Investigation of vascular limitations on floral water loss in temperate woody species

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Dedication

To my parents, who always tell me that education can never be taken away from you.

Abstract

In temperate biomes, limitations imposed by vascular physiology may influence floral water use in woody species. Freeze-thaw induced embolism in the xylem can reduce vascular transport capacity in the early spring, potentially limiting growth. To investigate whether xylem transport capacity impacts floral physiology, I quantified inflorescence water loss rates and stem hydraulic conductivity of five woody species that flower before producing leaves. I found inflorescence size and ambient temperature at flowering positively correlated with water loss. However, I detected no correlation between branch level floral water loss and stem hydraulic conductivity within species. Furthermore, a comparison of branch level water loss rates from inflorescences and leaves showed that leaf water loss is 2–4 orders of magnitude greater than that of flowers. To evaluate whether flowers were primarily phloem or xylem hydrated, I modeled the amount of water brought in during floral development and full bloom. Despite their relatively low rates of water loss, the model indicates that flowers in this study obtain the majority of their water from the xylem. Overall, the data suggest that within species floral water loss may not be limited by the xylem during flowering, but large differences in floral water loss and stem conductivity among species could explain hydraulic trait variation between large and small flowered plants.

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Introduction

Great steps have been taken to understand the diversity and development of flowers, but little is known about floral water usage (Gleason, 2018). On a basic level, flowers need water to grow and maintain turgor, but floral structural diversity convolutes our understanding of water requirements and hydration sources (Galen, 2005, Roddy et al., 2016; Savage et al., 2016; Roddy, Jiang, et al., 2018; Roddy, Simonin, et al., 2018). Additionally, water usage is different between flowers and leaves (Roddy et al., 2016; Roddy, Jiang, et al., 2018; Roddy, Simonin, et al., 2018), raising questions of how to measure floral water use. To begin to understand floral water use, drivers of floral water consumption must be addressed in more detail and floral structure and floral environment should be considered when investigating water loss. In this chapter, I will compare water use and rates of water loss between flowers and leaves to evaluate their similarity and assess hypotheses about floral hydration.

Water use in flowers and leaves

Beginning at development, leaves and flowers use water in essentially the same way. Water imbibition into cells increases turgor pressure, a driving force for cell wall expansion in plants (Lockhart, 1965). Cell expansion occurs in leaf and floral primordia prior to budburst (Tsukaya and Beemster, 2006), with water content increasing between dormant and budburst phenophases (Rinne et al., 1994; Savage, in press). Water influx to maintain turgor for cell elongation is required throughout budbreak, bud opening, and organ expansion for both flowers (Ram and Rao, 1984) and leaves (Dale, 1988). Once flowers and leaves are mature, water sustains cell turgor and physiological processes like

photosynthesis (Aschan and Pfanz, 2003) and circadian movements (Coté, 1995; Van Doorn and Kamdee, 2014). These physiological similarities are not surprising considering that floral organs may have evolved from leaves (Goethe, 1790; Weigel & Meyerowitz, 1994), with some proposing that ancestral flowers may have had structural traits more similar to leaves (Feild et al., 2009; Roddy et al., 2016; Roddy, Simonin, et al., 2018).

Despite apparent developmental similarities between leaves and flowers, these two organs differ dramatically in their primary functions. In most plants, leaves are optimized for photosynthesis. Photosynthetic rates can be limited by a number of factors—the rate of carbon dioxide diffusion into the leaf, concentrations of carboxylation substrate ribulose biphosphate (RuBP), energy carrier molecules (ADP and $NADP^+$), and the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), and light (Sharkey, 1985). As water is traded for carbon dioxide through stomata, stomatal conductance increases photosynthesis, as well as transpiration (Brodribb et al., 2004). High transpiration rates increase the risk of leaf desiccation to lethal levels (Brodribb et al., 2010), so maintaining photosynthetic rates necessitates coordination between gas exchanges rates and hydraulic conductance (Sperry, 2003; Sack et al., 2003). Traits associated with high rates of water movement in leaves, such as a short pathway through the mesophyll from veins to the epidermis and high vein density (Sack and Holbrook, 2006; Brodribb et al., 2007), are also correlated with high rates of photosynthesis (Brodribb and Feild, 2010; Brodribb et al., 2010). Xylem hydraulic conductivity, or the flow of water through a given length and area of sapwood under a

given pressure, also shows a positive correlation with stomatal conductance and maximum photosynthetic rate on a branch level (Saliendra et al., 1995; Tyree and Zimmermann, 2002; Santiago et al., 2004), indicating that gas exchanges rates can be restricted by vascular supply.

In contrast to leaves, the primary function of flowers is reproduction and they act as a resource sink. Flowers require nutrients, carbon, and water for pollen and ovule development. Additionally, many flowers produce pollinator rewards like nectar (Southwick, 1984; De la Barrera and Nobel, 2004) and waxes (Whitney and Glover, 2007), and organic compounds used as attractants and chemical defenses (Pichersky and Gershenzon, 2002; Galen et al., 2011). Some flowers are capable of photosynthesis (Bazzaz et al., 1979; Aschan and Pfanz, 2003), with many having rates that balance floral respiration throughout flowering (*Encelia*: Werk and Ehleringer, 1983; *Arctium*: Heilmeier and Whale, 1987; *Ranunculus*: Galen et al., 1993; *Spiranthes*: Antlfinger and Wendel, 1997; *Helleborus*: Aschan et al., 2005), but rates vary among species. Because photosynthesis in flowers rarely reaches rates comparable to leaves (Watson and Casper, 1984), water allocated to photosynthesis or tied to stomatal conductance may not make up the majority of floral water usage. Instead, water in flowers may be used for thermoregulation (Seymour and Schultze-Motel, 1999; Seymour, 2010), nectar secretion (Southwick, 1984; De la Berrara and Nobel, 2004), and scent diffusion (Maiti and Mitra, 2017).

Water loss from leaves and flowers

Water loss in leaves and flowers can occur through the epidermis or stomata. Cuticular conductance occurs through epidermal cells and the cuticle, a waxy layer on the surface of leaves and many other plant tissues. The cuticle protects against desiccation (Edwards et al., 1982) and the rate of cuticular conductance is largely determined by its chemical composition (Kersteins, 1996). In leaves, cuticular conductance can be low compared to the water lost through stomata (Pearcy et al., 1989; Boyer et al., 1997; Kersteins, 1996). Additionally, stomata in leaves can open and close in response to environmental triggers, thereby modifying gas exchange rates (Collatz et al., 1991). Generally, leaf stomatal closure is promoted under conditions where photosynthetic rates would be low, e.g. under low light levels and low carbon dioxide concentration, and when water loss would be high, such as under a high vapor pressure deficit (Farquhar and Sharkey, 1982).

Theoretically, leaf structural traits are optimized to minimize water loss and maximize carbon gain depending on environmental conditions (Brodribb, 2009). Current investigation seeks to know the impact of the impact of floral structural traits on floral water loss. Compared to leaves, flowers exhibit more diversity in water usage (Roddy, Simonin, et al., 2018). Studies on hydraulic traits in flowers have found that stomatal density (the number of stomata in a given area) varies substantially among species (*Persea*: Whiley et al., 1988; *Magnolia*: Feild et al., 2009; Roddy et al., 2016; Roddy, Simonin, et al., 2018), with many flowers lacking stomata entirely (*Salvia*: Lambrecht et al., 2011; Roddy et al., 2016). These results suggest floral water loss may primarily occur

through cuticular conductance. Therefore, flowers may be unable to adjust their water loss rates under conditions where leaves may have already closed their stomata. A study comparing leaf and floral water loss in *Salvia malifera*, which lacks floral stomata, found that floral water loss per area was 60% of that from leaves under optimal conditions for gas exchange and that floral water loss rates did not decrease under high vapor pressure deficits (Lambrecht et al., 2011). Comparative leaf and flower studies in species with floral stomata, such as *Persea americana* and *Calycanthus*, have also concluded that flowers may not reduce their water loss on a per area basis to the same degree as leaves, even when floral stomata closed in response to increased vapor pressure deficit (Whiley et al., 1988; Roddy, Jiang, et al., 2018). Whether floral stomata were present or not, most literature shows water loss from flowers in the range of ~25–82% of leaf water loss (Whiley et al., 1988; Lambrecht at al., 2011; Liu et al., 2017) and that floral water loss often increases in response to raised temperatures (Galen, 2006; Teixido and Valladares, 2014) and high vapor pressure deficit (Teixido and Valladares, 2014). Such evidence suggests that, although floral water use is often less than that of leaves, it can be "wasteful" since flowers appear to have little control over water loss.

