

How do trees make sweet edible sap in spring?

by

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A thesis submitted to the Faculty of Graduate and Postdoctoral
Affairs in partial fulfillment of the requirements for the degree of

Master of Science

in

Biology

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Ottawa, Ontario

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Abstract

How do trees make sweet edible sap in spring? At the end of winter, sap of temperate broadleaf eudicot trees provides energy-rich sugars for animals and peoples of North America. Here, I conduct a multi-disciplinary synthesis to elucidate a theory of sap production and movement at a ‘whole tree’ level. I begin by contrasting settler knowledge to Indigenous knowledge of maples, and describe how “two-eyed seeing” may be used to address settler appropriation of maples and begin to decolonize maple provisions. Second, with a synthesis of scientific literature I show that sugars are not just byproducts of sap movement, but are signaling molecules that reveal a blurred functional distinction between xylem and phloem. I propose numerous predictions from my theory of sap movement. Finally, when testing whether spring xylem sap sugar concentration correlates with number of cells in the width of xylem rays I found significant positive correlation.

Acknowledgements

First, I would like to acknowledge the Algonquin peoples in whose unceded territory I conduct research, reside and work. I would like to thank my supervisor for continued support and guidance, regardless of what path of intrigue I may have wandered down or roadblock that I encountered. The ability to conduct research without limitation to inquiry is a rare opportunity that I greatly valued. Thank you to my committee members for their stamina, guidance and direction. I would like to thank Joan Mallett whose open door and sense of humour has always made my time at Carleton more enjoyable. Thank you to Dani Fraser who has provided me with invaluable advice and guidance and to Zoe Panchen whose love for plants and welcoming manner I will miss. I would not have finished without the support of my partner, family and friends, and I would like to thank them for everything they do to encourage me in life. Finally, thank you to the Hibiscus Millennium Project Bursary through Carleton University for providing in-part funding for this research.

Preface

Chapter 2, 3 and 4 are unpublished work intended for future publication, in which the author, R. Young, was the principle investigator, responsible for data collection and analysis, manuscript preparation and revisions. R. Gorelick was the supervisory author on these chapters and was involved in project development and in revision of manuscripts. C. Harris and T. Xing provided preliminary comments to portions of Chapters 1 and 2 respectively.

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1 Chapter: Introduction

Land plants are remarkable. For hundreds of millions of years since diverging from green algae, land plants i.e. embryophytes have successfully moved water to prevent cell desiccation and death outside of aquatic environments. Water transport in embryophytes is facilitated over minute plant bodies in the case of mosses (and some embryophytes, such as *Lemna* L.), or scaled to heights of a hundred meters when moving water vertically from roots to leaves through stems of redwoods (Brown, 2013). The ability to move water, and therefore to tolerate drought, is a fundamental adaptation of mosses, ferns, cone-bearing and flowering plants, which can be traced in their evolution from green algae (Umezawa et al., 2010). What is so important about water? Water is required for basic functioning of cellular life (Lang and Waldegger, 1997), but also is required for the light reactions of photosynthesis conducted by plants. Plants are photoautotrophs that produce their own sugars by harnessing solar energy to split water (H_2O) and fix carbon dioxide (CO_2) to form oxygen (O_2) and sugars (e.g. glucose; $C_6H_{12}O_6$) (Rolland et al., 2006; Ramon et al., 2008). Many sugars on Earth are produced by plants and multicellular life uses sugars to develop carbon skeletons and fuel energy metabolism (Rolland et al., 2006; Ramon et al., 2008; Chen et al., 2015; Li and Sheen, 2016). The ability of plants to fix atmospheric carbon to energy-rich sugars has re-shaped the planet by providing the literal building blocks and energy for life (Ramon et al., 2008).

Xylem are cells that are dead when functional, serving as heavily-lignified (woody) conduits to move groundwater absorbed at roots to photosynthetic tissues, typically terminal organs such as leaves (Brown, 2013; Cookson et al., 2016; Venturas et al.,

2017). Humans expend tremendous energy to pump water to the top of skyscrapers, so one can only wonder at the amount of energy a redwood must exert to move water vertically in xylem up to heights of over a hundred metres. Amazingly, it seems that none of its own energy is needed. Gas exchange at open stomata of photosynthetic tissues releases water vapour, facilitating sap movement within the plant. Water can be considered “pulled” from roots to shoots due to the evaporative power of the sun. The effect of water loss to transpiration is so significant that trees can use anywhere from ten litres to one thousand litres of water a day depending on species, size, habitat and environmental conditions (Brown, 2013; Venturas et al., 2017). The chemistry of water imparts cohesive behaviour to water molecules because of hydrogen bonding from charge polarization (Venturas et al., 2017). Cohesion-tension theory thus describes how solar energy induces negative hydrostatic pressure (tension) in the xylem water column that drives sap ascent via transpiration at stomata transmitted by cohesion of water molecules from leaves to roots (Nardini et al., 2011; Brown, 2013; Sengupta and Majumder, 2014; Venturas et al., 2017).

Sugars produced from photosynthesis of leaves or stomata-laden stems are typically translocated within the plant body by distinct vascular conduits called phloem. Phloem cells are alive, and expend energy to maintain an osmotic gradient of sugars which facilitates sap movement and protects the system from leakage (Hacke and Sperry, 2001). Maintaining a high concentration of sugars in phloem facilitates osmotic influx of water to pressurize sap and direct movement of sugars based on source-sink status of plant tissues (Hacke and Sperry, 2001; Ramon et al., 2008). What is a source and what is a

sink? Sources are sugar-exporting tissues such as photosynthetic organs or sites of storage, and inversely sinks are sugar-importing tissues (Rolland et al., 2006; Ramon et al., 2008). The pressure-flow hypothesis describes how sugars loaded to phloem generate osmotic pressure and flow that subsequently will translocate sugars over long distances of the plant body, directed via sugar unloading and phloem pressure release at sink tissues (Sturm and Tang, 1999; Hacke and Sperry, 2001; Stanfield et al., 2017). Typical sinks include roots which store sugars, and also growing meristems (elongating branch or root tips) and cambia (circumferential growth) that use sugars for carbon and energy metabolism (Rolland et al., 2006). In plants, source-sink relations heavily govern translocation of sugars within the plant body (Stitt et al., 2012). Mobilization of sugar upon delivery to sink tissues may be for energy availability via respiration, long-term carbohydrate storage, freeze avoidance or tolerance, osmotic regulation, even pathogen and wound response, and is typically regulated by hormone cross-talk and carbon status of the plant (Jansen and Schenk, 2015; Plavcova and Jansen, 2015; Cookson et al., 2016; Li and Sheen, 2016).

Interestingly, some temperate broadleaf tree species in North America have sugary xylem sap that flows seasonally in late-winter/early-spring. How trees make sweet edible sap in spring is thought-provoking when considering leaves are not present to support xylem sap ascent, and sugars typically found in phloem are concentrated to xylem. Thus, the objectives of my research are, a multi-disciplinary synthesis, to understand how temperate trees make sweet edible sap in spring. I will first contrast Indigenous and settler knowledge of maple provisions, and then use physiological and anatomical data

from temperate trees, and molecular evidence from *Arabidopsis* Heynh. to elucidate a theory of sap production and movement at a ‘whole tree’ level. I show that sugars are not just byproducts of sap movement, but are signaling molecules that reveal a blurred functional distinction between xylem and phloem. Finally, I show that spring xylem sap sugar concentration correlates with width of xylem rays in temperate trees of three genera with spring sap flow. From these contributions, I make numerous predictions (some of which are testable now and some that should be testable in the near-future) that with corroboration or falsification may improve understanding of the dynamics of sugar and water flux between xylem and phloem of vascular plants.

1.1 Background and consequences

Improvements in knowledge of sap production and movement may lead to future research of sugar and water flux in plants for a variety of practical applications. Furthermore, understanding plant water relations and sugar signaling mechanisms is important for mediating impacts of climate change on plants, and especially crops, which face increased periods of drought exasperated by climate change (Jansen and Schenk, 2015; Venturas et al., 2017). Non-timber forest products in North America, although not considered crops still produce annual yields that support communities and industry (Emery, 2002; Murphy et al., 2012). For example, the making of maple syrup was significant in the development of North American culture and identity and still impacts various regional customs and economies today (Whitney and Upmeyer, 2004). Processing of maple sap to syrup for global export occurs primarily in North America, with Quebec accounting for near 80% of global exports (Whitney and Upmeyer, 2004; Li

and Seeram, 2011a; Zhang et al., 2014). Data from 2002 showed neighbouring Vermont as the next largest supplier, followed by Ontario, Maine, New York, New Hampshire, New Brunswick, Massachusetts, Connecticut, Pennsylvania, Ohio, Michigan and Wisconsin, which accounts for the remaining 20% of global maple syrup exports (Whitney and Upmeyer, 2004). Maple syrup is the more famous of foods and medicines produced from sap of temperate broadleaf trees that flows in late-winter/early-spring. Origins of maple sugaring may lie with squirrels, and North American Indigenous peoples who traditionally collected sap in late-winter from maple (*Acer* L.) species. These traditions may have developed from observational learning and peoples' interrelationship with squirrels and maples (Larsson and Jaciw, 1967; Zaczek et al., 2003; Ball, 2007; Turner and von Aderkas, 2012; Kimmerer, 2013).

1.2 Indigenous knowledge of maples

What is now known as the Ottawa region is unceded traditional territory of the Algonquin peoples (Tomiak Julie, 2016). Maple species are found throughout the region, and only an hour by car from Ottawa is Lanark County, one of the top two regional sites of maple syrup production in the province of Ontario (Murphy et al., 2012). When asking how trees make sweet edible sap in spring, it is important to consider the ethnobotany of tree saps. Indigenous peoples of the northeastern woodlands (for example Anishinaabe and Haudenosaunee peoples) traditionally collect the gift of sweet and healthy sap that maples provide in late-winter. Anishinaabe Indigenous knowledge is situated within a web of relationships that encompass all aspects of creation such as energy, physical landscapes and natural processes, spirits, living beings, and interactions (Geniusz, 2009;

Simpson, 2014), and so Anishinaabeg conduct themselves as stewards of the land which gives to them (Murphy et al., 2012; Kimmerer, 2013; Moody, 2015). A western or European way of knowing often sees humans and their interests to define occupants and “use” of land, which contrasts observation and relational understanding that provides meaning and direction characteristic of Anishinaabe Indigenous knowledge (Simpson, 2014; Moody, 2015).

“Two-eyed seeing” is the bringing together of two ways of knowing or worldviews to motivate a better understanding of world dynamics (Hauser et al., 2009; Bartlett et al., 2012; Forsyth and Giles, 2012; Martin, 2012). For example, values and beliefs of how humans relate to their environment directly impact a culture’s world perception and thus, how ecological knowledge is acquired, communicated and applied (Bussey et al., 2016). Judeo-Christian perceptions that reinforce dominion influence western capitalist societies (Mebratu, 1998; Geisinger, 1999), which often see the natural world and other living beings as a resource to be controlled and commoditized for the sake of producing capital. Western societies frame ecological knowledge through hypothesis testing and empirical analyses that isolate, measure and control natural processes (Bussey et al., 2016) often to inform “reductionist, materialist economic and political agendas” (Kimmerer, 2013: 368). Although western science may approach ecological knowledge differently than traditional ecological knowledge acquisition by Anishinaabe and Haudenosaunee Indigenous peoples, both ways of knowing rely on primary observation (Bussey et al., 2016). Indigenous knowledge is also known as Traditional Ecological Knowledge, TEK (Smith, 2012). Indigenous knowledge may employ a more holistic understanding that

integrates community, relationship and spirituality with other living beings (Simpson, 2014; Moody, 2015). However, both knowledge systems have the power to support and inform modern ecological management practices (Bussey et al., 2016).

1.3 Xylem recovery from embolisms

How trees make sweet edible sap in spring can also be understood through hypothesis testing and empirical analyses, typical of western science. Sugar and water flux that drives sap production and movement is inextricably linked with xylem pressure and refilling of xylem conduits. Most flowering plants (angiosperms) have developed large-diameter xylem conduits called vessels (Hacke and Sperry, 2001; Motomura et al., 2007; Carlquist, 2012). Tracheids are more narrow xylem conduits observed in vascular plants such as cone-bearing plants (gymnosperms). Although vessels may impart improved hydraulic conductance due to increased conduit diameter, vessels are more susceptible to freeze-thaw induced loss of hydraulic conductivity (Sakr et al., 2003; Carlquist, 2012; Charrier et al., 2014). Thus, xylem of temperate broadleaf eudicot trees can be vulnerable to cavitation, i.e. air bubble formation, induced from freeze-thaw events. As water freezes, dissolved gasses come out of solution and expand upon thawing to obstruct xylem conductivity (Sperry, 1993; Hao et al., 2013; Charrier et al., 2014). The same principle can be observed in one's home when making ice cubes – once water has frozen, an array of air bubbles or pockets are visible in newly-formed ice. What is the big problem about a few bubbles in sap? When trees thaw, air bubbles can expand, proliferate and block xylem conduits under increasing negative hydrostatic pressure (Hacke and Sperry, 2001; Hao et al., 2013; Venturas et al., 2017). Embolisms are when xylem

conduits become air-filled and are no longer hydraulically conductive, a state that cannot be reversed in some species (Holbrook and Zwieniecki, 1999; Brodersen and McElrone, 2013; Venturas et al., 2017). Maintenance of xylem hydraulic conductivity is critical to supply water for phloem transport of sugars to sinks and thus, for plant survival (Hölttä et al., 2009; Mencuccini et al., 2015; Hartmann and Trumbore, 2016). A means of restoring xylem hydraulic conductivity or to ‘prime’ vessel embolisms for refilling may be crucial to support cohesion-tension theory (Zwieniecki and Holbrook, 2000; Gouin, 2015; Trifilò et al., 2015; Secchi and Zwieniecki, 2016; Secchi et al., 2017).

1.4 Spring xylem sap sugar content

In late-winter/early-spring, xylem sap of some temperate broadleaf eudicot trees contains an unusually high concentration of sugars (Wiegand, 1906; Ewers et al., 2001; Améglio et al., 2004; Cirelli et al., 2008). Mature photosynthetic leaves are not developed by these trees for some weeks, and so sites of storage may provide the source of sugars loaded to xylem sap. Ray and axial parenchyma are formed at vascular cambia alongside xylem and phloem, are living, and provide sites of storage for sugars and water (Carlquist, 2013; Morris et al., 2016). Rays are ray and axial parenchyma cells that connect phloem to xylem allowing for radial transfer of solutes and water (Pfausch et al., 2015). During winter, rays have high concentrations of soluble sugars that contribute to spring xylem sap concentration (Sauter et al., 1973; Wong et al., 2003; Améglio et al., 2004). In the past, attempts to define the strength of correlation between ray parenchyma storage capacity and spring xylem sap sugar concentration were conducted with sugar maple (*Acer saccharum* Marsh.) (Morselli et al., 1978; Garrett and Dudzik, 1989). However, a

few temperate genera other than *Acer* make sweet edible sap in spring and do so at unique temperature thresholds, and with variable saccharide content (i.e. mono- or disaccharide) and concentration (Wiegand, 1906; Cruziat et al., 2002). Insight of how storage tissue capacity effects spring xylem sap sugar in species of multiple genera may prove relevant for producers of sap and syrup products.

2 Chapter: Decolonizing maple provisions through “two-eyed seeing”; contrasting settler knowledge to Indigenous knowledge of maples

2.1 Abstract

North-eastern North America and the Great Lakes region are dominated by maples (*Acer*) that produce most of the global supply of maple provisions (sap, syrup, sugar).

Anishinaabe and Haudenosaunee peoples traditionally harvested sugary maple sap in late-winter/early-spring. Following European colonization of North America, settler economies adopted Indigenous knowledge of maple sugar and syrup production. Settler knowledge and Indigenous knowledge of maples are acquired through different ways of knowing (world-views). First, I discuss the ethnobotany and ethnoecology of Anishinaabe and Haudenosaunee maple provisions. Second, I contrast settler knowledge with Indigenous knowledge of maple nutrition, time of gifting, and pharmacological and medicinal properties. I describe Indigenous ways of knowing with no counterpart in settler knowledge to emphasize consideration of relationality. Finally, I address how contemporary settler societies can make “two-eyed seeing” fundamental to mainstream activities, thereby encouraging decolonization in that Indigenous communities may reclaim cultural traditions and values.

Hello, my name is Ross Young. I am descended from English and Scottish settlers. I am from White Lake, a settler town in unceded territory of the Algonquins, encompassing the Ottawa and Mattawa River watersheds.

In accordance with Indigenous methodology, it is important to begin with a greeting and share about oneself, so that “knowledge can be situated within a web of relationships” (Ray and Cormier, 2012: 163). Most of my life has been spent in the place now known as the Ottawa region, unceded traditional Anishinaabeg territory. The Algonquin land claim process may soon restore Indigenous rights and title to the land, and serve to reverse settler politics of Indigenous dispossession and disappearance in the region (Tomiak Julie, 2016). As a graduate researcher of settler descent, an occupant of unceded land, and a student attending a post-secondary institute situated on unceded land, it is essential for me to consider Indigenous knowledge alongside western knowledge regarding maple trees.

2.2 Introduction

Late-winter and early-spring harvest of sugary maple (*Acer*) sap is a tradition of Indigenous peoples of northeastern Turtle Island (North America), especially of the Great Lakes region. Turtle Island is how many Indigenous peoples of the northeastern woodlands know North America (Lavallee et al., 2010). The ritual harvest of sap and manufacture of maple provisions (sap beverage, syrup, sugar) was readily adopted by European settlers of Turtle Island (Larsson and Jaciw, 1967; Zaczek et al., 2003; Ball, 2007; Turner and von Aderkas, 2012). The harvest and use of tree sap in Europe,

primarily from maple and birch (*Betula L.*), is common of north and eastern European countries (Svanberg et al., 2012). The shared practice of sap harvest was likely a factor in the high adoption rates of Indigenous traditions by European settlers upon the colonization of Turtle Island. To Indigenous peoples, the harvest of maple sap and production of syrup or sugar was an essential supplement of traditional diet and an important famine food following winter (Turner and von Aderkas, 2012; Gorelick, 2014). More importantly, maple sap was considered a vital medicine. Reducing the water content of collected sap concentrates sucrose to produce maple sugar or syrup, an important economic resource in eastern Canada and the northeastern United States to this day (Stuckel and Low, 1996; Ball, 2007; Turner and von Aderkas, 2012). To address settler appropriation of maple provisions, settlers can try to understand maple provisions not only through settler (western or European) knowledge, but also through an Indigenous way of knowing. “Two-eyed seeing” is emphasized in integrative science education, and brings together different ways of knowing to motivate a better understanding of world dynamics (Hauser et al., 2009; Bartlett et al., 2012; Forsyth and Giles, 2012; Martin, 2012). Thus, I will first discuss the ethnobotany and ethnoecology of Anishinaabe and Haudenosaunee maple provisions. Second, I will contrast settler knowledge with Indigenous knowledge of maples and highlight when ways of knowing are concordant, or when discordant. Third, I will describe Indigenous ways of knowing with no counterpart in settler knowledge to stress how “two-eyed seeing” can promote a settler world-view, where relationality is emphasized.

2.3 Ethnobotany and ethnoecology of Anishinaabe and Haudenosaunee maple provisions

Many Indigenous peoples of Turtle Island harvested maple sap in late-winter/early-spring as tradition to produce maple provisions, notably Anishinaabe and Haudenosaunee peoples of the Great Lakes region (Arnason et al., 1981; Keller, 1989; Kuhnlein and Turner, 1991; Turner and von Aderkas, 2012; Gorelick, 2014). The Ojibwe (Ojibwa, Ojibway, Chippewa), Potawatomi, Odawa (Ottawa) (Nichols and Nyholm, 1995), and Algonquin (Bohaker, 2006) peoples know themselves as the Anishinaabe. The Iroquois Six Nations (Mohawk, Oneida, Onondaga, Cayuga, Seneca and Tuscarora), the Wendat (Wyandot, Huron), the Lunaapeew (Lenape, Delaware) and the Tutela are represented by the Haudenosaunee Confederacy (Haudenosaunee Development Institute, 2017). The significance of maple provisions to Anishinaabe and Haudenosaunee peoples is emphasized in two land treaties signed in 1817 and 1819. Signed with the US federal government, these treaties specifically use language to grant Anishinaabe and Haudenosaunee the continued “right to take maple sugar on ceded land” (Keller, 1989: 126).

