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SYMPOSIUM

Diverse Developmental Responses to Warming Temperatures Underlie Changes in Flowering Phenologies

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Synopsis Climate change has resulted in increased temperature means across the globe. Many angiosperms flower earlier in response to rising temperature and the phenologies of these species are reasonably well predicted by models that account for spring (early growing season) and winter temperatures. Surprisingly, however, exceptions to the general pattern of precocious flowering are common. Many species either do not appear to respond or even delay flowering in, or following, warm growing seasons. Existing phenological models have not fully addressed such exceptions to the common association of advancing phenologies with warming temperatures. The phenological events that are typically recorded (e.g., onset of flowering) are but one phase in a complex developmental process that often begins one or more years previously, and flowering time may be strongly influenced by temperature over the entire multi-year course of flower development. We propose a series of models that explore effects of growing-season temperature increase on the multiple processes of flower development and how changes in development may impact the timing of anthesis. We focus on temperate forest trees, which are characterized by preformation, the initiation of flower primordia one or more years prior to anthesis. We then synthesize the literature on flower development to evaluate the models. Although fragmentary, the existing data suggest the potential for temperature to affect all aspects of flower development in woody perennials. But, even for relatively well studied taxa, the critical developmental responses that underlie phenological patterns are difficult to identify. Our proposed models explain the seemingly counter-intuitive observations that warmer growing-season temperatures delay flowering in many species. Future research might concentrate on taxa that do not appear to respond to temperature, or delay flowering in response to warm temperatures, to understand what processes contribute to this pattern.

Introduction

“Phenological shifts have been among the most obvious and thoroughly documented biological responses to the climate warming of the last 150 years” (Forrest and Miller-Rushing 2010). For plants, the timing of flowering is a critical life history event (Rathcke and Lacey 1985), especially for species dependent on interactions with animals for pollination and seed/fruit dispersal (e.g., Hegland et al. 2009; Singer and Parmesan 2010). Many angiosperms flower earlier in response to increased temperatures (e.g., Amano et al. 2010; meta-analysis in Wolkovich et al. 2012) but the responses can be complex. The magnitude of precocious flowering relative to seasonal temperatures can be highly variable even among closely related taxa in the same area (Fitter et al. 1995; Calinger et al. 2013; Iler et al. 2013; CaraDonna et al. 2014). And paradoxically, a significant number of species either do not respond or in fact delay flowering in association with elevated temperatures (e.g., Bradley et al. 1999; Sherry et al. 2007; Ge et al. 2011; Cook et al. 2012; Mulder et al. 2017). In some communities, plants show the expected advance in the onset of flowering in warm years, but no change in mean flowering date on a multi-decadal timescale, despite significant increases in summer temperatures (e.g., Hart et al. 2014; Davis et al. 2015). Such individualistic species

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responses have the potential to result in profound disturbances at the population and community levels, as patterns of co-flowering (e.g., CaraDonna et al. 2014) and interactions with animal pollinators and dispersers are disrupted (e.g., Calinger et al. 2013; Høye et al. 2013; Ovaskainen et al. 2013).

Temperature is clearly an important cue for flowering time, and accumulation of warm days (e.g., measurements of degree days) predicts flowering date reasonably well for many species (e.g., Chuine 2000; Pope et al. 2014). What then, can explain the many exceptions to the general pattern of advancing spring phenologies with warming temperatures? Other environmental cues such as photoperiod or soil moisture may oppose the effects of warming (e.g., Cook et al. 2012; Dorji et al 2013), increased snow fall may result in delayed snow melt (e.g., Semenchuk et al. 2013), or warmer winter temperatures may result in unmet chilling requirements that affect emergence from dormancy (e.g., Yu et al. 2010; Cook et al. 2012; but see Shen 2011). The environmental cues and physiological responses that determine flowering time, however, are not well understood. Research on phenological responses often measures or manipulates an environmental cue, such as photoperiod, mean temperature, or growing degree days, in relation to specific and easily observed events such as bud break and the onset or peak of flowering. Leaf emergence or anthesis, however, are but brief phases within a complex developmental trajectory that often begins one or more years previously (e.g., Foerste 1891; Diggle 1997): a process known as preformation.

Preformation

Preformation of leaf and flower primordia is characteristic of temperate forest trees, shrubs, and herbaceous perennials, and ubiquitous for high elevation and high latitude species (Sørenson 1941; Hodgson 1966; Billings and Mooney 1968). Much of the development of preformed leaves and flowers occurs during the year (or years) before maturation and function (Foerste 1891; Sørenson, 1941; Diggle 1997; Aydelotte and Diggle 1997; Meloche and Diggle 2001; Albani and Coupland 2010). Figure 1 summarizes a typical two-year developmental trajectory of flower development associated with preformation. Preformation begins as plants emerge from dormancy; meristems resume activity and initiate new flower primordia. Additional initiation and significant morphogenesis of structures occur as the season proceeds until ultimately development ceases with primordia packed into condensed apical and lateral buds. Following growth cessation, dormancy must be induced, maintained, and then released (typically by cold winter temperatures, followed by warming spring temperatures; Lang et al. 1987; Cooke et al. 2012; Ding and Nilsson 2016). Once plants are released from dormancy, preformed flower primordia resume development, and for most species, flower maturation occurs in this second year. How temperature influences the onset of meristem activity, the rate of primordium initiation, and morphogenesis (i.e., the speed of preformation), and the cessation of development prior to dormancy are not well studied. As a result, the potential for developmental responses in one year to affect flowering phenology in the following year are difficult to evaluate.

For this symposium contribution, we develop a series of conceptual models (Fig. 2) that explore potential developmental responses to warming growing-season temperatures and the effect of those responses on the timing of anthesis. We then synthesize information from a variety of studies to summarize what is known about the effects of temperature on flower development during preformation. Because the majority of large-scale analyses of changing phenologies focus on the woody perennials (mostly trees) that dominate temperate ecosystems, our synthesis concentrates on the most well studied tree systems: species of the genus *Populus*, and fruit-crop trees of the Rosaceae (*Malus*, *Prunus*, *Pyrus*). For each system, the summaries are based on studies of multiple species and cultivars. For some processes, information on preforming herbaceous perennials is also included.

Models of potential developmental responses to growing-season temperature (Fig. 2)

Our models explore the consequences of changes in onset, rate, and cessation of development across the two-year trajectory of flower development characteristic of preforming species.

Model 1: Early warm spring temperatures in year 2 result in early emergence of preformed flower primordium from dormancy, with no necessary change in rate of development, resulting in advanced timing of anthesis.

Model 2: Warm spring temperatures in year 2 increase the rate (with no necessary change in onset) of preformed flower development in year 2 and anthesis is advanced.

Model 3: Warm temperatures during the growing season of year 1 accelerate flower development in year 1 with no effect on cessation of development.

