Silicon dynamics in hardwood and conifer trees of temperate forests with a focus on interactions with lignin and tannin metabolism

Roxane Jaffray

Department of Natural Resource Sciences

McGill University, Montreal

April 2018

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Master of Science

ABSTRACT

Silicates, comprised of silicon (Si) and oxygen, are ubiquitous in natural systems and are increasingly well known to provide structural, physiological, and protective benefits for agricultural crops. It was shown to be 10-20 times energetically cheaper to biosynthesize than lignin while having a similar structural effect on cell wall rigidity (Schoelynck et al. 2010). Taken up as silicic acid in plants, Si is reported to have three uptake modes (active, passive, and rejective) (Raven 1983; Sangster and Hodson 1986; Takahashi et al. 1990; Ma and Takahashi 2002). Limited studies however have focused on trees in temperate forests. The objective of this study was to examine how foliar Si concentration varies among 17 species of hardwoods and conifers in relation to lignin, condensed tannins and calcium content. I hypothesized that trees that are high in leaf Si have lower leaf lignin and calcium (Ca), but higher condensed tannin concentrations. The study was conducted at the International Diversity Experiment Network with Trees (IDENT) plantation on the Macdonald Campus in Sainte-Anne-de-Bellevue, QC. Tree leaves were sampled from monoculture plots in each of the four blocks in the summer of 2014 and 2015. Leaf Si concentration was determined through NaOH digestion and colorimetry analysis, and lignin by acid detergent fiber and acid detergent lignin procedures. Condensed tannins were extracted with 50% methanol, followed by a proanthocyanidins assay with acid butanol. Based on Si concentrations and Si/Ca ratios of the different species and multivariate discriminant analysis, I grouped hardwoods into Si accumulators (active), intermediate (passive) and excluders (rejective), and conifers into Si intermediates and excluders. The two very shade tolerant and late successional native species American beech and sugar maple were respectively classified as active and passive Si accumulators. As hypothesized, the active Si accumulating American beech had low leaf lignin and Ca concentrations and high condensed tannin concentrations. Although high in Si, sugar maple was high in Ca and lignin concentration and lower in condensed tannins suggesting a different role of Si in that species. My results also support a different metabolic strategy for hardwoods and conifers with Si (-0.84) being negatively associated with lignin (0.72) and calcium (0.77) in hardwoods and Si (0.79) being positively associated with condensed tannins (0.71) in conifers. Further research is necessary to

determine the underlying mechanisms and functions of Si and how it is intertwined with phenol metabolism, Ca nutrition and plant cell wall components in different tree species.

RÉSUMÉ

Les silicates, composés de silicium (Si) et d'oxygène, sont omniprésents dans les systèmes naturels et sont de plus en plus bien connus pour fournir des avantages structurels, physiologiques et protecteurs pour les cultures agricoles. Il a été démontré être de 10 à 20 fois énergétiquement moins cher à biosynthétiser que la lignine tout en ayant un effet structural similaire sur la paroi cellulaire. Absorbés sous forme d'acide silicique chez les plantes, le Si est rapporté avoir trois modes de captation (absorption active, passive et rejective) (Raven 1983; Sangster & Hodson 1986; Takahashi et al. 1990; Ma &Takahashi 2002). Cependant peu d'études ont mis l'accent sur les arbres dans les forêts tempérées. L'objectif de cette étude était d'examiner comment la concentration de Si varie chez 17 espèces de feuillus et de conifères en ce qui concerne la lignine, tanins condensés et teneur en calcium. J'ai émis l'hypothèse que les arbres qui sont riches en Si foliaire ont des concentrations en lignine et en calcium (Ca) inférieures, mais des concentrations plus élevées de tannins condensés. L'étude a été réalisée dans a plantation du réseau international sur la diversité (IDENT) sur le Campus Macdonald à Sainte-Anne-de-Bellevue, QC. Les feuilles des arbres ont été échantillonnées dans les parcelles de monoculture dans chacun des quatre blocs à l'été 2014 et 2015. La concentration en Si des feuilles a été déterminée par colorimétrie suite à une digestion au NaOH, tandis que la lignine a été mesurée selon la méthode de la lignine détergente acide. Les tannins condensés ont été extraits au méthanol suivi d'un dosage des proanthocyanidines au butanol acide. La concentration de Si et les résultats de rapport Si/Ca ainsi qu'une analyse discriminante multidimensionnelle a permis un regroupement des feuillus selon les trois modes d'absorption du Si (actif, passif et rejective). Les deux espèces tolérantes à l'ombre et de fin de succession ont été classées respectivement comme accumulateur actif et passif. Le hêtre avait les associations foliaires prédites pour une accumulation active en Si avec de faibles concentrations de lignine et de Ca et des concentrations élevées en tannins. L'érable à sucre avec des concentrations élevées en Si avait des concentrations élevées de Ca et de lignine et des concentrations plus faibles en tannins condensés, indiquant une stratégie métabolique différente. Nos résultats suggèrent également un rôle différent pour la Si pour les feuillus et les conifères avec des relations négatives entre le Si (-0.84) et la lignine (0,72) et le calcium (0,77) chez les feuillus et une

