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RESEARCH ARTICLE

A temporal shift in resource allocation facilitates flowering before leaf out and spring vessel maturation in precocious species

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PREMISE OF THE STUDY: New growth in the spring requires resource mobilization in the vascular system at a time when xylem and phloem function are often reduced in seasonally cold climates. As a result, the timing of leaf out and/or flowering could depend on when the vascular system resumes normal function in the spring. This study investigated whether flowering time is influenced by vascular phenology in plants that flower precociously before they have leaves.

METHODS: Flower, leaf, and vascular phenology were monitored in pairs of precocious and non-precocious congeners. Differences in resource allocation were quantified by measuring bud dry mass and water content throughout the year, floral hydration was modelled, and a girdling treatment completed on branches in the field.

KEY RESULTS: Precocious flowering species invested more in floral buds the year before flowering than did their non-precocious congeners, thus mobilizing less water in the spring, which allowed flowering before new vessel maturation.

CONCLUSIONS: A shift in the timing of resource allocation in precocious flowering plants allowed them to flower before the production of mature vessels and minimized the significance of seasonal changes in vascular function to their flowering phenology. The low investment required to complete floral development in the spring when the plant vascular system is often compromised could explain why flowers can emerge before leaf out.

KEY WORDS budburst; buds; carbon transport; flower phenology; hydraulics; leaf phenology; preformation; sugar transport; vascular cambium; xylogenesis.

One of the early signs of spring in many temperate forests is the appearance of flowers on leafless, woody branches. Plants that display these flowers initiate growth before any other woody species in the forest and are often described as seasonally precocious. Despite potential advantages incurred from flowering early (e.g., avoiding pollen and/or pollinator competition and minimizing interference of leaves with pollen dispersal [Elzinga et al., 2007; Munguia-Rosas et al., 2011]), the prevalence of precocious flowering in seasonal habitats is challenging to explain in terms of resource accessibility. Floral development requires water, nutrients, and carbon to fuel growth, synthesize attractants (e.g., volatiles and pigments) and rewards (e.g., nectar) for pollinators, maintain turgor, and thermally regulate sensitive reproductive organs (Whiley et al., 1988; Ashman and Schoen, 1997; Galen et al., 1999; Patiño and Grace, 2002; Galen, 2005; Roddy et al., 2018). In the early spring, these resources may be limited or inaccessible because of insufficient vascular function. How then do precociously flowering plants support large blooms of flowers?

During the winter, transport in both the xylem and phloem can be compromised by cold temperatures. In the xylem, the main threat is the expansion of bubbles formed during freeze–thaw events. These bubbles can block conduits, resulting in low stem hydraulic conductivity in the spring. In species with a high susceptibility to freezing-induced embolism, full hydraulic recovery may not occur until after the formation of new vessels (i.e., xylogenesis) (Zimmermann and Brown, 1977). In the phloem, transport is reduced in the winter because of limited sink activity (e.g., growth and metabolism) and increased sap viscosity and callose deposition on sieve plates (Esau, 1950). However, little is known about how often the phloem reactivates before the initiation of cambial activity (Tucker and Evert, 1969; Lawton, 1972).

Because new growth relies on resources transported in the vascular system, Lechowicz (1984) proposed that there may be a relationship between spring phenology in deciduous species and the timing of vascular reactivation, specifically in the xylem. The idea is that species that rely on xylogenesis to restore hydraulic function have to wait until later in the season to support new growth, while species with resistant xylem can leaf out early. Although Lechowicz focused on leaves, a similar logic could be applied to flowers; late flowering species may rely on cambial reactivation to restore xylem function, while precocious flowering species have to maintain sufficient hydraulic conductivity early in the spring.

Another explanation for how plants can flower precociously is that flowers may require limited support from the xylem, especially if they are hydrated by the phloem (Münch, 1930; Trolinder et al., 1993; Chapotin et al., 2003). However, there is limited evidence that many flowers are exclusively phloem-hydrated (Feild et al., 2009; Roddy and Dawson, 2012; Roddy et al., 2016; Savage et al., 2016). Instead, anatomical evidence suggests that during anthesis, most flowers, including those from seasonally precocious species, are well connected to both the xylem and phloem through traces that enter the perianth, stamens, and carpels (Gill, 1933; Puri, 1951). What remains unclear is the extent that species rely on each part of the vascular system during different stages of development.

In this study, I investigated how species can flower precociously when low vascular function might reduce their ability to remobilize resources. I considered two different but not mutually exclusive hypotheses that could explain how species support precocious flowering in seasonally cold climates. The first hypothesis is that precocious flowering species have higher vascular function in the spring either because of limited freezing-induced embolism or earlier cambial initiation. The second hypothesis is that precocious flowering requires limited xylem transport capacity in subtending branches during floral development. The latter hypothesis would be the case if there is greater investment in reproductive tissue in the fall and/or if reproductive structures require minimal xylem input in the spring. To test these two hypotheses, I examined resource allocation and seasonal changes in the plant vascular system in pairs of precocious and non-precocious congeners. My goal was to better understand the relationship between flower and vascular phenology in this iconic group of plants that dominates the early spring landscape of temperate forests.

MATERIALS AND METHODS

Species sampling

I examined the phenology of 10 pairs of seasonally precocious and non-precocious congeners and the anatomy of a subset of five pairs of species (Table 1). A species was considered precocious if it was able to start flowering in the spring before it produced leaves. This classification does not necessitate that flowering is finished before leaf out or that individual plants produce flowers before leaf out every year. In one genus, *Cornus*, two species pairs were differentiated based on floral morphology: two species with large bracts (*C. florida* and *C. kousa*) and two species without large bracts (*C. mas* and *C. sericea*). I studied six individuals per species with the exception of *Salix pierotii*, for which only five individuals were available. For both *Salix* species, only male plants were selected. All the study plants were located at the Arnold Arboretum in Boston, Massachusetts, United States, and accession numbers are available in Appendix S1. All of the non-precocious species produce flowers in the spring and summer with the exception of *Hamamelis virginiana*, which flowers in the fall.

TABLE 1. Pairs of seasonally precocious and non-precocious congeners studied at the Arnold Arboretum, Boston, Massachusetts, United States.