One last barrier to water loss in leaves and flowers that could be influential to understanding water loss rates is the boundary layer. The boundary layer is a zone of still air close to a surface that affects rates of gas exchange. Morphological traits that increase leaf surface area enlarge the boundary layer, slowing water loss rates, as dense leaf hairs and rugose veins trap moisture close to the leaf surface (Schuepp, 1993). Considering the complexity of floral structure, scaling water loss rates up from petal area may be

overlooking the influence of floral morphology on whole flower water loss rates. For example, studies on *Magnolia* species have determined that transpiration rates from tepals in the innermost whorls of the flower are higher than those in the outermost whorls (Field et al., 2009; Liu et al., 2017), indicating that water loss rates are not uniform within floral tissues. There is also some evidence that water loss rates are affected by floral size and larger flowers necessitate higher water costs (Galen, 1999; Galen et al., 1999; Teixido and Valladares, 2014). For example, studies on *Polemonium viscosum* and desert rockrose species have shown that corolla area correlates positively with water loss rates within species (Galen, 1999; Galen et al., 1999; Teixido and Valladares, 2014). However, the relationship between flower size and water loss has yet to be rigorously tested across multiple taxa.

Water influx into flowers

Knowing which vascular tissue keeps flowers hydrated—the xylem, the phloem, or both—is key to understanding floral water use. The xylem primarily transports water and is responsible for delivering the bulk amount of water to leaves and organs that experience high rates of transpiration (Jarvis, 1976). However, the phloem, which transports sugars, also delivers water to organs as long as they are a carbon sink. The direction of water movement in the xylem is determined by the water potential gradient. For example, during the day, leaves have a more negative water potential than stem tissue. In turn, the stem tissue water potential is more negative than the root tissue, indicating that water flows from the roots into the leaves (Jarvis, 1976). Organs with greater water potentials than the stem cannot be xylem hydrated because water would

flow out of them into the stem. Consequently, these organs are hypothesized to be phloem hydrated (Nobel et al., 1994; Chapotin et al., 2003; De la Berrara and Nobel, 2004; Savage et al., 2016). In the phloem, sap movement is driven by a positive pressure gradient that is generated by local osmotic gradients at source and sink tissues (Münch, 1930; Knoblauch and Peters, 2010). The pressure gradient allows for sap flow into sink tissues even if the water potential is higher than in the xylem. Therefore, observations of water potential can be used to determine water relations of tissues throughout the whole plant.

Measures of floral water potential and calculations of floral maintenance costs suggest that flowers of some species may be xylem hydrated while others receive water from the phloem. Flowers of some *Magnolia* and *Calycanthus* species display more negative water potentials than the stem, meaning they are likely xylem hydrated (Feild et al., 2009; Roddy, Jiang, et al., 2018). On the other hand, floral water potentials of cotton (Trolinder et al., 1993) and several dry tropical forest trees (Chapotin et al., 2003) are more positive than the stem water potentials, suggesting that the flowers may be primarily phloem hydrated. Considering floral maintenance costs, it has been hypothesized that the balance of water to carbon costs may influence whether a flower could receive water primarily from the xylem or the phloem, or if both contribute (Savage et al., 2016; Savage, in press). High costs of nectar production (De la Berrara and Nobel, 2004; Chapotin et al., 2003) or low water costs (Roddy et al., 2016) are thought to drive exclusive phloem hydration in flowers. Clearly, which vascular tissue provides the bulk of floral hydration is highly species specific.

Conclusion

From this brief summary of comparative floral and leaf water use, it is clear that more information about floral physiology and morphology is needed to understand how flowers obtain, use, and lose water. While there are some similarities between floral and leaf water use, such as their ability to photosynthesize and the presence and responsiveness of stomata, they do not hold true for all species, which makes drawing direct comparisons difficult. Furthermore, the unknown impacts of the floral boundary layer complicate our understanding of floral water loss responses to temperature and vapor pressure deficit. Even attempts to attribute the hydration of flowers to xylem or phloem appear to be thwarted by floral diversity. Yet, it is the complexity of flowers that makes floral water use a fascinating question. Flowers represent the many routes that plants have taken to achieve the goal of sexual reproduction and what role water use plays can better our understanding of plant reproductive strategies and how the abiotic environment impacts plant fitness.

Chapter I: Stem hydraulic conductivity does not limit floral water loss in temperate woody species

INTRODUCTION

Plants move large amounts of water from their roots to their leaves and the rate of this movement depends both on the water potential gradient between these tissues and the conductivity of the transport pathway. Consequently, there is a tight correlation between leaf traits related to water loss and xylem hydraulic conductivity. For example, stomatal conductance rates are correlated with plant hydraulic conductance in the leaves (Sack et al., 2003; Sack and Holbrook, 2006) and in the xylem of subtending shoots and branches (Meinzer and Grantz, 1990; Saliendra et al., 1995). There is also evidence that maximum rates of water loss from leaves are limited by the rate at which the plant can supply them with water (Sperry 2000). However, leaves are not the only part of a plant that can contribute substantially to water loss. Floral water loss can be considerable in herbaceous annuals (Galen, 2005; Lambrecht, 2013), shrubs (Lambrecht et al., 2011; Teixedo and Valladeres, 2014), and angiosperms with plesiomorphic traits (Sauquet et al., 2017; De-Paula et al., 2018) like *Magnolia* and *Calycanthus* (Field et al., 2009; Roddy et al., 2016; Liu et al., 2017), but the mechanisms affecting their rates of water loss are poorly understood. Since xylem physiology imposes limitations on leaf water loss rates, could flowers be similarly limited?

Water is necessary to maintain cell turgor and promote cell elongation (Lockhart, 1965) and is therefore influential during floral development and expansion (Ram and

Rao, 1984). Still, information on how much water flowers use during full bloom and what vascular tissue it comes from is complicated by floral structural diversity (Roddy et al., 2016; Roddy, Simonin, et al., 2018). Floral water use is not uniform across species and some flowers may use water for photosynthesis (Watson and Casper, 1984; Aschan and Pfanz, 2003), thermoregulation (Seymour and Schultze-Motel, 1999; Seymour, 2010), transport of nutrients and chemical defenses (Pichersky and Gershenzon, 2002; Galen et al., 2011), nectar secretion (Southwick, 1984; De la Berrara and Nobel, 2004), and to facilitate diffusion of scents through the epidermis and stomata (Maiti and Mitra, 2017). Size may also affect floral water use since floral transpiration rate increases with corolla size within species, as shown in *Polemonium viscosum* (Galen, 1999; Galen et al., 1999) and two *Cistus* species (Teixido and Valladares, 2014).