A long-standing debate has existed as to whether the manufacture of maple provisions by Indigenous peoples of Turtle Island was a pre-colonial or a European innovation (Vogel, 1987; Munson, 1989). Henshaw (1890: 349) noted the unlikelihood of Indigenous peoples to “give a name to the sugar month unless sugar-making was of respectable antiquity among them”. Munson (1989: 169) clarified “that maple syrup and maple sugar are not synonyms”, and as such, persuasive evidence exists for Indigenous pre-colonial

production of maple syrup. Maple sap could be thickened to syrup through various techniques implemented by Indigenous peoples (discussed in a later paragraph), but maple syrup can be difficult to store and transport and thus was associated with a famine food or dietary supplement (Munson, 1989). From the 17th through 19th century however, Anishinaabe and Haudenosaunee implemented the use of metal kettles over open fires to boil and concentrate maple sap to syrup (Holman and Egan, 1985; Thomas, 2005). Metal kettles were utilized to further thicken syrup to a molasses-like consistency, to be cooled and hardened into transportable sugar cakes (Thomas, 2005). Vogel (1987: 24) specifically noted that “the doubts or denials of [Indigenous pre-colonial production of maple provisions] ... stems from inadequate research into the data, unwarranted rejection of affirmative evidence, or from a tendency to underestimate aboriginal attainments”.

Maple species are prominent in the forested regions of Anishinaabe and Haudenosaunee territory. Of the pre-colonial maple species tapped by Anishinaabe and Haudenosaunee peoples, the sugar maple (*Acer saccharum* Marshall) typically yields sap with the highest sucrose content. The red maple (*Acer rubrum* L.), black maple (*Acer nigrum* F. Michx.), silver maple (*Acer saccharinum* L.), and the Manitoba maple (box-elder maple; *Acer negundo* L.) provide sap of successively lower sugar content. Maples were encountered by Anishinaabeg across their territory, which was significant after expansion westward to Lake Superior from a region initially occupied along the mouth of the St. Lawrence river (Steen-Adams et al., 2010). The territory is dominated by broadleaf tree species typical of both the Deciduous and the Great Lakes - St. Lawrence forest region classifications. Northern territory of Anishinaabe peoples extends to the boreal forest region, which is

dominated by conifers. Broadleaf tree species are typically limited in the boreal to genera such as *Populus* L. and *Betula*. Territory of the Haudenosaunee is located primarily within the Great Lakes - St. Lawrence region that has a mixed composition; deciduous broadleaf tree species are dominant in the south and conifers more typical in northern ranges (Arnason et al., 1981). Documented in the literature is the cultivation of a stand of *Acer negundo* by Iskatewizaagegan (Shoal Lake) Anishinaabe (Turner et al., 2009). At one time, Iskatewizaagegan Anishinaabe would travel and produce sugar in stands of what is now known as southwest Manitoba. Transplanting of maple trees by the community instead established a sugar stand at Shoal Lake that increased maple provisions for gifting and sharing amongst the people, and for trade with settlers (Davidson-Hunt and Berkes, 2003).

Maple provisions were used by Indigenous peoples as an essential supplement of traditional diet and an important medicine for personal health and community well-being (Turner and von Aderkas, 2012; Gorelick, 2014). Indigenous peoples consumed maple sap as a cold beverage or tea, soured it to a vinegar or an intoxicant, or boiled sap to a syrup or sugar for seasoning (Arnason et al., 1981; Emery, 2002; Turner and von Aderkas, 2012; Bi et al., 2016). The collection and boiling of maple sap is labour-intensive, requiring group participation, and encouraging socialization after a long winter when Anishinaabe and Haudenosaunee families typically dispersed in search of game (Holman and Egan, 1985). Amongst the Haudenosaunee of Chemong Lake, Chamberlain (1888) noted that each family had its own sugar stand, with production of maple provisions typically managed by the women. Family or group participation was important

as many tasks were required to make sap into syrup and sugar. Historical accounts noted that Anishinaabe and Haudenosaunee women tapped trees for sap collection and directed work amongst sugar camps (Chamberlain, 1888; Holman and Egan, 1985). Men cut wood to fuel fires, and even children tended to boiling sap. In late-winter/early-spring when the syrup season began, families annually returned to the same sugar stand (Holman and Egan, 1985). In a 2003 interview, elder Rose Martin (Lac Vieux Desert Tribal Council) noted that family sugar camps were moved throughout a stand every few years to allow recently tapped trees to ‘rest’ (Thomas, 2005). Traditionally, production of maple provisions by Indigenous peoples began with making a cut in the tree, followed by insertion of a wood shunt into the cut to direct exuding sap into birch bark baskets for collection. A fire was prepared and birch bark baskets of sap placed over flames, or stones heated, and hot stones dropped into birch bark baskets or hollowed-out logs containing sap. Thermal evaporation of water present in maple sap was achieved as the heat from the flames or stones reduced water content, eventually yielding syrup over time (Vogel, 1987; Munson, 1989; Ray and Cormier, 2012; Kimmerer, 2013).

In the 20th century, Anishinaabe began to utilize flat metal pans to boil sap, a half-century after adoption by settler producers (Thomas, 2005). Flat pans were supported by a stone arch constructed to raise the pan out of the flames and intensify heat over a greater surface area. Anishinaabe boiling arches are apparent in the archaeological record of the early 20th century across Michigan and Wisconsin (Thomas, 2005). The adoption of boiling arches over traditional techniques coincided with the westernization of Indigenous communities and replacement of women by men in the sugar bush (Thomas,

2005). Indigenous peoples and communities are only recently reclaiming the harvest and use of maple sap as part of their traditional practices and culture (Chrétien, 2014).

2.4 Contrasting settler knowledge with Indigenous knowledge of maples

Maple provisions have impacted social and cultural aspects of Indigenous peoples and settlers of Turtle Island (Emery, 2002). All these societies have developed knowledge specific to maples and the late-winter/early-spring harvest of sap, yet Indigenous knowledge (if recognized at all) is appropriated by settlers each year in the context of capitalism (Simpson, 2014). Indigenous knowledge of maples is reduced by many settlers to “a process within a hyper-individualism that negates relationality” (Simpson, 2014: 9). Holistic ways of knowing are often overlooked in settler societies, where knowledge is typically framed in hypothesis testing and empirical analyses that can be isolationist and further economic interests of the state or individual. Settlers can address appropriation of maple provisions through consideration of Indigenous knowledge of maples, alongside settler knowledge, i.e. “two-eyed seeing”. “Two-eyed seeing” brings two ways of knowing together to improve understanding of world dynamics and may encourage decolonization in that Indigenous communities can reclaim cultural traditions and values. I will contrast settler knowledge to Indigenous knowledge of maple provision nutrition, time of gifting, and pharmacological and medicinal properties. Settler academic systems do not provide proper context (for many reasons) for Indigenous knowledge, and Indigenous authors have persevered despite this challenge (Ray and Cormier, 2012; Simpson, 2014; Bopp et al., 2017). Recognizing Indigenous knowledge within the framework of settler knowledge is for settlers (myself included) to address decolonization

in a structure familiar to us. As the Michi Saagiig Nishnaabeg scholar Simpson (2014: 14) noted, “not one time has an Elder ever told me to go to school to learn Indigenous knowledge”.

2.4.1 *Maple provision nutrition*

Settlers recognize maple provisions as a highly nutritious source of calcium, potassium, and a valuable source of carbohydrates (Arnason et al., 1981; Ball, 2007; Zhang et al., 2014). The phytochemistry of maple sap and syrup is well-documented; the primary organic compounds of maple sap are sucrose (98-100% fraction of total organic content, 2-2.5% actual concentration in sap), glucose, phenolic compounds, primary amines, peptides, amino acids, proteins, and other trace organic acids (Ball, 2007). The presence of oxalic, succinic, fumaric, malic, tartaric, citric, and aconitic acids results in a slightly acidic pH of sap (Stuckel and Low, 1996; Ball, 2007). Organic acid concentration is relatively low at the beginning of the sap season at 8 ppm and progressively rises to over 45 ppm, yielding a pH range of sap from 7.9-3.9 (Ball, 2007). The end of the sap flow season is also implicated in the greatest variety of amino acids present in sap including glycine, alanine, asparagine, threonine, leucine, isoleucine, valine, and methionine. Relatively high levels of amino acids, typical of late-season sap, is known to contribute to a “buddy” flavour of syrup (Ball, 2007). Minerals are also present in sap, predominantly calcium (8–56 ppm) and potassium (26–75 ppm) (Stuckel and Low, 1996; Ball, 2007). Maple syrup is achieved as water is evaporated from sap to a 66-67° Brix syrup (66–67% sucrose and 33–34% water) (Stuckel and Low, 1996; Ball, 2007; Li and Seeram, 2011a). The pH of maple syrup is on average 6.7, and can range from 5.64–7.90 (Stuckel and

Low, 1996). For a summary of nutritional information, see Table 2.1. Although maple stand characteristics and syrup preparation methods vary across regions, no significant impact to sugar content, moisture, organic acid or pH of the finished maple syrup has been reported (Stuckel and Low, 1996).

Table 2.1. Nutritional information of maple sap (early versus late collection; asterisk indicates comparable concentrations between early and late sap) and maple syrup (mean values); data summarized from Stuckel and Low (1996) and Ball (2007). Units are ppm, except for pH.

Organic compound	Early-sap	Late-sap	Syrup
NSCs			
- Sucrose	20,000–25,000	*	680,000
- Glucose	0	40	4,300
- Fructose	0	30	3,000
pH	7.9	3.9	6.7
- Malic acid	8	45 ppm	4,700
Minerals			
- Potassium	26–75	*	2026
- Calcium	8 – 56 ppm	*	775
- Magnesium	<10 ppm	*	167
Phenolic compounds	0 – 0.1 ppm	*	Increased variety and concentration; likely process-derived from thermal evaporation

Amino acids

- Glycine, alanine, asparagine, threonine, leucine, isoleucine, valine, methionine	Trace	Increased variety and concentration	Dependent on time of sap collection; increased concentrations lead to an off-flavour of syrup
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Anishinaabe Indigenous knowledge of nutrition is conceptually related to both physical and spiritual well-being, and fundamentally grounded from Indigenous peoples' relationship and experience with the land, or *aki* in Anishinaabemowin (Simpson, 2014; Moody, 2015). *Aki* is the word that describes all aspects of creation such as energy, physical landscapes and natural processes, spirits, living beings, and interactions that form broad ecological networks (Geniusz, 2009; Simpson, 2014). Drawing guidance from *aki* is known as *akinoomaage*, and it is through observations and a relational understanding that Anishinaabeg find direction (Simpson, 2014). Moody (2015) provided an eloquent synthesis of 'Indigenous knowledge' definitions, primarily from an Indigenous perspective, and noted that elders, healers, and hunter/gatherers share Indigenous knowledge in various ways. The sharing of stories, dances, food, medicine, ceremonies etc. are all ways in which Indigenous knowledge is transmitted to present and future generations (Moody, 2015). Meaning is found from observation of interdependent relationships between the land and plants and animals, which is often discordant to settler

data, content and theory techniques that decontextualize knowledge (Simpson, 2014). Indigenous knowledge is a relationship and understanding of the landscape, and of the plants and animals within it; an observation such that “Squirrel taps maple trees” (Kimmerer, 2003, 2013; Gorelick, 2014). Red squirrels selectively harvest sap from maple trees in late-winter/early-spring, the hungry time, when caches of nuts are spent (Kimmerer, 2013). Squirrels feed on sugar after gnawing and scraping bark of shoots, allowing evaporation to concentrate sugar from flowing sap to candied streaks (Heinrich, 1992; Kimmerer, 2013). In an Indigenous way of knowing, each living being was given a gift and responsibility by the Creator, a way to care for other living beings (Kimmerer, 2003). Maple was given the gift of sweet sap, but also the responsibility to share that gift. Sweet sap fed the people and other beings at a hungry time of year (Kimmerer, 2013). The Anishinaabe traditional story of Nanaboozhoo and the maple trees describes how on a visit to his Anishinaabe friends, Nanaboozhoo (or the trickster) discovered them idling under the maple tree and letting sweet syrup drip from branches into their mouths. Nanaboozhoo lamented that the people did not appreciate the sweet syrup that the Creator had given them, and that they would become lazy (Keller, 1989; Ray and Cormier, 2012). As such, he limited the flow of sweet sap to a single season and watered “it down so that the Anishnaabek must engage with the sap in order to reap it’s benefits” (Ray and Cormier, 2012: 165). At face-value, Nanaboozhoo and the maple trees can be viewed as a knowledge transfer story of maple sap sweetness, but Ray and Cormier (2012) illuminated how Indigenous learning is conceptually the same; a dynamic process that requires active and personal engagement with the land to reap understanding.

2.4.2 *Time of gifting*

Settler knowledge attributes sugary sap flow in temperate maples to stem pressure (Wiegand, 1906; Johnson and Tyree, 1992; Cirelli et al., 2008). Trees with stem pressure display sap flow from the cut surface of the trunk when cut down in late-winter, i.e. even when stems are severed from their roots (Wiegand, 1906; Cruiziat et al., 2002). Stem pressure in *Acer* is proportional to sap sucrose concentration (Johnson et al., 1987; Cirelli et al., 2008), and sap flow correlates with twig/stem temperature (Marvin, 1958). For example, sap flow from positive hydrostatic pressure occurs in maples when overnight air temperatures fall below 0°C and daytime air temperatures rise to between 5° and 10°C (Naughton et al., 2006). Settler knowledge describes the fundamental physical model of stem pressure as bi-phasic (freeze-thaw) in which an arrangement of gas-filled fibres and fluid-filled vessels allows for the generation of stem pressure in response to freeze-thaw cycles (Milburn and O'Malley, 1984; Johnson et al., 1987; Tyree, 1995; Cirelli et al., 2008). For a more detailed description of settler knowledge, see Chapter 3.6.

For Anishinaabeg time is cyclical, and many know March (or April depending on community) as *Iskigamizige Giizis* or *Zizibaskwet Giizis*, 'Maple Sugar Moon', and beginning of the new year (Corbin, 2004; Kimmerer, 2013). Indigenous knowledge understands that cold nights of the Maple Sugar moon makes sap run possible, and that cold nights themselves help make syrup (Kimmerer, 2013). Indigenous knowledge and settler knowledge are concordant in this understanding, and that freezing can be used to reduce water content of sap. Ice that forms in baskets, hollowed-out logs or buckets overnight will separate from more sugar-concentrated sap, and can be tossed away in the

morning, leaving a sugary solution for processing (Holman and Egan, 1985; Kimmerer, 2013; Gorelick, 2014). Maple Sugar Moon was a hungry time of year, but maples provided food to the people when they needed it most (Kimmerer, 2013). Community and togetherness was important during Maple Sugar Moon, and Anishinaabeg feasted in advance of maple sugaring to give thanks and ask the Creator for blessing (Keller, 1989; Geniusz, 2009). Haudenosaunee peoples similarly held a Maple ceremony to give thanks for the sweet and healthy sap gifted annually (Chafe, 2007; Kimmerer, 2013).

Anishinaabe and Haudenosaunee peoples are acutely aware of the time of year and the conditions that wakes maples to nourish the people and animals, and to lead the other plant beings out of winter and into spring. Explanation and prediction drives both western and Indigenous sciences. However, a greater emphasis may exist on explanation in Indigenous science in contrast to prediction in western science (Gorelick, 2014). The Anishinaabe traditional story of Nanaboozhoo and the maple trees (Ray and Cormier, 2012) demonstrates this concept, providing *explanation* for observed sugary sap in maple trees, and presenting a story of why, when and what kind of work is necessary for the people to yield syrup from sugary maple sap.

2.4.3 *Pharmacological and medicinal properties*

Settler knowledge has recently reported that phenolic-enriched maple syrup extracts could be beneficial to human health; extracts demonstrate antibacterial, antimutagenic, human-cancer-cell antiproliferative, antioxidant, and anti-inflammatory properties (Li and Seeram, 2010, 2011a; b; González-Sarrías et al., 2013; Bi et al., 2016; Cardinal et al., 2016). Antimutagenic activity of maple syrup phenolic extract metabolites was

demonstrated in experiments with *Salmonella typhimurium* following mutagenesis induced by potassium dichromate (Thériault et al., 2006; Bi et al., 2016). Mutations caused by potassium dichromate (a strong oxidant) can escape the repair mechanism of UV damage in bacteria and contribute to the SOS response, which is an error-prone repair mechanism that subsequently is associated with mutagenesis (Thériault et al., 2006). Treatment with metabolites of phenolic-enriched maple syrup extract inhibited the SOS response. Phenolic compounds in plants are known to prevent mutation from UV radiation damage, however antimutagenic bio-availability in other organisms may be restricted by metabolic activity (Thériault et al., 2006). Ginnalins A–C (gallotannins) are bioactive phenolic compounds isolated through enriched polyphenol extracts of sugar and red maple sap. In two cancer cell lines (colon and breast), ginnalins A–C inhibited cell growth compared to normal colon cell line controls. Ginnalins A–C inhibited S- and G2/M-cell cycle arrest, mediated by decreased levels of cyclins A and D1. At highest experimental concentrations, apoptosis was absent in cancerous cell lines and no cytotoxic effects observed, indicating the potential chemopreventative effects rather than chemotherapeutic effects of ginnalins A-C (González-Sarrías et al., 2013). Antioxidant potential of maple syrup butanol extract (BuOH) and phenolic isolates were evaluated in the diphenylpicrylhydrazyl (DPPH) radical scavenging assay against two controls: vitamin C, and the commercial, synthetic antioxidant BHT. Pure isolates from BuOH displayed superior antioxidant activity to BHT, and three phenolic isolates to that of vitamin C (Li and Seeram, 2010). Maple syrup ethanol acetate extract (EtOAc) displayed superior antioxidant activity to BHT, and several pure isolates not previously reported in BuOH extracts had superior activity to vitamin C (Li and Seeram, 2011a). Antioxidant

activity of phenolic derivatives and coumarins was greater when compared against stilbene molecules and lignans following evaluation of phenolic sub-classes identified in BuOH and EtOAc extracts (Li and Seeram, 2010, 2011a). Oxygen radical absorbance capacity (ORAC) values of maple syrup were less than blueberry juice ($24 \pm 1 \mu\text{mol}$ of TE/mL), but comparable to that of strawberry or orange juice (Legault et al., 2010). Although antioxidant activity is well-identified as a pharmacological effect of maple syrup extracts, human studies or animal models are lacking to provide direct evidence for benefits to human health.

In contrast to native or naturally occurring compounds, settler knowledge has identified other process-derived phenolic compounds present in maple syrup extracts from boiling/thermal evaporation (Ball, 2007; Li and Seeram, 2010), i.e. present regardless of whether traditional or modern techniques are employed. Quebecol is one such process-derived phenolic compound, not originally present in maple sap, and lacking a feasible biosynthetic pathway (Li and Seeram, 2011b). Anti-inflammatory properties have recently been attributed to quebecol in bioassays evaluating the inflammatory response of human macrophages exposed to bacterial lipopolysaccharides (LPS) (Cardinal et al., 2016). LPS typically lead to up-regulation of gene expression and secretion of pro-inflammatory cytokines in macrophages through activation of nuclear factor-kappaB (NF- κ B). Quebecol demonstrated an anti-inflammatory effect by reducing LPS-induced NF- κ B activation, and inhibited the secretion of two pro-inflammatory cytokines. Isolates of the north substructure of the quebecol molecule inhibited secretion of all four pro-inflammatory cytokines evaluated by Cardinal et al. (2016). Furthermore, LPS induce

overproduction of nitric oxide, a free radical, that leads to tissue inflammation. Maple syrup EtOAc extract inhibited LPS-induced nitric oxide overproduction; Legault et al. (2010) found that the period of sap harvest had no significant impact on antioxidant activity, but the greatest nitric oxide inhibitory activity occurred at the end of the syrup season in darker grade syrups in contrast to light syrups (Legault et al., 2010). At the end of the season, increased bioactivity in the tree and the sap bacterial community (Morselli and Whalen, 1991; Lagacé et al., 2004) may increase the abundance of oxidized compounds responsible for the nitric oxide inhibitory activity (Legault et al., 2010). Interestingly, antimicrobial activity and a synergistic interaction with selected antibiotics (fluoroquinolones) against Gram-negative bacterial strains has been reported in phenolic-rich maple syrup extract (Maisuria et al., 2015; Bi et al., 2016). Animal safety studies have been conducted to determine the toxicity of maple syrup extracts with no change in body mass, food consumption, hematology, coagulation, clinical chemistry, urinalysis, or organ mass reported. Maple syrup extract samples (prepared in FDA food-grade approved resin, Amberlite XAD-16, and food-grade solvents (water and denatured ethanol), and eluted from the resin with denatured ethanol) were administered once daily for seven consecutive days to rats via oral gavage and on day eight, following euthanasia, animals were subjected to a gross necropsy examination. Oral administration of maple syrup extract samples at doses of up to 1000 mg/kg/day, for seven consecutive days, did not result in signs of overt toxicity (Zhang et al., 2014).