Species	Abbreviation	Flowering type	*N*
Acer rubrum L.	ARU	Precocious	6
Acer tataricum L.	ATA	Non-precocious	6
Amelanchier nantucketensis E.P.Bicknell	ANA	Precocious	6
Amelanchier arborea (Michx. f.) Fernald	AAR	Non-precocious	6
Betula lenta L.	BLE	Precocious	6
Betula alleghaniensis Britt.	BAL	Non-precocious	6
Cornus florida L.*	CFL	Precocious	6
Cornus kousa Hance*	CKO	Non-precocious	6
Cornus mas L.	CMA	Precocious	6
Cornus sericea L.	CSE	Non-precocious	6
Hamamelis mollis Oliv.*	HMO	Precocious	6
Hamamelis virginiana L.*	HVI	Non-precocious	6
Magnolia salicifolia Maxim.*	MSA	Precocious	6
Magnolia virginiana L.*	MVI	Non-precocious	6
Quercus macrocarpa Michx.	QMA	Precocious	6
Quercus rubra L.	QRU	Non-precocious	6
Rhododendron vaseyi A.Gray*	RVA	Precocious	6
Rhododendron calendulaceum (Michx.) Torr.*	RCA	Non-precocious	6
Salix purpurea L.*	SPU	Precocious	6
Salix pierotii Miq.*	SPI	Non-precocious	5

Note: Accession numbers for each plant can be found in Appendix 1.
*Core species included in all analyses.

Flower and leaf phenology

Flower and leaf phenology was monitored weekly from September 2014 to May 2016 on the 10 core species and from January 2015 to May 2016 on the full set of species. I studied five phenophases: (1) bud swelling (i.e., when buds began to noticeably increase in size in the spring); (2) first flower (i.e., when the first flower had unfolded and anthers had started dehiscing), (3) end of flowering (i.e., when 75% of the flowers had senesced on a plant), (4) leaf out (i.e., when the first leaf was unfolding and petiole was visible), and (5) leaf senescence (i.e., when 75% of leaves had senesced on a plant). For the core species, six leaves were collected every week during leaf expansion and then once in August to determine the size of a fully expanded leaf. In the autumn, the percentage of the canopy that was still green was monitored weekly in 2014 and biweekly in 2015. I calculated the growing season length based on the time between leaf out and 75% leaf senescence.

Xylogenesis and vascular anatomy

Branch samples (typically 1–3 mm in diameter) were collected immediately below floral buds, weekly, starting at least 1 month before

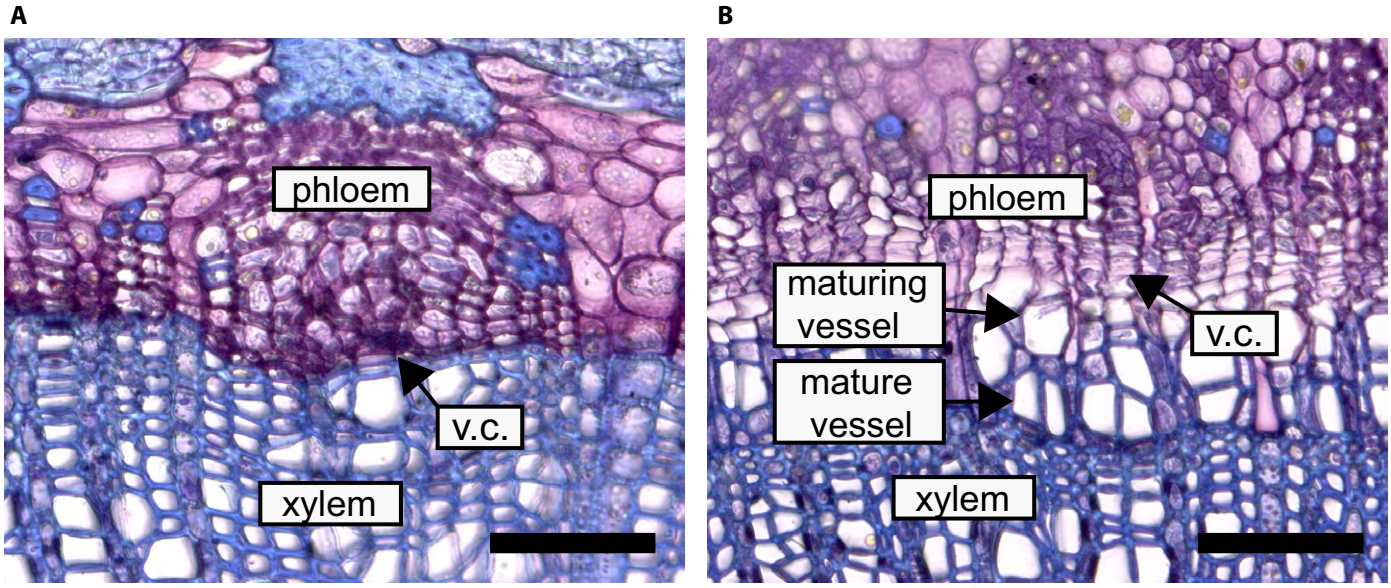

FIGURE 1. Vascular cambium in *Magnolia virginiana* branches (A) before the start of xylogenesis, when the vascular cambium (v.c.) is inactive, and (B) the first week that new vessels start to lignify. Tissue is stained with cresyl violet acetate, which turns from purple to blue when it stains lignin. Maturing vessels have some purple on their walls, but mature vessels are completely blue. Black bars = 50 µm.

the anticipated flowering date in 2015. Samples were put in 70% ethanol and later cut using a sliding microtome. They were stained with aqueous cresyl violet acetate (0.16% w/v), a metachromatic stain that differentiates lignified and nonlignified cells (Rossi et al., 2006). Because I was interested in determining the earliest point when new xylem could be conductive, I determined when the vessels first demonstrated cell wall maturation (Fig. 1). Maturation was characterized as the point when there was enough lignin to make the entire cell wall blue (Michelot et al., 2012; Rossi et al., 2012). It is important to note that this visual assessment of maturation does not determine when a vessel is conductive but the earliest date that it could become functional (Jacobsen et al., 2018). At the time of bud swelling and flowering, peduncles were also sectioned and stained with aniline blue (0.1% w/v aniline blue and 0.1% w/v calcofluor white in 10 mM CHES buffer with 100 mM KCL) to confirm that the presence of mature vessel and sieve tube elements.