relation positive entre le Si (0,79) et les tannins (0,71) chez les conifères. D'autres recherches sont nécessaires pour déterminer les mécanismes sous-jacents et les fonctions du Si dans le métabolisme des phénols, la nutrition en calcium et les composantes de la paroi cellulaire chez les différentes espèces d'arbres.

Dedicated to my parents

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Acknowledgements

First and foremost, I would like to express my thanks to my supervisor, Dr. Benoît Côté, for the opportunity to work with him on this project. His patience, encouragement and scientific teaching have all benefited and inspired me greatly. Furthermore, his guidance and attention to details greatly contributed to the success of this project as well as my overall graduate school experience at McGill.

Secondly, I would like to thank Hélène Lalande, the main NRS department technician, for her great tutoring in the laboratory. I owe a great deal to her help with plant digestion advice, as well as her involvement with the Si and calcium analyses. She was always present when I needed help and her help really guided my successful transition into this master's project.

I would also like to thank Dr. Jim Fyles for being on my committee and his guidance with my project. Many thanks to Stéphane Daigle for his help with data analysis and Emily Mitchell and Peter Tikasz for their help collecting field samples my first summer. Also, I would like to thank Dr. Arif Mustafa in Department of Animal Sciences for his assistance with the lignin analytical work. To Alain Paquette, I am grateful for all the data and information provided for the IDENT project, and the ability to have collected my samples from this research plantation.

My thesis was funded in part by my supervisor through a grant from the Natural Sciences and Engineering Research Council of Canada and by the Tomlinson Fellowship in Forest Ecology. My second year I was awarded a Graduate Research Enhancement and Travel award that permitted my traveling to New York for a Northeastern Ecosystem Research Cooperative conference. Without these generous funding sources, this research would not have been possible.

I am grateful to my network of friends, old and new, who welcomed and nurtured our friendships over the past few years. Many thanks to Greg, Sharni, Barbara, Jesse, Sam, Ashlee, Laura, Ekta, Aidan, Mitalie and many others for listening to my ramblings about silica and plants, and for helping me focus on the happy moments of great things past and still to come. I would like to extend sincere appreciation to fellow graduate students in the Natural Resource Sciences department for their continued encouragement during my stay at Macdonald Campus. I am

thankful for being an honorary guest of the neighboring Dr. E. Bennett's Ecosystem Services lab and Dr. G. Hickey's Sustainable Futures Research lab.

Finally, to my wonderful parents, my sister Amanda and my brother Arman, much love, I can never thank you enough for your unwavering support and encouragement and above all, your belief in me.

Preface

This thesis is original and is the result of independent work done by R. Jaffray in consultation with Dr. Benoît Côté. Some results from the 2014 sampling season (conifer samplings) were provided by Peter Tikasz. The thesis consists of six chapters, and is written as a traditional monograph. The first two chapters are a general introduction and literature review. The third chapter is a description of the materials and methods used in my study. In the fourth chapter, I report on 17 hardwood and conifer species leaf parameters (Si, lignin, condensed tannins, cellulose, calcium) concentrations and the associations between Si concentrations and lignin, condensed tannins and calcium concentrations. The final chapters consist of a general discussion, a conclusion/summary and recommendations for future research. All literature is cited at the end, following an appendix.

1 General Introduction

Canada is a forest nation made up of 3.47 million km^2 of forests (Statistics Canada 2018). Recent Intergovernmental Panel on Climate Change (IPCC 2007) publication reports that forests are severely stressed by disturbances such as insects, diseases, forest fires and climatic events including drought, wind, snow, ice and floods. In the context of ongoing climatic changes, there is the development of new constraints and challenges for plants, indicating that forest plant species could considerably benefit from the incorporation of an element with the ability to alleviate various stresses.