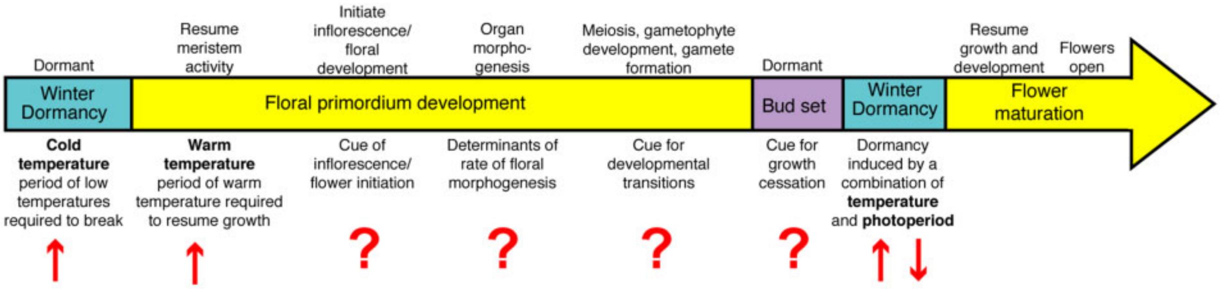


Fig. 1 Two-year trajectory of flower development and the potential effects of growing-season temperature. The text above arrow notes the processes of flower development, from initiation to anthesis. Below the arrow are possible cues that may affect the developmental processes. The red symbols below the cues show the potential effect of greater temperatures on the process. The symbol ? indicates that the effect is unknown. For many taxa a period of cold temperature is required for plants to emerge from dormancy and a period of warm temperature is required for meristems to resume activity.

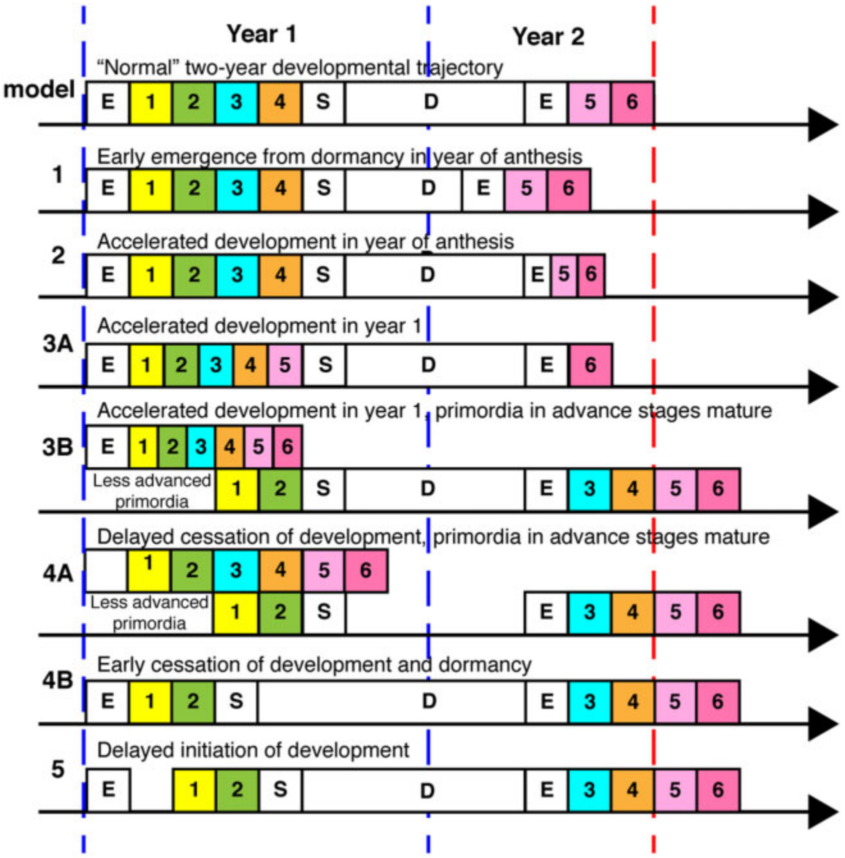


Fig. 2 Summary of models. Top panel shows expected 2-year flower development with emergence from dormancy (E) stages of development (1–6), cessation of development (S), dormancy (D) in year 1, and resumption of development (E) followed by anthesis (stage 6) in year 2. Lower panels show how variation in development could result in changes in the time of flowering in year 2. Models 3B and 4A require within plant variation in developmental stages of primordia, denoted by two trajectories in each panel. Vertical blue lines denote the beginning of the year. Vertical red line is anthesis of a "normal" year.

Accelerated development could either advance (3A) or delay (3B) flowering in year 2.

3A: Accelerated development in year 1, as long as developmental cessation and dormancy occur prior to flower maturation, would result in flower primordia in more advanced stages of development at dormancy. Following emergence from dormancy, primordia have less development to complete and could reach anthesis early in year 2, regardless of the temperature that year. Model 3A may explain the strong signal of phenological responses that lag warm temperatures by 1 year (Arft et al. 1999; Khorsand Rosa et al. 2015).

3B: Warm temperatures accelerate development of some (but, critically, not all) flower primordia such that they mature in the fall of the year they were initiated, leaving *less advanced* primordia to mature *later* in the following spring. This model requires that flower buds are in different stages of development. Model 3B is consistent with the occurrence of second flowering with warm temperatures (Foerste 1891; Ge et al. 2011 and anecdotal observations of species flowering in the fall during years with above-average summer temperatures, as well as phenological delays that lag warm temperatures by a year [Mulder et al. 2017]).

Model 4: Warm temperatures late in the growing season of year 1 affect the cessation of primordium development. Given the divergent physiological responses to late season temperatures (see section on "dormancy" below), growth cessation might be either delayed (4A) or accelerated (4B) by warming, depending on the species. Either developmental response in year 1 could result in later flowering in year 2.

4A. Warm temperatures delay growth cessation in year 1 to such an extent that some, but not all, flowers continue developing and mature before the onset of dormancy in late fall. As described for model 3B, an observed response of delayed flowering in year 2 requires flowers in different stages of development.