Investment in floral buds

Floral tissue was collected from each of the core plants during the winter (December 2014) and when flowers were in full bloom (spring 2015). Depending on the species and the size of their inflorescence, 2–15 buds/inflorescences were collected per plant. In the winter, the inflorescences were dissected, and all the floral tissue including the petals, sepals, androecium, gynoecium, and hypanthium was separated from other tissues in the bud. Care was taken to document which tissue was included for each species to standardize sampling in the dormant bud and the mature flower. Floral tissue was weighed and then freeze-dried to get a dry mass. For the *Salix* and *Cornus* species, samples were analyzed on an inflorescence basis, while all other species were analyzed on a per flower basis. Mature flowers were ground with a ball mill after drying and sent to the Earth System Center for Stable Isotopic Studies at Yale University (https://earth.yale.edu/yasic-yale-analytical-and-stable-isotope-center) for carbon analysis.

Girdling

To examine the role of phloem transport in floral development, I girdled one branch on each of the core plants approximately 20 cm below a floral bud before the buds began to swell in the spring of 2015 and 2016. I did not include *H. virginiana* because it flowers in the fall. Four to six individuals of six species flowered after girdling (*C. florida*, *C. kousa*, *M. salicifolia*, *R. calendulaceum*, *R. vaseyi*, and *S. purpurea*). After anthesis, 9–36 flowers were collected per species above the girdled branch, and the dry biomass and water content of the flowers compared to flowers from nongirdled branches. All the girdled branches were sectioned with a sliding microtome and examined with a dissecting microscope to confirm that the girdling treatment effectively removed the bark and did not visibly damage the xylem.

Model of floral hydration

I modeled carbon transport into buds between swelling and flowering (i.e., anthesis) based on the change in dry mass between these phenophases. I assumed that 46% of the dry mass was carbon, which was the average carbon content measured in the flowers (see above), and considered three scenarios for respiration. I chose to examine variation in respiration because it is not constant and can be variable among species. The first scenario assumed there is no respiration and/or that photosynthesis by the flower offsets respiration (Werk and Ehleringer, 1983). The two remaining scenarios had a low (10 nmol·CO_2·g^{-1}·s^{-1}) and moderate (30 nmol·CO_2·g^{-1}·s^{-1}) level of respiration (Bazzaz et al., 1979; Werk and Ehleringer, 1983; Williams et al., 1985). For each of the scenarios, I calculated total carbon flux and used it to estimate the volume of phloem sap entering the bud, assuming a sugar concentration of 18.2%, the average sugar concentration measured in plants (Jensen et al., 2013). Once I had an estimate of sap volume, I compared the amount of water that entered the bud between swelling and flowering (e.g., the difference

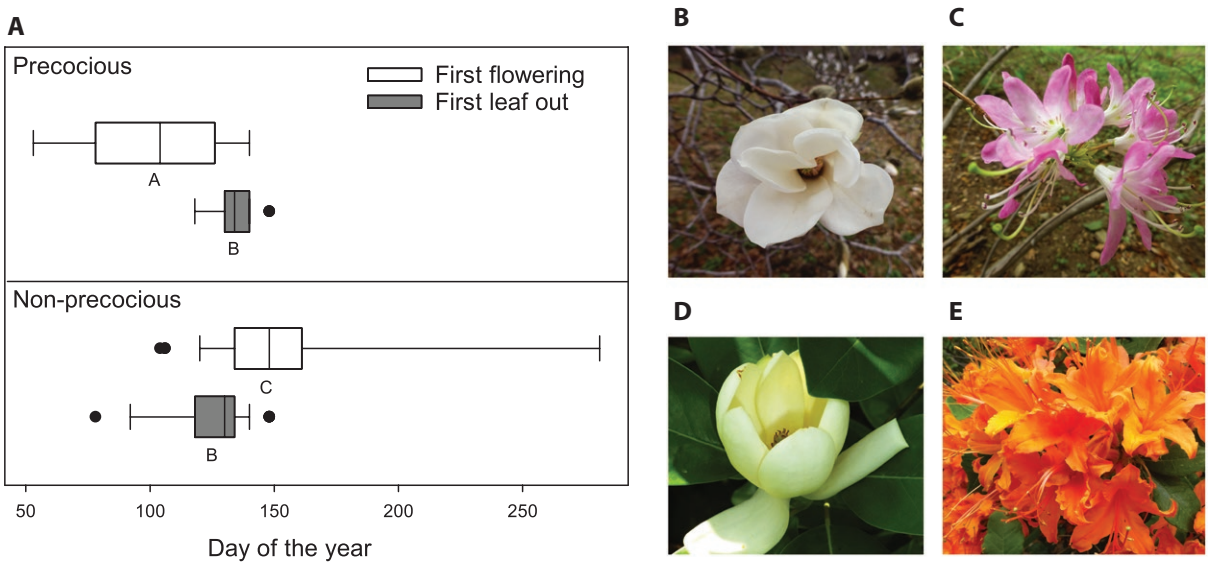

FIGURE 2. Flower and leaf phenology of 10 pairs of precocious and non-precocious congeners. (A) Box plot of the timing of flowering (white) and leaf out (gray) in 2016. Data is based on a survey of six plants per species, but one RCA, two QMA, and one QRU did not flower. Letters indicate significant differences among the phenophases (Tukey's HSD, α = 0.01). Photographs of flowers from (B, C) precocious and (D, E) non-precocious congeners of *Magnolia* and *Rhododendron*, respectively.

in water mass of the flowers at these two times). This comparison allowed me to test whether the phloem could theoretically provide all the water necessary for floral development before anthesis (see description of model in Appendix S2). Note that this analysis did not consider water loss from the flower or bud through evaporation or transpiration, only water retained in the tissue.

Fruit and seed production

Starting in late summer 2015, fruit development was monitored weekly on each of the core plants. For the *Magnolia* and *Cornus* species, fruit was considered mature when it was ripe (i.e., red and soft). For the *Rhododendron* and *Hamamelis* species, fruit was considered mature when it started to dehisce or abscise from the plant. Once the fruit had achieved maturity, I determined average fruit and seed mass, and the seed number per fruit for every species based on 20 fruits, and at least 50 seeds. For the *Rhododendron* species, at least 600 seeds per species were sampled. Whenever possible, the seeds were collected from six individuals per species. No fruit or seeds were collected from the *Salix* plants because they were male, and two of the *Hamamelis virginiana* plants did not produce fruit during the study period.