Silicon is known as a beneficial and bioactive element for some plants because of its ability to act as a physical protection to abiotic and biotic stress as well being a biological inducer of plant innate defence responses (Currie and Perry 2007; Van Bockhaven et al. 2013; Meharg et al. 2015; Guerriero et al. 2016). The benefits provided by silica in plants includes increased photosynthetic activity, increased insect and disease resistance, reduced mineral toxicity, improvement of nutrient imbalance, and enhanced drought and frost tolerance (Ma 2004; Fauteux et al. 2005; Balakhnina et al. 2013; Zhu et al. 2014). The application of silicon as a fertilizer (root or foliar supply) is a common supplement for agricultural crops in order to alleviate negative stresses and minimize losses in yield (Ma 2004).

A cell wall bound polyphenol compound, lignin, is responsible for plant structural support. Silicon has the potential to offer functional plant support as a cheaper energy option compared to lignin (Schoelynck et al. 2010). The nature of the association between silica and the cell wall components such as lignin is, however, not fully understood. Herms and Mattson (1992) claimed that when environmental conditions are favourable, plant growth usually has

resource priority over specialized metabolism, such that production of specialized defense compounds and growth become in direct competition for carbon. Plants produce specialized metabolites under specific conditions or environmental stimuli (Ignea et al. 2017), though these are not necessarily synonymous with unfavourable conditions. Rather when carbon is no longer needed for primary metabolism, specialized compounds are more actively synthesized (Bourgaud et al. 2001). The growth differentiation balance (GDB) hypothesis (Herms and Mattson 1992) predicts that environmental factors (such as elevated carbon dioxide CO_2 & tropospheric ozone O_3 concentrations) that favour carbon fixation through photosynthesis exceeding the requirements for plant growth, then there will be an enhanced production of C-based specialized compounds (King et al. 2001; Liu, King and Giardina 2005). This suggests that the reduced competition between plant growth and differentiation can produce a larger concentration and variety of specialized compounds. Plants secondary metabolism pathways produce carbon-based defensive compounds (e.g. polyphenols) that have a wide range of functions including structural support, protection against herbivory and signalling and defence against stresses (Herms and Mattson 1992; Bennett and Wallsgrove 1994). Plant silicon and phenolic studies have been foremost dedicated to agricultural systems with fewer studies examining forest systems. Therefore, this gap in knowledge is where this present study fits in with the greater scientific literature on the role of Si in tree metabolism, defense mechanisms and nutrition.

In this study, I monitored the concentration of Si, condensed tannin, lignin and calcium in leaves of 17 temperate forest tree species in Sainte-Anne-de-Bellevue, Quebec. Leaves were sampled over the growing season of 2015 (June-September) for hardwoods, and in August 2014 for conifers. The primary research question was whether Si-accumulating species have higher

concentrations of tannins and lower lignin compared to non Si-accumulating species. Specifically, I investigated if phenolic concentration in different tree species was a function of Si concentrations. Being likely Si-accumulators and common hardwoods of southern Quebec, *Acer saccharum* Marsh. (sugar maple) and *Fagus grandifolia* Ehrh. (American beech) were of main focus. I hypothesized that Si-accumulating species would have lower lignin levels as a result of benefiting from the potential cheaper energy requirement of Si and its capacity as a strong structural compound (Schoelynck et al., 2010). The substitution of lignin with silicates could result in a decrease in lignin concentration and enable the production of other specialized metabolite compounds (e.g. proanthocyanidins) that contribute beneficial properties to plants as defense against biotic and abiotic stresses.