Since photosynthesis is the primary function of leaves, water use is thought to be coordinated with water transport through the xylem to maximize carbon gain and minimize water loss (Brodribb 2009). Water is exchanged for carbon dioxide during stomatal conductance, so plants assimilate carbon while risking leaf desiccation (Brodribb et al., 2010). Traits that increase the rate of water movement through the leaf (i.e. leaf hydraulic conductance)—such as high vein density and short mesophyll pathways from veins to the epidermis (Sack and Holbrook, 2003; Brodribb et al., 2007)—may offset the risk of desiccation and are often correlated with high photosynthetic rates (Brodribb et al., 2010; Brodribb and Feild, 2010). Hydraulic conductance from the xylem to the leaves is also positively correlated with leaf water use traits like transpiration, stomatal conductance, leaf area, and maximum photosynthetic rate on a branch level

(Saliendra et al., 1995; Tyree and Zimmermann, 2002; Santiago et al., 2004). Furthermore, it is possible to reduce stomatal conductance and leaf transpiration by injecting air into transport conduits (i.e. vessels and tracheids) to block water flow and reduce xylem hydraulic conductance (Sperry and Pockman, 1993; Sperry et al., 1993), indicating that leaf physiology can be limited by vascular transport.

Flowers and leaves are functionally different but could share hydraulic traits that cause their water use to be similarly limited by the xylem. Traits associated with high water costs in leaves, such as high vein and stomatal density (Brodribb et al., 2010), are more variable across species in flowers than in leaves (Roddy et al., 2016; Roddy, Simonin, et al., 2018). Consistent with these findings, measures of water loss from flowers are also highly variable across species, with rates in the range of ~25–82% of leaf water loss (Whiley et al., 1988; Lambrecht at al., 2011; Liu et al., 2017). Additionally, some flowers may not be able to control water loss by closing stomata like leaves (Lambrecht et al., 2011; Teixido and Valladeres, 2014), making them more vulnerable than leaves under dry conditions.

There is also on-going debate about whether flowers are primarily hydrated by the xylem. While some *Magnolia* and *Calycanthus* flowers have been shown to be connected to the xylem (Feild et al., 2009; Roddy, Jiang, et al., 2018), studies on nectar producing flowers have proposed that the overall carbon cost of floral maintenance and nectar production may draw in enough phloem sap to support floral water needs (Trolinder et al., 1993; Chapotin et al., 2003; De la Barrera and Nobel, 2004). A study on *Magnolia* species that flower before producing leaves (a flowering habit referred to as ‘precocious’)

found that sap flow through the sapwood area of branches was ~22–55% lower during flowering for *Magnolia* species than when the branches bore leaves, and that floral tepal conductance was ~27–65% of leaf stomatal conductance (Liu et al., 2017). These results suggest that floral water loss may be limited by reduced xylem transport rates or that floral water loss does not require substantial xylem transport capacity.

To examine whether floral water loss is limited by the hydraulic transport capacity of subtending branches, I characterized the relationship between branch level water loss rates and stem specific hydraulic conductivity in plants that flower precociously before they have leaves. Precocious flowering woody species present a unique opportunity to study the relationship between floral water loss and xylem transport capacity because flowers are the first new growth in the spring. During the spring, air bubbles generated during freeze-thaw events can expand and block transport conduits, reducing xylem hydraulic conductivity, or the flow of water through a particular area and length of xylem under a given pressure (Sperry et al., 1988a). If a plant experiences substantial freeze-thaw induced embolism in the spring, then its floral water loss may be limited by reduced xylem hydraulic conductivity. If so, then (i) a positive correlation should be detected between branch level floral water loss rates and stem hydraulic conductivity within a species, similar to what is observed with leaf water loss rates and stem hydraulic conductivity (Tyree and Zimmermann, 2002). I also tested whether differences in inflorescence water loss among species can be explained by variation in their hydraulic transport capacity. I hypothesized that (ii) larger inflorescences should lose water more rapidly and require greater stem hydraulic

conductivity. Lastly, I examined whether inflorescences were primarily hydrated by the xylem, phloem, or both, during anthesis, with the hypothesis (iii) that flowers with low inflorescence water loss rates may be primarily phloem hydrated.

METHODS

Study species and sampling

I selected a combination of five horticultural and native species that exhibit precocious flowering in Minnesota (Table 1). I considered a species precocious if it could flower before it produced leaves. Categorizing a species as precocious does not require the plant to finish flowering before leaf out or that flowering occurs before leaf out every year. Study populations were located on two of the University of Minnesota campuses—Duluth (*Alnus, Salix, and Rhododendron*) and the Twin Cities Saint Paul campus (*Magnolia*)—as well as Wolf Ridge Environmental Learning Center in Finland, MN (*Prunus*). I performed all floral analyses on an inflorescence level, taking into consideration that the majority of our species have multiple flowers in an inflorescence, except *Magnolia,* which has single flowers. I also restricted our observations to female individuals in *Salix* and male catkins of *Alnus.*

Table 1. Study species and their sexual system, inflorescence type, and approximate flowering time at Minnesota study sites.

Species	Sexual System	Inflorescence	Flowering Time
Alnus incana (L.) Moench ssp. *rugosa* (Du Roi) R.T. Clausen	Monoecious, unisexual	Terminal catkin	April
Salix discolor Muhl.	Gynodioecious	Axillary catkin	Late April to early May
Magnolia stellata (Siebold & Zucc.) Maxim.	Monoecious, bisexual	Single or paired terminal flowers	Late April to early May
Prunus americana Marshall	Monoecious, bisexual	Axillary umbel of 2–5 flowers	May
Rhododendron L. 'Rosy Lights' Pellett	Monoecious, bisexual	Terminal umbel of 8–12 flowers	Late May to June

Rates of inflorescence and leaf gas exchange in the field

I measured water loss and respiration on inflorescences during anthesis with a portable infrared gas exchange analyzer (LI 6400, LI-COR Biosciences, Lincoln, NE) similar to Galen (1993) and Lambrecht et al. (2011). Inflorescence measurements were made on 6–8 individuals per species when the flowers were receptive and/or had dehiscing anthers. The only exception was *Rhododendron* which had 3 individuals sampled twice during anthesis (n=6). Measurements were made using an opaque LI-COR conifer chamber with an RGB light source attached (6400-22 and 6400-18/A) under ambient CO_2 (concentration = 410 ± 2.8 ppm [sd]), temperature, and humidity (Table 2) using a 20-L carboy as an air buffer. I measured inflorescences during the warmest part of the day, between 10h00 and 15h00, to maximize their rate of water loss. I used a light level of 1200 PAR ($\mu mol*m^{-2}*s^{-1}$) and flow rate of 400 $\mu mol*s^{-1}$. I sampled

inflorescences on mid-canopy, partial sun branches with minimal side branching. To take a measurement, an entire terminal inflorescence was enclosed in the chamber where the peduncle or pedicel met the shoot. For axillary inflorescences, the chamber encompassed the branch and multiple inflorescences. I waited until water flux stabilized before taking an average of 4–7 measurements. All measurements are reported on a per inflorescence basis. For *Alnus*, only male catkins were counted even though both sexes were present in the chamber because small female catkins are only 7.3 ± 2.5% [sd] of the total floral fresh weight and are thus unlikely to contribute significantly to evaporative water loss.

In July and early August, I performed spot measurements of leaf transpiration using the same light level and flow rate as the inflorescences with the standard broadleaf LI-COR chamber (6400-02B). Leaf measurements occurred between 8h00 and 11h00 with a set CO_2 concentration (400 ppm) and ambient temperature and humidity (Table 2). I measured 1–3 newly matured leaves on 6–8 individuals per species, taking the average of 3–5 stabilized leaf transpiration measurements for each plant. When possible, the same individuals measured for floral water loss were also used for leaf transpiration measurements.

Table 2. Ambient field conditions and chamber vapor pressure deficit during water loss measurements (mean ± sd) in the field.