Statistics

Anatomical and phenological traits were compared using generalized linear models that examined taxa, flowering type (i.e., precocious or non-precocious), and taxa × flowering type interactions with a maximum likelihood estimate method and results reported as a χ^2 statistic. Multiple comparisons were done with Tukey's honestly significant difference (HSD) test with an $\alpha = 0.01$. I used a negative binomial distribution for the phenology and count data and either a normal or lognormal distribution for the remaining data sets. All data that were a proportion or percentage were log-transformed after adding one. Results of the floral hydration model were examined on a species basis to determine whether the water required for the xylem was significantly different than zero.

RESULTS

Flower and leaf phenology

Phenology data confirmed that precocious flowering species flowered before they had leaves, while the non-precocious species flowered after they had leaves (Fig. 2). The timing of these phenophases (i.e., leaf out and flowering) was impacted by genus ($\chi^2 = 70.4$, df = 9, $P < 0.0001$), organ ($\chi^2 = 61.8$, df = 1, $P < 0.0001$), and an interaction between flowering type and organ ($\chi^2 = 107$, df = 1, $P < 0.0001$). This interaction occurred because congeners exhibited similar leaf out times, but precocious species flowered earlier in the spring than their non-precocious congeners. There was no relationship between leaf out and flowering time ($F_{1,8} = 0.08$, $P = 0.8$) across species. Note that in 2016, five of the 119 monitored plants did not flower, leading to an $N = 233$.

Many of the precocious flowering species began leaf expansion before they stopped flowering (Fig. 3), but their leaves were less expanded at the end of flowering (75% floral senescence) than their non-precocious congeners except for the two *Salix* species (Fig. 4A). As a result, there were significant genus ($\chi^2 = 128$, df = 4, $P < 0.0001$) and genus × flowering type effects on leaf expansion ($\chi^2 = 92.9$, df = 4, $P < 0.0001$). Nonetheless, the timing of leaf senescence and length of growing season were not significantly different among genera and flowering types. Phenology data for 2015–2016 are available in Appendix S3.

Xylogenesis and vascular anatomy

Vessel size varied among genera (Table 2; $\chi^2 = 54.7$, df = 4, $N = 59$, $P < 0.0001$), but this variation could not be explained by flowering type

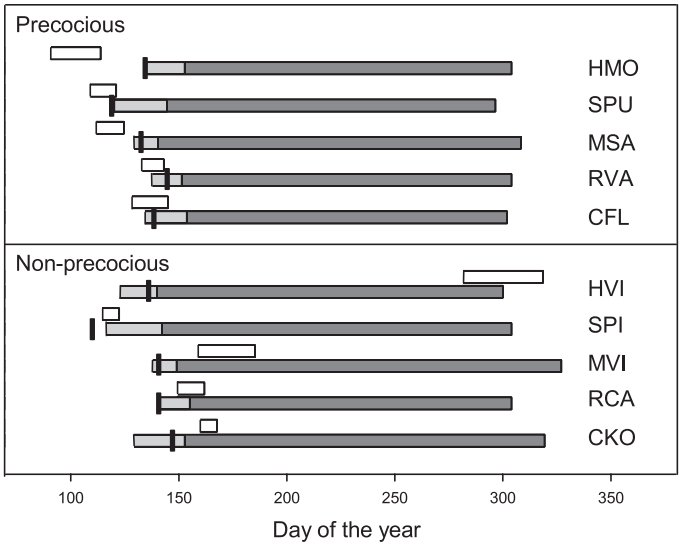

FIGURE 3. Visualization of major phenophases in each of the core species in 2015. The start and end of flowering (white) and leaves (gray) are noted with bars. Xylogenesis is marked with a black line. The period of leaf expansion is noted with light gray. The end of leaf and flower phenophases are marked at 75% senescence. Species are abbreviated according to Table 1, and genera are ordered based on flowering time of precocious species. For all species, $N = 6$ except for the non-precocious *Salix* species, which only had $N = 5$.

($\chi^2 = 5.5$, df = 1, $N = 59$, $P = 0.02$). Nevertheless, there was a genus × flowering type effect ($\chi^2 = 25.4$, df = 4, $N = 59$, $P < 0.0001$) driven by the difference between the *Salix* species. In contrast, the timing of vessel maturation was similar across species and was not impacted by genus, flowering type, or their interaction (Fig. 3 and Table 2) and not correlated with flowering phenology ($F_{1,8} = 0.51$, $P = 0.5$). However, the timing of vessel maturation did correlate with the timing of leaf out (Fig. 4B; $F_{1,8} = 17.3$, $P = 0.003$). As a result, all the precocious flowering species produced flowers before vessel maturation, while the non-precocious species flowered after the formation of the first new vessels in subtending branches. Mature sieve and vessel elements were present in the peduncles of all 10 core species at bud swelling and flowering (Appendix S4).

Investment in floral buds

Precocious flowering plants invested a greater proportion of their floral biomass into their buds the year before blooming on a per flower or inflorescence basis than did non-precocious congeners (Fig. 5A; $N = 59$, genus: $\chi^2 = 206$, df = 4, $P < 0.0001$, flowering type: $\chi^2 = 236$, df = 1, $P < 0.0001$, and interaction: $\chi^2 = 115$, df = 4, $P < 0.0001$). Differences between flowering types were noticeable as early as July, and precocious species consistently had more floral tissue per bud during winter dormancy (Fig. 6). However, bud size differences between the flowering types disappeared or reversed in the spring. For two of the species pairs (i.e., *Magnolia* and *Rhododendron*), high spring growth rate resulted in greater floral mass in the non-precocious species at flowering. Carbon content of the floral tissue was the same across species (46% ± 1 SD) regardless of flowering type, and there was no genus × flowering type interaction.

The average water content of floral tissue increased from 62% ± 10 SD in a dormant bud to 78% ± 12 SD at anthesis, but precocious flowering species overwintered with more of the water needed for flowering already in the floral tissue than in that of their precocious congeners (Fig. 5B; genus: $\chi^2 = 8060$, df = 4, $P < 0.0001$, flowering type: $\chi^2 = 73.9$, df = 1, $P < 0.0001$, and interaction: $\chi^2 = 4270$, df = 4, $P < 0.0001$). *Hamamelis* represented the most extreme case because the precocious species, *H. mollis*, had less than a 1% increase in water content during the spring before anthesis, indicating that most of water used during floral expansion entered the floral tissue the previous year.