2 Literature review

2.1 Silicon cycle

The Si cycle is strongly linked with the inventory of CO_2 in the atmosphere due to the weathering of silicate rocks being a primary sink for CO_2 over multimillion-year timescales (Royer 2014; Struyf and Conley 2012). Si plays an important role in primary productivity and carbon cycling in earth systems such that siliceous marine diatoms and terrestrial Si-accumulating plants account for about half of the net primary production for marine and terrestrial systems, respectively (Conley and Carey 2015). Trembath-Reichert et al. (2015) demonstrated that the effects of plants on terrestrial silica cycling dates back to more than 400 million years ago and suggest that in contrast to present day where modern grasses dominate substantially high amounts of silica uptake was carried out by the early plant lineages of bryophytes, lycophytes and some vascular plants. The dominance of diatoms and grasses over the biological cycle appeared over the past 65 million years, and consequently has played a role in the biological filter of silica between land and sea (Conley and Carey 2015). As a result, the vast majority of silica research has been focused on diatoms and graminaeous species, well known as species accumulating large quantities of Si (Ma and Takahashi 2002; Cornelis et al. 2010a; Sahebi et al. 2015), while overlooking Si dynamics in forests species (Struyf and Conley 2012; Cornelis et al. 2010b). Plants largely contribute to the terrestrial Si reservoir with annual soil biogenic silica (BSi, phytoliths) production by plants ranging between 60 and 200 Tmol $year^{-1}$ (Conley 2002), which closely compete with that of diatoms in oceans (240 Tmol -year-1) (Treguer et al.1995). In temperate forests, Bartoli (1983) estimated that the biological cycle of Si is an important factor characterizing deciduous forest ecosystems with 26 kg ha^{-1} yr^{-1} absorbed

through biological uptake (compared to only 8 kg ha $^{-1}$ yr $^{-1}$ in coniferous forest ecosystem) and 85% of the soluble Si derived from biogenic silica (BSi, $SiO_2 \cdot nH_2O$).

2.2 Silica in soils

The primary source of soil Si is from weathered rock and usually is in the form of silicon dioxide and various aluminosilicates where the main Si-rich compounds are inert quartz or crystalline silicates (Epstein 2001; Balakhinina et al. 2013). Silicon is commonly combined with other elements, usually forming oxides or silicates. As such, silicates comprised of silicon and oxygen are ubiquitous in the biosphere.

Granite rock is estimated to contain 74.51% silica, SiO_2, (Jenny 1980) and elemental Si consists about 28% of the Earth's crust mass (Singer and Munns 1999). The soil minerals undergo chemical and biological weathering that enable Si to become a solute in the soil solution (Epstein 2001). The physically and chemically active forms of soluble silicon in the soil are monosilicic acids, polysilicic acids, and organosilicon compounds (Matichenkov et al. 1996; Balakhinina et al. 2013). Soils in the tropics are typically silicon-deficient because of the increased release and leaching of silicic acid due to extreme weathering conditions such as high rainfall and temperatures (Van Bockhaven et al. 2013). Silicic acid is created when silicates are dissolved in aqueous solutions, as shown by the equation: $SiO_2 + 2\ H_2O = H_4SiO_4$.

2.3 Silicon in plants

Silicon is absorbed by plants in the form of uncharged silicic acid, $Si(OH)_4$, a very weak acid (pka >9.5), and is transported upwards in the plant transpiration stream via the xylem (Kaufman et al. 1981). Once absorbed and assimilated by plant roots, the silicic acid is constantly polymerized (biomineralization) into insoluble silica which accumulates in the cell walls, intercellular spaces and epidermis tissues via distillation, as amorphous silica (SiO_2–nH_2O; also referred to as 'opal', silica gel, or phytoliths) (Yoshida 1975; Muir et al. 2001; Ma 2004; Piperno 2006; Ranganathan et al. 2006). Piperno (2006) defines phytoliths as the "silicified remains of plants arising from biological and physical processes of solid silica deposition in intracellular and extracellular regions in higher living plants after the absorption of soluble silica". Phytoliths or 'silica bodies' are hydrogen bonded to cellulose molecules in the cell wall (Epstein 2001; Piperno 2006; Trembath-Reichert et al. 2015). Phytoliths provide an immediate source of Si in soil solution, along with the Si derived from chemical weathering of silicate minerals.

2.4 Si accumulation in plants

Plant species differ greatly in their ability to absorb and accumulate silicon, ranging from 0.1% to 10.0% Si (dry weight) in leaves (Ma and Takahashi 2002). To receive benefits from Si, plants are required to transport silicic acid from a soil solution to different tissues. Two types of Si transporters have been identified thus far, 1) influx and 2) efflux transporters (Ma and Yamaji 2008; Ma 2010). In gramineous plants including rice, barley and maize, both influx (*Lsi1*, low silicon 1) and efflux (*Lsi2*, low silicon 2) transporters for silicic acid have been identified (Ma 2010). *Lsi1* and its homologs are responsible for the transport of Si from the external solution to

the root cells, while *Lsi2* and its homologs transport Si out of the cells toward the xylem (Ma 2010).