	Inflorescences			Leaves		
Genus	Temperature (°C)	Relative Humidity (%)	Chamber VPD (kPa)	Temperature (°C)	Relative Humidity (%)	Chamber VPD (kPa)
Alnus	18 ± 1.6	25 ± 0.9	1.4 ± 0.2	25 ± 2.8	43 ± 6.7	1.4 ± 0.3
Salix	15 ± 0.7	18 ± 0.5	1.1 ± 0.2	24 ± 2.8	50 ± 0.7	1 ± 0.2
Magnolia	31 ± 1.1	16 ± 1	2.6 ± 0.3	26 ± 2	52 ± 3.6	1 ± 0.2
Prunus	33 ± 1.2	28 ± 1.5	3.6 ± 0.4	27 ± 1	51 ± 1.1	1 ± 0.1
Rhododendron	30 ± 0.9	18 ± 1.8	3.0 ± 0.2	21 ± 0.8	51 ± 0.9	1 ± 0.1

Native stem specific conductivity

I collected branches for stem hydraulic conductivity measurements in the early morning the day after floral water loss and leaf transpiration measurements. Branches were cut underwater two maximum vessel lengths away from the segment selected for conductivity measurements. Maximum vessel lengths for each species were determined from the literature and confirmed through either the air injection method or by progressively cutting back stem segments and noting the lengths at which conductivity destabilized (Melcher et al., 2012). The branches were relaxed in a bucket of water covered with black plastic bags for several hours. All samples were processed within 24 hours. When possible, I used the branch that bore the inflorescences and leaves used for gas exchange measurements.

In the laboratory, I excised straight, unbranching stem segments underwater and shaved the ends with a fresh razor blade. The final segment length for each species was in the range of the maximum vessel lengths (Table 3). I measured the flow rate of the stem segment by gravimetrically perfusing partially degassed, filtered (0.22 μm) 10 mM KCl solution at a standard temperature of 20°C through the stem under a driving pressure of 1–1.5 kPa (Sperry et al., 1988b). The flow rate was recorded with a liquid flow sensor (SLI-0430, Sensirion, Switzerland) upstream of the stem segment (Wason et al., 2018). I averaged the flow rate once the measurement had stabilized and subtracted out the background noise of the system when no flow was occurring. I then calculated stem specific conductivity (K_s: $kg*m^{-1}*MPa^{-1}*s^{-1}$) which corrects for stem length and cross-sectional xylem area.

Table 3. Maximum vessel length and average length of stem segments (n = 12–16) used for stem specific conductivity measurements.

Genus	Vessel Length (cm)	Source	Length of Stem Segments (cm)
Alnus	3–15	Tognetti and Borghetti (2002)	13.9 ± 0.6
Salix	20	Savage and Cavender-Bares (2011)	19.8 ± 0.7
Magnolia	7.5 ± 3.4 [sd]	Air injection method	10.9 ± 0.5
Prunus	10–15	Jeje and Zimmerman (1981), Scholz et al. (2013)	11.9 ± 0.7
Rhododendron	2–8	Cordero and Nilsen (2002)	10.2 ± 0.4

K_s per inflorescence and branch water loss rates

During stem specific conductivity (K_s) measurements, I counted the inflorescences and collected the leaves downstream of the segment. I weighed and then dried the inflorescences used for water loss measurements shortly after collecting the branches for stem specific conductivity measurements. I digitally scanned (CanonScan

LiDE 600F) and measured the downstream leaf area with ImageJ (version 1.48). To quantify water transport capacity per inflorescence, I divided K_s by the number of inflorescences downstream of the stem segment. To relate K_s to the rate of inflorescence water loss, I calculated floral water loss on a whole branch basis (rate of branch water loss: mmol* s^{-1}) by multiplying average inflorescence water loss by the total number of inflorescences downstream of the stem segment used for stem specific conductivity measurements. For branch water loss rates when leaves were present, I multiplied average leaf transpiration by the total leaf area downstream of the segment.

Floral heating experiment

To account for impacts of abiotic conditions that may result from measuring inflorescences in the spring and leaves in the summer, I performed a laboratory heating experiment on cut branches of three study species (*Rhododendron*, *Salix*, and *Prunus*). Before the treatment, I assessed the impact of cutting branches on the rate of inflorescence water loss. I selected 12–14 inflorescence-bearing branches and excised half of them from an individual plant underwater and placed them next to the plant with their cut ends in containers of water. After at least 12 hours, I measured water loss in the cut and intact inflorescences using a clear LI-COR conifer chamber (6400-05) under ambient field conditions (Table S1) with a flow rate of 500 μmol*s^{-1} using a 20-L carboy as an air buffer. I standardized the rate of water loss by inflorescence fresh mass. Cutting did not cause a decrease in inflorescence water loss in any of the species. Instead, there was no change in water loss for *Rhododendron* or *Prunus* and a significant increase in

Salix (Table S2), likely due to the increase in water potential gradient that occurred when the branch was placed in an open container of water.

Using the clear LI-COR conifer chamber, I measured the rate of water loss for 7 cut inflorescences per species at four temperatures: ambient (~20–23°C), 26°C, 28°C, and 30°C. An additional measurement was taken after the highest temperature was held for ten minutes. I heated the chamber using an infrared light (125R40/HT, No. 0390700, Westinghouse Lighting, Philadelphia, PA) and the temperature of the chamber was monitored with the LI-COR chamber thermocouple and a thermocouple (TC6-T, HOBO UX100-014M, Onset Computer Corporation, Bourne, MA) touching the flower. The measurements were taken under a grow light (SunBlaze T5HO-44, Sunlight Supply, Inc., Vancouver, WA), at a flow rate of 500 $\mu mol*s^{-1}$ using a 20-L carboy as an air buffer (Table 4). Note the chosen temperatures are substantially higher than historical average high temperatures (~10–22°C) in Duluth, MN for the months of April–June and more similar to average temperatures in July and August, when I measured leaf transpiration (Climate.gov, 2018).

Table 4. Laboratory conditions throughout floral heating experiments (mean ± sd).

Genus	Reference CO_2 (ppm)	PAR ($\mu mol*m^{-2}*s^{-1}$)	Relative Humidity (%)
Salix	420 ± 4	240 ± 33	30.9 ± 4.5
Prunus	430 ± 4	200 ± 30	30 ± 4
Rhododendron	426 ± 16	180 ± 28	20 ± 4

Water flux into inflorescences during development and anthesis

I modeled total water influx per day into floral buds in the late winter and spring to determine when the greatest water use occurred. I monitored the whole tree floral phenology of six individuals of our study species on a weekly basis in spring 2017,

starting in late February. I collected at least three floral tissue samples from each individual at the following four phenophases; (1) bud dormancy in late winter, (2) bud swelling when the bud enlarges considerably and bud scales may separate slightly, (3) floral expansion where the bud scales begin to fall off and floral tissue emerges and unfurls or the catkin begins to elongate, and (4) anthesis when stigmas are receptive and/or anthers are dehiscent. A total of 18 inflorescences per species were collected for each phenophase. Note *Alnus* and *Prunus* do not exhibit a swelling stage, and *Prunus* dormant bud samples were collected in November 2018, a year later than the other species. The date of the last observation of dormant *Prunus* buds was based on phenology data from the National Phenology Network (Usanpn.org, 2018). For collection, I sampled the entire bud or inflorescence, including the pedicel or peduncle. Samples were refrigerated in plastic bags prior to measuring fresh mass, and then dried and reweighed. I calculated the rate of water movement into the buds by dividing the difference in water content of subsequent phenophases by the number of days between them.

I evaluated the potential contribution of phloem sap to floral hydration during anthesis using the phloem hydration model described in Savage (in press). I estimated water influx per day for each species using measured respiration and water loss rates, assuming no change in the mass of the inflorescence over time. From the respiration rates, I calculated potential phloem sap influx in grams per day by converting the grams of carbon used in respiration into grams of sucrose that could be carried in by the phloem given a sap sucrose concentration of 18% (Jensen et al., 2013). Phloem sap influx was calculated with a respiration rate of 0 $\mu mol \bullet s^{-1}$ for *Salix* since gas exchange

measurements showed that photosynthesis rates balanced respiration rates. I then calculated total water influx in grams per day from the observed water loss rates and the percentage of phloem sap that contributed to the total water influx.