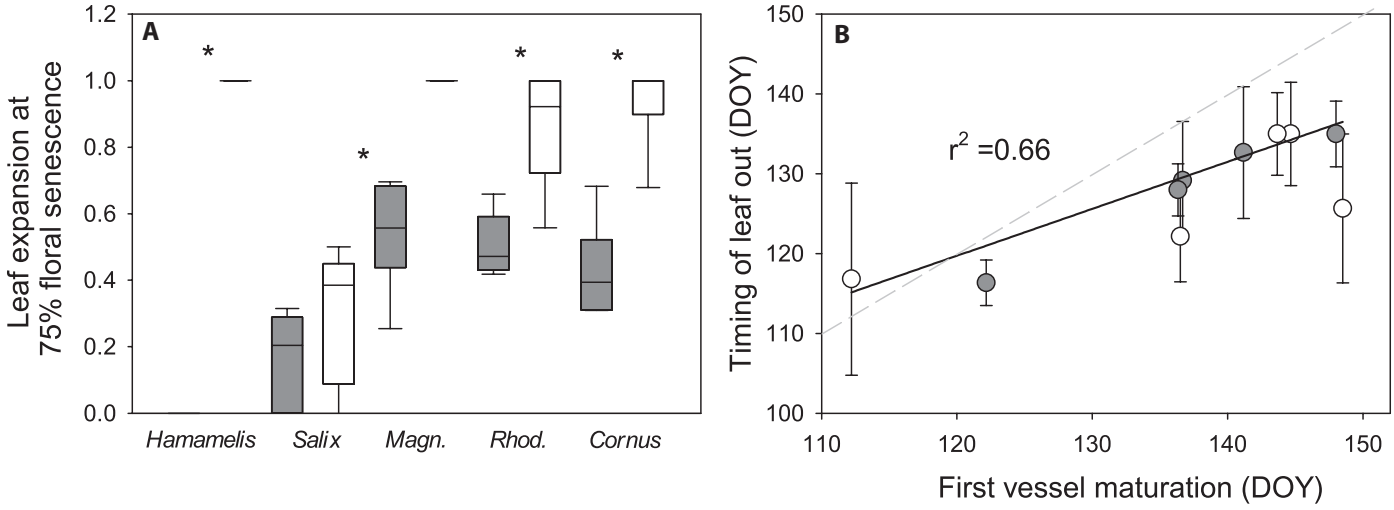

FIGURE 4. Relationship between leaf phenology, flower phenology, and vessel maturation in precocious (gray) and non-precocious (white) congeners. (A) Box plot of the amount of leaf expansion at the end of flowering (75% floral senescence) in 2015. Leaf expansion is given as ratio of current leaf size to final leaf size. Asterisks mark congeners with significantly different values (Tukey's HSD, $\alpha = 0.01$). Genera are ordered for each species based on flowering time of precocious species. (B) Correlation between average time of leaf out and first vessel maturation ($N = 10$, $F = 15.3$, df = 9, $P = 0.004$). Dashed lines denote a 1:1 line. Error bars are ± one standard deviation.

TABLE 2. Distal branch wood anatomy and phenology in precocious (P) and non-precocious (NP) congeners.

Species	Type	Vessel radius (μm)	Timing of xylogenesis (DOY)
HMO	P	7.3 ± 0.9	137 ± 7
HVI	NP	7.6 ± 0.7	137 ± 6
SPU	P	8.1 ± 1*	122 ± 3
SPI	NP	10.7 ± 1.1*	112 ± 12
MSA	P	8.0 ± 0.8	136 ± 3
MVI	NP	8.4 ± 1.1	145 ± 6
RVA	P	6.8 ± 0.7	148 ± 4
RCA	NP	6.3 ± 0.5	144 ± 5
CFL	P	8.7 ± 0.7	141 ± 8
CKO	NP	8.2 ± 0.8	149 ± 9

Notes: Species are abbreviated according to Table 1. Data are averages ± one standard deviation. $N = 6$ except for species SPI, for which $N = 5$. DOY, day of year.
*Within a column, congeners differed significantly based on Tukey's HSD test ($\alpha = 0.01$).

Girdling

In precocious flowering species, girdled branches produced flowers with a fraction of the biomass of flowers on nonmanipulated branches (Table 3; Species: $\chi^2 = 45.7$, df = 3, $P < 0.0001$; Girdling: $\chi^2 = 23.7$, df = 1, $P < 0.0001$; Interaction: $\chi^2 = 6.2$, df = 3, $P = 0.10$). A similar trend was observed for water content, but treatment was marginally nonsignificant ($\chi^2 = 6.62$, df = 1, $P = 0.0101$), and there was no species ($\chi^2 = 9.6$, df = 3, $P = 0.02$) or species × treatment effect on percentage of water in flowers ($\chi^2 = 1.32$, df = 3, $P = 0.72$).

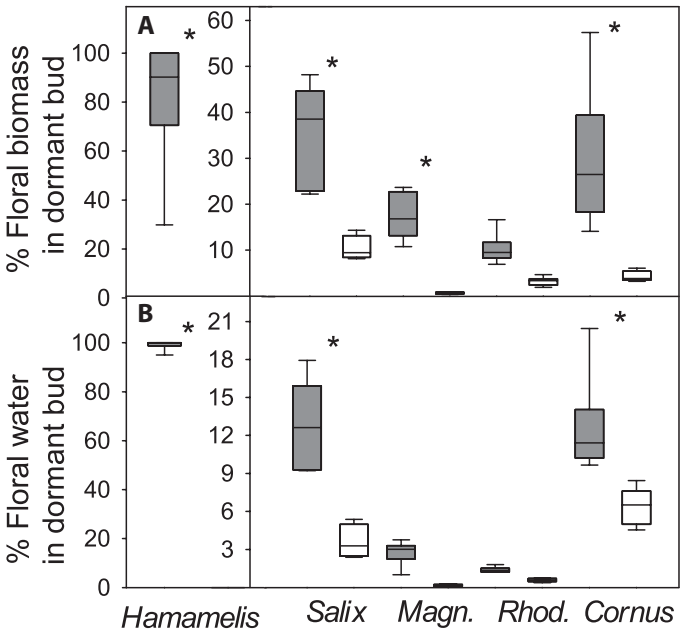

FIGURE 5. Investment in floral tissue before winter dormancy as a percentage of final floral mass in precocious (gray) and non-precocious (white) congeners. (A) Box plots of the percentage of total floral biomass and (B) total water in floral tissue in dormant bud during the winter. Dormant bud mass was measured in December. Asterisks mark congeners with significantly different values (Tukey's HSD, $\alpha = 0.01$). For all species, $N = 6$ except the non-precocious *Salix* species, which had $N = 4$. Note the non-precocious *Hamamelis* does not overwinter floral buds. Genera are ordered based on flowering time of precocious species. Magn., *Magnolia*; Rhod., *Rhododendron*.