Grasses may accumulate Si via mechanisms of passive uptake, and species-specific efflux transporters (Sangster and Hodson 1986; Ma et al. 2007). A specific transport system for the active uptake of silicic acid seems to exist in the roots of Si-accumulating plants (Ma 2003). In rice two different transport mechanisms have been identified for the uptake of Si in solution: 1) a low- affinity transporter (*Lsi1*) discovered on the lateral roots responsible for the uptake of silicic acid from the external solution to the root cortical cells (Ma and Yamaji 2006), and 2) a transporter responsible for xylem loading of Si (Mitani and Ma 2005). In contrast, the latter mechanism of xylem loading occurs via passive diffusion in cucumber and tomato plants (Mitani and Ma 2005).

The difference in Si accumulation between species has been attributed to differences in the Si uptake ability of the roots (Takahashi et al. 1990; Ma and Takahashi 2002; Yang et al. 2014). The active uptake is done via a metabolic process, the passive uptake with the flow of groundwater via plant transpiration, and the rejective uptake actively excludes silica from plant tissues (Raven 1983; Mitani and Ma 2005). The mechanisms responsible for these different uptake modes are not entirely understood since the cell-specificity of Si transporters seem to vary between species (Ma 2010). The difference in the uptake system observed between rice, maize and barely is suggested to be attributed to the root structures, such that in rice roots there are two Casparian strips at the exodermis and endodermis in contrast to one typically present at the endodermis of maize and barley roots (Ma 2010). It has been suggested that low Si accumulating plants have either a defective or non-existent Si transporter from cortical cells into the xylem (Ma and Yamaji 2008). In certain species referred to as Si-excluders, a silica rejection

mechanism possibly exists via a barrier on the outer surface of the roots, a waxy substance that may serve as a protective coating and denying entry to soluble substances (Parry and Winslow 1977; de Bakker 1999; Carey and Fulweiler 2014).

Takahashi et al. (1990) performed an extensive survey of nearly 500 plant species grown on the same soil and determined that plants can be distinguished into Si-accumulating plants and Si-non accumulating plants based on the Si content of the top (mostly leaves) and molar silicon-to-calcium ratio. Si-accumulating plants are discriminated from non-accumulating plants based on a leaf Si content higher than 1.0 % and a Si/Ca molar ratio larger than 1.0 (Takahashi and Miyake 1977; Raven 1983; Agarie et al. 1996). Plants with Si content lower than 0.5% and a Si/Ca molar ratio less than 0.5 are defined as Si-excluders (non-accumulating plants). While, plants with a Si content in between 0.5 – 1% and a Si/Ca molar ratio < 1 are classified as Si-intermediate types.

Within the Si-accumulating species, the accumulated silica can account for 5-20% of the shoot dry-weight (Lewin and Reimann 1969). Plants that accumulate substantially more Si in their shoots are typically from the monocot families Poales, Arecales, Equisetaceae and Cyperaceae, while the Cucurbitales, Urticales and Commelinaceae tend to be intermediate Si accumulators (Ma and Takahashi 2002; Hudson et al. 2005; Currie and Perry 2007). The Poaceae family (grasses) are known to mobilize large amounts of Si and are said to dominate the terrestrial ecosystem (Trembath-Reichert et al 2015). Overall, Si accumulation in the shoots varies considerably among plant species, in general ranging from 0.1 to 10% in dry weight, although values outside this range have been reported (Epstein 1999; Ma and Takahashi 2002). Ma and Yamaji (2006) suggest that most plants, particularly dicots, are unable to accumulate high levels of Si in their shoots. Compared to graminaceous plants especially within agricultural

systems, there is much less data available for the Si contents of forest plant species. Generally it has been found that conifers accumulate low amounts of Si in their shoots relative to hardwoods (Geis 1973; Bartoli and Souchier 1978; Hodson et al. 2005; Tikasz 2016). Recently, sugar maple was found to accumulate high leaf Si in both a monoculture and mixed plot plantation (Tikasz 2016).