Indigenous peoples have long considered maple sap and maple provisions to have medicinal qualities and uses. Still, Indigenous knowledge is discordant from settler

knowledge in how health benefits of maple provisions are directly attributed. Maple provisions have a dual function in Indigenous society. Maple provisions are not just food and medicine in a physical sense, but also provide spiritual “healing that accompanies the process of making it, using it, and sharing it” (Chrétien et al., 2014). Maple provisions are recognized as a medicine for overall well-being and health (physical and spiritual) (Keller, 1989; Chrétien et al., 2014; Moody, 2015). Anishinaabe traditional medicine centers on seven tenets of a purposeful life: an active lifestyle, healthy food, cultural continuity, spirituality, a connection to the land, and community and family relationships (Simon Brascoupe, pers. comm. 2017). The collection of maple sap provides purpose after winter, keeps the people active, and encourages community engagement allowing for cultural transmission of harvest and preparation techniques. Consumption of maple provisions provides nutritional value, and is a direct connection to the land and the living beings (maple trees) that nourish the people after a long winter (Kimmerer, 2013). The harvest of maple sap, and the manufacture and consumption of maple provisions are considered medicinal (physical and spiritual healing) from an Indigenous perspective.

2.5 Indigenous ways of knowing with no counterpart in settler knowledge

In Anishinaabemowin, the name given to sugar maple is *a'nina'tig* or *anenemik*, the man tree, and Haudenosaunee peoples know maple as the leader of the trees (Geniusz, 2009; Kimmerer, 2013). It is recognized that the peoples living in the Great Lakes region “are in the embrace of the Maple Nation”, i.e. maples are acknowledged as the dominant denizens of the region (Kimmerer, 2013: 168). This Indigenous way of knowing has no counterpart in settler knowledge. Relationality with the land and other beings is often

detached in a western or European way of knowing, i.e. humans and their interests define the occupants of the land, or humans are often seen as to operate outside the “natural” world. Consider that settler knowledge first accounts for Indigenous production of maple provisions in the 17th century, but it was not until European interest in sugar developed in the 18th century that Indigenous knowledge/relationships with maples were commoditized (Whitney and Upmeyer, 2004). Again when western or European interests changed upon commoditization of sugar cane, maples were relegated from rich, well-drained lands where they preferentially-occurred to exposures too steep for cultivation in favor of commercial crop production (Whitney and Upmeyer, 2004). Settler farm families accounted for significant production of maple provisions during this time (Whitney and Upmeyer, 2004), and over generations often developed notions near to Indigenous knowledge of maples. As Corbin (2004: 57) writes, “each spring, the [maple] trees came back to life and ran sap that was sweet and clear, sap that could be turned through the alchemy of ice and fire into honey. Each spring for generations, the [settler] family took some of this and savored it, until finally the winters turned warm and the sap stopped flowing and the [settler] family, like the trees, disappeared.” What settler knowledge and Indigenous knowledge does share is the concern that with changes to land-use, maples will be lost from a region where they have created entire ecosystems and complex relationships with the beings around them (Kimmerer, 2013). Settlers and Indigenous peoples have always had close association with maples. Seeds of sugar maple, although wind dispersed, fall a short distance and their dispersal can be attributed in part to selectivity by humans for sugaring that alters regional forest composition (Bouchard and Domon, 1997; Whitney and Upmeyer, 2004). With “two-eyed seeing”, Kimmerer (2013)

relays how “we are what we eat, and with every golden spoonful maple carbon becomes human carbon... traditional thinking [Indigenous knowledge] had it right: maples are people, people are maples.” Indigenous knowledge encourages thought of other beings/species, and encourages care for their well-being as we would our own. To consider our actions and relationship with the species/beings around us allows us to respect and preserve the land and the life it supports for future generations.

2.6 Concluding remarks

Settler knowledge and Indigenous knowledge of maples can be concordant. Each way of knowing however, uses very different language to communicate observational findings, i.e. data or theory vs. relational transmission of knowledge. The sharing of stories, dances, food, medicine, and ceremonies that define Indigenous knowledge is fundamentally relational not only in how observations of the natural world are recorded, but also in the way knowledge is transmitted to present and future generations. A western or European way of knowing is often subject to hyper-individualism or -speciesism that innately overlooks fundamentals of Indigenous knowledge. Instead, “two-eyed seeing” can be used to consider relationality to the land, and the plants and animals within it. The limited ethnopharmacological data in western literature regarding maple provisions exemplifies the general settler understanding of maple provisions as food. In settler societies, food is typically considered a product or vector to nourishment with little consideration to the process of producing food as a health benefit. To understand medicine through an Indigenous way of knowing is to reconsider food and medicine as a dynamic process. One must consider *aki*, the landscape and natural processes that

nourished plants and animals incorporated into food, techniques used by the people to prepare food, and to understand this interrelationship as necessities of physical and spiritual nourishment provided through connection to other living beings.

Many barriers exist for the lack of reciprocity between settler knowledge and Indigenous knowledge. Canadian post-secondary institutes and business organizations that implement programs to address needs of Indigenous students or communities typically do so as a special project, initiative or department that often operates and is viewed as outside mainstream activities (Bopp et al., 2017). Compartmentalizing Indigenous knowledge is paradoxical to core Indigenous ways of knowing where understanding is derived from relationship and interconnection. Compartmentalization of Indigenous peoples and Indigenous knowledge by settler societies is not new. To break from western tradition, contemporary settler societies can make “two-eyed seeing” fundamental to mainstream activities. For example, rectifying and reconciling the appropriation of Indigenous knowledge of maples by settler societies could begin through inclusion of Indigenous groups and their knowledge alongside settler knowledge of maples (i.e. within curriculum material, at settler sugaring operations open to the public, etc.). Fostering mentorship and collaboration between Indigenous peoples and settlers may not only encourage reconciliation (following The Truth and Reconciliation Commission of Canada’s 94 “Calls to Action” in 2015 (Bopp et al., 2017)), but encourage decolonization insofar as Indigenous communities may reclaim cultural traditions and values. Current sites of settler maple syrup “production” may be transformed to regions where stewards

(through Indigenous and settler collaboration) gather the gift of maples for benefit of communities, global or regional.

3 Chapter: How does xylem recover from embolisms? A review of seasonal sugar-water flux between xylem and phloem of temperate broadleaf eudicot trees

3.1 Abstract

Cavitation (bubble formation) in xylem of temperate broadleaf eudicot trees limits hydraulic conductivity and can lead to embolisms, where hydraulic conductivity is zero. The ‘whole tree’ is threatened by desiccation when xylem embolisms proliferate, thus ‘whole tree’ mobilization of resources to recover xylem from embolisms is not surprising. Xylem pressure and vessel-loading of soluble non-structural carbohydrates are inextricably linked and restore hydraulic conductivity of xylem conduits following freeze- or drought-induced embolisms. I hypothesize that what works in spring (refilling air-filled vessels), also works in summer (dissolving tiny air bubbles), i.e. sugars will refill xylem across scales and seasons. In the leafless period, I hypothesize that trees have stem pressure and not just root pressure if pits do not occur (or pits are blind) between fibres and vessels. Finally, I hypothesize that xylem refilling and spring xylem movement of sugars to shoot apical meristems is mediated by long-distance sugar signaling via source-sink gradients, revealing a blurred functional distinction between xylem and phloem.

3.2 Introduction

When photosynthetic, xylem hydraulic conductivity of temperate broadleaved eudicot trees is generally restricted to negative hydrostatic pressure (tension) that requires the presence of leaves or stomata-laden photosynthetic stems per cohesion-tension theory. Sap flow from roots to shoots is driven from water lost to evaporation at stomata via capillary force generated by mesophyll cell walls (Venturas et al., 2017). Under increasing evaporative tension, or when tissue is damaged by herbivory or natural events – such as fire, storms and leaf or root senescence – air bubbles in xylem sap can proliferate, expand and obstruct xylem hydraulic conductivity leading to a state of embolism (Sperry et al., 1988; Hacke and Sperry, 2001; Brodersen and McElrone, 2013; Venturas et al., 2017). Xylem embolisms during the photosynthetic season may be common, even under conditions of low water stress (Holbrook and Zwieniecki, 1999; Secchi and Zwieniecki, 2010; Trifilò et al., 2015). Consequently, a means of restoring xylem hydraulic conductivity or to ‘prime’ xylem embolisms for refilling may be crucial to support cohesion-tension theory (Zwieniecki and Holbrook, 2000; Gouin, 2015; Trifilò et al., 2015; Secchi et al., 2017). Debate exists regarding frequency of embolisms and the importance of xylem refilling (Hartmann and Trumbore, 2016), but as Vogel (2012: 133) noted, “trees clearly can re-establish broken water columns each spring, but how they do it isn’t entirely clear”. Xylem hydraulics of temperate broadleaf eudicot trees is lacking a holistic or ‘whole tree’ level understanding (Secchi et al., 2017; Tixier et al., 2017; Venturas et al., 2017). The ‘whole tree’ is threatened by desiccation when xylem embolisms proliferate, and so ‘whole tree’ mobilization of resources (water and sugars)

may serve as the response, i.e. sugars and source-sink regulation recovers xylem from embolism.

Sap ascent in xylem conduits, as described by cohesion-tension, is subject to transpiration and water loss at stomata, however conduits require ‘priming’ (i.e. are ‘born’ wet) typically via growth that increases water content as sugars concentrate at sink tissues (Brown, 2013). I will show that xylem and phloem, acting as a unified network of sugar and water flux, provides a mechanism for sensing embolisms and for xylem recovery from embolisms when trees are leafless or when photosynthetic. I hypothesize that pits impact seasonal bulk xylem pressure in that trees will have stem pressure if pits do not occur between fibres and vessels instead of just root pressure only. Finally, I hypothesize that spring xylem movement of sugars to shoot apical meristems is mediated by long-distance sugar signaling via source-sink gradients. I highlight each hypothesis and provide takeaway points as direction for future research to corroborate or falsify my hypotheses. I provide support through review of sugar and water flux in temperate broadleaf eudicot trees in distinct seasons.

3.3 Hypothesis 1: What works in spring (refilling air-filled vessels), also works in summer (dissolving tiny air bubbles), i.e. sugars will refill xylem across scales and seasons.

Evidence is found by examining common elements of xylem refilling when leafless or when leaves are present and have acquired photosynthetic independence. For example, when leaves are present: starch degradation in ray and axial parenchyma, and loading of

soluble sucrose to vessels initiates local refilling/‘priming’ and restoration of xylem hydraulic conductivity (Nardini et al., 2011; Secchi and Zwieniecki, 2011, 2016). Plasma-membrane aquaporin mobilization (Secchi and Zwieniecki, 2010, 2011, 2016) and water recirculation via phloem Münch water coincides with refilling events (Hölttä et al., 2006, 2009; Mencuccini et al., 2015). Unloading of sugars from vessel sap and deposition of starch in ray and axial parenchyma occurs following restoration of xylem hydraulic conductivity (Secchi and Zwieniecki, 2011, 2016). And finally, subsequent to transport of sucrose across vessel-associated-ray-parenchyma-cell membranes, low pH of vessel cell walls develop and is associated with cleavage of sucrose to monosaccharides (Hacke and Laur, 2016; Secchi and Zwieniecki, 2016).

Now consider that loading of soluble sugars to vessels following dormancy-release and when trees are still leafless generates xylem pressure and restores xylem hydraulic conductivity (Sauter et al., 1973; Johnson et al., 1987; Tyree, 1995; Cirelli et al., 2008). Plasma membrane aquaporin mobilization (Sakr et al., 2003) and water recirculation via phloem Münch water (Tixier et al., 2017) aids refilling. Following restoration of xylem hydraulic conductivity, NSCs are unloaded from vessel sap and starch deposition occurs in ray and axial parenchyma (Sauter, 1981, 1982, 1983, 1988, Améglio et al., 2000, 2001, 2004; Decourteix et al., 2006). Subsequent to vessel-loading of sucrose, sucrose cleavage to hexoses such as maltose and glucose is known to occur in vessel sap via acid invertase as a response to low pH (Sauter, 1983, 1988; Sauter and Ambrosius, 1986). Thus, I hypothesize that vessel-regulation (loading and unloading) of soluble sugars is how temperate, broadleaf eudicot trees restore xylem hydraulic conductivity when leafless or

when leaves are present and have acquired photosynthetic independence. To my knowledge, nobody has previously linked the mechanism of xylem refilling (recovery from embolism) in the photosynthetic and leafless periods and across scales and seasons, i.e. refilling of entire conduits in spring, refilling of cavitation events in summer. There is no single theory of xylem recovery from embolism in vascular plants, and it is likely that gymnosperms (specifically conifers) have distinct refilling strategies (Secchi et al., 2017) hence my hypothesis applies solely to temperate broadleaf eudicot trees.

3.4 Support for Hypothesis 1

Below, I will provide evidence from the literature in support of Hypothesis 1. I describe patterns of starch regulation, and sugar and water flux between xylem and phloem beginning in mid- to late-summer. I review autumn starch synthesis, winter freeze avoidance and finally xylem recovery from embolisms in late-winter to early-spring.

3.4.1 *Mid- to late-summer: NSC allocation to carbon sinks including xylem embolisms*

Chloroplasts of leaves or stomata-laden photosynthetic stems use sunlight to convert carbon dioxide and water to sugars and oxygen (Ramon et al., 2008). Photoassimilates are mobilized for transient starch production in chloroplasts, or for export to the cytosol as triose-phosphates that in a series of reactions are converted to hexose-phosphates for carbon and energy metabolism (Ramon et al., 2008; Stitt et al., 2012; Hartmann and Trumbore, 2016). *Arabidopsis* deposits 30-50% of photoassimilates as transient starch during the day to be remobilized the subsequent night (Ramon et al., 2008; Stitt et al.,

2012). Transient starch deposition is directly linked to the Calvin-Benson cycle via three enzymatic steps (Stitt et al., 2012). In the final reaction, glucose-1-phosphate is phosphorylated by ADP-glucose pyrophosphorylase to produce ADP-glucose which is the glycosyl donor for synthesis of amylose or amylopectin, the components of starch (Stitt et al., 2012; Hartmann and Trumbore, 2016). Starch, whether linear and helical (amylose) or branched (amylopectin), is osmotically inactive, allowing plants to accumulate large quantities in storage tissues without disrupting regular cell functions (Hartmann and Trumbore, 2016). Starch is a polymer of glucose, but maples were once thought to convert starch directly to soluble sucrose or cellobiose via “sugrogen-amylose” and “cellbiose-amylose” respectively (Haq and Adams, 1961). The presence of these enzymes in maple sap has since been refuted with identification of cellular starch degradation to maltose and then sucrose (Haq and Adams, 1961; Wolfgang et al., 1994; Secchi and Zwieniecki, 2016). Maltose is first catalyzed by disproportionating enzymes to produce hexose-phosphates involved in sucrose synthesis (Stitt et al., 2012). Starch is critical for normal growth, and sugars produced from network reactions involved in starch degradation drive signaling that coordinates responses to environmental changes (Ramon et al., 2008; Stitt et al., 2012; Hartmann and Trumbore, 2016).

Degradation of starch remobilizes soluble sugars in source tissues (photosynthetic leaves or stems, or sites of storage such as ray and axial parenchyma) via cytosolic accumulation of hexose-phosphates and sucrose (Ramon et al., 2008; Stitt et al., 2012). Synthesis of sucrose specifically occurs for export to recipient carbon sinks (Rolland et al., 2006; Ramon et al., 2008; Stitt et al., 2012; Li and Sheen, 2016), such as apical meristems,

cambia, or sites of storage. Delivery of sucrose or glucose is generally accomplished via the phloem pathway (Stitt et al., 2012; Hartmann and Trumbore, 2016). Phloem transport is driven as sugars are actively loaded against, or passively loaded along, a sucrose gradient between mesophyll and phloem companion cells (Hartmann and Trumbore, 2016). Water moves from xylem and surrounding cells to generate positive hydrostatic pressure due to low osmotic and water potential of sugar-concentrated phloem. Positive hydrostatic pressure moves sugars to sink tissues where unloading of soluble sugars relieves pressure (Hartmann and Trumbore, 2016). Thus, maintenance of xylem hydraulic conductivity is critical to supply water for phloem transport of sugars to sinks (Hölttä et al., 2009; Mencuccini et al., 2015; Hartmann and Trumbore, 2016).

Phloem transport capacity in the photosynthetic period is limited specifically by drought-induced xylem embolisms. Although freeze-induced xylem embolisms can occur during either the leafless or photosynthetic periods, drought-induced embolisms only occur in the photosynthetic period. Hydrostatic pressure of sap greater than atmospheric pressure, known as xylem pressure, can recover xylem from embolism and restore xylem hydraulic conductivity that supports phloem transport (Holbrook and Zwieniecki, 1999; Nardini et al., 2011; Brodersen and McElrone, 2013). In the photosynthetic period, ‘novel refilling’ or ‘priming’ of embolized xylem conduits occurs against negative bulk xylem pressure, and is hypothesized to be accomplished by a localized osmotic force, such as the accumulation of soluble sugars to embolized vessels (Sperry et al., 1988; Hacke and Sperry, 2001; Evert and Eichhorn, 2006; Venturas et al., 2017). Lignification of vessel walls, composition of inter-vessel pit membranes, and a bordered (by cell wall lignin

deposition) pit structure may allow for the hydraulic compartmentalization required for local refilling of vessels via concentration of soluble non-structural carbohydrates (Holbrook and Zwieniecki, 1999; Zwieniecki and Holbrook, 2000). Non-structural carbohydrates (NSCs) are the products of photosynthesis, including mono-, di-, oligo-, and poly-saccharides, and can be stored in various plant tissues including secondary xylem (Plavcova and Jansen, 2015; Plavcova et al., 2016). Secondary xylem of temperate broadleaf eudicot trees includes xylem conduits (tracheids and vessels), libriform fibres, ray parenchyma (and vessel-associated-ray-parenchyma cells) and axial parenchyma (Evert and Eichhorn, 2006). Ray and axial parenchyma (RAP) are derived from fusiform and ray cambial initials, are alive at maturity and have been implicated in storage of NSCs (Plavcova and Jansen, 2015; Morris et al., 2016; Plavcova et al., 2016). Loading of soluble NSCs stored in RAP to vessel sap by vessel-associated-ray-parenchyma cells (VACs) increases osmolarity, generating low osmotic potential and water potential of vessels, thereby drawing water from surrounding hydraulically conductive xylem conduits (under tension) to establish localized xylem pressure in embolized conduits. Disjointed pressure theory can explain possible phase equilibria in the stem during contact between liquid water at negative pressure in hydraulically conductive conduits and a liquid thin-film at positive pressure in embolized conduits (Gouin, 2015). Availability of water in response to localized osmotic forces could be a function of distribution of xylem conduits with varying diameters, thereby providing a local water reservoir via smaller conduits that are less susceptible to cavitation and are therefore more likely to remain functional (Brodersen and McElrone, 2013). Angiosperm trees are prone to embolisms because their vessels have large diameters in comparison to small

diameter tracheids of gymnosperms (Hacke and Sperry, 2001). Increased xylem conduit size leads to a higher concentration of dissolved gas in vessel sap that can form large diameter air bubbles under drought-stress (Hacke and Sperry, 2001; Hao et al., 2013). In the photosynthetic period, refilling against negative bulk xylem pressure has been observed in both angiosperms and gymnosperms (Hacke and Sperry, 2001; Nardini et al., 2011; Hacke and Laur, 2016). Establishing localized xylem pressure results in the dissolution and expulsion of air bubbles to restore xylem hydraulic conductivity of an embolized vessel. Xylem pressure in embolized conduits may be facilitated when the majority of water in adjacent xylem is under tension, or will soon be under tension (Sperry et al., 1988; Yang and Tyree, 1992; Zwieniecki and Holbrook, 2000; Nardini et al., 2011; Secchi et al., 2017).