On average, floral water mass of girdled branches was 27% ± 21 SD, and the dry biomass was 43% ± 26 SD of what was measured on nonmanipulated branches. Variation in water content was also higher in two of the species (i.e. MSA and SPU) after girdling.

Model of floral hydration

I modeled water import into the bud via the phloem by assuming a constant sugar concentration in the phloem (18.2%) and a low carbon loss from the bud (e.g., no respiration or the respiration is offset by carbon fixation). Under these conditions, only three species had significantly lower water input from the phloem than they needed for floral expansion (Table 4). Assuming a slightly higher level of floral respiration (i.e., 10 pmole $CO_2 \cdot g^{-1} \cdot s^{-1}$), only one of these species required xylem hydration, and under a moderate level of respiration (i.e., 30 pmol $CO_2 \cdot g^{-1} \cdot s^{-1}$), none of the species required supplemental water from the xylem.

Fruit and seed production

The timing of fruit maturation was similar among all the core species, but their reproductive investment varied (Table 5). Seed mass differed significantly among all species pairs, leading to significant genus ($N = 48$; $\chi^2 = 574$, df = 3, $P < 0.0001$), flowering type ($\chi^2 = 561$, df = 1, $P < 0.0001$) and interaction effects ($\chi^2 = 563$, df = 3, $P < 0.0001$). Similarly, seed number ($N = 218$; genus: $\chi^2 = 1335$, df = 3, $P < 0.0001$; flowering type: $\chi^2 = 145$, df = 1, $P < 0.0001$; interaction: $\chi^2 = 311$, df = 3, $P < 0.0001$) and fruit mass ($N = 8$; genus: $\chi^2 = 259$, df = 3, $P < 0.0001$; flowering type: $\chi^2 = 21.7$, df = 1, $P < 0.0001$; interaction: $\chi^2 = 85$, df = 3, $P < 0.0001$) varied across species. In three of the four species pairs, the precocious species had heavier seeds, but this trend toward larger reproductive investment did not hold for fruit size or number of seeds per fruit.

DISCUSSION

Scattered across the angiosperm phylogeny from the Magnoliales to the Fagales, there are deciduous species that flower early in the spring before they have leaves (Fig. 2). While this flowering strategy may provide a fitness advantage in seasonal climates (Elzinga et al., 2007; Munguia-Rosas et al., 2011), its success could be limited by a plant's ability to remobilize resources early in the spring. In this study, I found that precocious flowering is not facilitated by earlier reactivation of the vascular system (Fig. 3 and Table 2) but instead a greater investment in floral structures the year before flowering (Figs. 5, 6). I hypothesize that this change in resource allocation is necessary for precocious flowering species because it reduces the amount of resources that are remobilized when vascular function is potentially reduced.

Precocious flowering does not require early xylogenesis

All the precocious flowering species in this study supported developing flowers exclusively on vessels produced during the previous growing season (Fig. 3). Thus, their flowers required little, if any input from the xylem before floral anthesis, and the phloem provided most of the water needed for floral development in the spring (Table 4). Similarly, non-precocious species appeared to rely heavily on the phloem during development, despite the fact that they flowered after or concurrently with vessel maturation. However, because there