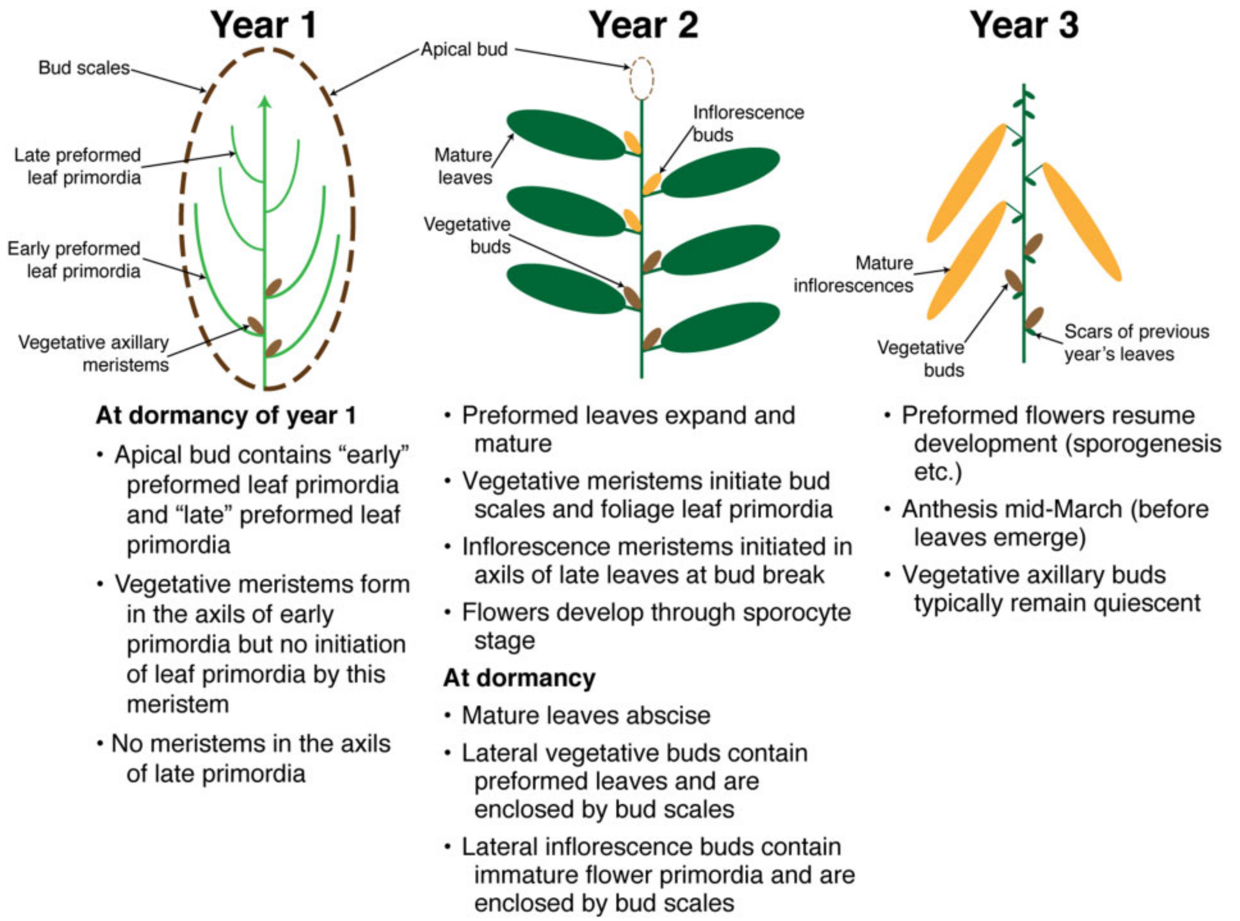
4B: Warm temperatures induce early cessation of development and dormancy in year 1. Consequently, flower primordia are in earlier stages of development at dormancy and require longer to reach anthesis in year 2.

Model 5: Warm temperatures early in the growing season of year 1 delay initiation of reproductive development, but with no effect on rate, such that flowers are in earlier stages of development at dormancy. Following resumption of development in year 2, flowers require a longer time period to reach anthesis.

Preformation in *Populus* and Rosaceous fruit-crop trees

Below we summarize what literature is available to evaluate the models. Because our summary of the literature will concentrate on *Populus* and Rosaceous fruit-crop trees as exemplars of temperate woody trees, we first provide a summary of their reproductive development as context. For both *Populus* and Rosaceous fruit-crop trees, anthesis is the end point of a 3-year developmental trajectory (Figs. 3 and 4). Inflorescences of *Populus* are lateral on long or short shoots (Fig. 3; based on Boes and Strauss 1994; Yuceer et al. 2003). Development of an annual increment of a shoot that will ultimately bear inflorescences begins with the initiation of bud scales and leaf primordia by the apical meristem, forming an apical bud. Vegetative axillary meristems are initiated in association with the basal most ("early") leaf primordia before the bud, enclosed by the bud scales, becomes dormant. In year 2, as the preformed leaf primordia begin to expand at bud break in early spring, inflorescences are initiated in the axils of the distal ("late") preformed leaves. Those inflorescence meristems initiate a succession of bracts, and approximately 2 months after inflorescence initiation, individual flower primordia form in the bract axils. At dormancy of year 2 flowers have initiated all floral organs. The gynoecium consists of ovary, style, and stigma, and within the ovary ovules have been initiated, however, meiosis has not yet occurred. Stamens are differentiated into filament and anthers, with clearly defined sporocytes in each locule. In year 3, flowers undergo the final stages of morphogenesis and reach anthesis in the early spring, before foliage emerges.

Inflorescences of Rosaceous fruit-crop trees are terminal and, for most taxa, are borne primarily on sympodial short shoots. As for *Populus*, each annual shoot increment undergoes a 3-year process of preformation (Fig. 4; based on Foster et al. 2003); however, the timing and relationship between vegetative and reproductive structures are quite different. In year 1, within an apical bud, a renewal-shoot meristem is initiated in the axil of a foliage leaf primordium (the subtending leaf and meristem are initiated in the same year). The preformed leaf and axillary meristem then undergo a period of dormancy still within the apical bud. In year 2, as the subtending leaf expands and matures, the renewal-shoot meristem in its axil initiates a set of protective bud scales and foliage leaf primordia, and then makes a transition from vegetative to reproductive capacity. In *Malus* the transition from vegetative to inflorescence meristem occurs in late summer, ~100 days after full bloom of the preformed inflorescences that had developed during the preceding year (Foster et al. 2003), however this interval differs among genera, cultivars, and location (Forshey and Elfving 1989; Fulford 1966; Rivero et al. 2017). Following the transition to reproductive activity, floral primordia are initiated. Similar to *Populus*, flowers of most taxa have all organs initiated and development has proceeded to the presence of sporocytes within ovules and anthers prior to dormancy



At dormancy of year 1

- Apical bud contains "early" preformed leaf primordia and "late" preformed leaf primordia
- Vegetative meristems form in the axils of early primordia but no initiation of leaf primordia by this meristem
- No meristems in the axils of late primordia

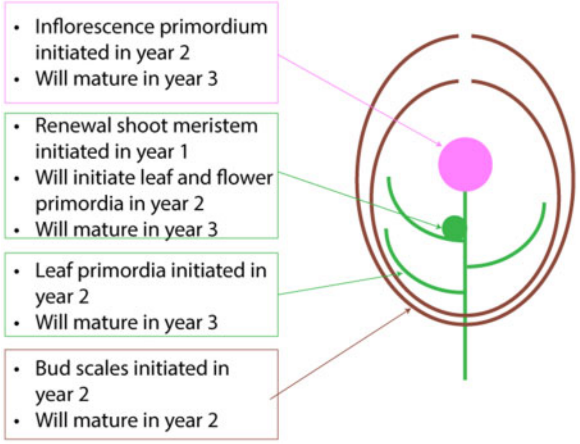
- Preformed leaves expand and mature
- Vegetative meristems initiate bud scales and foliage leaf primordia
- Inflorescence meristems initiated in axils of late leaves at bud break
- Flowers develop through sporocyte stage

At dormancy

- Mature leaves abscise
- Lateral vegetative buds contain preformed leaves and are enclosed by bud scales
- Lateral inflorescence buds contain immature flower primordia and are enclosed by bud scales

- Preformed flowers resume development (sporogenesis etc.)
- Anthesis mid-March (before leaves emerge)
- Vegetative axillary buds typically remain quiescent

Fig. 3 Three-year developmental trajectory of a reproductive shoot of *Populus*. Based on Boes and Strauss (1994) and Yuceer et al. (2003).