Drought-induced embolisms can develop as cavitation events occur via (a) bubble nucleation within hydraulically conductive conduits or (b) through air seeding where water vaporizes by cavitation and proliferates to adjacent conduits under increasing tension (Ponomarenko et al., 2014). In hydraulically conductive vessels, xylem sap (per cohesion-tension theory) is under tension and in a metastable state (Ponomarenko et al., 2014; Hacke and Laur, 2016). Embolisms can be detected from ultrasonic acoustic emissions that are emitted from a sudden relaxation of tension when bubbles nucleate in xylem conduits (Ponomarenko et al., 2014). Secchi and Zwieniecki (2011) identified a feedback mechanism of soluble NSC allocation to vessel sap that implicates embolisms as a common event in the photosynthetic period, with refilling as the physiological response. In California poplar (*Populus trichocarpa* Torr. & Gray), up-regulation of a

gene involved in sucrose transport, *PtSUC2.1*, and of genes involved in starch degradation was observed in response to artificially induced embolisms and to sucrose perfusion treatments. In both experimental treatments, high sucrose concentration of vessel lumens developed and signaled gene up-regulation. In the embolism treatment, a return to a hydraulically functional state diluted sucrose of vessel sap and initiated a feedback mechanism that down-regulated gene expression and further lowered vessel sap sucrose concentration (Secchi and Zwieniecki, 2011). Mechanical stimuli such as the vibration of vessel walls (Salleo et al., 2008) or loss of turgor pressure in VACs have been suggested to trigger NSC accumulation to vessel sap during refilling events (Nardini et al., 2011). However, Secchi and Zwieniecki (2011) speculated that vessel sucrose concentration itself regulated the refilling response i.e. sucrose is a signaling molecule (Rolland et al., 2006). During the photosynthetic period, the source of vessel-loaded sucrose may be NSC allocation by phloem to xylem conduits (Secchi and Zwieniecki, 2011). A continual ‘leak’ of trace sucrose levels from phloem via symplastic transport in RAP to xylem conduits is inferred from low levels of carbohydrates detected in xylem sap throughout the photosynthetic period (Johnson et al., 1987; Secchi and Zwieniecki, 2011). The signal for refilling may occur when sucrose concentration increases on the lumen-side of vessel cell walls in the absence of vessel sap (Nardini et al., 2011; Secchi and Zwieniecki, 2011). Drought and cavitation can remove a large volume of water from vessels, which may result in accumulation of sucrose along vessel walls and trigger signal pathways related to refilling (Secchi and Zwieniecki, 2011). Starch degradation in local RAP is signaled in response to embolized conduits [signaling mechanisms are discussed in ‘Support for Hypothesis 3’, but probably involve sucrose/*T6P/SnRK1* signaling

system], allowing RAP cells to become sinks for NSC allocation by phloem. In response, unloading of both sugars and water via bulk flow from phloem is speculated to be directed first to RAP, then to VACs, and finally via pit membrane pores to the refilling conduits (Nardini et al., 2011). Phloem unloading of NSCs to vessel sap relies on the ‘pit membrane osmosis hypothesis’, where solutes of vessel sap may osmotically regulate VACs via pit membrane pores (Hacke and Sperry, 2003). Accumulation of solutes as a thin sap on the inner vessel pit membrane would lower vessel osmotic potential (Hacke and Sperry, 2003; Nardini et al., 2011). During refilling experiments, concentrations of inorganic solutes were shown to be too dilute in stems of laurel (*Laurus nobilis* L.) (Tyree et al., 1999), implicating soluble NSCs from phloem-unloading in generating osmotic pressure (Nardini et al., 2011). The ‘hydraulic priming’ that results from accumulation of soluble NSCs may allow for rapid refilling or recovery from embolisms when drought stress is temporarily relieved (Secchi and Zwieniecki, 2016).

Water transport processes, in association with NSC transport, are evident in xylem from VAC-localized gene up-regulation of *PIP1* aquaporin proteins (Sakr et al., 2003; Secchi and Zwieniecki, 2010, 2011). In conducting phloem, changes in localization of *PIP1* proteins have been associated with osmotic fluctuations and water flow into or out of the cell (Stanfield et al., 2017). Stanfield et al. (2017) thus speculated that in phloem sieve elements, *PIP1s* support *PIP2* aquaporin proteins as regulators of membrane permeability and hydrostatic pressure. *PIP1s* may similarly support membrane permeability and water flow to xylem vessels when refilling against negative bulk xylem pressure, as transcriptional profiles of *Populus trichocarpa* indicate over-expression under water-

stress (Secchi et al., 2017). The phloem-unloading/‘pit membrane osmosis hypothesis’ as a mechanism for refilling against negative bulk xylem pressure emphasizes aquaporin localization to VACs and availability of water from sites of storage (Nardini et al., 2011). Re-circulated water between xylem and phloem (Münch water) thus replenishes the water reservoir in RAP (Hölttä et al., 2006, 2009; Mencuccini et al., 2015; Pfautsch et al., 2015) required for local xylem refilling (Nardini et al., 2011). The secretion of water into vessels may depend on solute-water co-transport, a process similarly identified in mammalian epithelia, which could extend to localized refilling against negative bulk xylem pressure (Wegner, 2013).

3.4.2 *Autumn: NSC storage via starch deposition*

Cooling temperatures and shorter days of autumn initiate starch deposition in amyloplasts of RAP (Wong et al., 2003). As winter nears, freeze-thaw cycles can induce the formation of air bubbles in xylem vessels leading to embolisms. In species with stem pressure, vessels are typically fluid-filled over winter and air bubbles formed in freezing events can persist until above-zero temperatures initiate bubble proliferation and embolisms. In contrast, species with only root pressure seemingly have gas-filled vessels over winter months that completely refill in spring via root pressure. Spring conditions are often unpredictable and freeze-thaw events can occur, producing air bubbles in recently refilled conduits, i.e. creates xylem embolisms. Xylem pressure and starch deposition in autumn (Améglio et al., 2001, 2002, 2004; Ewers et al., 2001; Hao et al., 2013) occurs presumably as a response to environmental conditions, regulated by source-sink gradients that occur like those in spring. I will discuss these gradients in the late-

winter to early-spring phase, as they may be more pertinent as a physiological response to environmental conditions following dormancy release.

3.4.3 *Early- to mid-winter: Freeze avoidance or tolerance via soluble NSCs*

Most temperate broadleaf eudicot trees, including sugar maple, display deep supercooling of RAP cells as a means of freeze avoidance (Sakai and Larcher, 1987; Byard et al., 2010). Intracellular ice formation in xylem is prevented by extending the temperature at which water transitions from a liquid to a solid phase (Kasuga et al., 2007). Freeze avoidance can be achieved to the homogenous nucleation temperature of water (-40°C), and still further depression of the freezing point can be reached depending on solute concentration (Kasuga et al., 2007; Byard et al., 2010; Gorelick et al., 2015). Freeze avoidance is maintained through high soluble NSC concentration of RAP acting as an osmoregulating substance and lowering the freezing point of xylem tissue (Wong et al., 2003). A high concentration of soluble sucrose in RAP depresses the freezing point and nucleation temperature of intracellular fluid up to threefold, permitting deep supercooling (Kasuga et al., 2007). In Japanese white birch (*Betula platyphylla* Sukat. var. *japonica* Hara), freeze avoidance is positively correlated with intracellular accumulation of soluble NSCs in RAP. Much higher concentrations of soluble NSCs, specifically sucrose and/or its galactosides, were found in RAP (Kasuga et al., 2007).

Freeze tolerance vis-à-vis the extracellular formation of ice prevents intracellular freezing and subsequent cell membrane rupture. Ice crystallization is preferentially initiated in extracellular spaces in response to high solute concentrations depressing the freezing

point of RAP cells (Sakai and Larcher, 1987; Gorelick et al., 2015). Intercellular spaces are largely absent in ray parenchyma of many temperate broadleaf eudicot trees, hindering the ability for freeze tolerance (Wiegand, 1906; Milburn and O'Malley, 1984; Johnson et al., 1987; Sakai and Larcher, 1987). Only some species of *Betula*, *Populus* L. and *Salix* L. demonstrate xylem freeze tolerance and extracellular freezing of ray parenchyma, presumably due to thin cell walls permitting freeze dehydration (Sakai and Larcher, 1987).

3.4.4 Late-winter to early-spring: Recovery from embolisms via seasonal bulk xylem pressure

By late-winter, freeze-thaw cycles can be common and easily induce the formation of air bubbles in xylem vessels of temperate broadleaf eudicot trees. As freezing occurs, dissolved gasses are forced out of vessel sap and form bubbles that expand in those vessels and obstruct hydraulic conductivity upon thawing (Sperry et al., 1988; Hacke and Sperry, 2001; Brodersen and McElrone, 2013). Re-establishment of xylem hydraulic conductivity following freeze-thaw induced embolisms can be accomplished with xylem pressure, dependent on a solute reservoir of ions or soluble NSCs (Furukawa et al., 2011; Carlquist, 2012). Following dormancy release, temperate trees secrete ions and sugars into the sap of vessels in their roots from surrounding parenchyma cells, generating positive hydrostatic pressure in xylem known as root pressure (Furukawa et al., 2011). Above-zero temperatures to initiate root pressure may be perceived at the shoot (Furukawa et al., 2011). However once initiated, root pressure is positively correlated with soil temperatures (Améglio et al., 2004). Root pressure relies on dormancy release to

initiate root growth. As water and mineral uptake occurs at root tips, osmotic and water potential of vessels is lowered, generating xylem pressure (Furukawa et al., 2011). Temperate genera with only root pressure are described by Wiegand (1906) as “late-bleeders”, and include *Carya* Nutt., *Malus* Mill., *Crataegus* L., *Salix*., *Ulmus* L. (Wiegand, 1906), *Vitis* L., *Betula*, *Ostrya* Scop. (Marvin, 1958), *Alnus* Mill., *Fagus* L. and *Quercus* L. (Améglio et al., 2004). Root pressure in *Vitis* was described early in the literature (Hales et al., 1727), and is critical for re-establishing hydraulic conductivity of vessels in spring (Sperry et al., 1987). New vessels do not differentiate until after leaf expansion, and vessel diameters of *Vitis* spp. are amongst the widest-known, implicating the importance of root pressure as a refilling mechanism (Sperry et al., 1987). When cut down late in the leafless period, temperate species with only root pressure exhibit sap flow from the root stump, while the cut surface of the trunk dries over time (Wiegand, 1906). In contrast to root pressure, trees with stem pressure display sap flow from the cut surface of the trunk when cut down in late-winter, i.e. even when stems are severed from their roots (Wiegand, 1906; Cruiziat et al., 2002). Seasonal bulk xylem pressure includes both root and stem pressure. However, stem pressure sap flow correlates better with twig/stem temperature (Marvin, 1958). For example, sap flow from positive hydrostatic pressure occurs in *Acer* when overnight air temperatures fall below 0°C and daytime air temperatures rise to between 5° and 10°C (Naughton et al., 2006). The dependence of stem pressure on freeze-thaws appears in the literature by the late-nineteenth century (Clark, 1875). North American genera that have sap flow in response to freeze-thaws are categorized by Wiegand (1906) as “early bleeders”, and display stem pressure that exceeds root pressure. Genera with stem pressure include *Acer*, *Juglans* L., *Cladrastis*

Raf., *Nyssa* Marsh., and *Prunus* L. (Wiegand, 1906). Stem pressure in *Acer* and *Juglans* is proportional to soluble NSC concentration, and more specifically, sucrose concentration of vessel sap (Johnson et al., 1987; Cirelli et al., 2008).

As the leafless period ends, residual embolisms from freeze-thaw events hinder hydraulic conductivity of xylem conduits (Hacke and Sperry, 2001). In some temperate broadleaf eudicot trees, hydraulic conductivity of conduits is never restored and new functional conduits in spring are produced. Trees with seasonal bulk xylem pressure restore hydraulic conductivity and likely experience fewer residual embolisms in early-summer (Cruziat et al., 2002). Seasonal bulk xylem pressure and vessel-loading of soluble NSCs are inextricably linked. Sugar maple (*Acer saccharum*) maintain a reservoir of soluble sucrose in RAP over the leafless period (Gregory and Hawley, 1983), and winter sub-zero temperatures signal catabolism of starch to soluble sucrose, thereby concentrating solutes to RAP (Sauter et al., 1973; Sakai and Larcher, 1987; Sakr et al., 2003; Wong et al., 2003; Kasuga et al., 2007). Temperature-dependent loading of sucrose to xylem sap from RAP was observed in stems of *Acer saccharum* incubated at temperatures from 0°C to 22°C (Sauter et al., 1973). Incubation at still higher temperatures (22°C to 40°C) resulted in a rapid decrease (unloading) of sucrose from vessel sap (Sauter et al., 1973). Stems of European walnut (*Juglans regia* L.) similarly displayed a temperature-sensitive threshold of sucrose regulation. Stems incubated at temperatures slightly above 0°C resulted in starch deposition in RAP and loading of sucrose to vessels; at 15°C, unloading of sucrose back to VACs from vessel sap occurred followed by starch deposition in RAP (Améglio et al., 2000, 2004; Ewers et al., 2001; Decourteix et al., 2006). *JrSUT1* is a

putative sucrose transporter identified in European walnut, and shares 69% homology with the sucrose transporter, *PtSUC2.1*, identified in California poplar (*Populus trichocarpa*) (Secchi and Zwieniecki, 2011). Both genes were implicated in loading of sucrose to vessel sap and xylem refilling. *Betula pendula* Roth. is a species with only root pressure, and abundant sucrose accumulates to vessel sap in stems incubated at 0°C (Sauter and Ambrosius, 1986). Hexoses such as glucose and fructose were more prominent as temperatures increased, and, near 20°C, a rapid decrease of sugars in vessel sap occurred (Sauter and Ambrosius, 1986). Temperate *Salix* spp. only have root pressure (Wiegand, 1906), and sucrose accumulated to vessels in stems incubated at 2°C. A reduction of sugars via unloading of NSCs (hexoses and sucrose) to VACs occurred at 21°C (Sauter, 1981). Sauter (1982) found that in *Salix*, sucrose accumulation to vessel sap was dependent on:

- a) low temperatures resulting in abundant starch to sucrose conversion in RAP;
- b) loading of sucrose to xylem conduits at low, positive temperatures;
- c) an absence of NSC unloading to VACs at low, positive temperatures;
- d) and a lack of starch re-synthesis in RAP at low, positive temperatures.

Although seasonal bulk xylem pressure has not been reported in temperate *Populus* spp., temperature-dependent soluble NSC allocation to vessel sap has been demonstrated therein. Vessel sap sucrose concentration in *Populus × canadensis* Moench cv. ‘Robusta’ was reported to increase at low temperatures (0°C and -2°C) with an inverse relationship to starch content of RAP. At a temperature of 10°C, sucrose of vessel sap was rapidly converted to hexoses such as maltose (Sauter, 1988). Similarly, in black poplar (*Populus*

nigra L.), glucose accumulates in vessel sap at low positive temperatures (Furukawa et al., 2011). NSC storage and mobilization in temperate broadleaf eudicot trees clearly is

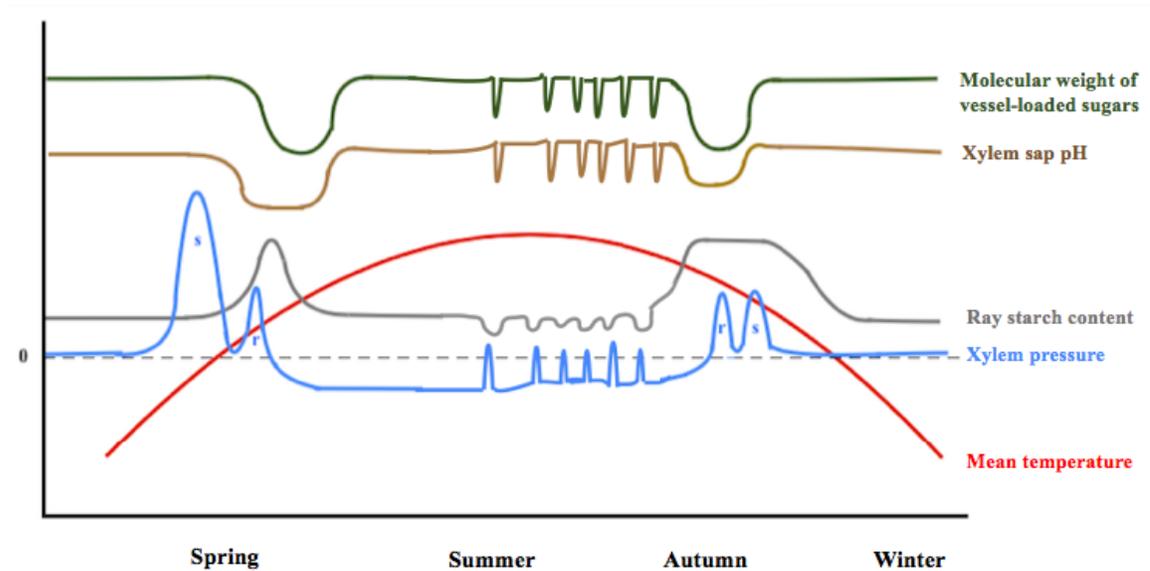


Figure 3.1 Time-series of typical seasonal temperature, xylem pressure (r=root pressure, s=stem pressure), ray starch content, xylem sap pH, and molecular weight of vessel-loaded sugars from theory of sap production and movement by a ‘whole tree’. Time-series loosely based on data from the following sources:

Seasonal temperature and ray starch content: Sauter, 1981, 1982, 1983, 1988, Améglio et al., 2000, 2001, 2004; Decourteix et al., 2006; Bonhomme et al., 2010; Secchi and Zwieniecki, 2016, 2011.

Xylem hydrostatic pressure: Johnson et al., 1987; Johnson and Tyree, 1992; Améglio et al., 2001, 2004; Sakr et al., 2003; Cirelli et al., 2008; Secchi and Zwieniecki, 2011, 2016; Secchi et al., 2017.

Xylem sap pH: Hacke and Laur, 2016; Secchi and Zwieniecki, 2016; Stanfield et al., 2017.

Molecular weight of vessel-loaded NSCs: Sauter, 1983, 1988; Sauter and Ambrosius, 1986; Améglio et al., 2004; Decourteix et al., 2006, 2008, Secchi and Zwieniecki, 2011, 2016.

sensitive to seasons and temperature (see Figure 3.1), which may have ecological implications – when compared to tropical trees, temperate tree species exhibit increased concentrations of stored NSCs in xylem despite lower RAP fractions (Hartmann and Trumbore, 2016; Morris et al., 2016; Plavcova et al., 2016). Temperature-dependent loading and unloading of NSCs (sucrose and hexoses) to vessel sap likely plays a more critical role in temperate broadleaf eudicot trees through seasonal bulk xylem pressure and repair of freeze-thaw induced embolism.

3.5 Hypothesis 2: Trees will have stem pressure, not just root pressure, only if pits do not occur (or pits are blind) between fibres and vessels.

Seasonal bulk xylem pressure in temperate broadleaf eudicot trees increases dramatically in late-winter and early spring, because of root and/or stem pressure. Although the mechanism of stem pressure is dynamic, I hypothesize that temperate broadleaf eudicot trees with fibres sequestered from vessels by a lack of pits (or pits are blind) will have stem pressure. Pits are small holes in cell wall lignin deposition between secondary xylem cells and contain a pit membrane composed of the modified primary walls of adjacent (paired) cells and a middle lamella (Melcher et al., 2003; Rabaey et al., 2006). Pits that are considered blind lack a complimentary structure or pair of openings (Evert and Eichhorn, 2006). Furthermore, I also hypothesize that if pits are present then trees will only have root pressure. Anatomical characteristics are important to the mechanism of stem pressure (Johnson et al., 1987), yet fibre-vessel pit pair information is lacking from most wood anatomical descriptions (Ohta et al., 2008). Pit features and pit arrangement affect both xylem hydraulic conductivity and resistance to

cavitation/embolism (Miller and Johnson, 2017). Tyree (1995) and Cirelli et al. (2008) previously hypothesized that pits must be absent between fibres and vessels for lignin to provide an effective semipermeable barrier of sucrose, but did not explicitly conclude the absence/presence of pits to be the feature to distinguish the relative timing of sap flow in plants with only root pressure. To my knowledge, I am the first to hypothesize that pit arrangement produces the temporal distinction in sap flow between root and stem pressure in temperate broadleaf eudicot trees. Cirelli et al. (2008) provided anatomical evidence that fibres are not connected by pits to vessels nor tracheids in *Acer saccharum* and *Juglans cinerea* L., yet for other temperate broadleaf eudicot species with stem pressure this data is lacking. Pit arrangement in other temperate species with stem pressure (*Acer*, *Juglans*, *Cladrastis*, *Nyssa*, *Prunus* spp.) should be investigated for a similar fibre-vessel pit arrangement reported in *Acer saccharum* and *Juglans cinerea*. Other temperate species with only root pressure (*Carya*, *Malus*, *Crataegus*, *Salix*, *Ulmus*, *Betula*, *Ostrya*, *Alnus*, *Fagus* and *Quercus*) should be investigated for fibres and vessels connected by pits as reported in *Betula papyrifera* Marshall by Cirelli et al. (2008). Light microscopy observation of stem cross-sections could determine if blind fibre-vessel pitting is a trait of species with stem pressure. My hypothesis would be falsified if species with stem pressure do not commonly display blind fibre-vessel pitting.