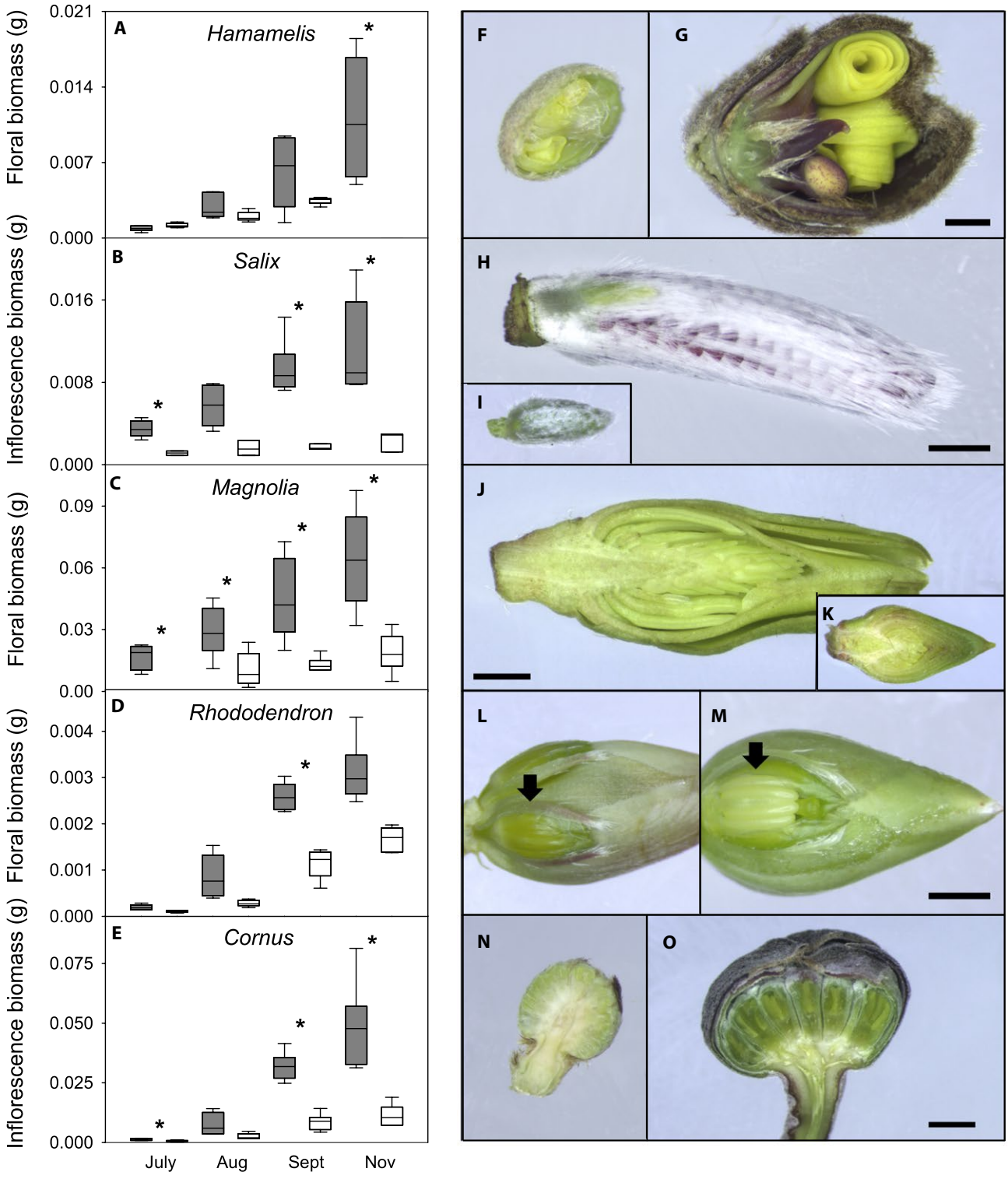

FIGURE 6. Investment in floral tissue the year before flowering in precocious (gray) and non-precocious (white) congeners. (A–E) Box plots of dry biomass on a per flower or per inflorescence basis from July to November. For the *Cornus* and *Salix* species, mass is on an inflorescence level. Asterisks mark congeners with significantly different values (Tukey HSD, $\alpha = 0.01$). Genera are ordered based on the flowering time of precocious species. (F–J) Flowers dissected out of dormant buds in December except for HVI, which was imaged in October. Note: For *Rhododendron* buds, the whole inflorescence is shown, and an arrow points to an individual flower. Black bars in all images are 2 mm, except for *Hamamelis*, for which it is 1 mm.

TABLE 3. Water content (WC) and dry mass (DM) of dormant buds and flowers at anthesis from girdled and nongirdled branches of precocious (P) and non-precocious (NP) congeners.

Species	Type	Bud WC (%)	Floral WC (%)	Girdled WC (%)	Bud DM (g)	Floral DM (g)	Girdled DM (g)
SPU	P	55 ± 2^{A}	77 ± 2^{B}	45 ± 30^{A}	0.01 ± 0.002^{a}	0.027 ± 0.003^{b}	0.021 ± 0.006^{a}
MSA	P	61 ± 5^{A}	89 ± 0.5^{B}	79 ± 9^{B}	0.06 ± 0.02^{a}	0.4 ± 0.1^{b}	0.08 ± 0.05^{a}
RVA	P	60 ± 1^{A}	91 ± 3^{B}	86 ± 3^{B}	0.003 ± 0^{a}	0.028 ± 0.009^{b}	0.008 ± 0.002^{a}
RCA	NP	62 ± 0.9^{A}	90 ± 2^{B}	83 ± 5^{B}	0.002 ± 0^{a}	0.05 ± 0.01^{b}	0.01 ± 0.01^{a}
CFL	P	63 ± 7^{A}	78 ± 4^{A}	75 ± 4^{A}	0.04 ± 0.01^{a}	0.16 ± 0.03^{b}	0.08 ± 0.01^{a}
CKO	NP	80 ± 2^{B}	73 ± 1^{A}	72 ± 4^{A}	0.012 ± 0.004^{a}	0.26 ± 0.09^{b}	0.11 ± 0.05^{a}

Notes: Species are abbreviated according to Table 1. Four of the species did not successfully bloom after girdling: HMO, HVI, SPI, and MVI. Data are averages ± one standard deviation. Number of plants sampled are as follows: SPU ($N = 6$), MSA ($N = 6$), RVA ($N = 5$), RCA ($N = 6$), CFL ($N = 5$) and CKO ($N = 4$). For each plant, between 9 and 36 flowers were sampled. Uppercase letters indicate when WC is significantly different among treatments for each species, and lowercase letters indicate when DM is significantly different among treatments for each species based on Tukey's HSD test ($\alpha = 0.01$).

TABLE 4. Amount of water (g) required for the last stage of floral expansion (i.e., bud swelling to flowering) that is not provided by the phloem based on three floral hydration models.

		Water flux (g)		
Species	Type	Model 1 (no respiration)	Model 2 (low respiration)	Model 3 (moderate respiration)
HMO	P	0.00 ± 0.01	−0.02 ± 0.01*	−0.04 ± 0.02*
SPU	P	−0.02 ± 0.01*	−0.03 ± 0.01*	−0.04 ± 0.00*
SPI	NP	−0.01 ± 0.02	−0.04 ± 0.03	−0.05 ± 0.02*
MSA	P	**0.6 ± 0.2***	**0.4 ± 0.2**	−0.5 ± 0.1*
MVI	NP	**1.1 ± 0.6***	**0.3 ± 0.6**	−1.8 ± 0.5*
RVA	P	**0.12 ± 0.04***	**0.10 ± 0.04***	−0.04 ± 0.01*
RCA	NP	**0.2 ± 0.1**	**0.2 ± 0.1**	−0.05 ± 0.01*
CFL	P	−0.1 ± 0.2	−0.2 ± 0.1	−0.31 ± 0.07*
CKO	NP	−0.9 ± 0.3*	−1.1 ± 0.3*	−0.36 ± 0.09*

Notes: The three models assume different rates of respiration. Low respiration was 10 nmol·CO2·g^{-1}·s^{-1}, and moderate respiration was 30 nmol·CO2·g^{-1}·s^{-1}. Species are abbreviated according to Table 1. Data are averages ± one standard deviation. $N = 6$ for all species except SPI and SPU ($N = 5$). HVI was not included in the analysis because it blooms in the fall. Values for samples that require xylem input (positive numbers) are in boldface.

*Values that are significantly different than zero ($\alpha = 0.01$)

is often a lag between when vessels are lignified, and when they become functional (Jacobsen et al., 2018), these species probably did not have functional xylem until later in the spring. Angiosperms also reactivate their cambium basipetally (Priestley, 1930), and cambial initiation in distal branches will not necessarily coincide with the reactivation of the entire vascular system. Instead, early in xylogenesis, water transport may primarily come from water stores in branches.