Data analysis

I performed statistical analyses in R (v. 3.3.3; R Core Team, 2017). When comparing seasonal data within species, I used two-sample t-tests or Welch's t-tests (for unequal variance). Comparisons across species and across phenophases within species were evaluated with one-way ANOVAs or Welch's one-way ANOVAs (for unequal variance) followed by a post-hoc Tukey HSD test with an $\alpha = 0.05$. I examined relationships between branch water loss and K_s using Pearson's correlation coefficient. I used a simple linear regression to assess the dependence of branch water loss on K_s for significant correlations.

RESULTS

Effect of inflorescence size on water loss

Fresh floral mass differed significantly among species ($F_{4,31}$= 110.2, P <0.0001) with species falling into two distinct groups; *Salix*, *Alnus*, and *Prunus* had similar averages and *Magnolia* and *Rhododendron* were significantly heavier than the other species (Fig. 1A). Overall, species with heavier inflorescences had a higher rate of water loss than those with small inflorescences (Fig. 1B), resulting in significant differences between species ($F_{4,30}$= 125.5, P <0.0001). However, *Magnolia* lost the most water, despite having lighter inflorescences than *Rhododendron*. Within species, only *Prunus*

($F_{1,4}$ = 11.1, P = 0.03) and *Salix* ($F_{1,6}$ =157.7, P < 0.001) showed a positive correlation between inflorescence water loss and fresh mass (Fig. 1D & 1E). Heavier inflorescences also showed a greater K_s per inflorescence than lighter inflorescences (Fig. 1C; $F_{4,30}$= 17.8, P <0.0001). In *Rhododendron*, K_s per inflorescence was greater than in *Prunus* (P = 0.03) but not significantly different from *Salix* (P = 0.5) or *Alnus* (P = 0.6). The high variation in *Rhododendron* K_s per inflorescence may be partially driven by greater variation in inflorescence mass.

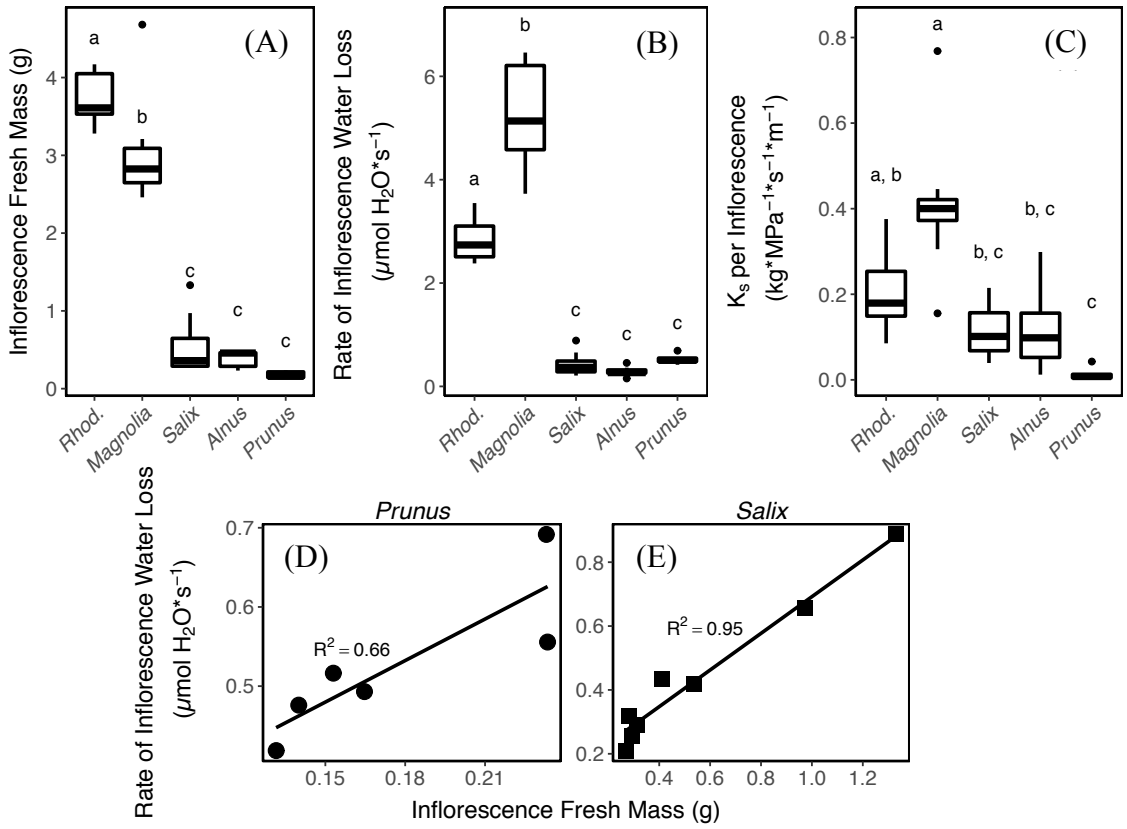

Figure 1. Inflorescence mass impacts floral water use within and across species. In (A) through (C), species are ordered based on inflorescence fresh mass (*Rhododendron* is abbreviated as *Rhod.*). Lowercase letters represent species that are significantly different from each other according to a post-hoc Tukey HSD analysis (α = 0.05). In within species (D) *Prunus* and (E) *Salix* scatterplots, points are from individual plants (n=6–8).

Branch level water loss rates and native stem specific conductivity

Rates of water loss from leaf-bearing branches are higher than the water lost from flower-bearing branches for all species (Table 5). Native stem specific conductivity (K_s) is significantly greater in the summer for *Prunus, Salix,* and *Alnus*, but I detected no difference between the seasons for *Magnolia* and *Rhododendron* (Table 6). In all species, I found no correlation between floral branch water loss rates and K_s ($\alpha = 0.05$, Fig. 2). In contrast, leaf branch water loss rates showed a positive relationship with K_s for *Salix* ($F_{1,5} = 9.3$, $P = 0.02$) and *Magnolia* ($F_{1,4} = 70.01$, $P = 0.001$), and a weak positive correlation for *Prunus* ($F_{1,4} = 5.2$, $P = 0.08$). However, there was no correlation for *Alnus* and *Rhododendron* (Fig. 2).

Table 5. Comparison of branch level water loss rates of inflorescences to leaves in the field during the spring and summer respectively. †

Genus	Floral Branch Rate (mmol H_2O*s^{-1})	Leaf Branch Rate (mmol H_2O*s^{-1})	df	t	P
Prunus	0.008 ± 0.005	33 ± 22	5	-3.7	0.01
Alnus	0.002 ± 0.001	31 ± 13	7	-7.0	0.0002
Salix	0.003 ± 0.001	22 ± 11	6	-5.2	0.002
Magnolia	0.007 ± 0.003	10 ± 6	5	-4.1	0.009
Rhododendron	0.01 ± 0.01	6 ± 1	5	-14.1	<0.0001

† Welch's t-test was used to account for unequal variance. Reported values are the mean ± standard deviation.

Table 6. Native stem specific conductivity (K_s) during floral anthesis compared to K_s during mid-summer when mature leaves were present on branches. †

Genus	K_s Floral Anthesis ($kg*m^{-1}*MPa^{-1}*s^{-1}$)	K_s Summer ($kg*m^{-1}*MPa^{-1}*s^{-1}$)	df	t	P
Prunus	0.2 ± 0.01	1 ± 0.5	5	-5.8	0.002
Alnus	0.8 ± 0.6	2 ± 0.5	14	-3.1	0.007
Salix	1 ± 0.5	2 ± 0.7	13	-4.5	<0.0001
Magnolia	0.6 ± 0.2	0.5 ± 0.1	12	0.9	0.3
Rhododendron	0.7 ± 0.01	0.7 ± 0.1	10	0.1	0.9

† A Welch's t-test was used to account for unequal variance in *Prunus*. Reported values are the mean ± standard deviation.