3.6 Support for Hypothesis 2

I will demonstrate that the temporal distinction between sap flow from root and stem pressure may simply result from water availability dictated by pit arrangement. As the mechanism of stem pressure is often debated, I will first synthesize prevailing stem

pressure models and physiology in *Acer* and *Juglans*, then contrast this with structural differences in xylem anatomy of *Betula* (root pressure only).

3.6.1 *Physical model of stem pressure*

The fundamental physical model of stem pressure is bi-phasic (freeze-thaw) in which an arrangement of gas-filled fibres and fluid-filled vessels allows for the generation of bulk xylem pressure in response to freeze-thaw cycles (Milburn and O'Malley, 1984; Johnson et al., 1987; Tyree, 1995; Cirelli et al., 2008). In winter, vessels of both *Acer* and *Juglans* are filled with sap (water and solutes) and very little gas, whereas fibres are gas-filled (Johnson and Tyree, 1992). The opposite distribution of fluid and gas is observed during winter in temperate genera with only root pressure, including *Vitis*, *Salix*, *Populus*, *Ulmus*, *Fraxinus* and *Quercus* (Wiegand, 1906; Tyree, 1995). In Milburn and O'Malley's (1984) physical model of stem pressure, ice crystallization is thermodynamically favoured in gas-filled fibre lumens during the freeze phase (see Figure 3.2). Ice growth is fueled by water stored in microcapillaries of fibre cell walls (Tyree, 1995). Microfibrils group together and form an interfibrillar system of microcapillaries, which primarily store colloidal pectic compounds that absorb and hold water (Bailey, 1939). In the freeze phase, freezing point depression of fibre cell wall microcapillaries is increased due to surface absorption effects (Milburn and O'Malley, 1984) and freezing point depression of vessel sap is increased as a function of solute concentration. Fluid in fibre lumens thus freezes prior to fluid in fibre cell walls and vessels, permitting vapour distillation to locally extract water from sites of storage (microcapillaries and voids left from intercellular spaces) to crystallize as ice in fibre lumens. A process known as cryostatic

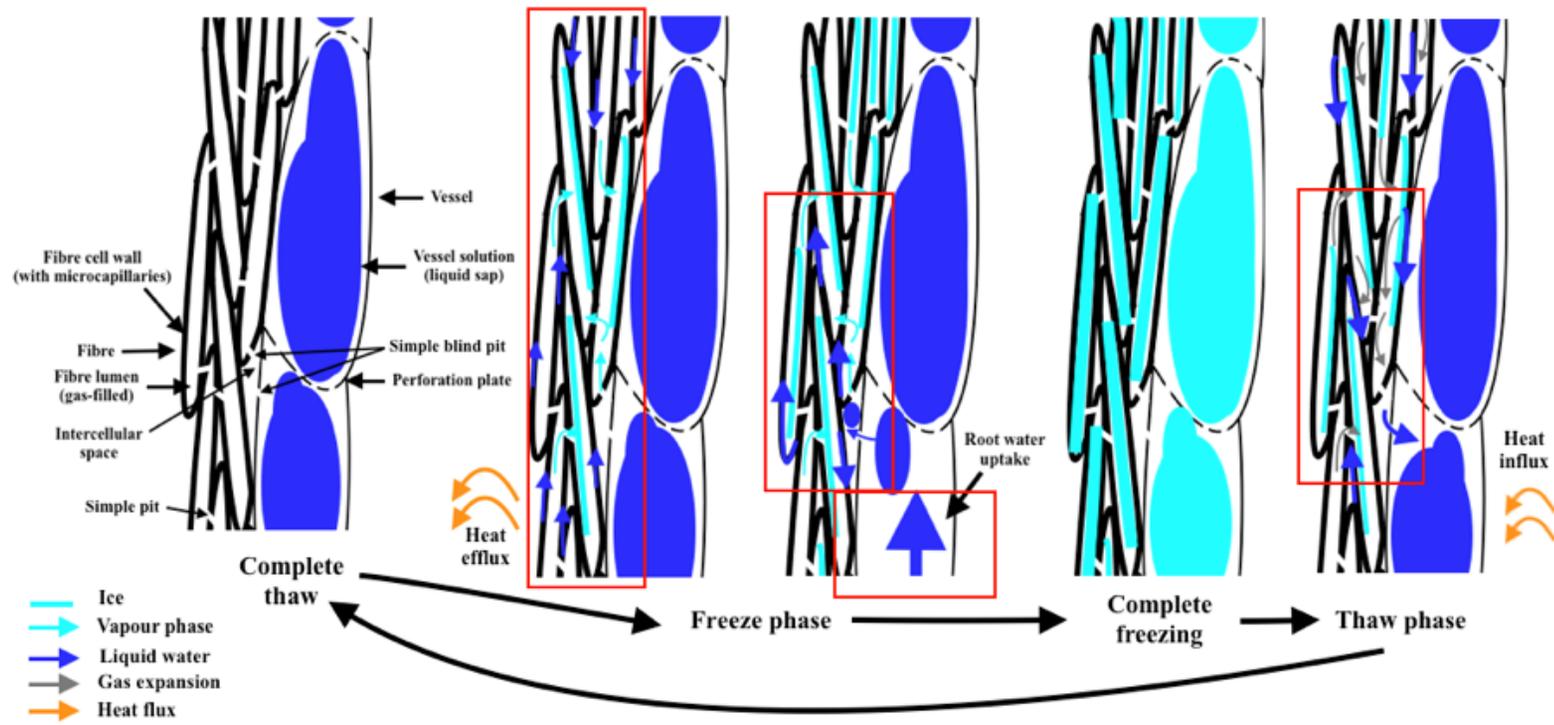


Figure 3.2 Freeze-thaw response in *Acer* when leafless. This figure is loosely based on ideas from Milburn and O'Malley (1984) and includes blind fibre-vessel pits that restrict sucrose to vessel sap, identified by Cirelli et al. (2008). Red box during initial heat efflux highlights movement of liquid water via microcapillaries to crystallize as ice in fibre lumens. As heat efflux continues, and because of vessel sap freezing point depression, water moves from vessel sap to replenish water in microcapillaries. Subsequent root water uptake to vessel sap fuels continued ice growth and compresses gas in fibre lumens as freezing continues. Heat influx initiates thawing allowing for expansion of gas in fibre lumens and water movement back to vessels promoted by vessel sap osmotic potential to produce xylem pressure.

suction subsequently extracts systemic water from vessel sap to replace local water depleted at microcapillaries. Cryostatic suction, or the movement of unfrozen water through a medium to a site of freezing (or lens), is well-described in regards to frost heaving (Milburn and O'Malley, 1984; Graf et al., 2015). Cryostatic suction fuels ice crystal growth in fibre lumens and promotes negative hydrostatic pressure (tension) in vessels. Water movement to fibre lumens and the generation of fluid tension in vessels induces root water uptake to vessels via cohesion-tension prior to vessels completely freezing. Water movement from vessels to adjacent cells is primarily accomplished via pit connections (Zwieniecki and Holbrook, 2000). Vessel pit pairing with adjacent xylem conduits and/or RAP allows for water movement from hydraulically conductive conduits or sites of storage respectively. Water movement between RAP cells occurs via plasmodesmata, and VACs adjacent to (and in direct contact with) vessels have pit connections that permit water to be drawn from RAP to xylem conduits. Cryostatic suction in the freeze phase leads to water accumulation in the vessel solution (sap), which can enter voids left from intercellular spaces adjacent to fibres via unpaired fibre-vessel pits. Accumulating water in voids can replenish water lost at cell wall microcapillaries before entering fibre lumens. Vapour distillation of water from microcapillaries promotes further ice crystallization in lumens and compresses gas. Fibres are dead at maturity with a heavily lignified secondary wall that reduces damage from ice crystal growth and allows for the compression of gas. In the thaw phase, compressed gas expands and forces water out of fibre lumens back to vessels, generating stem pressure and initiating sap flow (Milburn and O'Malley, 1984; Johnson et al., 1987; Tyree, 1995; Cirelli et al., 2008; Graf et al., 2015).

3.6.2 *Progression of the physical model after 1990*

The stability of air bubbles in fibre lumens is critical to the physical model of stem pressure. In a confined space at pressure, such as in fibre lumens, air bubbles are predicted to be unstable and to dissolve over time (Tyree, 1995; Cirelli et al., 2008). Surface tension increases pressure within the air bubble in comparison to the surrounding fluid pressure. Constant diffusion of air from the bubble surface out of solution will therefore occur over time, leading to bubble dissolution and expulsion of gasses (Yang and Tyree, 1992; Tyree, 1995; Cirelli et al., 2008). During the freeze phase, solute concentration of vessel sap induces an osmotic gradient on fluid in adjacent fibre lumens, thus equalizing fluid pressure of the fibre lumen and surface tension pressure of the air bubble. Equilibrium between air bubble surface tension pressure and the surrounding fluid pressure promotes the stable growth of air bubbles in fibre lumens (Johnson et al., 1987; Johnson and Tyree, 1992; Tyree, 1995). Sucrose concentrated vessel sap provides a sufficient solute concentration to stabilize air bubble growth in adjacent fibre lumens (Cirelli et al., 2008). When sucrose was absent from vessel sap, air bubbles compressed by infusion dissolved over time (Cirelli et al., 2008).

Stem water content also plays a crucial role in sap flow. If stem water content is greater than 70% (w/v), even with sucrose present in vessels at the appropriate concentrations, root water uptake will not occur during the freeze phase, thereby preventing sap flow upon thawing (Milburn and O'Malley, 1984; Johnson and Tyree, 1992; Tyree, 1995). Sap flow volumes rely on the process of freeze dehydration (several days of exposure to sub-freezing temperatures) as pre-conditioning. Dehydration at near-zero temperatures leads

to water loss from vessels via vapour distillation at lenticels along the stem surface, increasing the occurrence of air bubbles that impede fluid uptake from the roots upon thawing. By contrast, prolonged dehydration at sub-zero temperatures initiates water vapour migration from ice crystals of fibre lumens to large, thermodynamically-favoured crystals in vessels, replacing water lost to vapour distillation and reducing the occurrence of air bubbles in vessels (Johnson and Tyree, 1992; Tyree, 1995). In sugar maple, Marvin (1958) noted that sap flow volume was not related to temperature rise nor sucrose concentration on the day of sap flow, but to temperature of the preceding cooling (dehydration) period, i.e. the number of hours twig temperature was below freezing prior to a thaw event.

3.6.3 *Vitalistic model of stem pressure*

Vitalistic models of the stem pressure mechanism emphasize the role of living cells (Jones et al., 1903; Johnson, 1945; Marvin and Greene, 1951; Canny, 1995). Tyree (1995) refined the physical model to support the role of living cells and high molecular weight solutes (specifically sucrose) in developing an osmotic differential between vessels and fibres, listing a series of testable hypotheses:

- a) lignified cell walls in maple xylem must be semipermeable to high molecular weight solutes, but permeable to small molecular weight solutes;
- b) during freezing, low molecular weight solutes can pass with water into fibre lumen, but high molecular weight solutes are restricted;
- c) when ice melts during the thaw, high molecular weight solutes loaded to vessel sap draw water from fibre lumens osmotically to generate xylem pressure. Low

molecular weight solutes would be at equal concentrations within both xylem and fibre lumens and therefore have no osmotic effect.

To successfully lower water potential of vessel sap through osmoregulation, a semipermeable barrier was expected to be present in vessels. If pits between fibres and vessels were absent, lignin was anticipated to prevent diffusion of sucrose into surrounding cells but allow water to move freely in and out of vessels (Tyree, 1995; Cirelli et al., 2008). Fluorescein-perfusion experiments by Cirelli et al. (2008) with stems of *Acer saccharum*, *Juglans cinerea* and *Betula papyrifera* demonstrated that:

- a) pit membranes are porous to sucrose,
- b) lignified secondary cell walls of vessels provide an effective barrier for molecules of a similar or greater mass than sucrose (pores in the secondary wall restrict the movement of molecules with a molecular mass greater than 300 g mol^{-1}),
- c) blind simple pits in fibres of sugar maple occur when facing a vessel, providing anatomical evidence that neither vessels nor tracheids are connected by pits to libriform fibres.

Perfusion experiments with stems of *Acer* spp. demonstrated sap flow when sucrose (a disaccharide) was present in vessel sap, but no sap flow when solely hexoses (monosaccharides) were present (Marvin, 1958; Johnson et al., 1987; Johnson and Tyree, 1992). Perfusion of stems with raffinose, sucrose, maltose, and lactose at equal osmotic concentrations produced sap flow (exudation) upon thawing. In contrast, the absorption of sap (rather than exudation) occurred upon thawing in stems perfused with hexoses, including glucose, fructose and mannitol (see Table 3.1). It is unexpected that perfusion with inositol (a hexose) would induce sap flow upon thawing because Cirelli et al. (2008)

results suggest that diffusion would be unrestricted due to a molecular mass less than 300g mol^{-1} . However, replicated experiments demonstrated similar results (Marvin, 1958; Johnson et al., 1987). Even though inositol does induce sap flow, specific volume is much lower than induced by sugars of greater molecular mass, e.g. disaccharides (see Table 3.1). Compared with other hexoses, sap flow may result from a reduced inositol diffusion rate from vessels into surrounding cells. In salt-stressed plants, inositol has

Table 3.1. Solute perfusion and response (absorption or exudation) of sugar maple (*Acer saccharum*) stems to thawing; reproduced from (Johnson et al., 1987). Methods of Marvin (1958) unknown, but perfusion experiments of Marvin (1958) and Johnson et al. (1987) yielded identical qualitative results (absorption or exudation). Sap flow specific volume reported by Johnson et al. (1987).

Sugar (NSC)	Molecular mass (g mol^{-1})	Absorption or exudation upon thawing	Sap flow specific volume (ml/kg) at equal solute concentrations
Glucose	180	Absorb	29 ± 3
Fructose	180	Absorb	31 ± 3
Mannitol	182	Absorb	15 ± 2
Inositol	180	Exude	9 ± 2
Sucrose	342	Exude	66 ± 8
Maltose	342	Exude	61 ± 5
Lactose	342	Exude	58 ± 8
Raffinose	504	Exude	47 ± 5

been implicated in leaf-to-root signaling of sodium uptake through regulation of vessel inositol concentration (Nelson et al., 1999). The association of inositol as a signal response suggests that xylem concentration of inositol is regulated, potentially through diffusion rates. Therefore, sap flow/exudation upon thawing observed in sugar maple stems perfused with inositol may be independent of molecular mass, and reliant upon the process in which xylem inositol concentration is regulated in stress signaling.

Hydrolysis of starch to sugars (primarily sucrose) in temperate broadleaf eudicot trees occurs as a response to sub-zero temperatures (Sauter et al., 1973; Sakr et al., 2003; Wong et al., 2003; Spicer, 2014), and is attributed to amylase isoforms stored in amyloplasts of RAP (Wolfgang et al., 1994). Above-zero spring temperatures can also contribute to the solute reservoir by initiating cell dehardening i.e. membranes and cytoplasmic material produced during autumn cold acclimation are converted to sugars (Sakai and Larcher, 1987; Wong et al., 2003, 2005). Solute flux, including of soluble NSCs, between RAP and vessel sap occurs at VACs (Sauter et al., 1973; Decourteix et al., 2006, 2008; Evert and Eichhorn, 2006) that also have an active role in the allocation of water from storage sites to vessels (Sakr et al., 2003). The mode of transport of soluble NSCs from RAP to VACs has yet to be confirmed (Brodersen and McElrone, 2013), but likely relies on symplastic transport through an abundance of plasmodesmata connections in rays (Sokolowska and Zagórska-Marek, 2012; Pfautsch et al., 2015). From VACs, half-bordered (by cell wall lignin deposition) pit pairs in contact with vessels of sugar maple allow for direct sucrose loading to vessel sap (Cirelli et al., 2008). VACs are localized to uniseriate xylem rays and uniseriate end-portions of multiseriate rays in

contact with vessels. VACs are concentrated to rays adjacent to vessels, including vascentric ray parenchyma that forms a sheath around a single vessel or a group of multiple vessels (Sauter et al., 1973; Cirelli et al., 2008). Larger multiseriate rays are probably mostly involved in storage of starches and sugars, whereas narrow uniseriate rays are probably mostly involved in the loading and unloading of solutes. Over winter, an increase in respiratory and phosphatase activity occurs in maple VACs (Sauter et al., 1973; Evert and Eichhorn, 2006). A respiratory inhibitor, p-chloromercuribenzoate, in experimental treatments drastically reduced vessel sucrose accumulation, demonstrating the dependence of sucrose-loading on respiratory activity of VACs (Sauter et al., 1973). Sauter et al. (1973) thus speculated that active loading or facilitated diffusion of sucrose occurs from living cells to vessel sap. Sap flow in maple stems was also prevented by physical damage to cells (steam treatment), which demonstrated that living cells must play a role in maintaining stem pressure (Marvin, 1958). The dependence of sap flow on living cells is consistent with sap flowing from recently cut maple logs (in late-winter), but no sap flow in dead maple trees. Thus, sap flow resulting from stem pressure and loading of sucrose to vessels cannot occur if there is physical or metabolic damage to adjacent cells (Secchi and Zwieniecki, 2011).

3.6.4 *Incorporation of physical processes to the vitalistic model*

A multi-scale mathematical model developed by Graf et al. (2015) describes the importance of three physical mechanisms in stem pressure:

- a) gas bubbles in vessel sap,
- b) freezing point depression of vessel sap, and

c) root water uptake to vessel sap prior to freezing.

Gas bubbles in vessel sap are necessary to account for the fluid transfer and pressure exchange between fibres and vessels during the thaw phase. Vessels fully saturated with sap would behave as an incompressible fluid (Graf et al., 2015). Furthermore, the osmotic potential of sap in the stem pressure mechanism has been disputed (Cortes and Sinclair, 1985; Johnson et al., 1987; Graf et al., 2015). Graf et al. (2015) thus speculate that freezing point depression is likely the primary role of high-molecular weight solutes in driving the sap flow mechanism. Freezing point depression of vessel sap resulting from sucrose accumulation, via a lack of fibre-vessel pits, is sufficient to allow for ice crystal growth in fibre lumens adjacent to liquid sap in vessels. Even though disaccharides such as sucrose may not be more effective at depressing the freezing point of gels compared with monosaccharides (Kim et al., 2004), restriction of sucrose to vessel sap not only stabilizes bubble growth in fibre lumens, but through freezing point depression of vessel sap allows for phase partitioning between fibres when freezing. Cryostatic suction can then initiate water flow from liquid vessel sap to solid ice crystal growth occurring in fibre lumens via vapour distillation. Subsequent root water uptake to vessels fueling ice crystal growth in fibre lumens during the freeze phase is well-documented in the literature (Wiegand, 1906; Marvin, 1958; Milburn and O'Malley, 1984; Johnson et al., 1987; Johnson and Tyree, 1992; Tyree, 1995; Graf et al., 2015).

Structural differences in xylem anatomy have been investigated in only few temperate broadleaf eudicot trees with seasonal bulk xylem pressure. The flow of sap (water and solutes) between adjacent xylem conduits occurs through minute pores of pit membranes

that also aid in limiting the passage of air bubbles (Zwieniecki and Holbrook, 2000; Melcher et al., 2003; Rabaey et al., 2006). Pit membrane composition is diverse across species. Pits of conifers contain pit membranes of cellulose with pectin, whereas temperate broadleaf eudicot trees may have pit membranes consisting of cellulose embedded in a lignin matrix, at least as identified in hybrid poplar (*Populus tremula* L. x *alba* L.) (Herbette et al., 2015). In sugar maple, half-bordered pits occur between vessels and ray parenchyma, and bordered pits between vessels and adjacent fibre-tracheids (see Figure 3.3 reproduced from Cirelli et al., 2008). Axial parenchyma is formed from fusiform initials (Morris et al., 2016), as are fibre-tracheids. Fibre-tracheids are claimed to grade into axial parenchyma (Carlquist, 2013), but unlike axial parenchyma are likely dead when fully mature. Blind pits occur in sugar maple between fibre(-tracheid)s and vessels (Cirelli et al., 2008). Blind pits can occur against cell wall sections that are exceedingly or irregularly thickened, but more commonly occur opposite to intercellular spaces (Laming, 1974). As lignin deposition to cell walls is hydrophobic and water-impermeable (Whetten and Sederoff, 1995; Sperry, 2003), intercellular spaces facing blind pits of both fibres and vessels is the likely path of water to replenish fibre cell wall microcapillaries during the freeze phase. Blind fibre-vessel pits effectively isolate fibres from the interconnected RAP-vessel system, and so fibres can be considered impermeable to sucrose (Sauter et al., 1973; Cirelli et al., 2008). The restriction of sucrose by lignin and exclusion of fibre-vessel pit pairs, therefore, establishes an osmotic differential between vessels and fibres (Cirelli et al., 2008). Low osmotic potential of vessels (in contrast to fibres) in the thaw phase is likely synergistic with expanding gas in

fibre lumens driving the movement of water from fibres back to vessels. For the potential pathways of water and sucrose in secondary xylem of *Acer saccharum*, see Figure 3.3.