In this study, floral hydration was examined using a model of phloem transport that makes assumptions about respiration, photosynthesis, and transpiration. One critical assumption is that photosynthesis is either the same as or lower than respiration on a whole-flower basis. If this assumption is incorrect and flowers have high rates of carbon assimilation, then phloem input into the flower would be lower than predicted, and all the species would require more xylem input. However, if the flowers have high rates of photosynthesis, we would expect to see similar growth between girdled and nongirdled branches. Instead, girdled flowers did not exhibited significant growth before anthesis (Table 3). These results suggest that respiration and photosynthesis are equal or that respiration is higher in these species and that flowers are accessing stored carbon in the stem above the girdled point. Either way, our calculations show that the water required for floral expansion is relatively low and will likely place minimal constraint on the hydraulics of the plants before anthesis.

Different than flowers, leaves appear more tightly tied to seasonal changes in xylem function. Across species, vessels began maturing before leaf out and/or during early stages of leaf expansion (Fig. 3), which led to a relationship between leaf and vascular phenology (Fig. 4B). This relationship was largely driven by the *Salix* species but is consistent with previous research (Wang et al., 1992; Takahashi et al., 2013). These results suggest that leaves are less likely than flowers to emerge at a time when hydraulic transport could be compromised.

Because this study focused only on flower development and expansion, an important next step is to consider what happens after anthesis. A recent study examining sap flux in two precocious magnolias found that mature flowers can easily require 20–60% of the

TABLE 5. Fruit phenology and reproductive investment in precocious (P) and non-precocious (NP) congeners.

Species	Type	First fruit maturation (DOY)	Seed mass (g)	Seeds per fruit	Fruit mass (g)	Ratio of seed to fruit mass
HMO	P	290 ± 4	0.06 ± 0.01*	4.00 ± 0	0.5 ± 0.2	0.48
HVI	NP	284 ± 5	0.04 ± 0.01*	4.00 ± 0	0.3 ± 0.06	0.53
MSA	P	264 ± 0	0.16 ± 0.02*	3 ± 3*	1.18 ± 0.66	0.41
MVI	NP	249 ± 9	0.05 ± 0.01*	26 ± 8*	5 ±1	0.26
RVA	P	256 ± 0	3E-5 ± 3E-5*	200 ± 100*	0.06 ± 0.02	0.1
RCA	NP	283 ± 4	1.7E-4 ± 8E-5*	50 ± 40*	0.14 ± 0.04	0.06
CFL	P	260 ± 12	0.12 ± 0.02*	3 ± 2	0.7 ± 0.4	0.51
CKO	NP	257 ± 3	0.09 ± 0.02*	2 ± 1	0.24 ± 0.47	0.75

Notes: Species are abbreviated according to Table 1. Data are averages ± one standard deviation. For fruit maturation, $N = 6$ for all species except SPI ($N = 5$) and HVI ($N = 4$) because two plants did not produce fruit. Note that SPI and SPU were not included because only male plants were studied. DOY, day of year.

*Within columns, congeners differed significantly based on Tukey's HSD test ($\alpha = 0.01$).

water used by leaves (Liu et al., 2017). If this is true for the species in this study, access to water may be more of a concern after anthesis and explain why some flowers have a similar hydraulic conductance to leaves (Roddy et al., 2016).

Precociousness is marked by increased resource investment in floral buds

One of the largest differences between precocious and non-precocious congeners was the timing of resource allocation into floral buds. All the species in this study (except for *H. virginiana,* which flowers in the fall), overwinter with preformed flowers, but the precocious flowering species demonstrated greater resource investment during the previous growing season than their non-precocious congeners (Fig. 6). The observed pattern is suggestive of heterochronic evolution, where changes in the timing of different developmental stages led to diversification of floral type and phenology (Li and Johnston, 2000; Box and Glover, 2010; Ronse De Craene, 2018). However, more research is needed to understand the development of these flowers, their genetic control and the ancestral form of the different congeners.

Although few studies have examined the timing of phloem reactivation (Begum et al., 2007; Gričar et al., 2017), the phloem was probably functional in the study species before flowering. The production of new sieve tubes often precedes vessel maturation by several weeks (Tucker and Evert, 1969; Davis and Evert, 1970), and flowering in most of our species occurred less than a month before xylogenesis. The only species that flowered over a month before xylogenesis was *Hamamelis mollis*. It is possible that flowers of this species do not require phloem input before anthesis because they show minimal increase in biomass and water content in the spring (Fig. 5). This behavior is consistent with what Gill (1933) observed in two precocious flowering species in a classic study on floral vascular differentiation. Gill hypothesized that these two species, which did not increase their biomass in the spring, do not require vascular input. It was also noted that they were the only precocious species that did not show evidence of phloem reactivation in the pedicel before flowering. These results suggest that there is variation in how much input is required from the phloem in the spring and that not all precocious flowering species require a fully functional vascular system.

CONCLUSIONS

Precocious flowering plants minimize the impact of vascular phenology on floral development by investing more in their buds during the previous growing season. This shift in allocation allows precocious species to flower when they may not be able to support leaves. Although precocious flowering is a common strategy in temperature climates, it is only viable if there is a low risk of losing reproductive material in the winter. As climatic conditions become more variable and killing frosts increase in frequency (Inouye, 2008; Augspurger, 2013), the fate of precocious flowering species may change. In recent years, I have observed premature blooming in many precocious species and because flowers are often more sensitive to cold temperatures than other parts of a plant (CaraDonna and Bain, 2016), the blooms rarely survive. If we want to understand the implications of climatic changes on plant survival and reproduction, we need to better understand the biotic and abiotic factors that regulate and limit the timing of flower and leaf phenology (Chuine and Beaubien, 2001; Savage and Cavender-Bares, 2013).

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AUTHOR CONTRIBUTIONS

J.S. designed and carried out the research and wrote the manuscript.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

APPENDIX S1. Plants sampled and/or monitored at the Arnold Arboretum of Harvard University in Boston, MA, USA from 2014 to 2016.

APPENDIX S2. Model of floral hydration prior to anthesis.

APPENDIX S3. The average timing of phenophases in 2015-2016 of precocious (P) and non-precocious (P) congeners.

APPENDIX S4. Sieve tubes in peduncles of swollen floral buds prior to anthesis.

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