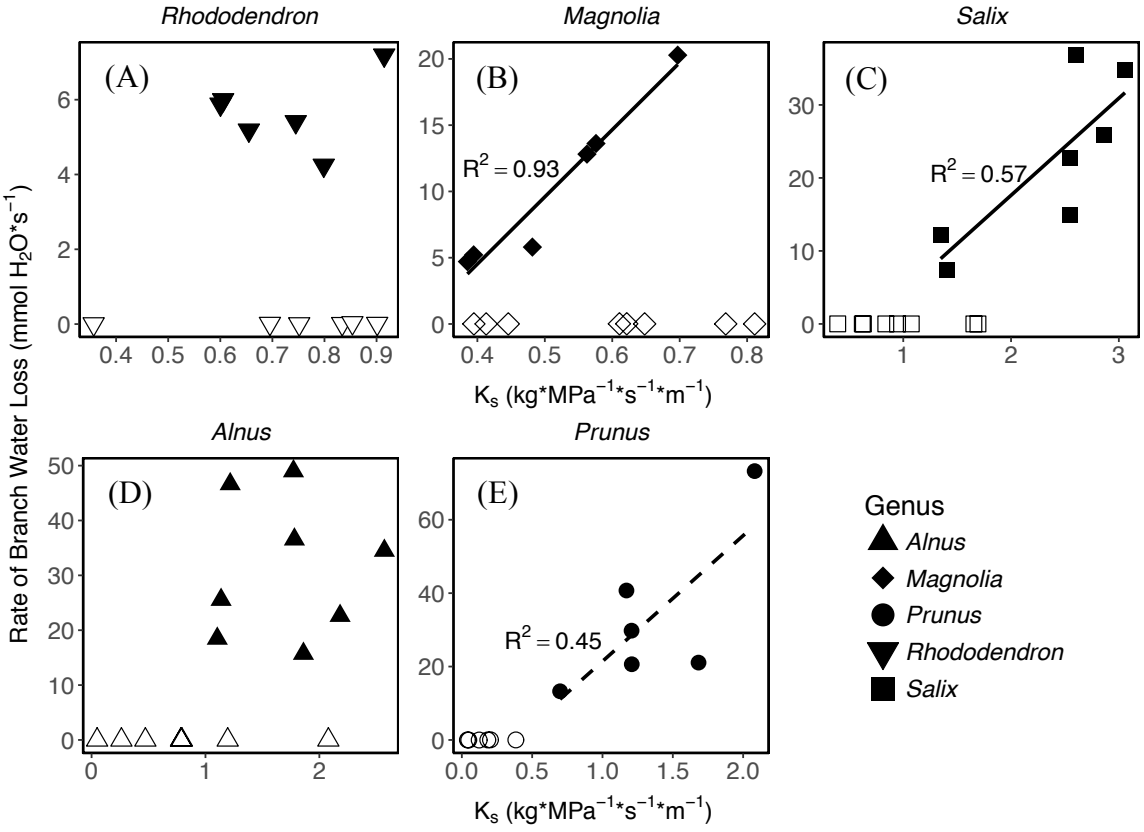

Figure 2. Relationship between the native stem specific conductivity (K_s) and the total water lost from downstream inflorescences (open) and leaves (filled) in (A) *Rhododendron*, (B) *Magnolia,* (C) *Salix,* (D) *Alnus*, and (E) *Prunus* measured in the field. Each point represents measurements from a single branch collected during anthesis for inflorescences and mid-summer for leaves. Measurements were taken once from 6–8 individuals at each timepoint in 2018 with the exception of *Rhododendron*, which had 3 individuals sampled twice during anthesis in 2017 (n=6).

Effect of temperature on the rate of floral water loss

The rate of floral water loss increased significantly at higher temperatures for every species. *Salix* ($F_{1,33}$= 17.5, P < 0.0001) and *Prunus* ($F_{1, 33}$ = 19.2, P = 0.0001) each experienced 0.2 µmoles increase in water loss per gram of fresh tissue per degree, while *Rhododendron* ($F_{1, 33}$ = 37.6, P < 0.0001) increased 0.1 µmoles of water loss per gram per degree (Fig. 3). The rate of water loss for inflorescences held at 30°C were not significantly different from our field measurements of *Prunus* (ambient: 3 ± 0.3 µmol $H_2O*g^{-1}*s^{-1}$ [sd], 30°C: 3 ± 0.6 µmol $H_2O*g^{-1}*s^{-1}$ [sd], t_{11} = 0.6, P = 0.7), which were collected under similar temperature conditions (Table 2). *Salix* field measurements of water loss, which were taken on a day with an average temperature of 15°C, were significantly lower than that of inflorescences held at 30°C (ambient: 0.8 ± 0.1 µmol $H_2O*g^{-1}*s^{-1}$ [sd], 30°C: 3 ± 0.3 µmol $H_2O*g^{-1}*s^{-1}$ [sd], t_{13} = -14.1, P < 0.0001). *Rhododendron* inflorescence water loss rates were also lower in the field than at 30°C (ambient: 0.7 ± 0.1 µmol $H_2O*g^{-1}*s^{-1}$ [sd], 30°C: 1 ± 0.3 µmol $H_2O*g^{-1}*s^{-1}$ [sd], t_{11} = -9.7, P < 0.0001) even though average field temperature was ~29°C. This difference may be attributed to the faster flow rate used in the chamber during the heating trials.

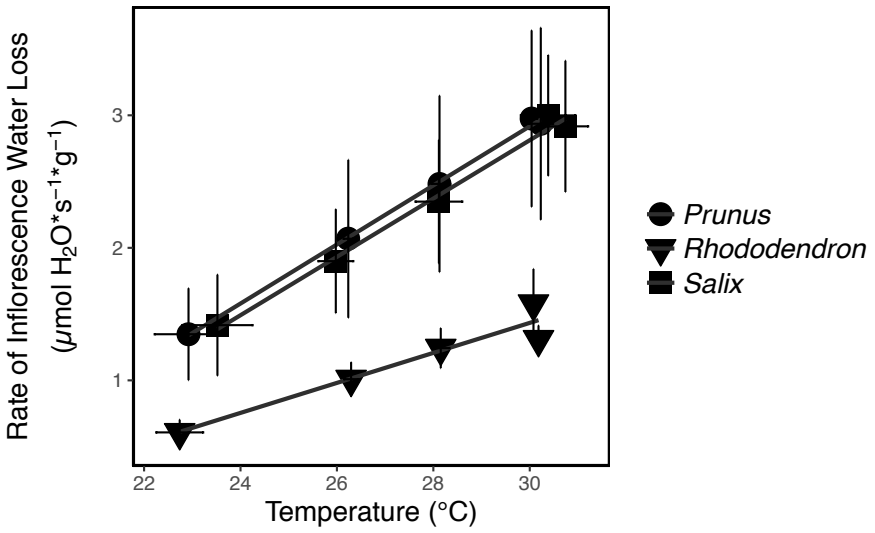

Figure 3. Floral heating experiments show that inflorescences of *Prunus* (Adj. $R^2 = 0.34$), *Salix* (Adj. $R^2 = 0.32$), and *Rhododendron* (Adj. $R^2 = 0.51$) lost water more rapidly at higher temperatures. Each point is an average of 7 inflorescences and error bars represent one standard deviation.