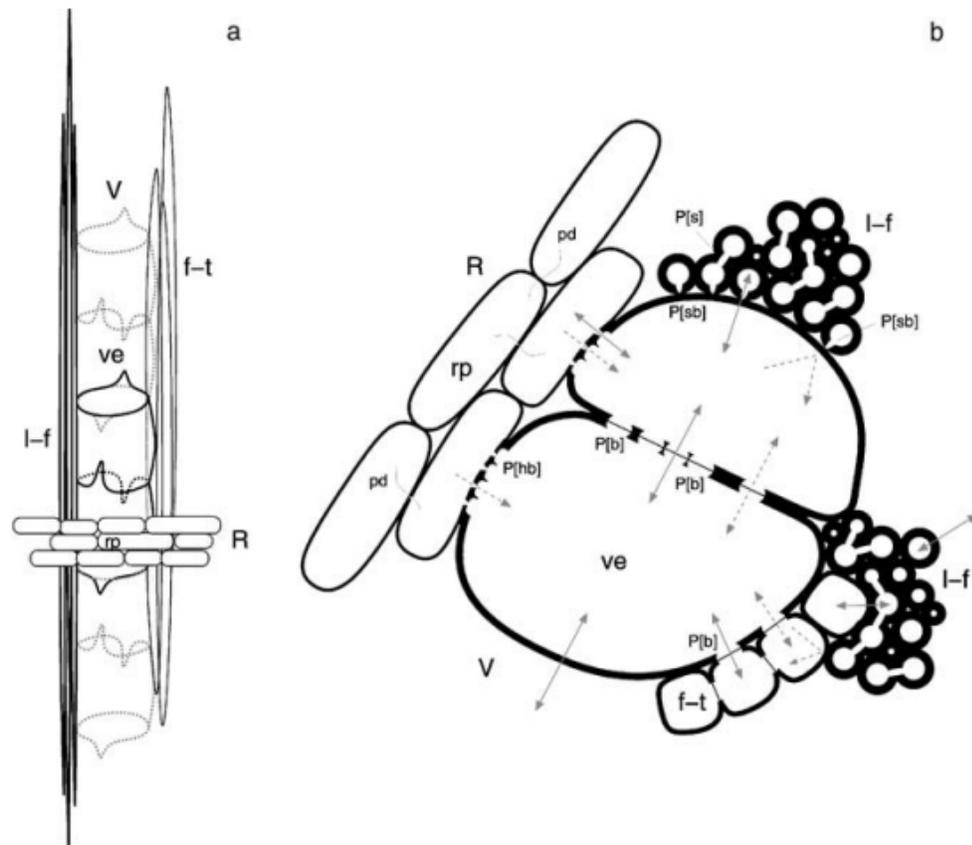


Figure 3.3 Potential pathways of water and sucrose in secondary xylem of *Acer saccharum*, reproduced from Cirelli et al. (2008)*. Abbreviations: V, vessel; ve, vessel element; R, ray; rp, ray parenchyma (only living cells); l-f, libriform fibers; f-t, fibre-tracheids. (a) Radial view of a stack of vessel-elements surrounded by fibers (l-f) and f-t and in contact with a ray. (b) Cross section of an arrangement analogous to (a) showing pit connections (P[type]) (where [hb] = half-bordered; [b] = bordered; [s] simple; and [sb] = simple blind); plasmodesmata connections (pd) between living ray parenchyma; and potential pathways of H₂O (solid line arrows) and sucrose (dashed line arrows).

* Cirelli et al. (2008), Toward an improved model of maple sap exudation: the location and role of osmotic barriers in sugar maple, butternut and white birch, *Tree Physiology*, 2008, Volume 28, Issue 8, pages 1145-1155, by permission of Oxford University Press.

In contrast to *Acer saccharum* and *Juglans cinerea* (stem pressure exceeds root pressure), simple pit pairs (un-bordered by secondary wall lignin deposition) occur between libriform fibres and vessels of paper birch (*Betula papyrifera*; see Figure 3.4) allowing for diffusion of sap (water and soluble NSCs) from vessels to fibre lumens (Cirelli et al., 2008). Fibres of *Betula* spp., which only exhibit root pressure, can therefore be considered permeable to sucrose. Fibres are joined with the interconnected RAP-vessel system by simple pits allowing for sucrose diffusion across pit membranes in *Betula*. The osmotic differential developed in *Acer* spp. from the restriction of sucrose from fibres cannot be established in *Betula* spp. This likely accounts for the opposite distribution of fluid and gas in winter xylem (fluid-filled fibres; gas-filled vessels) of species with only root pressure when compared to species with stem pressure (gas-filled fibres; fluid-filled vessels) and for the temporal distinction in sap flow i.e. early-spring versus late-winter. Fluid in fibres and (any) fluid in vessels would thus be expected to freeze and thaw in unison (see Figure 3.5), and so root water uptake, which is critical for generating stem pressure in a freeze-thaw cycle, is unlikely to occur.

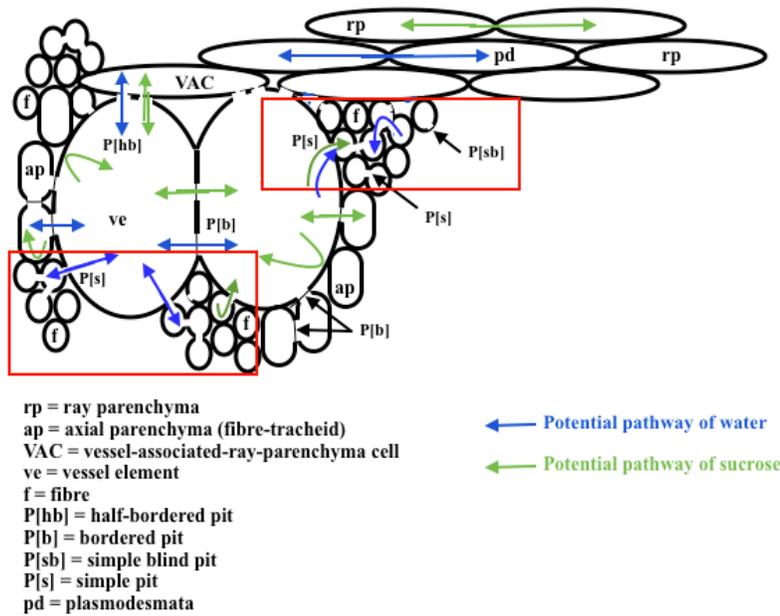


Figure 3.4 Potential pathways of water and sucrose in secondary xylem of *Betula papyrifera*; inferred and modified from Cirelli et al. (2008).

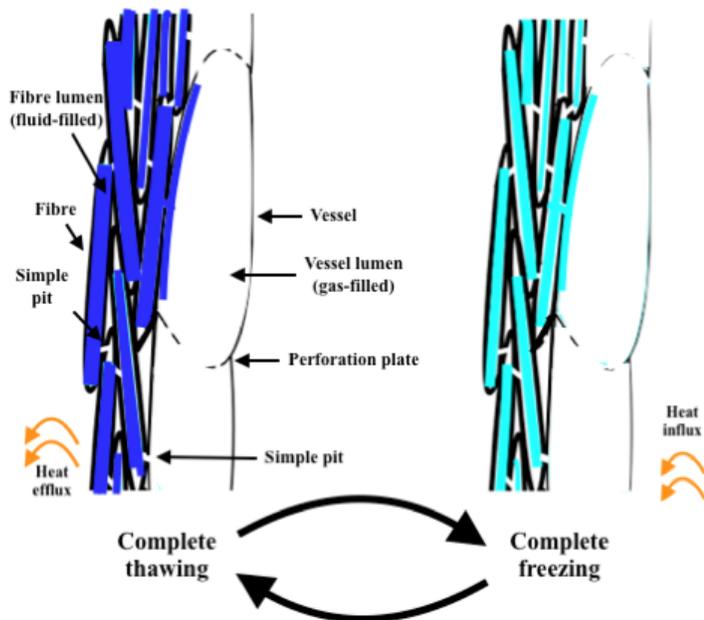


Figure 3.5 Hypothesized fluid and gas distribution in vessels and fibres of *Betula papyrifera* and freeze-thaw response when leafless, resulting from fibre-vessel pits.

3.7 Hypothesis 3: Spring xylem movement of sugars to shoot apical meristems is mediated by long-distance signaling via source-sink gradients

Xylem recovery from embolism in temperate broadleaf eudicot trees demonstrates a highly regulated flux of sugar and water via ray parenchyma between xylem and phloem. Xylem and phloem acting as a unified network of sugar and water flux provides a mechanism for sensing embolisms, for xylem recovery from embolisms, and rapid movement of soluble sugars to vascular cambia or shoot apical meristems following dormancy. I hypothesize that spring xylem movement of sugars to shoot apical meristems is accomplished through long-distance sugar signaling via source-sink gradients maintained at the ‘whole tree’ level. Contributions from molecular biology demonstrate dynamic and diverse signaling of sugars in plants (Li and Sheen, 2016) and so I use molecular evidence from *Arabidopsis* to support the notion that xylem and phloem act as a single network in trees to maximize hydraulic and energy efficiency via soluble sugar and water flux. Tixier *et al.* (2013) provide compelling arguments for *Arabidopsis* as a model for secondary growth, and support is detailed in Sengupta & Majumder's (2014) review of plant vasculature. It is likely that sugar signaling (conserved from archaeobacteria to plants and animals (Chen *et al.*, 2015)) and specifically source-sink gradients, governs the functional role of xylem and phloem as a response to diurnal, environmental or seasonal stimuli. In spring, xylem moves sugars via bulk transport to sink tissues at apical branches. Reversal of functional state to bulk water transport occurs when growth of shoot apical meristems produces leaves that are photosynthetically independent and act as source tissues that signal bulk movement of sugars via phloem (see Figure 3.8). Furthermore, consider ‘whole tree’ responses when xylem recovers from

embolisms via seasonal bulk xylem pressure: (i) high concentration of soluble sucrose in RAP following winter freeze avoidance or tolerance leads to high efflux or loading of sucrose to vessels via facilitated diffusion at low positive temperatures (Sauter, 1982; Améglio et al., 2004; Plavcova and Jansen, 2015), (ii) at apical branches, unloading of sucrose from vessel sap via upregulation of *SUT* transport genes in RAP (Améglio et al., 2000, 2001, 2004; Decourteix et al., 2006) likely initiates a signal to begin a source-sink gradient, and (iii) after sucrose unloading to RAP, starch is deposited before bud break (Wong et al., 2003; Bonhomme et al., 2010) maintaining the source-sink gradient (see Figure 3.6). Environmental conditions that stimulate bud break then signal degradation of starch reserves to sinks, the strongest being developing shoot apical meristems and cambia.

3.8 Support for Hypothesis 3

I hypothesize that seasonal bulk xylem pressure (root or stem) may not only be associated with xylem recovery from embolisms when leafless, but also with moving sugars to shoot apical meristems and that these mechanisms are coordinated by source-sink gradients. To provide support, I will describe sugar and water flux in temperate broadleaf eudicot trees following dormancy i.e. mid-spring to early summer. Phloem is typically the means for bulk transport of NSCs to carbon sinks. However, xylem sap can also provide a route for NSCs following dormancy and prior to leaves acquiring photosynthetic independence (Essiamah and Eschrich, 1986; Aloni, 1987). Rapid carbon transport can be accomplished via xylem sap and loading of soluble NSCs to vessels (Heizmann et al., 2001; Evert and Eichhorn, 2006; Mason et al., 2014). Soluble NSC concentration of vessel sap is typically

highest in spring due to the strong carbon sinks (sugar demand) generated by shoot apical meristems (Heizmann et al., 2001).

Notably, after sap movement from stem or root pressure and prior to growth of shoot apical meristems, NSCs are unloaded from xylem sap and deposited as starch in RAP of apical branches (Sauter et al., 1973; Wong et al., 2003). In European walnut, *Juglans regia*, after restoration of xylem hydraulic conductivity via bulk xylem pressure, the sucrose transporter *JrSUT1* decreases sucrose concentration of vessel sap via unloading of sucrose to RAP (Decourteix et al., 2006). Low levels of soluble sugars in spring vessel sap of European walnut coincided with starch synthesis in RAP (Decourteix et al., 2008). Notably again, starch was rapidly degraded to soluble sugars with growth onset of shoot apical meristems coinciding with minimums of starch content observed after leaf expansion (Bonhomme et al., 2010). Walnut VACs of apical shoots demonstrated localization of *JrSUT1* before bud break (Decourteix et al., 2008), implicating reabsorption of sucrose from vessel sap (via *SUT*) and potentially even symplastic transport between parenchyma cells (via plasmodesmata) (Plavcova and Jansen, 2015). Bud traces consist of two strands of procambial cells going to the shoot apical meristem, which serve as conducting channels early in spring development (Evert and Eichhorn, 2006). The location of local NSC reserves for shoot apical meristem carbohydrate availability may therefore be in the bud itself or neighbouring tissues of the stem (Bonhomme et al., 2010). When vascular cambial growth slows, NSCs unloaded from vessel sap probably move radially via symplastic transport to shoot apical meristems (Améglio et al., 2002; Decourteix et al., 2008). NSCs unloaded from vessel sap include

sucrose and hexoses, but European walnut seemingly has limited active hexose (glucose) import (Decourteix et al., 2008). Putative hexose transporters (*JrHT1* and *JrHT2*) in European walnut have been identified, but are regulated at low levels prior to growth of shoot apical meristems. Temperate species may uniquely regulate sucrose and hexose transporters, depending on concentrations in sap or as a response to environmental conditions.

Importantly, evidence exists for sucrose cleavage to hexoses such as maltose and glucose in vessel sap. Following loading to vessels, sucrose is apparently cleaved by cell wall invertase and as a response to low pH (Sauter, 1983, 1988; Sauter and Ambrosius, 1986; Secchi and Zwieniecki, 2011, 2016). Unloading NSCs from vessel sap apoplastically requires energy. Thus, ATPase-dependent unloading of soluble NSCs via *HT* and probably more specifically *SUT* transporters (Plavcova and Jansen, 2015) up-regulates H⁺ ATPase's leading to an accumulation of H⁺ in vessel lumens that lowers pH (Sauter, 1988; Wolfgang et al., 1994; Améglio et al., 2000; Secchi and Zwieniecki, 2016). Initial unloading of sucrose (i.e. before active hexose unloading) from vessel sap with proton-coupled sucrose transport could decrease pH, which is typically greater than pH=6, triggering cell wall (acid) invertase activity and leading to accumulation of hexoses in the vessel solution (Secchi and Zwieniecki, 2016).

Molecular evidence from *Arabidopsis* may provide insight of physiological processes observed in temperate trees following sap movement from bulk xylem pressure. In *Arabidopsis*, sucrose import to cells can be symplastic (through plasmodesmata via

cytoplasmic continuum) or apoplastic (across the cell membrane; ATPase-dependent) transport (Rolland et al., 2006). In leaves of *Arabidopsis*, cytoplasmic sucrose and trehalose-6-phosphate (T6P; involved in endogenous glucose signaling and sensing) can signal starch synthesis from hexose phosphates via upregulation of the large sub-unit of AGP (ADP-glucose pyrophosphorylase), promoting redox-dependent activation of AGP (Ramon et al., 2008; Li and Sheen, 2016). Hexose-phosphates for starch deposition are produced following sucrose cleavage by cytoplasmic (neutral or alkaline) invertase to produce glucose and fructose, or hydrolysed by sucrose synthase (SUS) in the presence of UDP to produce fructose and UDP-glucose (Sturm and Tang, 1999; Rolland et al., 2006). SUS can be both soluble in the cytoplasm and also plasma membrane-associated when dephosphorylated (Sturm and Tang, 1999). Even before intracellular import, sucrose can be cleaved by cell wall invertase to produce glucose and fructose (Rolland et al., 2006). Extracellular glucose and fructose enter the cell via hexose transporters that are co-expressed with cell wall invertase (Sturm and Tang, 1999; Rolland et al., 2006). The primary glucose sensor, HXK, is implicated in signaling and metabolism of glucose in the cytoplasm to hexose-phosphates that will be used for starch synthesis (Li and Sheen, 2016). Thus, it can be speculated that in temperate broadleaf eudicot trees, following unloading of sucrose to the cytosol of RAP via *SUT* at apical shoots, sucrose metabolism by SUS leads to accumulation of hexoses that can yield hexose-phosphates including UDP-glucose (see Figure 3.6). Similarly, hexoses cleaved from sucrose at the cell wall are transported to the cytosol, where their metabolism and that of UDP-glucose yields hexose-phosphates to fuel starch synthesis or regulate other sugar signal networks. Glucose-6-phosphate (G6P) and UDP-glucose can be synthesized to T6P, which

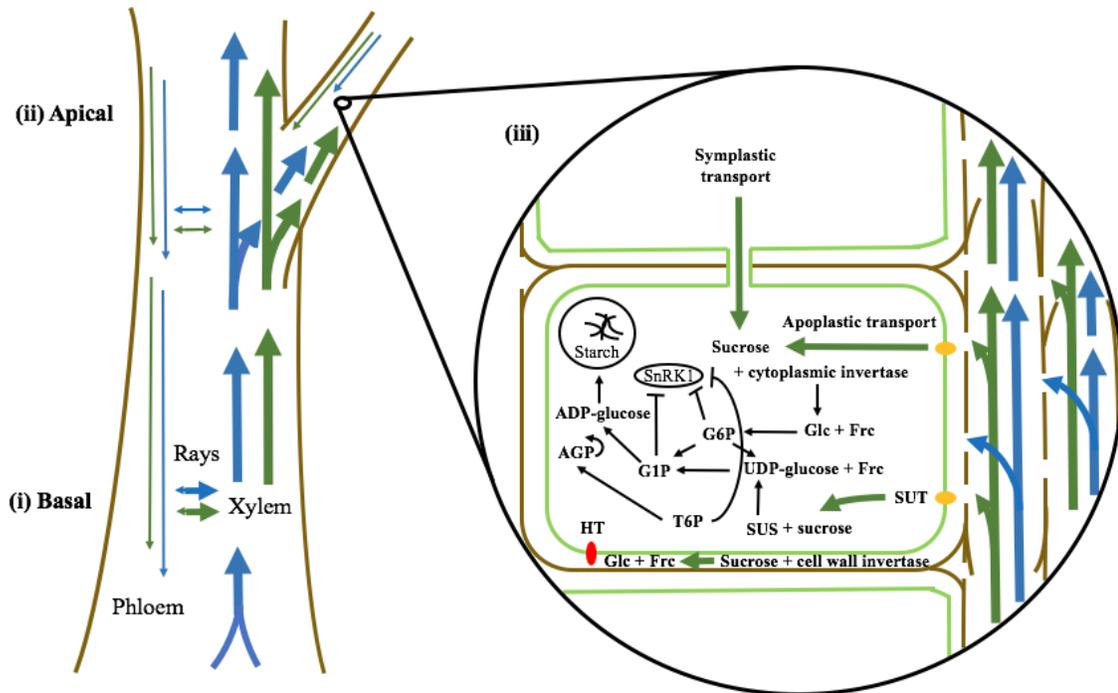


Figure 3.6 Theoretical model of how seasonal bulk xylem pressure (stem and/or root pressure) in temperate broadleaf eudicot trees moves sucrose (green arrows) and water (blue arrows) to apical branches for starch synthesis prior to bud break; supported with molecular evidence from *Arabidopsis* reported by Ramon et al. (2008), Stitt et al. (2012), and Li and Sheen (2016). (i) High concentration of soluble sucrose in ray and axial parenchyma leads to efflux or loading (facilitated diffusion) of sucrose to vessels via ray parenchyma (rp), refilling xylem conduits (teal arrow) via root water uptake (blue two-tailed arrow). (ii) High concentration of vessel-loaded sucrose lowers water and osmotic potential, generating bulk xylem pressure and sap movement to apical branches. (iii) Unloading of vessel-loaded sucrose at apical branches is apoplastic via *SUT* (orange oval) sucrose symporters. Cytoplasmic sucrose (which can be transported symplastically via plasmodesmata) is cleaved by cytoplasmic invertase to glucose (Glc) and fructose (Frc) or hydrolyzed by sucrose synthase (*SUS*) to UDP-glucose and fructose. Apoplastic sucrose is also cleaved by cell wall invertase to produce glucose and fructose that enters the cytoplasm via *HT* hexose symporters (red oval). In the cytoplasm, UDP-glucose or glucose can be metabolized to hexose-phosphates to fuel starch synthesis or regulate other sugar signal networks. Brown lines represent stem of tree and cell wall lignin deposition, light green the cell membrane.

upregulates AGP in the presence of cytosolic sucrose and in association with Sucrose NonFermenting1-Related Kinase1 (*SnRK1*), an energy sensor, to induce starch deposition and sucrose-induced upregulation of SUS (Rolland et al., 2006; Ramon et al., 2008; Stitt et al., 2012; Li and Sheen, 2016). SUS activity, which is positively correlated with starch content in potato tubers (Sturm and Tang, 1999) and associated with sucrose/*T6P/SnRK1* signaling (Rolland et al., 2006; Ramon et al., 2008; Li and Sheen, 2016), likely governs sink strength via starch deposition.