- Inflorescence primordium initiated in year 2
- Will mature in year 3

- Renewal shoot meristem initiated in year 1
- Will initiate leaf and flower primordia in year 2
- Will mature in year 3

- Leaf primordia initiated in year 2
- Will mature in year 3

- Bud scales initiated in year 2
- Will mature in year 2

Fig. 4 Three-year developmental trajectory of a reproductive shoot of *Malus*. Dormant bud enclosed by bud scales contains leaf and flower primordia on a renewal shoot that had been initiated in the preceding year and that will mature in the following year. Based on Foster et al. (2003).

(Pratt 1988; Fadón et al. 2018 [specific time to rest]; Felker et al. 1983; Julian et al. 2011). The inflorescence and the subjacent foliage leaves, protected by the basal most bud scales, become dormant in the fall. Flower and leaf maturation occur in year 3.

What is known about the effect of temperature over the 3-year course of development for these two systems? Development during preformation is difficult to study. It occurs deep within axillary and apical buds, and analyses generally require destructive sampling and light or scanning-electron microscopy.

Temperature effects on inflorescence and flower initiation

In *Populus*, inflorescences are initiated in the axils of preformed leaves at bud break (Fig. 3). Whether meristem initiation follows leaf expansion inevitably, or is triggered by a separate cue, is not known. If initiation invariably follows leaf expansion, then the timing of inflorescence initiation would be directly related to the environmental cues, including temperature, that affect vegetative budburst. Conversely, if bud break and inflorescence initiation respond to (at least partially) separable cues then initiation could vary with respect to bud burst.

The transition from vegetative to reproductive development in Rosaceous fruit trees has been described as "autonomous" (e.g., Wilkie et al. 2008). Some reports, however, are suggestive of a role for external environmental cues. Because inflorescences are terminal on vegetative shoots (Fig. 4), environmental cues such as temperature that affect vegetative vigor (duration of leaf initiation) may also affect the timing of inflorescence initiation. Fulford (1965, 1966) and Luckwill (1974) suggest that the transition from vegetative to inflorescence development is dependent on the initiation of a particular number of leaves (and this varies among cultivars). Evidence for an effect of temperature on the rate of vegetative growth (the length of time to initiate the prescribed number of leaves), however, is contradictory (compare Fulford 1966; Verheij 1996). Other work shows that decreasing temperatures in late summer may be required for the transition to reproductive development (Tromp 1976; Zhu et al. 1997; Rivero et al. 2017), suggesting that warm temperatures could delay this transition. Considering all of the evidence for Rosaceous fruit trees, warmer temperature might either advance initiation indirectly by a positive effect on the rate of vegetative development, or delay inflorescence initiation by affecting induction of reproductive development.

Insight into the relationship between temperature and the timing of inflorescence initiation in woody species may soon come from molecular genetics. In *Arabidopsis*, regulation of flowering time, including inflorescence initiation, is well understood (e.g., Amasino and Michaels [2010] and references therein). Although the pathway is complex and affected by both photoperiod and temperature, one protein, FT, appears to be a "master integrator" of various pathways that converge to control the transition from vegetative reproductive development. The expression of FT in *Arabidopsis* is temperature sensitive and levels of FT link variation in inflorescence initiation to temperature (e.g., Blázquez et al. 2003; Amasino and Michaels 2010). Orthologs of FT have been found in both *Populus* and *Malus* (Böhlenius et al 2006; Hsu et al. 2011; Mimida et al. 2011). PtFT1 expression in *Populus* is activated by cold temperatures and suppressed by warm temperatures. While temperature and PtFT1 levels affect meristem identity (vegetative vs. reproductive) in *Populus*, we do not know whether the timing or level of PtFT1 expression might, in turn, influence the *timing* of meristem initiation. In *Malus*, inflorescence initiation is associated with increased expression of MdFT (also an ortholog of the *Arabidopsis* FT) in leaf primordia and the shoot apex (Mimida et al. 2011) at the time of transition to reproductive development. Whether MdFT expression affects the timing of this transition and whether expression is affected by temperature is not known.

Temperature effects on the rate of flower development following initiation (Year 1)

Temperature is well-known to affect the rate of biological processes such as cell division and expansion (e.g., Hänninen and Tanino 2011; Körner 2015). The effects of temperature on these processes form the basis for many models of phenology (e.g., Chuine et al. 2016); however, such models focus on the year of flower maturation, not the preceding year(s). The effects of temperature on the rate of development during the first year have not, to our knowledge, been studied.

Temperature effects on the transition to dormancy

Physiologists have long known that critical short photoperiods trigger the cessation of growth and the onset of dormancy in many species (e.g., Kramer 1936; Vaartaja 1954; Wareing 1956; Nitsch 1957; Weiser 1970; reviewed in Singh et al. 2017). Recent experimental evidence, however, demonstrates more complex and interactive effects of photoperiod and temperature on dormancy in a large number of woody species (e.g., 25 studies summarized in Table 1 of Tanino et al. 2010; Kalcsits et al. 2009; Hänninen and Tanino 2011). This research finds that during the short day-lengths that are characteristic of late summer, high temperatures can induce *earlier* growth cessation and deeper dormancy for many species (references in Tanino et al. 2010; Rohde et al. 2011). In contrast, warm temperatures and short days *delay* the onset of dormancy in *Populus* (Rohde et al. 2011; Rinne et al. 2018). Other species, including Rosaceous fruit trees, are insensitive to photoperiod and require low night temperatures to induce growth cessation and dormancy (Häbørg 1972; Junttila 1980, 1982; Heide and Prestrud 2005; Svendsen et al. 2007; Heide 2008; Wilkie et al. 2008; Tanino et al. 2010; Hänninen and Tanino 2011; Cooke et al. 2012). In such non-photoperiodic species, warmer temperatures may cause an *extension* of the growing season into the autumn. Given these divergent physiological responses, dormancy might be either delayed or accelerated by late season warming, depending on the species.

What is the relationship between flower development, growth cessation, and dormancy?

Prior to dormancy, both reproductive and vegetative development cease. The relationships among the visible cessation of vegetative growth, the appearance of apical or axillary buds (typically enclosed by bud scales; this stage is often recognized as bud set), and the developmental processes occurring within those buds are not often examined. When vegetative and floral development actually cease, and what environmental cues (if any) might affect that timing relative to visible signs of bud set or the induction of dormancy are largely unknown.

In Rosaceous fruit trees, the maturation of foliage leaves on the reproductive short shoots is completed (i.e., apparent vegetative growth stops) about 4 weeks after full bloom (Forshey and Elfving 1989), yet preformation of leaf and flower primordia on that same shoot continues well into late fall without visible changes to the enclosing bud (Fadón et al. 2018). Thus, termination of visible vegetative growth (at least for reproductive shoots) occurs well before cessation of leaf and floral development within buds. The cues that determine the time that development stops, and whether leaf and flower primordia, which are on the same shoot in the same bud in these taxa (Fig. 4), respond to the same cue, is not known.

