 in Figure 3) in numerous fruit tree species, with important roles demonstrated for *PpeDAM1*, *PpeDAM2*, *PpeDAM3*, and *PpeDAM4* in peach [71,74]; for *MdoDAM2* in apple (*Malus x domestica*) [94,95]; and for *PmuDAM1*, *PmuDAM2*, and *PmuDAM3* in Japanese apricot (*Prunus mume*) [72,96], as well as for *EesDAM1* in the perennial weed leafy spurge (*Euphorbia esula*) [97,98]. The expression of these genes was shown to be related to shifting flowering times and to abnormalities in floral development [30].

In perennials, changes in expression patterns of dormancy-associated genes in response to environmental signals have been postulated to involve epigenetic regulation [26,75]. Changes in the expression of DNA and histone modification genes, as well as in the levels of genomic DNA methylation and histone acetylation, suggest a role for chromatin remodeling in coordinating global changes in gene expression during growth-dormancy cycles [23,77]. In hybrid aspen, for instance, genes encoding *FERTILIZATION-INDEPENDENT ENDOSPERM (FIE)*, a component of the Polycomb repressive complex2 (PRC2) linked to the trimethylation of H3 lysine 27 (H3K27me3), putative histone deacetylases, and a histone lysine methyltransferase were shown to be up-regulated during the transition to the dormant state [77].

Temperate woody perennials prevent starvation during winter by seasonal storage and reallocation of carbohydrates (process a.4 in Figure 3). During the growing season, carbohydrates are transported via the phloem from source (i.e., mainly leaves) to sink structures (i.e., fruits, new growth units, storage organs). In late autumn and winter, the synthesis of carbohydrates declines progressively until leaf fall. During this period, nitrogen is remobilized from leaves and transported to the storage organs [99]. Main storage structures are roots and stems, which reach a maximum level of reserves before leaf fall [100]. In sweet cherry twigs, which present maximum starch accumulation after autumn, nonstructural carbohydrates act as reserve molecules and may support future growth [101]. In flower primordia of reproductive sweet cherry buds, starch is only available in very low concentrations in ovule and anther tissues [102,103]. Regarding morphological changes inside the buds, flower primordia only present slight growth during dormancy establishment.

Taken together, all the processes described above lead to the cessation of growth and the establishment of the dormant state.

5. Physiological Processes during Endo-Dormancy

One of the biggest challenges that dormancy research faces is the elucidation of chilling perception. This requires deep knowledge of the molecular and supramolecular structures perceiving temperature shifts, as well as of the mechanisms employed to accumulate this signal. As noted above, the available information focuses on annual green plants but the mechanisms by which temperate woody perennials perceive temperature during winter remain unclear. Interestingly, research on dormancy has revealed several processes related with growth capability that respond to winter chill accumulation (Figure 3b). Among these processes are the restoration of the symplastic field by cell-to-cell communication in the shoot apical meristem [82,83] (process b.1 in Figure 3), expression of *DAM5* and *DAM6* genes [73] (process b.3 in Figure 3), and starch accumulation in the ovary primordia [102] (process b.4 in Figure 3).

During endo-dormancy, cell-to-cell communication is progressively restored by continuous exposure to chilling temperatures, which trigger callose degradation [82,83] (process b.1 in Figure 3). Exposure to chilling also leads to formation of proteins of the GH17 family (1,3-β-glucanases).

This group of proteins has been linked to the degradation of callose in the plasmodesmata to recover cell-to-cell communication. The restoration of the symplastic continuum in the shoot apical meristem does not imply direct growth resumption, but it facilitates the transition of buds into a quiescent state, in which they maintain freezing-tolerance [29]. Meanwhile, sap flow remains completely interrupted by heavy callose plugs in the phloem and air bubbles and cavitation in the xylem.

Phytohormones appear to be playing an important role in endo-dormancy maintenance and release (process b.2 in Figure 3). Continued in situ ABA synthesis may be a requirement for the maintenance of bud dormancy, and a decrease of ABA levels below a certain threshold contributes to the release of dormancy [104]. This has been demonstrated by the external application of an ABA synthesis inhibitor (fluridone) to dormant buds, which caused the initiation of new leaf primordia growth [105]. Consistent with this finding, bud-breaking chemicals act via activation of ABA catabolism in grapevine, involving a reduction in VvXERICO and VvNCED transcript levels and an induction in levels of VvABA8'OH homologues [106]. GA levels, which remain low during maintenance and increase towards release of endo-dormancy, seem to be regulated by the expression of GA-synthetic (GA20ox and GA3ox) and GA-catabolic (GA2ox) genes. GA catabolism was shown to be crucial for the induction and maintenance of bud dormancy [104] in woody species such as rose (*Rosa* sp.) [107], Japanese apricot [108], and grapevine [86]. The presence of GA, in turn, seems to promote dormancy release. GA4, a specific type of gibberellic acid, was shown to be able to replace the effect of chilling on dormancy release in *Populus* [83].