Recently observed spatial gradients further implicate sugar signaling networks in temperate broadleaf eudicot trees. In apical shoots of European walnut, Tixier et al., (2017) found that water content, starch and soluble NSC concentrations increased closer to shoot apical meristems and that the spatial gradient is disrupted by girdling of phloem. Tixier et al., (2017) concluded that phloem Münch flow, i.e. shoot to root flow supported by phloem loading of water and NSCs at shoot apices, maintained the spatial gradient to shoot apices (see Figure 3.7). They hypothesized that phloem loading removes water at shoot apices, and releases water from remote sites of starch degradation ensuring a gradient is maintained. Recirculation of NSCs and water between xylem and phloem thus generates an osmotic gradient that promotes mobilization and delivery of NSCs via xylem to shoot apical meristems (Tixier et al., 2017). Interestingly, girdling also delayed bud phenology and reduced biomass production, implicating long-distance transport via bulk pressurized sap at the end of winter to expedite movement of sucrose to newly growing shoot apical meristems of temperate broadleaf eudicot trees. Thus, Tixier et al., (2017) demonstrated that maintaining high starch and soluble NSC content of tissues at

shoot apices was critical for growth of shoot apical meristems and mobilization of NSCs from remote sites of storage in spring.

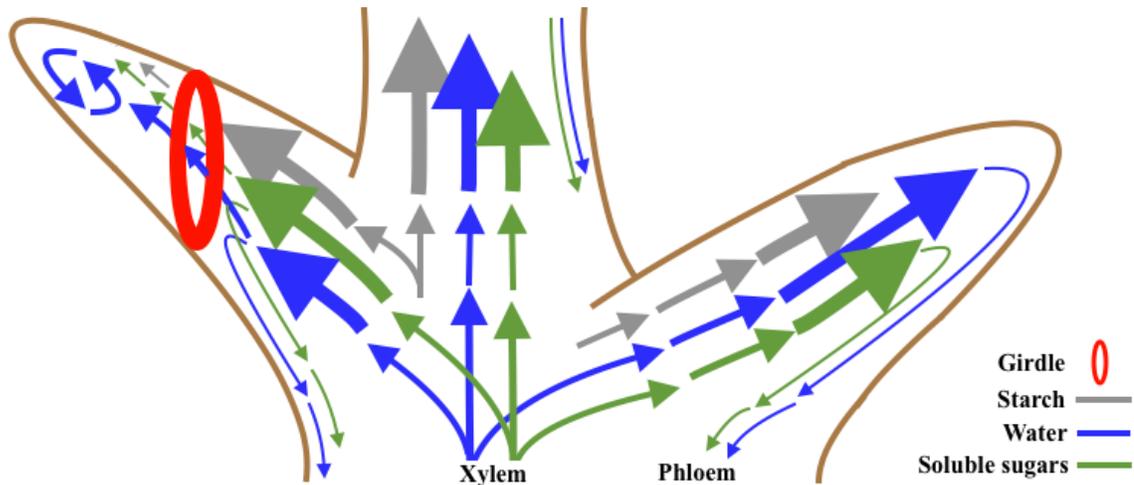


Figure 3.7 Spatial gradient of increased water content, starch and soluble sugar concentrations to shoot apical meristems, as reported by Tixier et al. (2017) in *Juglans regia*.

Consequently, I hypothesize that source-sink gradients regulate starch deposition at apical branches and fuel the gradient of water and sugars to shoot apices following dormancy release. I hypothesize that sugar signaling via change in source-sink status of tissues will signal functional reversal of xylem to bulk water transport and phloem to bulk transport of sugars (see Figure 3.8). In spring, xylem functions in bulk transport of NSCs to shoot apical meristems. When shoot apical meristems and vascular cambia have limited growth (i.e. in early spring, prior to favourable growing conditions), starch synthesis and phloem backflow at apical shoots maintains a source-sink gradient of sugar and water flux between xylem and phloem and NSC delivery to shoot apical meristems or vascular cambia to fuel growth once favourable growing conditions start later in spring.

When growth of shoot apical meristems produces photosynthetic leaves, the source status of tissues changes from starch/soluble NSCs stored in RAP of apical branches to photoassimilates produced by newly photosynthetic tissues. Spring starch synthesis is observed in weeks of reduced meristematic and cambial growth before bud break.

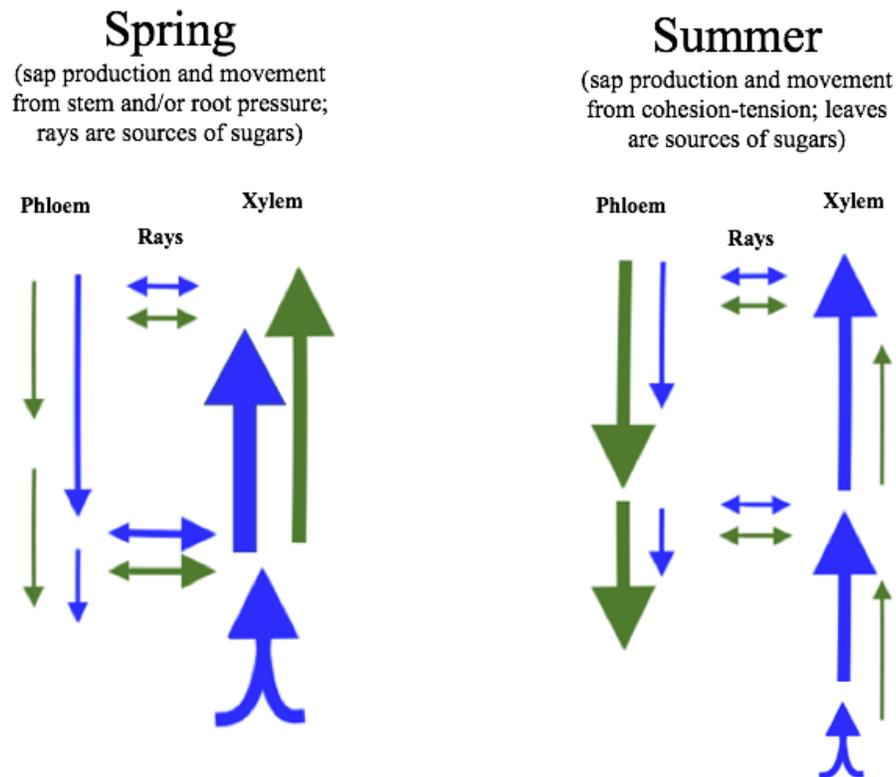


Figure 3.8 Bulk movement of sugar (green arrows) and water (blue arrows) in xylem and phloem (blue two-tailed arrow indicates root water uptake) in spring versus summer. In spring, the functional state of xylem is bulk sugar transport, supported by phloem backflow reported by Tixier et al. (2017). The hypothesis here is that as summer begins, photosynthetic leaves become sources that signal functional state reversal of xylem to bulk water transport and phloem to bulk sugar transport. Thickness of arrows indicates concentration of sugars and volume of water during movement.

Likewise, autumn starch synthesis occurs when meristems and cambia have limited growth due to the onset of dormancy (see Figure 3.1). Rather than source activity, upregulation of sucrose-6-phosphate-synthase (SPS) leads to starch degradation and autumn production of soluble sucrose for freeze tolerance or avoidance. SPS likewise mobilizes starch reserves (i.e. sources) to sinks at bud break (Plavcova and Jansen, 2015), the strongest being developing shoot apical meristems and cambia (Decourteix et al., 2008; Bonhomme et al., 2010; Plavcova and Jansen, 2015). Vascular cambium of ring-porous species represent more important sinks than developing shoot apical meristems because significant cambial growth occurs prior to leaves acquiring photosynthetic independence (Panchen et al., 2014; Plavcova and Jansen, 2015). Ring-porous *Robinia pseudoacacia* L. has upregulated SUS at vascular cambium to produce UDP-glucose from soluble sucrose for cell wall synthesis of new xylem conduits (Plavcova and Jansen, 2015). SUS isoforms have developmental- and organ-specific expression but are largely known for feeding sink metabolism (Sturm and Tang, 1999), and probably are active in cell expansion at shoot apical meristems of diffuse-porous species as well. Thus, I expect that source-sink status of tissues and sugar signaling determines the functional state of phloem and xylem (bulk water versus sugar transport), and of RAP (NSC storage versus mobilization). This blurred functional distinction between xylem and phloem makes sense insofar as they are homologous cells, i.e. arise from the same vascular cambia.

3.9 Concluding Remarks

Sugary sap flow in xylem of temperate broadleaf eudicot trees is an epiphenomenon of xylem recovery from embolisms and long-distance sugar signaling that humans and some

other mammals benefit from a few weeks of the year. In trees with stem pressure, pits are absent between fibres and vessels, isolating vessel-loaded sucrose from gas-filled fibres to generate phase partitioning (via freezing point depression), and cryostatic suction as a response to freeze-thaw temperatures (not observed in species with only root pressure). I propose the temporal distinction between sap flow from root and stem pressure may simply result from water availability dictated by pit arrangement. Liquid water would not be available to species with only root pressure during freezing as equal solute concentrations in fluid of fibres and (any) fluid in vessels would impede phase partitioning, and thus restrict water availability to a warming period.

Temperate broadleaf eudicot trees are not only capable of xylem recovery from embolism via seasonal bulk xylem pressure when leafless, but likely repair embolisms in the photosynthetic period against negative bulk xylem pressure as well via local loading of soluble sugars to ‘prime’ or generate water movement from adjacent hydraulically conductive conduits. I propose that the functional distinction between xylem and phloem is blurred and that soluble sugar and water flux is highly regulated by source-sink gradients and sugar signaling. Physiology of seasonal bulk xylem pressure in trees is well understood, with molecular biology well-understood in *Arabidopsis*. As Hartmann & Trumbore (2016: 397) remarked, “directly addressing storage regulatory mechanisms in mature forest trees will remain a major – and likely impossible – challenge.” Thus, contrasting molecular evidence from *Arabidopsis* to observed physiology in temperate broadleaf eudicot trees may improve understanding of broader mechanisms and source-sink sugar signaling complexes at a ‘whole tree’ level. I provide predictions in Table 3.2

that with corroboration or falsification may lead to further research on sugar and water flux in plants, which will have a variety of practical applications related to non-structural carbohydrates (NSCs) including signaling, metabolism, defense, osmoregulation, transport or storage.

Table 3.2. Hypotheses/predictions elucidated from theory of sap production and movement by a ‘whole tree’.

Hypothesis 1: What works in spring (refilling air-filled vessels), also works in summer (dissolving tiny air bubbles), i.e. sugars will refill xylem across scales and seasons.

- Sugars will pressurize and refill xylem across scales, i.e. will pressurize and refill wide vessels, narrow tracheids, narrow fibres, and diminutive cavitation events.
- Multiseriate xylem rays will facilitate storage of sugars (see chapter 4), whereas uniseriate rays and uniseriate end-portions of multiseriate rays will facilitate sugar loading and unloading to vessels.

Hypothesis 2: Trees will have stem pressure, not just root pressure, if pits are absent or blind between fibres and vessels.

- Trees with stem pressure will not have pits between fibres and vessels. Trees with only root pressure will have functional pits between fibres and vessels.
- Because of pits:
 - Trees with stem pressure will have fluid-filled vessels in winter, whereas trees with only root pressure will have mostly gas-filled vessels.
 - Trees with only root pressure will have fibres that are fluid-filled in winter. Fluid in fibres will freeze in unison with any fluid in vessels.

- Trees with stem pressure in late-winter will always also have root pressure in spring.

Hypothesis 3: Spring xylem movement of sugars to shoot apical meristems is mediated by long-distance signaling via source-sink gradients of sugars.

- In spring, sugar signaling initiates xylem refilling and loading (versus unloading of sugars by rays) at both small scales and over long-distances.
- Source-sink gradients via sugar signaling regulate spring starch deposition at apical branches and fuel the gradient of water and sugars to shoot apices.
- Sugar signaling via change in source-sink/sugar status of tissues determines functional state of xylem, phloem and rays (i.e. bulk water vs. sugar transport, storage vs. mobilization).

4 Chapter: Non-structural carbohydrate content of spring xylem sap is correlated with seriate number of rays

4.1 Abstract

Ray and axial parenchyma is a site of storage for non-structural carbohydrates, at least in temperate broadleaf eudicot trees. Rays can have high concentrations of soluble sugars that contribute to spring xylem sap sugar concentration. In the past, attempts to define the strength of correlation between ray parenchyma storage capacity and spring xylem sap sugar content were conducted with sugar maple (*Acer saccharum*). However, a few temperate genera other than *Acer* make sweet edible sap in spring and do so at unique temperature thresholds, and with variable saccharide content (i.e. mono- or disaccharide) and concentration. I compared temperate species with known seasonal xylem pressure (root and/or stem pressure) in *Acer*, *Juglans*, and *Betula*. Spring xylem sap sugar content is positively correlated with the number of cells in the width of multi-seriate rays after accounting for phylogenetic non-independence of traits.

4.2 Introduction

Ray and axial parenchyma (RAP) are derived from fusiform and ray cambial initials respectively, are alive at maturity, and are implicated with storage of non-structural carbohydrates (NSCs); the products of photosynthesis, including mono-, di-, oligo-, and poly-saccharides (Plavcova and Jansen, 2015; Morris et al., 2016; Plavcova et al., 2016). Flowering in sugar maple (*Acer saccharum*) and red maple (*Acer rubrum*) occurs prior to vegetative bud break, suggesting that stored NSCs provide the primary source of

carbohydrates for developing flowers (Rapp and Crone, 2015). Rapp and Crone (2015) hypothesized that increased NSC stores may provide the cue for flowering in sugar maple. In high-flowering years, they showed that depletion of total NSC stores following masting (seeding) events coincided with a decline in maple syrup yields the subsequent spring. Sap sugar content was thus reduced after a year of heavy seed production, suggesting that the primary path of soluble NSCs stored in RAP to developing flower buds is xylem sap (Rapp and Crone, 2015). Correlation between ray parenchyma storage capacity and spring xylem sap sugar concentration has mostly been investigated in sugar maple (*Acer saccharum*) (Morselli et al., 1978; Garrett and Dudzik, 1989; Rapp and Crone, 2015). However, temperate broadleaf eudicot trees of multiple genera have seasonal xylem pressure and load soluble NSCs from RAP to xylem sap in response to late-winter freeze/thaw or early-spring temperatures (Sauter et al., 1973; Sauter, 1981, 1982, 1988; Sauter and Ambrosius, 1986; Améglio et al., 2000, 2004; Ewers et al., 2001; Decourteix et al., 2008; Furukawa et al., 2011). It is my expectation that spring xylem sap sugar content in temperate species of genera with seasonal xylem pressure (root pressure and/or stem pressure) will similarly reflect RAP NSC storage capacity.

Seasonal xylem pressure, i.e. stem and root pressure, is prominently discussed in three temperate genera: *Acer* (maple) and *Juglans* (walnut) with stem pressure greater than root pressure, and *Betula* (birch) with only root pressure (e.g. Wiegand, 1906; Johnson et al., 1987; Tyree, 1995; Améglio et al., 2001; Cirelli et al., 2008). Sucrose is the primary NSC in spring xylem sap of species with stem pressure, i.e. temperate maple (Ball, 2007) and walnut species (Matta et al., 2005). The maple syrup industry recognizes variation in sap

sugar content between species, with sap of sugar maple having the greatest sucrose concentration, followed by sap of red maple, black maple (*Acer nigrum*), silver maple (*Acer saccharinum*), and Manitoba maple (or box-elder maple; *Acer negundo*) (Randall, 2010). In contrast to maple and walnut, monosaccharides such as glucose and fructose are the primary NSCs in spring xylem sap of temperate birch species (Johnson, 1944; Kallio and Ahtonen, 1987). Low pH of late spring xylem sap upregulates invertase activity, splitting sucrose to hexoses that typically concentrate in xylem sap of species with only root pressure (Sauter, 1983, 1988; Sauter and Ambrosius, 1986; Secchi and Zwieniecki, 2011, 2016). Here, I compared spring xylem sap sugar content for temperate *Acer*, *Juglans*, and *Betula* spp. with the number of cells in the width of multi-seriate rays. I predict xylem sap sugar content to positively correlate with n-seriate number of rays, i.e. the number of cells wide each ray is, and thus demonstrate that spring xylem sap sugar content reflects RAP NSC storage capacity of temperate broadleaf eudicot trees.

4.3 Materials and Methods

I conducted a literature review to determine spring xylem sap sugar content (mean and standard error) for temperate *Acer*, *Juglans*, and *Betula* spp. Sources for sugar content data can be found in Appendix A. The width of rays in number of cells, i.e. n-seriate number per ray, was obtained from the InsideWood database (InsideWood, 2004; Wheeler, 2011) and analyzed as factors i.e. “1-3”, “2-5”, “3-8” and “4-10”. Distribution of parenchyma between a species roots, stems, trunk or branches displays little variability (Morris et al., 2016). Using the updated megaphylogeny of plants (Qian and Jin, 2016), I built a pruned phylogenetic tree of species identified in my literature review with R 3.4.1

(<http://cran.r-project.org/>) using the package *phytools*. Quantifying correlation between biological traits requires consideration of phylogenetic non-independence of species (Felsenstein, 1985). Thus, I conducted phylogenetic independent contrasts of sugar content (dependent variable) against the number of cells in the width of multi-seriate rays (independent variable) and tested for phylogenetic signal using packages *ape*, *nlme*, *geiger*, and *phyolm*.

4.4 Results

I used a generalized linear model to compare spring xylem sap sugar concentration to the number of cells in the width of multi-seriate rays, which indicated slope of the regression line was not significantly different from zero (adjusted $R^2 = 0.19$, $p = 0.2387$; Appendix A). Spring xylem sap sugar content demonstrated high phylogenetic signal ($\lambda = 0.81$) and no signal in the number of cells in the width of multi-seriate rays ($\lambda = 0$). I then conducted phylogenetic generalized least squares to infer trait evolution through comparison of AIC values, which indicated that Ornstein-Uhlenbeck motion (AIC=21.10) was of marginally poorer fit than Brownian motion (AIC=19.26). Finally, I used phylogenetic independent contrasts to remove the effect of trait phylogenetic non-independence, which demonstrated positive correlation (adjusted $R^2 = 0.48$, $p = 0.011$; Figure 4.1). Error bars were omitted from Figure 4.1 because data for both axes ($n = 11$ species) was transformed into node contrast values ($n = 10$ contrasts arising from speciation nodes) that were independent and identically distributed (see Table 4.1 for standard error of mean spring xylem sap sugar concentrations). An R^2 of 0.48 corroborates my hypothesis about the relationship between seriate number and sugar

storage, especially considering data for both axes was obtained from different individual plants and from different studies. The P-value of 0.011 indicates that slope (of 0.46) of the regression line departed significantly from zero.

Table 4.1. Number of cells in the width of multi-seriate rays, mean spring xylem sap sugar concentration (and standard error) of temperate *Acer*, *Juglans* and *Betula*.