For *Populus*, because preforming reproductive and vegetative buds are spatially separated (Fig. 3), termination of their development may be quite independent. When vegetative shoots of *Populus deltoides* are induced experimentally into dormancy by transfer to short days, the stipules of the first leaf primordia initiated after transfer differentiate into bud scales, and visible signs of vegetative growth cease. Initiation of leaf primordia within the buds continues, however, and an average of 16 leaf primordia are present before vegetative development ceases prior to dormancy (Goffinet and Larson 1981). In field trials of *Populus nigra*, vegetative growth stopped in mid-August in northern Europe and mid-September in the Mediterranean, while the stage recognized as bud set, with mature, hardened bud scales, took place about 2 months later (Rohde et al. 2011). How long leaf primordium initiation continued within those buds, and whether bud set corresponds to termination of development within the buds was not examined. Inflorescence development was not monitored in these trials, but studies of trees from the NW USA find that floral development within lateral buds continues well into late fall (Boes and Strauss 1994; Yuceer et al. 2003). Again, the cues for cessation of inflorescence development are not known. Based on these studies of *Populus*, the environmental cues associated with the cessation of leaf and flower development within buds clearly cannot be inferred from the termination of visible vegetative growth or even from the stage typically recognized as bud set.

Temperature effects on developmental stage at dormancy

Development of preforming flower primordia ceases at some point prior to dormancy. *Populus* and Rosaceous fruit trees enter dormancy with all floral organs well developed but before meiosis has occurred in ovules or anthers (see description of preformation above). Julian et al. (2014) suggest that for most temperate woody species, including both flowering plants and conifers, flower development ceases just prior to, or just after meiosis. However, the number of taxa that have been examined is quite small. The stage at which development ceases appears to be characteristic for a particular species or cultivar, suggesting that stage may not be subject to the effects of temperature. Conversely, the uniformity of stage at dormancy may indicate that flower primordia are incapable of responding to the environmental cues that induce growth cessation until they have reached a particular developmental stage.

In contrast to the prevalence of pre-/post-meiosis stages at dormancy of temperate trees, Sørenson's (1941) comprehensive study of the arctic tundra flora showed that the developmental stage of flower primordia at dormancy varies widely among species, ranging from floral meristems with no evidence of organ initiation to fully developed (but unexpanded) flowers with mature pollen. Similarly, Mark (1970) found many different stages of development at dormancy in a survey of alpine plants of New Zealand. These studies suggest that no particular stage of development is particularly favored for persistence over winter; however, it must be noted that dormant buds of tundra species were at or below ground level and may be insulated from extreme cold temperatures by snow.

Variation among flowers and inflorescences at dormancy

Although variation in developmental stage among flowers on an individual is rarely reported, it may be more common than published studies suggest.

Flowers within inflorescences are initiated sequentially, and at least early in development must differ in developmental stage. Inflorescences of an individual may also begin developing at different times. Similarly, flowers and inflorescences on the same individual often reach anthesis at different times. Given this variation at the initial and final stages of flower development, it would not be surprising to find variation in developmental stage at dormancy. Among the few studies to explicitly analyze such variation, Mirgorodskaya et al. (2015) found that flowers of distal inflorescences of *Rhododendron ledebourii* are in more advanced stages of development at dormancy and that these flowers may reach anthesis in the autumn of unusually warm years. Reports of anomalous fall flowering are increasingly common (Ge et al. 2011; personal observation and many anecdotal reports). The frequency of variation in developmental stage among flowers and whether this is associated with anomalous fall flowering cannot be evaluated with the available data.

Temperature effects on release from dormancy and subsequent bud burst

For many species, release from dormancy and the expansion of overwintering buds in the spring are clearly sensitive to temperature (e.g., Singh et al. 2017 and references therein). For most temperate perennials, some period of low temperature is required before plants are capable of responding to warming spring temperatures. Following release from dormancy, bud break occurs after some accumulation of warm temperatures. The specific temperature requirements to release dormancy and then to resume growth differ among taxa, and even among individuals within species that are distributed at different latitudes.

Early flowering in warm springs is commonly attributed to early release from dormancy, and release from dormancy is often assessed by bud break. What is known about the relationship between release from dormancy, resumption of development of preformed structures, and bud break? Bud break, recognized as the separation of bud scales and emergence of green leaf tips (e.g., Ettinger et al. 2018), must necessarily be preceded by some period of growth (cell division and/or expansion) of preformed structures within those buds. Information about when development within preformed buds actually resumes, however, is scarce, and it is difficult to evaluate the extent to which early onset of development explains advanced phenologies. We also do not know whether the resumption of vegetative and reproductive development occur at the same time and in response to the same environmental cues. Or, do flower primordia require a specific environmental cue to resume growth?

An association between the timing of bud burst and flowering across years might suggest that resumption of vegetative and reproductive development respond to the same cues. The temporal relationship among various phenological events, however, is rarely examined. In fact, a meta-analysis of phenological studies showed that a mere 5 of 51 studies assessed both vegetative and reproductive phases (Wolkovich et al. 2012, cited by Ettinger et al. 2018). Two recent studies provide critical evidence that resumption of vegetative and flower development may be independent. Mulder and Spellman (2019) examined bud burst and flowering for 41 boreal understory species over 3 years. They found no correlation between dates of bud burst and flower emergence, and only a marginally significant correlation between changes in the onset of these two events across years that had very different early spring temperatures. Similarly, data from 25 temperate tree species recorded over a single year (Ettinger et al. 2018) show only a very weak association between the time of bud burst and flowering. The lack of relationship between the two is consistent with a different onset or rate of development for vegetative and reproductive development.

Identifying the time at which development actually resumes within preformed buds requires destructive sampling over time. In Rosaceous fruit trees, such analyses show that considerable development of preformed floral primordia occurs before any external changes are visible. For example, in *Prunus avium* flowers develop for nearly 3 weeks before buds begin to swell and bud scales separate. During this time, meiosis occurs and microsporogenesis is completed within the anthers (Fadón et al. 2018). The effects of early spring temperatures on the resumption of preformed flower development in *Prunus* or other Rosaceous fruit trees have not, to our knowledge, been examined. For *Populus*, the onset of flower development in the year of anthesis has not been studied (Brunner et al. 2014).