DAM genes present an expression pattern that is linked to endo-dormancy maintenance (process b.3 in Figure 3). These genes show high expression levels during endo-dormancy and are progressively repressed upon exposure to chilling temperatures. This expression pattern was first described for *PpeDAM5* and *PpeDAM6* in flower buds of peach trees, but further studies have consolidated its role in dormancy maintenance in other species, for example, *PmeDAM4, PmeDAM5,* and *PmeDAM6* in Japanese apricot [96], and *PseDAM5* and *PseDAM5* in sour cherry [109], reviewed in [30]). The physiological mechanisms regulating the expression of these genes in different genotypes in response to chilling accumulation require further examination. However, several recent studies indicate involvement of epigenetic mechanisms in environmental control of *DAM* gene expression during bud endo-dormancy release. In leafy spurge, cold exposure induces a decline in trimethylation of H3 lysine 4 (H3K4me3) and a rise of H3K27me3 in the promoter of *DAM1* concomitant with dormancy release [98]. The expression profiles of micro-RNAs, small non-coding RNAs, changed during chill accumulation in buds of peach [110]. The acetylation and methylation pattern associated with *DAM6* gene repression has been reported to change after dormancy release [111].

Temperate woody plants present maximum levels of reserves during endo-dormancy establishment (process b.4 in Figure 3) [67,100]. This pattern has been described in buds [112,113], as well as in storage organs such as twigs [101]. High sugar concentrations in the cytoplasm and an ABA-mediated rise in dehydrin concentrations prevent freezing injuries by facilitating osmotic regulation [114]. They also maintain plant metabolism, although at low rates. Detailed microscopic observations of starch in specific tissues of the flower primordia within reproductive sweet cherry buds have revealed that starch progressively accumulates in the tissue of ovary primordia during exposure to chilling. The ovary cells appear full of starch at the end of endo-dormancy [102]. This occurs specifically in this tissue, whereas the anthers do not appear to be accumulating starch during endo-dormancy [103].

Carbohydrate metabolism and chilling have been reported to directly affect oxidative stress at the cellular level. An elevated concentration of reactive oxygen species generated by gluconeogenesis and/or oxidative phosphorylation processes may lead to endo-dormancy release. Similarly, the absence of cellular communication due to blockage of the plasmodesmata may result in oxygen deprivation inside meristem cells. The latter phenomenon has been reported to increase glycolysis and ethanolic fermentation, which could lead to rising levels of reactive oxygen species [115].

6. Physiological Processes during Eco-Dormancy

Buds progressively regain their capacity to grow. During eco-dormancy, this capacity is completely restored, although growth is not resumed until the end of this phase. The transition to eco-dormancy does not directly lead to any morphological or developmental changes (Figure 3c). However, some of the endo-dormancy-regulating processes described above may serve as indicators of this transition. The re-opening of the symplastic communication pathway in the apical meristem appears to unlock the meristem's ability to grow (process c.1 in Figure 3) [82,83]. The expression of *DAM5* and *DAM6* genes, which act as growth repressors, is low (process c.3 in Figure 3) [30]. The ovary primordia cells appear filled with starch that can support subsequent growth and development (process c.4 in Figure 3) [102,116].

Cell-to-cell communication has already been restored in the shoot apical meristem. Communication at the whole-plant level, that is between different organs of the plant, is restored by removal of callose plugs from the sieve plates in the phloem vessels during eco-dormancy (process c.1 in Figure 3). Sap flow enables growth resumption, as it allows transport of water, reserves, and growth regulators to the buds. This restoration of the communication both between and within the organs of the plants enables phytohormones to facilitate growth resumption in early spring. External application of auxins has been shown to accelerate sap flow reactivation [117]. During eco-dormancy, concentrations of ABA drop and levels of growth-promoting hormones such as auxin and gibberellin increase [104].

Restoration of the hydraulic transport capacity of the xylem (process c.1 in Figure 3) depends on the starch stored in the surrounding cells [118] (process c.4 in Figure 3). The embolism repair mechanism is osmotically driven and supported by the degradation of starch into soluble sugars. Sugars are transported across membranes into the vessels, establishing a gradient that drives water movement [119,120]. Decreases in starch concentrations accompanied by increasing levels of simple sugars have been reported in sweet cherry twigs at the end of eco-dormancy [101]. These changes could be functionally associated with hydraulic transport capacity.