Water influx into floral buds during development and anthesis

Water influx rate into floral buds was low during floral development when compared to the rate during anthesis (*Rhododendron*: $F_{3, 5.7} = 81.7$, $P < 0.0001$, *Magnolia*: $F_{3, 8.4} = 64.2$, $P < 0.0001$, *Salix*: $F_{3, 6.1} = 11.6$, $P = 0.006$, *Prunus*: $F_{2, 7.3} = 40.4$, $P = 0.0001$). I excluded *Alnus* from this analysis because floral mass dropped between expansion and anthesis. This decrease could be attributed to either rapid desiccation of the catkin or to substantial pollen dehiscence. Of the remaining species, daily water influx remained constant between dormant and swelling, swelling and expansion, and expansion and anthesis but increased significantly during anthesis (Fig. 4). Calculations of total influx and phloem sap influx during anthesis showed that all species required substantial daily water input that could not be satisfied by the phloem alone (Fig. 5). I

estimated that less than 10 % of daily inflorescence water intake could be supported by the phloem in all species, assuming constant respiration and water loss rates and no change in floral mass.

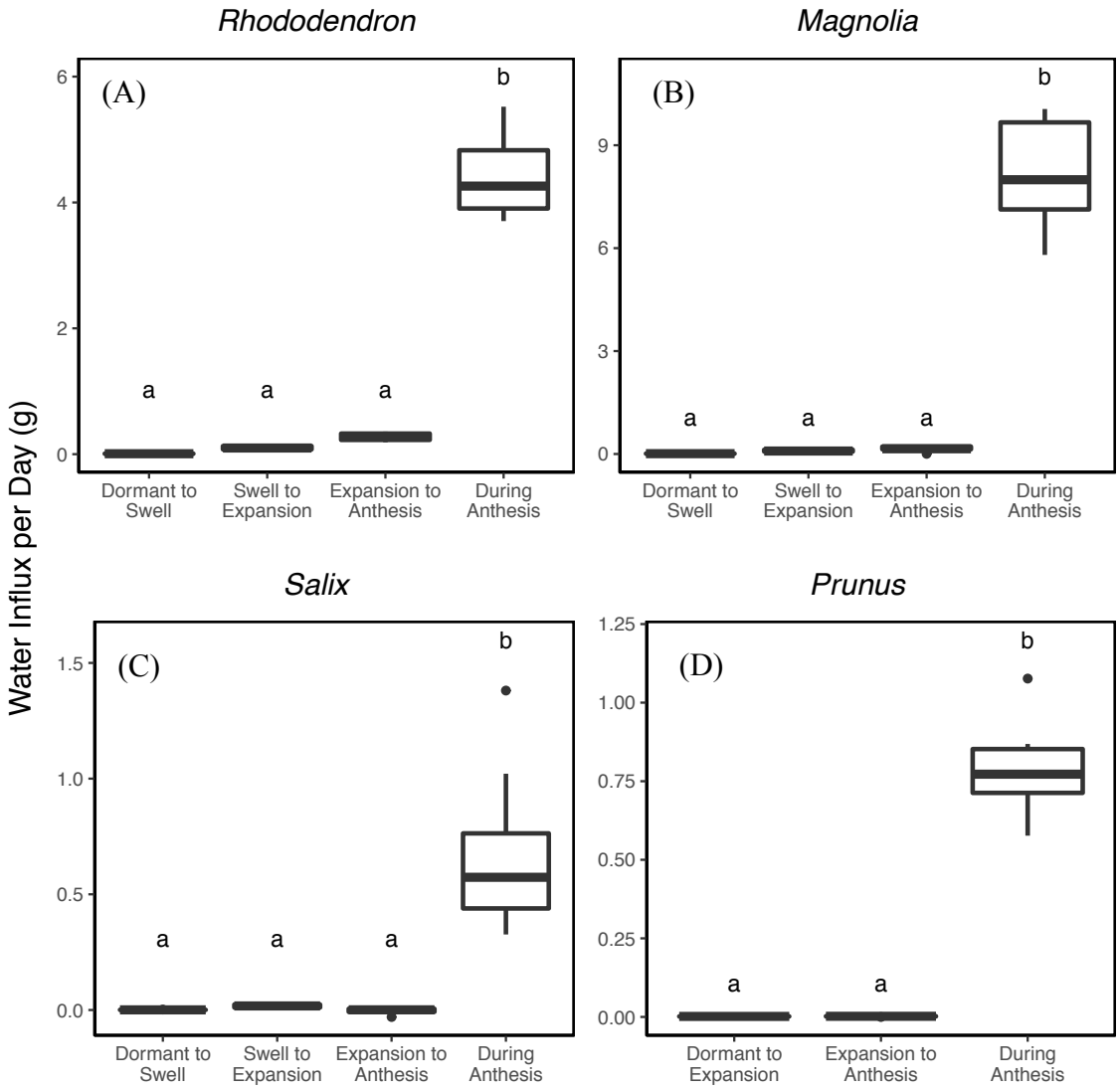

Figure 4. Water influx into floral buds differs between phenophases in (A) *Rhododendron*, (B) *Magnolia*, (C) *Salix*, and (D) *Prunus*. *Alnus* was not included in this analysis because male catkins experience desiccation and pollen dehiscence in the expansion stage. Lowercase letters show significant differences in water influx between phenophases within a species according to a post-hoc Tukey HSD analysis ($\alpha = 0.05$).

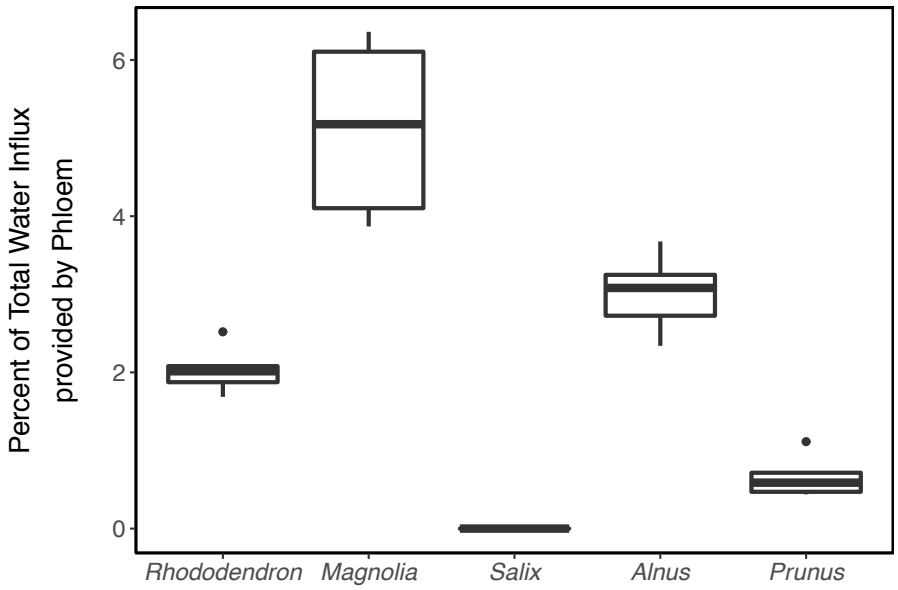

Figure 5. Water influx from phloem makes up less than 10% of the total water influx required to support water loss during anthesis. Each bar shows the percentage of phloem sap that is contributing to total water influx in each species, which was calculated from field measurements of respiration and water loss respectively.

DISCUSSION

I found no evidence that xylem hydraulic supply limits floral water loss within individual precocious flowering species (Fig. 2), despite these flowers' high dependence on the xylem for hydration (Fig. 5). I hypothesize that the flowers in these species are not limited by the xylem because their rates of water loss are low regardless of environmental conditions (Fig. 3). These results contrast with what I found for leaves, in which leaf water loss and branch hydraulic conductivity were positively correlated in three of the five species. Although the data show floral water loss is not influenced by variation in xylem hydraulic conductivity within species, there are consistent differences across

species in inflorescence water loss (Fig. 1B) and K_s per inflorescence (Fig. 1C) between large and small flowered plants. Overall, these data suggest that floral size and water loss traits may be influenced by xylem transport capacity across taxa.