2.5 Silicon as an essential element for higher plants

Plant growth and development necessitates the involvement of many different chemical elements. Three criteria formulated by Arnon and Stout (1939) establish if an element is essential: 1) the element must be required for the completion of the life cycle of the plant, 2) it must not be replaceable by another element, and 3) it must be directly involved in the plant metabolism. Primary metabolism consists of the biological reactions essential to maintaining life in living organisms, for example when plants convert sunlight energy to chemical energy and synthesize carbohydrates that are stored and used for differentiation of plant tissues. While secondary metabolism is not directly related to maintaining life, specialized metabolite compounds participate in the reinforcement of tissue, protection against pathogens and plant regulation. Calcium is an essential nutrient for plants due to its involvement in mechanisms such as cell wall stabilization and ion exchange properties (Demarty et al. 1984). Depletion of calcium has been found to contribute to physiological disruptions of fundamental aspects of tree function like photosynthesis and carbohydrate metabolism in red spruce and paper birch species (Halman et al. 2008). In contrast, silicon has not been included in the list of elements considered essential for plants. The dismissal of Si from consideration as an essential element, and the subsequent

minimal literature available on the element, has received criticism over the years (Epstein 1999; Ma 2004; Morikawa and Saigusa 2003; Yang et al. 2014; Sivanesan and Park 2014). The issue of whether the presence of silica in plants confers a benefit has been a subject of considerable debate (Piperno 2006). Studies have shown that silica is an indispensable element for some plant species, for example horsetails or scouring rushes (*Equistem*), rice (*Oryza sativa*) and beets (*Beta vulgaris*) (Piperno 2006; Takahashi and Miyake 1977; Ma and Yamajii 2015). Epstein (1999) explained that the strict definition of essentiality used by plant pathologists who were skeptical of the possible roles played by silica in plants was confusing the issue. For example, it was stated to be difficult in laboratory studies to create and preserve an experimental growth solution that is truly absent of silica. Epstein and Bloom (2005) reassessed the original definition and proposed that an element is essential if it fulfills either one or both of two criteria, (1) the element is part of a molecule which is an intrinsic component of the structure or metabolism of the plant, and (2) the plant can be so severely deficient in the element that it exhibits abnormalities in growth, development, or reproduction. According to this modified definition, Si is considered a "quasi-essential element" for it fits essentiality for some, though not all plants (Epstein and Bloom 2005). Several studies on plant growth have reported that plants deprived of silicon are often structurally weaker and more susceptible to abnormalities of growth, development and reproduction (Epstein 1999; Ma and Takahashi 2002; Currie et al. 2007).

The uptake of silicon by plant species has been reported to alleviate abiotic and biotic stress (Epstein 1999; Ma and Yamaji 2006), and consequently enhance plant resistance (Ma. 2004; Cooke and Leishman 2011; Sahebi et al. 2015). Since the 1950s, silica has been widely used in Japan as a form of fertilizer for rice plants (Ma 2004). Si applications resulted in a significant increase in production and yield of rice plants due to beneficial factors such as

increased canopy photosynthesis resulting from erect leaf blades (Ishizuka 1971), increased resistance to fungi and bacteria (Takijima et al 1949) and decreased accumulation of toxic heavy metals (Agarie et al. 1996). Silicon deposited in the culms, leaves and hull of rice enhance the strength and rigidity of cell walls and deceases transpiration from the cuticle, which strengthens the stem and decreases damage from electrolyte leakage (Ma and Yamaji 2006; Sahebi, et al. 2015). Results of a study on the function of Si over controlling powdery mildew in cucumber displayed that Si is able to produce inactive phytoalexins that are activated by fungal infection of Si-treated plants and lead to cell death of fungi (Fawe et al. 1998). Several studies have researched and reviewed the role of Si on plant tolerance to biotic (Van Bockhaven et al. 2013; Sivanesan et al. 2014) and abiotic stresses (Balakhnina et al. 2013; Zhu et al. 2014) demonstrating that plants of different types attain a number of benefits to growth and reproduction that can be attributed directly to the presence of silica in either soluble or solid form (Epstein 1994; Piperno 2006). Sangster et al. (2001) characterized these major functions as structural, physiological, and protective.

2.6 Si, Lignin and plant cell walls

In the grass family, deposited Si occurs as a layer immediately beneath the cuticle layer with a Si – cuticle double layer being found in the leaf blades of rice (Currie and Perry 2007). The silicification of cells is not restricted to the leaf blades and in rice can also be found within the epidermis and vascular tissues of the stem, leaf sheath and hull (Prychid et al. 2004). The three regions of silicification most recognized are the cell wall, the cell lumen and the intercellular spaces in root or shoot tissues or in an extracellular layer (Sangster et al. 2001).