Temperature effects on rate of development in year 2

Is early flowering in response to warm spring temperatures associated with more rapid development? As noted above, temperature generally affects the rate of biological processes such as cell division and expansion and it would be surprising if warm temperatures did not increase at least the final processes of cell expansion responsible for emergence of flowers from buds. In a study of earlier processes of development, Julian et al. (2014) compared flower development of five cultivars of *Prunus armeniaca* over the course of one cold and one warm year. For all five cultivars, preformed flowers resumed development later following the warm winter due to unmet chilling requirements, but subsequent development (meiosis and microsporogenesis) was more rapid and flowering time was ultimately unchanged compared with the colder year. Spring temperatures were also higher following the warm winter and may have caused the rapid pre-anthesis development (J. Rodrigo, personal communication). We found no information on the rate of floral development in year 2 for *Populus.*

The studies of *Prunus* relied on destructive sampling over time to identify onset and rate of flower development. Another approach is to focus on an environmental cue associated with the onset of development and ask whether the interval between resumption of development and anthesis varies with temperature. The difficulty with this approach is identifying a reliable estimator of when development within buds resumes. In tundra environments, growth is often assumed to begin at snow melt. In a meta-analysis of 41 studies of snow and temperature manipulation of tundra species Wipf and Rixen (2010) found that the time interval between snow melt and anthesis was quite plastic for many taxa (these were manipulative experiments that could infer plasticity as opposed to genetic differences). The later the snow melt, and by inference the later that development resumed, the more rapidly plants reached anthesis following bud break. In contrast, when snow was removed earlier (simulating earlier snow melt), anthesis was earlier, but the rate of pre-anthesis development was unchanged compared with controls. In these snow removal experiments, earlier flowering is due to earlier onset of spring snow melt, and by inference earlier resumption of development, but not faster development. Although the effects of ambient temperatures were not analyzed, presumably development following late snow melt occurs at higher temperatures than controls, which may explain the greater developmental rate. Conversely, development following early snow removal may occur at lower than usual temperatures. In an experiment that manipulated spring temperature independently of snow melt, warm spring temps accelerated flowering date regardless of snowmelt treatment (and by inference, onset of growth; Aerts et al. 2004).

For herbaceous perennials of the tundra, the stage of development at dormancy varies among species and is negatively correlated with the time of anthesis the following spring, with more advanced stages associated with earlier flowering (Sørenson 1941; Molau et al 2005; Wagner et al. 2012). Among 12 tundra species studied by Wagner et al. (2012), regardless of the developmental stage at dormancy, the time between flower initiation and anthesis (not including dormancy) was 6–8 weeks. That is, there is little difference among species in duration/rate of development even though development may occur at very different times and temperatures; later flowering species likely complete development in warmer air and soil temperatures than earlier flowering species. In contrast, experimental warming of grassland species (Sherry et al. 2007) showed that warm summer temperatures prolonged the pre-anthesis developmental stages of late blooming perennials. The authors speculated that unusually warm summer temperatures may have actually suspended reproductive development in these plants. For the herbaceous perennials summarized here, evidence of temperature effects on flower development in year 2 is mixed. Snow melt experiments suggest that developmental rate responds positively to increased temperature, but comparison of species that develop at different times suggests that rate is insensitive, and finally, high temperatures may even inhibit development.

Conclusion

Although fragmentary, the existing data suggest the potential for temperature to affect all aspects of flower development in woody perennials. None of the models can be rejected based on the existing evidence. Temperature may affect initiation (model 1), rate of development in year 1 (model 3A, B), cessation of development (model 4A, B), emergence from dormancy in year 2 (model 1), and rate of development in year 2 (model 2). Note that these models explore the consequences of changing single variables, while warming temperatures are likely to change multiple aspects of development, and changes in one process may well affect other processes. For example, if initiation is delayed, flower primordia will be developing later in the season, perhaps with higher temperatures than usual. *Populus* and Rosaceous fruit trees respond to warm temperatures by flowering earlier (e.g., Beaubien and Freeland 2000; Wolfe et al. 2005; Rivero et al. 2017). But, even for these relatively well studied taxa, the critical developmental responses that underlie this pattern are difficult to identify. Clearly, far more research on the processes occurring within preforming buds is required. Our proposed models explain the seemingly counter-intuitive observations that warmer growing season temperatures delay flowering in many species. Future research might compare developmental dynamics of taxa with divergent responses to temperature under controlled temperature and daylength conditions. In addition, the actual temperature regime experienced by meristems could be explored in greater detail. The temperature, and temperature fluctuations, in the crown of a large tree may be quite different from the conditions in a shrub understory, or for herbaceous perennials that are insulated by soil or snow.

Understanding and predicting species' responses to climate change is a critical imperative of ecological research. The discovery of a strong role for development could dramatically improve predictability of phenological responses of the temperate and boreal communities dominated by preforming species. Species-specific shifts in flowering phenology can lead to novel patterns of co-flowering (e.g., CaraDonna et al. 2014) and can have profound effects on the pollinator and florivore communities. Shifts in flowering phenology also are likely to affect the timing of fruit production, and late flowering may even preclude complete ripening of fruit and cause declines in plant fitness. A mechanistic understanding of how flowering phenology responds to temperature over multiple years will allow for better predictions of changing community dynamics.

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References

Aerts R, Cornelissen JHC, Dorrepaal E, van Logtestijn RSP, Callaghan TV. 2004. Effects of experimentally imposed climate scenarios on flowering phenology and flower production of subarctic bog species. Glob Change Biol 10:1599–609.

Albani MC, Coupland G. 2010. Comparative analysis of flowering in annual and perennial plants. Curr Top Dev Biol 91:323–48.

Amano T, Smithers RJ, Sparks TH, Sutherland WJ. 2010. A 250-year index of first flowering dates and its response to temperature changes. Proc R Soc B Biol Sci 277:2451–7.

Amasino RM, Michaels SD. 2010. The timing of flowering. Plant Physiol 154:516–20.

Arft AM, Walker MD, Gurevitch J, Alatalo JM. 1999. Responses of tundra plants to experimental warming: meta-analysis of the international tundra experiment. Ecol Monogr 69:491–511.

Aydelotte AR, Diggle PK. 1997. Analysis of developmental preformation in the alpine herb, *Caltha leptosepala*. Am J Bot 84:1648–57.

Beaubien EG, Freeland HJ. 2000. Spring phenology trends in Alberta, Canada: links to ocean temperature. Int J Biometeorol 44:53–9.

Billings WD, Mooney HA. 1968. The ecology of arctic and alpine plants. Biol Rev 43:481–529.

Blázquez MA, Ahn JH, Weigel D. 2003. A thermosensory pathway controlling flowering time in *Arabidopsis thaliana*. Nat Genet 33:168–71.

Boes TK, Strauss SH. 1994. Floral phenology and morphology of black cottonwood, *Populus trichocarpa* (Salicaceae). Am J Bot 81:562–7.

Böhlenius H, Huang T, Charbonnel-Campaa L, Brunner AM, Jansson S, Strauss SH, Nilsson O. 2006. CO/FT regulatory module controls timing of flowering and seasonal growth cessation in trees. Science 312:1040–3.

Bradley NL, Leopold AC, Ross J, Huffaker W. 1999. Phenological changes reflect climate change in Wisconsin. Proc Natl Acad Sci U S A 96:9701–4.

Brunner AM, Evans LM, Hsu C-Y, Sheng X. 2014. Vernalization and the chilling requirement to exit bud dormancy: shared or separate regulation? Front Plant Sci 5:732.

Calinger KM, Queenborough S, Curtis PS. 2013. Herbarium specimens reveal the footprint of climate change on flowering trends across North-Central North America. Ecol Lett 16:1037–44.