Larger inflorescences lose more water and require a greater stem transport capacity

In the study, species with large inflorescences had greater rates of water loss than species with small inflorescences and, within some species, floral water loss increased with floral mass (Fig. 1B). Larger inflorescence size also corresponded with higher stem specific conductivity (K_s) per inflorescence on a coarse level (Fig. 1C). However, *Magnolia* showed the greatest water loss and K_s per inflorescence despite have a smaller inflorescence than *Rhododendron*. This discrepancy may be a result of differences in the species' floral structure. *Rhododendron* inflorescences are composed of an average of 8–12 flowers while *Magnolia* inflorescences contain one. Furthermore, *Magnolia* flowers have greater stomatal density and vein length per area than eudicot flowers (Roddy et al., 2016). These traits are associated with high water costs in leaves (Brodribb et al., 2010).

The species with large inflorescences in this study also have different origins than the small inflorescence species. The study populations of *Magnolia* and *Rhododendron* are horticultural varieties of species native to warmer, moister climates and have experienced artificial selection for both cold hardiness and floral display, while *Salix*, *Alnus*, and *Prunus* are native to temperate regions. Interestingly, the horticultural species appear to be more resistant to freezing-induced embolism than the native species, which exhibit a greater decline in K_s during the spring (Table 6). Overall, differences in K_s and K_s per inflorescence indicate that species experiencing a high degree of embolism invest

less water into flowering than the species resistant to embolism. While I cannot determine whether compromised spring hydraulic capacity necessitates small inflorescences, assessing xylem embolism resistance in relation to floral water use could be influential in understanding floral size in woody species.

Anthesis requires xylem hydration but is not constrained by stem hydraulic capacity on a branch level

Flowers exhibit the greatest water influx into reproductive structures during anthesis (Fig. 4), and the majority of this water comes from the xylem (Fig. 5). These results are surprising, given the range of floral sizes represented in the study species. Large flowers, like those of *Magnolia* and *Calycanthus,* have been shown to be connected to the xylem (Field et al., 2009; Liu et al., 2017; Roddy, Jiang, et al., 2018) but it has been hypothesized that smaller flowers may acquire sufficient water from the phloem (Field et al., 2009; Savage et al., 2016; Roddy et al., 2016). My results are more consistent with the idea that the role of the xylem and phloem in floral hydration may be species specific and more tied to costs of floral nectar production (De la Barrera and Nobel, 2004) or floral development and phenology (Savage, in press) than floral size.

Despite the necessity of xylem hydration, I found no correlation between the rate of branch level floral water loss and stem hydraulic conductivity (Fig. 2). I attribute this lack of correlation to the low rate of water loss by flowers of the study species. Under field conditions during anthesis, water loss rates were 2–4 orders of magnitude less in inflorescences than leaves for every species (Table 5). While there was a positive effect

of temperature on water loss rates, many of the inflorescences were measured on warm days (Table 2) and increasing temperature would only double their water loss (Fig. 4).

This disparity in water loss rates between leaves and flowers is likely driven by complex differences in their morphology. We are only beginning to understand how traits like stomatal density and cuticular conductance impact floral transpiration rates (Gleason, 2018), and we have yet to delve into floral boundary layer effects on floral water loss. Stomatal density on flowers varies significantly among species (Roddy et al., 2016), with some species lacking stomata entirely (Lambrecht et al., 2011), indicating that in some water loss may occur primarily through cuticular conductance. Meanwhile, for species with floral stomata, environmental cues may help to mitigate water loss rates. For example, *Persea americana* and *Calycanthus* species close their stomata in response to high vapor pressure deficit (VPD), reducing stomatal conductance rates by 50% or more (Whiley et al. 1988; Roddy, Jiang, et al., 2018). Whiley et al. (1988) found floral transpiration decreased simultaneously with stomatal conductance, but Roddy, Jiang et al. (2018) concluded that high cuticular conductance of *Calycanthus* tepals could maintain high rates of floral transpiration even when the stomata were closed. Although great steps have been taken to quantify floral water loss and to determine where and how flowers lose water, more work is needed to flesh out common mechanisms of floral water loss given the great diversity of floral forms in the world.

Unlike flowers, leaf water loss is limited by stem hydraulic conductivity in many species

I found that stem hydraulic conductivity was higher when plants had leaves than when they had flowers for three species (Table 6), indicating that xylem capacity is more

coupled to leaf physiology than floral physiology. These results are consistent with the findings in Liu et al. (2017), which showed that sap flow in two precocious flowering *Magnolia* species was 22–55% less during flowering than when the trees had leaves. Granted, a positive correlation between branch level leaf transpiration and stem hydraulic conductivity was not found in all study species but this could be due to microhabitat variation among species. Shading has been shown to weaken relationships between water transport capacity and leaf area (Schulz and Matthews, 1993; Brodribb and Jordan, 2008), and to reduce leaf water use efficiency and photosynthetic capacity in many species (Givnish, 1988; Lemoine et al. 2002). The *Prunus, Rhododendron*, and *Alnus* populations measured in this study that showed weak or no correlation (Fig. 2) between branch level leaf transpiration and stem hydraulic conductivity, were located in the understory. Therefore, partial shading could explain why leaf water loss was decoupled from xylem hydraulic capacity for some species.

CONCLUSION

Although floral water loss rates correlate positively with inflorescence size and ambient temperature, water loss from inflorescences are not limited by xylem hydraulic conductivity within individual species because branch level water loss rates are approximately three orders of magnitude less for flowers than leaves. While much is known about the role of water loss in the shaping of leaf morphology and physiology in angiosperms (Boyce et al., 2009; Brodribb & Field 2010; Brodribb et al., 2010; Nicotra et al., 2011), more work is needed to understand the impact of water loss on floral structure

and function. My results indicate that traits influencing floral water loss are not coordinated with variation in xylem hydraulic conductivity within species, but large, precocious flowers might require greater spring transport capacity than smaller flowers. Further studies that consider floral hydraulic traits in relation to the vascular system are needed to elucidate whether there is a size relationship between floral structure and xylem transport capacity and how floral water loss varies across species and biomes.

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Appendices

Table S1. Environmental conditions and chamber vapor pressure deficit (mean ± sd) for cut versus intact inflorescence Li-Cor measurements.

	Cut				Intact			
Genus	Temperature (°C)	Relative Humidity (%)	Chamber VPD (kPa)	PAR ($\mu mol*m^{-2}*s^{-1}$)	Temperature (°C)	Relative Humidity (%)	Chamber VPD (kPa)	PAR ($\mu mol*m^{-2}*s^{-1}$)
Salix	17.4 ± 0.9	21.4 ± 1.3	1.3 ± 0.2	1171 ± 447	16.8 ± 1.1	21 ± 1	1.3 ± 0.1	991 ± 355
Prunus	30.2 ± 0.2	25.0 ± 0.7	3.2 ± 0.1	210 ± 40	32.8 ± 1.1	27.9 ± 0.2	3.6 ± 0.4	1199.6 ± 0.2
Rhododendron	29.8 ± 0.9	33 ± 2	1.7 ± 0.2	1298 ± 646	24.8 ± 1.1	31.1 ± 2.4	1.7 ± 0.2	1413 ± 556

Table S2. Results of student's t-tests comparing cut and intact inflorescence water loss rates.

Genus	Cut Inflorescence Water Loss ($\mu mol\ H_2O*g^{-1}*s^{-1}$)	Intact Inflorescence Water Loss ($\mu mol\ H_2O*g^{-1}*s^{-1}$)	df	t	P
Salix	1.5 ± 0.3	0.8 ± 0.1	25.8	7.8	<0.0001
Prunus	2.9 ± 0.7	3.0 ± 0.3	17.1	-0.2	0.8
Rhododendron	0.7 ± 0.1	0.9 ± 0.2	17.8	-1.7	0.1