The high expression levels of *SHORT VEGETATIVE PHASE (SVP)* genes, i.e. *SVP1*, *SVP2*, and *SVP3*, during early spring function as growth inhibitors that prevent premature growth before bud break [30,121,122] in apple [110] and kiwifruit [109] (process c.3 in Figure 3).

Starch is also involved in supporting early-season development (process c.4 in Figure 3). Starch accumulates in the ovary during endo-dormancy, but its concentrations in the stamens only build up during eco-dormancy. The subsequent depletion of starch then leads to ovary growth resumption [102] and anther meiosis [103]. Thus, starch in flower primordia tissues acts as carbon and energy supply for growth and development, as described in the annual model plant *A. thaliana* [116], and in various flower structures of temperate fruit trees before key reproductive events [123–126].

7. Conclusions

This work aimed to integrate the current knowledge on dormancy into a general conceptual framework. In pursuing this goal, we attempted to strike a reasonable balance between the need for disciplinary depth and the ambition to capture the "big picture" of tree dormancy. To achieve an integrated understanding of the dormancy process, linkages between the various aspects that have been investigated need to be established and interrelationships clarified. We believe that such integration is crucial for understanding how trees adapt to the winter season in temperate regions, an adaptation that is critical for enabling their perennial life cycle.

The studies reviewed here can be divided into those that look for factors that regulate dormancy, such as hormones or genes, and those that focus on the effect of dormancy on physiological processes, such as cell-to-cell and whole-tree communication, management of reserves, and flower development. Establishing links and interconnections between both categories is fundamental for understanding how dormancy works. For example, the expression of some *DAM* genes and high concentrations of ABA might trigger the cold acclimation process. This cold acclimation is achieved by synthesis of dehydrins

and the production of callose, which in turn is involved in the interruption of both cell-to-cell and whole-tree communication.

This conceptual model aims to help researchers in focusing further studies to develop better tools to estimate and anticipate dormancy-related phenomena:

- How dormant trees perceive temperature, translate this perception into a signal and "accumulate" or record it: Chilling temperatures have clearly been established as one of the most important environmental factors that regulate dormancy. However, one of the main knowledge gaps in tree dormancy concerns the mechanisms by which plants perceive temperature. Although this knowledge gap persists, several factors have been related with the accumulation of chilling and dormancy completion.
- How the complex interactions of numerous physiological processes contribute to dormancy dynamics: The significance of each factor appears to vary according to species and location, and there is similar variation in plants' responsiveness to environmental signals (i.e. temperature and photoperiod). Photoperiod and temperature appear to contribute to dormancy establishment in species-specific ways. The most effective chilling temperatures that contribute to dormancy completion may also differ between species and cultivars.
- Dormancy model improvement: In general, the dormancy models that are currently available are composed of combinations of chill and heat sub-models, both of which are only based on temperature and phenological observations. A modeling approach based on the whole process, rather than on just a single environmental parameter (i.e., temperature), could offer not only a tool to predict flowering dates but also a reliable framework of dormancy progression.

In contrast to most other modeling challenges in agricultural research, dormancy modeling attempts to explain a process during which buds do not show any visible growth. Modeling dormancy is further complicated by apparent differences in dormancy-related processes across species, cultivars, and possibly climates. Dormancy modeling thus remains a substantial challenge. Nevertheless, considering that all of the commonly used chill models, which constitute the heart of all dormancy models, were developed before 1987, major progress should be within reach. None of these models includes the significant progress in our understanding of dormancy physiology that has emerged during more than three decades of highly productive dormancy research across multiple disciplines. At the same time, tools for integrating various types of information and for modeling complex processes have greatly expanded, opening opportunities that were not available to the pioneers of chill and dormancy modeling.

Given the prospects of increasingly warm winters, which may have serious implications for many growers of deciduous fruit and nut trees, tools to anticipate the impacts of global warming are urgently needed. Warm winter temperatures may compromise dormancy release and modify the phenology of temperate trees. More generally, climate change may limit the suitable ranges of temperate fruit and nut trees, possibly with devastating impacts on well-established industries. These challenges call for renewed efforts to model tree dormancy and to narrow remaining knowledge gaps through targeted experimentation. They also highlight the need for international collaborations to elucidate, generalize, and validate temperature responses across the wide range of climatic settings in which deciduous trees are cultivated.

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