CaraDonna PJ, Iler AM, Inouye DW. 2014. Shifts in flowering phenology reshape a subalpine plant community. Proc Natl Acad Sci U S A 111:4916–21.

Chuine I. 2000. A unified model for the budburst of trees. J Theor Biol 207:337–47.

Chuine I, Bonhomme M, Legave J-M, García de Cortázar-Atauri I, Charrier G, Lacointe A, Améglio T. 2016. Can phenological models predict tree phenology accurately in the future? The unrevealed hurdle of endodormancy break. Glob Change Biol 22:3444–60.

Cook BI, Wolkovich EM, Parmesan C. 2012. Divergent responses to spring and winter warming drive community level flowering trends. Proc Natl Acad Sci U S A 109:9000–5.

Cooke J, Maria EK, Eriksson E, Junttila O. 2012. The dynamic nature of bud dormancy in trees: environmental control and molecular mechanisms. Plant Cell Environ 35:1707–28.

Davis CC, Willis CG, Connolly B, Kelly C, Ellison AM. 2015. Herbarium records are reliable sources of phenological change driven by climate and provide novel insights into species' phenological cueing mechanisms. Am J Bot 102:1599–609.

Diggle P. 1997. Extreme preformation in alpine *Polygonum viviparum*: an architectural and developmental analysis. Am J Bot 84:154.

Ding J, Nilsson O. 2016. Molecular regulation of phenology in trees—because the seasons they are a-changin'. Curr Opin Plant Biol 29:73–9.

Dorji T, Totland Ø, Moe SR, Hopping KA, Pan J, Klein JA. 2013. Plant functional traits mediate reproductive phenology and success in response to experimental warming and snow addition in tibet. Glob Change Biol 19:459–72.

Ettinger AK, Gee S, Wolkovich EM. 2018. Phenological sequences: how early-season events define those that follow. Am J Bot 105:1771–80.

Fadón E, Rodrigo J, Herrero M. 2018. Is there a specific stage to rest? Morphological changes in flower primordia in relation to endodormancy in sweet cherry (*Prunus avium* L.). Trees 32:1583–94.

Fadón E, Herrero M, Rodrigo J. 2019. Anther and pollen development in sweet cherry (*Prunus av*ium L.) in relation to winter. Protoplasma 256:733–44.

Felker FC, Robitaille HA, Hess FD. 1983. Morphological and ultrastructural development and starch accumulation during chilling of Sour Cherry flower buds. Am J Bot 70:376–86.

Fitter AH, Fitter RSR, Harris ITB, Williamson MH. 1995. Relationships between first flowering date and temperature in the flora of a locality in Central England. Funct Ecol 9:55–60.

Foerste AF. 1891. On the formation of the flower buds of spring-blossoming plants during the preceding summer. Bull Torrey Bot Club 18:101–6.

Forrest J, Miller-Rushing AJ. 2010. Toward a synthetic understanding of the role of phenology in ecology and evolution. Philos Trans R Soc B Biol Sci 365:3101–12.

Forshey CG, Elfving DC. 1989. The relationship between vegetative growth and fruiting in apple trees. Hort Rev 11:229–87.

Foster T, Johnston R, Seleznyova A. 2003. A morphological and quantitative characterization of early floral development in apple (*Malus* × domestica Borkh.). Ann Bot 92:199–206.

Fulford RM. 1966. The morphogenesis of apple buds. II. the development of the bud. Ann Bot 30:25–38.

Ge Q, Dai J, Zheng J, Bai J, Zhong S, Wang H, Wang W-C. 2011. Advances in first bloom dates and increased occurrences of yearly second blooms in eastern China since the 1960s: further phenological evidence of climate warming. Ecol Res 26:713–23.

Goffinet MC, Larson PR. 1981. Structural changes in *Populus deltoides* terminal buds and in the vascular transition zone of the stems during dormancy induction. Am J Bot 68:118–29.

Häbørg A. 1972. Effects of photoperiod and temperature on growth and development of three latitudinal and three altitudinal populations of *Betula pubescens* Ehrh. Sci Rep Agric Univ Norway 51:1–27.

Hänninen H, Tanino K. 2011. Tree seasonality in a warming climate. Trends Plant Sci 16:412–6.

Hart R, Salick J, Ranjitkar S, Xu J. 2014. Herbarium specimens show contrasting phenological responses to Himalayan climate. Proc Natl Acad Sci U S A 111:10615–9.

Hegland SJ, Joar S, Nielsen A, Lázaro A, Bjerknes A-L, Totland Ø. 2009. How does climate warming affect plant–pollinator interactions? Ecol Lett 12:184–95.

Heide OM, Prestrud AK. 2005. Low temperature, but not photoperiod, controls growth cessation and dormancy induction and release in apple and pear. Tree Physiol 25:109–14.

Heide OM. 2008. Interaction of photoperiod and temperature in the control of growth and dormancy of *Prunus* species. Sci Horticult 115:309–14.

Hodgson HJ. 1966. Floral initiation in Alaskan Gramineae. Bot Gaz 127:64–70.

Høye TT, Post E, Schmidt NM, Trojelsgaard K, Forchhammer MC. 2013. Shorter flowering seasons and declining abundance of flower visitors in a warmer arctic. Nat Clim Change 3:759–63.

Hsu C, Adams J, Kim H, No K, Ma C, Strauss SH, Drnevich J, Vandervelde L, Ellis JD, Rice BM, et al. 2011. *FLOWERING LOCUS T* duplication coordinates reproductive and vegetative growth in perennial poplar. Proc Natl Acad Sci U S A 108:10756–61.

Iler AM, Hoye TT, Inouye DW, Schmidt NM. 2013. Long-term trends mask variation in the direction and magnitude of short-term phenological shifts. Am J Bot 100:1398–406.

Julian C, Herrero M, Rodrigo J. 2014. Anther meiosis time is related to winter cold temperatures in apricot (*Prunus armeniaca* L.). Environ Exp Bot 100:20–5.

Julian C, Rodrigo J, Herrero M. 2011. Stamen development and winter dormancy in apricot (*Prunus armeniaca*). Ann Bot 108:617–25.

Junttila O. 1980. Flower bud differentiation in *Salix pentandra* as affected by photoperiod, temperature and growth regulators. Physiol Plant 49:127–34.

Junttila O. 1982. The cessation of apical growth in latitudinal ecotypes and ecotype crosses of *Salix pentandra* L. J Exp Bot 33:1021–9.

Kalcsits LA, Silim S, Tanino K. 2009. Warm temperature accelerates short photoperiod-induced growth cessation and dormancy induction in hybrid poplar (*Populus* × spp.). Trees 23:971–9.

Kramer PJ. 1936. Effect of variation in length of day on growth and dormancy of trees. Plant Physiol 11:127–37.

Lang GA, Early JD, Martin GC, Darnell RL. 1987. Endodormancy, paradormancy, and ecodormancy—physiological terminology and classification for dormancy research. HortScience 22:371–7.

Luckwill LC. 1974. A new look at the process of fruit bud formation in apple. Warszawa, Poland: XIX International Horticultural Congress. p. 237–45.