Early studies on sorghum (*Sorghum bicolor*) plants recognized that not only are large amounts of silicon absorbed, rapidly transported and deposited in leaves but also found silicon deposits to be associated with cellulose layers of the cell walls indicating interactions with polyphenols in xylem cell walls that affect lignin deposition and biosynthesis (Grib and Lanning 1970; Parry and Kelson 1975). Under growth conditions of varying silica treatments [0, 20, 40, 100 ppm of SiO2], the cell walls of the long epidermal cells in Si-treated rice leaves were found to be thicker than in non-treated leaves, which demonstrated the binding of polymerized silicic acids with cellulose forming silico-cellulose membranes (Agarie et al. 1996).

In an attempt to assess long-term silica cycling, Trembath-Reichert et al. (2015) examined Si levels of early diverging land plant clades and with results from electron microscopy and x-ray spectroscopy showed that the most common silica-mineralized tissues include the vascular system, epidermal cells and stomata. With visualization tools like scanning electron microscopy, studies have confirmed the presence of silicified cell wall structures in diverse taxa, including Equisetum, Selaginella, Gnetum and Vaccinum corymbosus species (Trembath-Reichert et al. 2015; Morikawa and Saigusa 2003). Species that invest more in the deposition of amorphous Si have been shown to differ in lignin and cellulose metabolism compared with species that exclude Si (Schorlynck et al. 2010). This follows an earlier idea proposed by Raven (1983) that silica, substituting carbon for some plant functions, can be a compression-resistant alternative to lignin and cellulose. Guerriero et al. (2016) published a recent review on the interaction between Si and plant cell wall components (cellulose, hemicelluloses, callose, pectins, lignin, and proteins).

Lignin polymers are particularly abundant in plant cells that undergo secondary cell wall thickening such as tracheids and vessels (Wagner et al. 2012; Harris 2006). The lignin in woody plants comprise up to 30% of the organic matter of trees that is responsible, along with cellulose, for the stiffness and rigidity of plant stems (Harborne 1988). Primary functions of lignin include plant cell wall reinforcement, water transport facilitation (in xylem), providing compressive strength to conducting tissues and acting as a mechanical barrier to pathogens (Wagner et al. 2012; Boudet 2007). In addition, plants exposed to abiotic and biotic stresses such as pathogen infection, metabolic stress, wounding and perturbations in cell wall structure can induce the biosynthesis of lignin thus changing lignin composition and content (Vanholme et al. 2010; Moura et al. 2010).

By interacting with other molecules of the cell wall, lignin has the functional role of strengthening the plant cell wall and subsequently the whole structure. The structural accomplishment of lignin and cellulose is energetically expensive with values of 6957 kcal kg^{-1} and 4000 kcal kg^{-1}, respectively (Jung et al.1999); in contrast, silica (3.7% the cost of lignin) is indicated to be 10-20 times energetically cheaper while having a similar effect on the cell wall (Raven 1983; Schoelynck et al.2010). This is suggestive of a competitive advantage for plants using silica deposits for support in comparison to lignin-supported plants. Why then are silica deposits not found in large quantities in all species and why do plants make lignin at all? Raven (1983) suggests that there is a trade-off occurring between structural biomolecules within the plant. Because if a plant incorporates silica in leaves, then the added density of SiO_2 (heavier and less versatile once deposited) must be adjusted to, or "paid" for by silicifying the cells of structures below the leaves, possibly a more expensive strategy than simply using lignin.

A recent study by He et al. (2013) confirmed the deposition of Si within the cell walls of *in vitro* cultured rice cells. Si improves the structural stability of cell walls during cell elongation and division and thereby maintained cell shape, which can be considered significant for the function and survival of cells (He et al. 2013). Currie and Perry (2009) demonstrated in *Equisetum* that an increase in the silicon levels in cell walls coincided with material (hydroxyl containing amino acids threonine and serine) of increased positive charge and hydrogen bonding potential. The latter process has an increased likelihood of stabilizing silicic acids by hydrogen bonding interactions (Currie and Perry 2009). These studies however, and few others have not been conclusive on the complex interactions between the components of wall phenols (e.g. lignin) with the components of Si-containing species, leading us to question whether silica can adequately substitute for lignin in the plant cell wall. This present study will help elucidate on whether leaf silica concentration is correlated with lignin and cellulose in temperate forest trees.