Species	Common number of cells in the width of rays (InsideWood)	Mean spring xylem sap sugar concentration (Table 6.1 for sources)	Standard error of mean spring xylem sap concentration
<i>Acer negundo</i>	2-5	2.27	±0.288
<i>A. platanoides</i>	4-10	2.92	±0.253
<i>A. sccharinum</i>	2-5	2.85	±0.336
<i>A. saccharum</i>	3-8	2.78	±0.125
<i>A. rubrum</i>	2-5	2.66	±0.208
<i>Betula alleghaniensis</i>	2-5	0.84	±0.148
<i>B. papyrifera</i>	1-3	0.88	±0.069
<i>B. pendula</i>	2-5	1.25	±0.196
<i>B. populifolia</i>	1-3	0.61	±0.132
<i>B. pubescens</i>	2-5	1.44	±0.596
<i>Juglans nigra</i>	1-3	2.01	±0.108

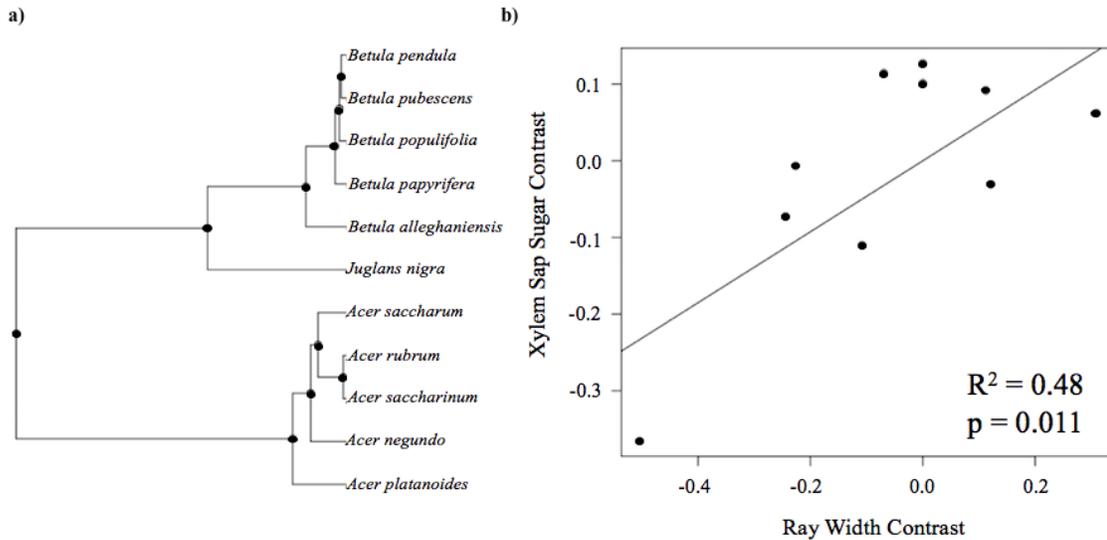


Figure 4.1 Phylogenetic independent contrasts of spring xylem sap sugar concentration versus the number of cells in the width of multi-seriate rays. a) Branch lengths reflect seven gene regions available in GenBank (<http://www.ncbi.nlm.nih.gov/genbank>) and fossil data, circles indicate node contrasts in the phylogeny of the pruned 100-bootstrapped tree. b) Regression of (n=10) contrasts identified in the phylogeny of the pruned tree (Xylem Sap Sugar Contrast = phylogenetic independent contrast of spring xylem sap sugar concentration; Ray Width Contrast = phylogenetic independent contrast of the number of cells in the width of multi-seriate rays) with regression line plotted. Axes scales derived from transformation of original tip data (spring xylem sap sugar concentration and the number of cells in the width of multi-seriate rays) into values that are independent and identically distributed.

4.5 Discussion

Positive correlation between spring xylem sap sugar content and n-seriate number of rays has direct application for syrup producers when targeting species for tapping. High NSC content of spring xylem sap reduces energy input for producers when boiling sap down to syrup. This could further influence tree breeding programs to enhance xylem ray density (storage cells) in progeny of targeted populations. R^2 of 48% is consistent with spring

xylem sap sugar content being caused by RAP NSC storage capacity. A larger sample size would likely increase significance of this relationship by enhancing statistical power when contrasting the number of cells in the width of multi-seriate rays as factors. More rigorous correlative analysis could be conducted by collecting data points for both variables (spring sap sugar content and n-seriate number of rays) from the same individual trees. Furthermore, causation could be shown by analyzing spring sap sugar content (over time) of selected progeny of individuals with known high- and low-n-seriate number of rays. Progeny should be grown in environments with equal nitrogen availability, which has been demonstrated to enhance sap sweetness (Wild and Yanai, 2015). If progeny with a higher n-seriate number of rays were to produce less sweet sap significantly over time, the hypothesis here would be falsified.

High phylogenetic signal reported in spring xylem sap sugar content may be related to saccharide type (i.e. mono- versus di-saccharide). Lignin is impermeable to disaccharides, restricting vessel-loaded sucrose to xylem sap (Cirelli et al., 2008). In contrast, monosaccharides, which rapidly accumulate in xylem sap at positive temperatures (Sauter, 1983, 1988; Améglio et al., 2004), may result in lower observed xylem sap sugar content due to lignin-permeability that enables rapid diffusion into wood tissues. Furthermore, confluent bands of parenchyma that overlay rays in some genera (Cirelli et al., 2008; Carlquist, 2013) may impact NSC storage availability not considered in this study.

5 Chapter: Discussion

How do trees make sweet edible sap in spring? Western (European) and North American (Turtle Island) Indigenous peoples have both developed knowledge of maples related to late-winter/early-spring harvest of sweet edible sap. Western knowledge has been reductionist, whereas Indigenous knowledge has been relational in its understanding of world dynamics. Both western and Indigenous knowledge systems describe temperate broadleaf eudicot trees (gifted) with sweet sap in late-winter or early-spring. Along with maples, Indigenous peoples know birch and walnut trees gift sweet sap between the winter and spring equinox at distinct times and conditions (Naughton et al., 2004; Suzukovich et al., 2015), observations that settler societies began to quantify early in the twentieth century (Clark, 1875; Wiegand, 1906). A reductionist view of this temporal distinction in sap flow can be based on secondary xylem anatomy and pit arrangement that may explain why temperate maple and walnut only have late-winter stem pressure and not just root pressure in early-spring like birch. Further reductionist evidence correlates xylem sap sugar concentration with sites of storage, which are sinks for sugars when trees are photosynthetic and sources of sugar when loaded to spring xylem sap for transport to shoot apical meristems. Thus, trees that can store more sugar can make/gift more sweet sap in spring.

An Indigenous way of knowing explains that each living being was given a way to care for other living beings. Trees that received the gift of sweet sap in spring are also responsible to share that gift by feeding other living beings at a hungry time of year (Kimmerer, 2003, 2013). From a more reductionist view, how trees make sweet edible

sap in spring is implicated with tree vascular status and sugar status following winter dormancy. Capitalist settler societies have lost understanding of interrelationship with maples in effort to reduce the sweet sap that is gifted by maples to a commodity. Large-scale regional exploitation of maples by settler economies is particularly prevalent in Canada, with Quebec regulating market supply and demand by managing stockpiles and quotas (Whitney and Upmeyer, 2004). The science employed by western societies utilizes testable predictions to forward understanding, and can be concordant with Indigenous knowledge that is derived from observation of and relation to the natural world. Often, Indigenous knowledge is either overlooked or underestimated by western societies, partly due to policies of dispossession and disappearance (Tomiak Julie, 2016), but probably more so from fundamental differences in worldview and therefore communication of the natural world. Worldview fundamentally shapes ecological understanding and the nature of interrelationship, how interrelationship is measured, communicated and thus valued. Judeo-Christian teachings that are fundamental to western societies assert a hierarchy of control over nature, with humans at the top and nature beneath to be harnessed for purposes of survival and prosperity, i.e. *scala naturae* (Mebratu, 1998; Geisinger, 1999). Indigenous peoples may consider humans not as separate or superior to other living beings, but instead situated within a web of relationships that provide insight and example for how to live and understand the world around oneself. In the hierarchy of species, Indigenous peoples consider humans as younger siblings of Creation, and so must learn from the elder beings that were here first and have had longer to figure things out (Kimmerer, 2013). As Kuhn (1970: 148–150) remarked, there are “... several reasons why the proponents of competing paradigms

must fail to make complete contact with each other's viewpoints.... Their standards or their definitions of science are not the same.... The proponents of competing paradigms practice their trades in different worlds." Nevertheless, Indigenous knowledge may be used to reshape the imperial and capitalist interrelationship settlers currently maintain with maples and decolonize maple provisions so that Indigenous peoples and communities can reclaim cultural traditions.

Western and Indigenous understandings of the world may be reconciled and with "two-eyed seeing" can be concordant in their way of knowing. For example, Indigenous knowledge explains that although maples are to care for the people at a hungry time of year, they also care for their own survival and spring re-growth. Hungry buds that sense signals from the light-world relay those signals to roots in the underworld (Kimmerer, 2013). A reductionist way of communicating this understanding could state that sink status in spring is typically governed by growing shoot apical meristems that require delivery of sugars for growth. Indigenous knowledge communicates that sugars move upwards to feed buds and are dissolved in water that roots of maple absorb from spring-wet earth (Kimmerer, 2013). Western science instead describes rapid delivery of sugars to shoot apical meristems via the xylem pathway, which experiences recovery from embolisms and restoration of hydraulic conductivity following spring loading of sugars to xylem sap. Indigenous knowledge communicates that only in the time of year when sugars are needed most are they moved this way, and only once leaves have emerged and can make their own sugars will sap return to a watery state (Kimmerer, 2013). Western knowledge again communicates similar findings in that unloading of sugars from xylem

sap at shoot apical meristems is facilitated by phloem Münch flow seemingly until leaves have acquired photosynthetic independence, signaling a functional return of xylem to water transport rather than long-distance transport of sugars. From either way of knowing, it is evident that notions of xylem and phloem as separate systems (with fixed roles) can be dispelled, and that the ‘whole tree’ mobilizes sugars to feed hungry buds in spring.

Consideration or incorporation of Indigenous knowledge alongside mainstream western practices may encourage more robust partnership between Indigenous peoples and settler communities. Following The Truth and Reconciliation Commission of Canada’s 94 “Calls to Action” in 2015 (Bopp et al., 2017), decolonizing maple provisions so that Indigenous peoples and communities can reclaim traditions and cultural values, seems especially pertinent. Notably, multiple “Calls to Action” request various settler governing or religious bodies to repudiate concepts used to justify European sovereignty over Indigenous peoples and lands. Although the Doctrine of Discovery and *terra nullius* are written documents to affirm western notions of dominion (Moody, 2015; Tomiak Julie, 2016), it is also the unwritten worldview of western societies that must be reconciled with Indigenous ways of knowing. Decolonizing maple provisions is a small contribution to this effort. Larger projects to consolidate Indigenous knowledge within mainstream western practices can support Indigenous knowledge systems and advance western worldview to motivate a better understanding of world dynamics.

6 Chapter: Appendices

Appendix A: Supplemental Information for Chapter 4

- Output from generalized linear model:

Call: `lm(formula = sugarC ~ rayW)`

Residuals:

Min	1Q	Median	3Q	Max
-1.0466	-0.4980	0.0000	0.5817	0.9621

Coefficients:

	Estimate	Std. Error	t value	Pr(> t)
(Intercept)	1.1706	0.4614	2.537	0.0388 *
rayW2 to 5	0.7126	0.5651	1.261	0.2477
rayW3 to 8	1.6102	0.9229	1.745	0.1245
rayW4 to 10	1.7527	0.9229	1.899	0.0993

Residual standard error: 0.7992 on 7 degrees of freedom

Multiple R-squared: 0.4325, Adjusted R-squared: 0.1892

F-statistic: 1.778 on 3 and 7 DF, p-value: 0.2387

- Output from phylogenetic generalized least squares fit by REML:

Model: `sugarC ~ rayW`

Data: DF.rosid

AIC	BIC	logLik
19.26081	18.99036	-4.630403

Correlation Structure: `corBrownian`

Formula: ~1

Parameter estimate(s): numeric(0)

Coefficients:

	Value	Std.Error	t-value	p-value
(Intercept)	1.5389993	0.7770195	1.980644	0.0881
rayW2 to 5	0.5785622	0.1876979	3.082412	0.0178
rayW3 to 8	0.8088380	0.4693211	1.723421	0.1285
rayW4 to 10	1.0302352	0.6297281	1.636000	0.1459

Correlation:

	(Intr)	ryW2t5	ryW3t8
rayW2 to 5	-0.156		
rayW3 to 8	-0.132	0.394	
rayW4 to 10	-0.181	0.286	0.294

Standardized residuals:

Min	Q1	Med	Q3	Max
-1.0878361	-0.6571812	0.1306760	0.3849440	0.6181516

Residual standard error: 1.17747

Degrees of freedom: 11 total; 7 residual.

- Output from phylogenetic independent contrasts:

Call: `lm(formula = ContrastsugarC ~ ContrastrayW - 1)`

Residuals:

Min	1Q	Median	3Q	Max
-----	----	--------	----	-----

-0.13236 -0.07552 0.04022 0.10047 0.14641

Coefficients:

Estimate Std. Error t value Pr(>|t|)

ContrastrayW 0.4630 0.1455 3.182 0.0111 *

Residual standard error: 0.1032 on 9 degrees of freedom

Multiple R-squared: 0.5294, Adjusted R-squared: 0.4772

F-statistic: 10.13 on 1 and 9 DF, p-value: 0.01115

Table 6.1. Spring xylem sap sugar content (species listed alphabetically and literature sources sorted from highest to lowest sap sugar content reported) of temperate *Acer*, *Juglans*, and *Betula*. Various analytical methods were used to measure sap sugar content.

*Data collected for *Acer nigrum* was analyzed as a subspecies of *Acer saccharum*.

Species	% sugar content w/v	Source
<i>Acer negundo</i> L.	3.2	Kort and Michiels, 1997
	2.9	Luczaj et al., 2014
	2.7	Blouin, 1992
	2.4	James and Baker, 1933
	2	Munson, 1989
	1.7	Brown, 1914
	1	Randall, 2010
<i>A. nigrum</i> F. Michx*	2.7	Brown, 1914
	2.5	Randall, 2010
	2.5	Gabriel, 1990

	2.5	Munson, 1989
	2.25	Ball, 2007
	1.81	Kriebel, 1955
<i>A. platanoides</i> L.	3.24	Luczaj et al., 2014
	3.1	Blouin, 1992
	2.43	James and Baker, 1933
	2.2	Brown, 1914
<i>A. rubrum</i> L.	3.5	Stevens and Eggert, 1945
	3.25	Morse and Wood, 1895
	2.81	James and Baker, 1933
	2.4	Jones and Alli, 1986
	2.4	Blouin, 1992
	2.25	Ball, 2007
	2	Munson, 1989
<i>A. saccharinum</i> L.	5.15	James and Baker, 1933
	4.3	Kort and Michiels, 1997
	4.01	Luczaj et al., 2014
	3.38	Morse, 1895
	3.02	Morse and Wood, 1895
	3	Williams, 1980
	2.1	Blouin, 1992
	2.1	Brown, 1914
	2	Munson, 1989

	1.75	Zaczek et al., 2003
	1.71	Crum et al., 2004
	1.625	Randall, 2010
<i>A. saccharum</i> Marsh.	5.5	Larochelle et al., 1998
	4.45	Jones and Alli, 1986
	4.2	Kriebel, 1989
	3.8	Kriebel, 1990
	3.25	Marvin et al., 1967
	3.23	Gibbs, 1969
	3	Cortes and Sinclair, 1985
	3	Cameron, 2001
	2.98	Tate, 1973
	2.93	Bryan et al., 1940
	2.88	Morse and Wood, 1895
	2.8	Gregory and Hawley, 1983
	2.79	Johnson, 1945
	2.7	Brown, 1914
	2.66	Jones et al., 1903
	2.62	Wilkinson, 1985
	2.6	Marvin, 1958
	2.6	Blouin, 1992
	2.5	Randall, 2010
	2.5	Safford et al., 1990

	2.5	Gabriel and Seegrist, 1977
	2.5	Munson, 1989
	2.5	Muhr et al., 2016
	2.25	Ball, 2007
	2.2	Saupe, 2010
	2.16	Wilmot et al., 1995
	2.1	Nyland and Rudolph, 1969
	2.08	Kim and Leech, 1985
	1.79	Baker et al., 2009
<i>Betula alleghaniensis</i>		
Britton	1.11	Johnson, 1944
	0.8	Kallio and Ahtonen, 1987a
	0.6	Jones and Alli, 1986
<i>B. papyrifera</i> Marshall	1.1	Brown, 1914
	1	Tate, 1973
	1	Cameron, 2001
	0.9	Ganns et al., 1982
	0.9	Jones and Alli, 1986
	0.68	Johnson, 1944
	0.59	Haq and Adams, 1962
<i>B. pendula</i> Roth	2.46	Luczaj et al., 2014
	1.6	Kallio and Ahtonen, 1987b
	1.2	Kok et al., 1978

	1.15	Kalinichenko, 1974
	0.97	Westhoff et al., 2008
	0.95	Piispanen and Saranpää, 2001
	0.95	Kallio and Ahtonen, 1987a
	0.7	Kallio et al., 1985
<i>B. populifolia</i> Marshall	0.76	Gibbs, 1940
	0.74	Kok et al., 1978
	0.35	Sheldrake and Northcote, 1968
<i>B. pubescens</i> Ehrh.	2.62	Luczaj et al., 2014
	1	Kallio et al., 1989
	0.7	Kallio et al., 1985
<i>Juglans nigra</i> L.	2.2	Matta et al., 2005
	2.08	Naughton et al., 2004
	2.07	Naughton et al., 2006
	1.7	Williams, 1980

A.1 Sub-Appendix A: Bibliography for Appendix A

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7 Chapter: Glossary

ADP glucose pyrophosphorylase – glycosyl donor in the synthesis of starch.

Anishinaabe – Algonquin, Ojibwe (Ojibwa, Ojibway, Chippewa), Potawatomi and Odawa (Ottawa) peoples.

Cytoplasmic invertase – cleaves sucrose to glucose and fructose within the cytoplasm at neutral or alkaline pH.

Cell wall invertase – cleaves sucrose to glucose and fructose at the cell wall and at acidic pH.

Decolonization – a process in which Indigenous peoples and communities affected by colonial expansion, genocide and cultural assimilation, can reclaim cultural values and traditions, while, at the same time, considering the future (Smith, 1999).

Diffuse-porous – trees with little to no distinction in diameter of early-wood versus late-wood vessels.

Fibre – heavily lignified secondary xylem cell, dead at maturity, and often with prominent pitting.

Indigenous knowledge – elders, healers, and hunter/gatherers transmit knowledge to present and future generations typically through sharing of stories, dances, food, medicine and ceremonies. See (Moody, 2015) for definitions, primarily from an Indigenous perspective.

Haudenosaunee Confederacy – Representative of the Iroquois Six Nations (Mohawk, Oneida, Onondaga, Cayuga, Seneca and Tuscarora), Wendat (Wyandot, Huron), Lunaapeew (Lenape, Delaware) and Tutela peoples.

HT – hexose proton-symporter; active transport of hexoses (glucose, fructose etc.) across

the cell membrane.

Münch water – movement of water directed by an osmotically generated pressure gradient between sites of sugar loading and unloading or source to sink.

NSCs (non-structural carbohydrates) – the products of photosynthesis, including mono-, di-, oligo-, and poly-saccharides.

Pitting (simple) – small openings in cell walls of adjacent secondary xylem, unbordered by cell wall lignin deposition, and containing a pit membrane composed of the modified primary (cellulose) walls of adjacent (paired) cells and a middle lamella.

Pitting (blind) – Pits that are considered blind lack a complimentary structure or pair of openings.

Pitting (bordered) – pit has margin of cell wall lignin deposition on both sides of the pit membrane.

Pitting (half-bordered) – pit has margin of cell wall lignin deposition on single side of the pit membrane.

RAP (ray and axial parenchyma) – secondary xylem cells derived from vascular cambia (fusiform and ray cambial initials) that form an extensive three-dimensional network interwoven amongst vascular tissues, and connecting xylem and phloem via rays.

Rays (xylem) – ray parenchyma cells that connect phloem to xylem allowing for radial transfer of solutes and water.

Reductionist – hypothesis testing and empirical analyses that isolate, measure and control natural processes.

Ring porous – trees with large diameter vessels distinctly evident in early-wood.

Shoot apical meristem – region of meristematic growth at the apex of a shoot.

Starch – polymer of glucose that can be linear (amylose) or branched (amylopectin), and can accumulate in large quantities within cells due to osmotic inactivity.

Sucrose-phosphate synthase – plays a role in sucrose synthesis following degradation of starch.

Sucrose synthase (SUS) – cleaves sucrose to fructose and UDP-glucose.

SUT/SUC – sucrose proton-symporter; active transport of sucrose across the cell membrane.

Trehalose-6-phosphate (T6P) – involved in regulation of glucose sensing and signaling within the cell.

Turtle Island – a term used by many Indigenous peoples of the Northeastern Woodlands when referring to North America.

Two-eyed seeing – the bringing together of two ways of knowing to motivate a better understanding of world dynamics.

Vessel-associated-ray-parenchyma cells (VACs) – ray parenchyma cells in contact/adjacent to vessels and involved in solute and water flux between the RAP network and vessel sap.

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