Khorsand Rosa R, Oberbauer SF, Starr G, La Puma IP, Pop E, Ahlquist L, Baldwin T. 2015. Plant phenological responses to a long-term experimental extension of growing season and soil warming in the tussock tundra of Alaska. Glob Change Biol 21:4520–32.

Körner C. 2015. Paradigm shift in plant growth control. Curr Opin Plant Biol 25:107–14.

Mark AF. 1970. Floral initiation and development in New Zealand alpine plants. N Z J Bot 8:67–75.

Meloche CG, Diggle PK. 2001. Preformation, architectural complexity and developmental flexibility in *Acomastylis rossii* (Rosaceae). Am J Bot 88:980–92.

Mimida N, Ureshino A, Tanaka N, Shigeta N, Sato N, Moriya-Tanaka Y, Iwanami H, Honda C, Suzuki A, Komori S, et al. 2011. Expression patterns of several floral genes during flower initiation in the apical buds of apple (*Malus* × domestica Borkh.) revealed by in situ hybridization. Plant Cell Rep 30:1485–92.

Mirgorodskaya OE, Koteyeva NK, Volchanskaya AV, Miroslavov EA. 2015. Pollen development in *Rhododendron* in relation to winter dormancy and bloom time. Protoplasma 252:1313–23.

Molau U, Nordenhäll U, Eriksen B. 2005. Onset of flowering and climate variability in an alpine landscape: a 10-year study from Swedish Lapland. Am J Bot 92:422–31.

Mulder CPH, Iles DT, Rockwell RF. 2017. Increased variance in temperature and lag effects alter phenological responses to rapid warming in a subarctic plant community. Glob Change Biol 23:801–14.

Mulder CPH, Spellman KV. 2019. Do longer growing seasons give introduced plants an advantage over native plants in Interior Alaska? Botany 97:347–62.

Ovaskainen O, Skorokhodova S, Yakovleva M, Sukhov A, Kutenkov A, Kutenkova N, Shcherbakov A, Meyke E, Delgado MM. 2013. Community-level phenological response to climate change. Proc Natl Acad Sci 110:13434–9.

Pope K, Da Silva D, Brown P, DeJong T. 2014. A biologically based approach to modeling spring phenology in temperate deciduous trees. Agric For Meteorol 198–199:15–23.

Pratt C. 1988. Apple flower and fruit: morphology and anatomy. Horticult Rev 10:273–308.

Rathcke B, Lacey EP. 1985. Phenological patterns of terrestrial plants. Annu Rev Ecol Syst 16:179–214.

Rinne PLH, Paul LK, van der Schoot C. 2018. Decoupling photo- and thermoperiod by projected climate change perturbs bud development, dormancy establishment and vernalization in the model tree *Populus*. BMC Plant Biol 18:220.

Rivero R, Sønsteby A, XHeide OM, Måge F, Remberg DF. 2017. Flowering phenology and the interrelations between phenological stages in apple trees (*Malus domestica* Borkh.) as influenced by the Nordic climate. Acta Agric Scand B Soil Plant Sci 67:292–302.

Rohde A, Bastien C, Boerjan W. 2011. Temperature signals contribute to the timing of photoperiodic growth cessation and bud set in *Poplar*. Tree Physiol 31:472–82.

Semenchuk PR, Elberling B, Cooper EJ. 2013. Snow cover and extreme winter warming events control flower abundance of some, but not all species in high arctic Svalbard. Ecol Evol 3:2586–99.

Shen M. 2011. Spring phenology was not consistently related to winter warming on the Tibetan Plateau. Proc Natl Acad Sci U S A 108:E91–E92.

Sherry RA, Zhou X, Gu S, Arnone JA, Schimel DS, Verburg PS, Wallace LL, Luo Y. 2007. Divergence of reproductive phenology under climate warming. Proc Natl Acad Sci U S A 104:198–202.

Singer MC, Parmesan C. 2010. Phenological asynchrony between herbivorous insects and their hosts: signal of climate change or pre-existing adaptive strategy? Philos Trans R Soc B Biol Sci 365:3161–76.

Singh RK, Svystun T, AlDahmash B, Jönsson AM, Bhalerao RP. 2017. Photoperiod- and temperature-mediated control of phenology in trees—a molecular perspective. New Phytol 213:511–24.

Sørenson T. 1941. Temperature relations and phenology of the northeast Greenland flowering plants. Meddel Grønland 125:1–305.

Svendsen E, Wilen R, Stevenson R, Liu R, Tanino KK. 2007. A molecular marker associated with low-temperature induction of dormancy in Red Osier Dogwood (*Cornus sericea*). Tree Physiol 27:385–97.

Tanino KK, Kalcsits L, Silim S, Kendall E, Gray GR. 2010. Temperature-driven plasticity in growth cessation and dormancy development in deciduous woody plants: a working hypothesis suggesting how molecular and cellular function is affected by temperature during dormancy induction. Plant Mol Biol 73:49–65.

Tromp J. 1976. Flower-bud formation and shoot growth in apple as affected by temperature. Sci Horticult 5:1–10.

Vaartaja O. 1954. Photoperiodic ecotypes of trees. Can J Bot 32:392–9.

Verheij FA. 1996. Morphological and physiological aspects of the early phases of flower bud formation of apple [thesis]. Agricultural University Wageningen. p. 147.

Wagner J, Ladinig U, Steinacher G, Larl I. 2012. From the flower bud to the mature seed: timing and dynamics of flower and seed development in high-mountain plants. In: Lutz C, editor. Plants in alpine regions. Vienna: Springer-Verlag/Wien. p. 135–52.

Wareing PF. 1956. Photoperiodism in woody plants. Annu Rev Plant Physiol 7:191–214.

Weiser CJ. 1970. Cold resistance and injury in woody plants. Science 169:1269–78.

Wilkie JD, Sedgley M, Olesen T. 2008. Regulation of floral initiation in horticultural trees. J Exp Bot 59:3215–28.

Wipf S, Rixen C. 2010. A review of snow manipulation experiments in arctic and alpine tundra ecosystems. Polar Res 29:95–109.

Wolfe DW, Schwartz MD, Lakso AN, Otsuki Y, Pool RM, Shaulis NJ. 2005. Climate change and shifts in spring phenology of three horticultural woody perennials in northeastern USA. Int J Biometerol 49:303–9.

Wolkovich EM, Cook BI, Allen JM, Crimmins TM, Betancourt JL, Travers SE, Pau S, Regetz J, Davies TJ, Kraft NJB, et al. 2012. Warming experiments underpredict plant phenological responses to climate change. Nature 485:494–7.

Yu H, Luedeling E, Xu J. 2010. Winter and spring warming result in delayed spring phenology on the Tibetan Plateau. Proc Natl Acad Sci U S A 107:22151–6.

Yuceer C, Land SB, Kubiske ME, Harkess RL. 2003. Shoot morphogenesis associated with flowering in *Populus deltoides* (Salicaceae). Am J Bot 90:196–206.

Zhu LH, Borsboom O, Tromp J. 1997. The effect of temperature on flower-bud formation in apple including some morphological aspects. Sci Horticult 70:1–8.