2.7 Specialized metabolites

Many specialized metabolites have been observed to protect plants against a wide variety of pests, pathogens and herbivores (Bennett and Wallsgrove, 1994; Wink, 1988). Specialized metabolites are natural substances that are characteristic of plants and appear throughout the plant kingdom. As of yet, these metabolites appear to lack a primary function (i.e. growth, photosynthesis, reproduction) for the organism that produces them. The largest distributed class of specialized metabolites are polyphenols with several thousand different identified compounds (Hassanpour et al. 2011). Polyphenols constitute a distinctive and unique group of higher plant metabolites, and include two broad groups: condensed and hydrolysable tannins more

specifically described as the proanthocyanidins (previously referred to as leucoanthocyanins) and the galloyl and hexahydroxydiphenol esters, respectively (Haslam 1989). Phenols are the monomer units for both lignins and tannins. Apart from lignin, proanthocyanidins represent the most abundant class of natural phenolic compounds (Hummer and Schreier 2008). The word tannin has a long established and extensive usage in chemical and botanical literature, giving rise to a medley of descriptions. The most accepted definition is that of Bate-Smith and Swain (1962) adopted from the earlier ideas of White (1956) which classifies vegetable tannins as water-soluble phenolic compounds having molecular weights between 500 and 3000 g mol^{-1}, and with a capacity to precipitate alkaloids, gelatin and other proteins (Haslam 1989).

Belonging to the broader family of polyphenolic compounds, tannins have the ability to bind and precipitate proteins, creating tannin-protein complexes. Typically located in the vacuoles of the plants, Cannas et al. (2015) suggested that tannins do not interfere with plant metabolism until after cell breakdown and death where they can act and have metabolic effects. Maksimovic et al. (2007) reported that Si nutrition modulates the metabolism and utilization of phenols by stimulating the formation of Si polyphenol complexes; moreover, the polymerization of phenols were found to induce the co-precipitation of Si and lignin in higher plants (Fang et al. 2003). These Si-polyphenol complexes facilitate the synthesis of phenolic alcohols as precursors of lignin biosynthesis (Cakmak and Romheld, 1997). Plant growth typically has resource priority over differentiation such that production of specialized defense compounds and growth are in competition for carbon. The growth differentiation balance (GDB) hypothesis (Herms and Mattson 1992) predicts that when carbon fixation exceeds the requirements for plant growth, then the production of C-based specialized compounds will be enhanced (King et al. 2001; Liu,

King and Giardina 2005). This suggests that the reduced competition between plant growth and differentiation can produce a larger concentration and variety of specialized compounds. Studies by King et al. (2001 & 2005) focused on these predictions in terms of litter quality and decomposition. Cooke and Leishman (2012) tested the association between foliar silica and leaf physical and chemical traits on two different soil types, finding that plants with a high Si concentration had relatively lower concentration of total phenols and tannins, indicating a trade-off in defence investments. In contrast, a study of 18 grass species by Massey et al. (2007) showed a trade-off with growth rate. Many relevant studies articulate the need for further elucidation both of the role of Si in polyphenol metabolism in the plant as a whole as well as the associated structural changes in cell walls.

Objectives

1. Classify common hardwoods and conifers of eastern Canada into three categories of Si-accumulation: active, passive or rejective accumulation
2. Examine the relationship between foliar Si concentration and other leaf components: lignin, condensed tannins, calcium and cellulose

Hypotheses

- Sugar maple and beech will have the highest leaf Si concentration among species tested
- The accumulation of Si in leaves of common hardwoods of eastern Canada over the growing season is correlated with an increase in condensed tannin concentrations
- Si concentration in mature leaves is positively correlated with condensed tannins and negatively correlated with leaf lignin, cellulose and calcium concentrations

3 Materials and Methods

3.1 Site description

My main study site was the International Diversity Experiment Network with Trees (IDENT) plantation located in the Morgan Arboretum of McGill University on the island of Montreal (latitude: 45.4247N, longitude: 73.9390W, elevation: 39 m). Established in spring 2009 on a previously high-input agricultural field, the IDENT plantation (~0.6 ha) has nearly 10,000 trees representing North American temperate forest species. The plantation is fenced to protect trees from herbivory. The IDENT plantation was designed to study the effect of gradients of species richness and functional diversity. Based on a block design with monocultures combinations of two-species and four-species mixtures (Tobner et al. 2013), each community block (6 x 9) was replicated four times for a total of 216 plots (Figure 1). Within plots, trees in mixtures were planted at random with restrictions (at least two of the eight neighbors had to be different species). The trees were planted at 50 cm intervals in plots (5 x 5) of 64 individuals (8 x 8 rows). Local temperature and precipitation measurements for 2014 and 2015 are illustrated in Fig. 2.

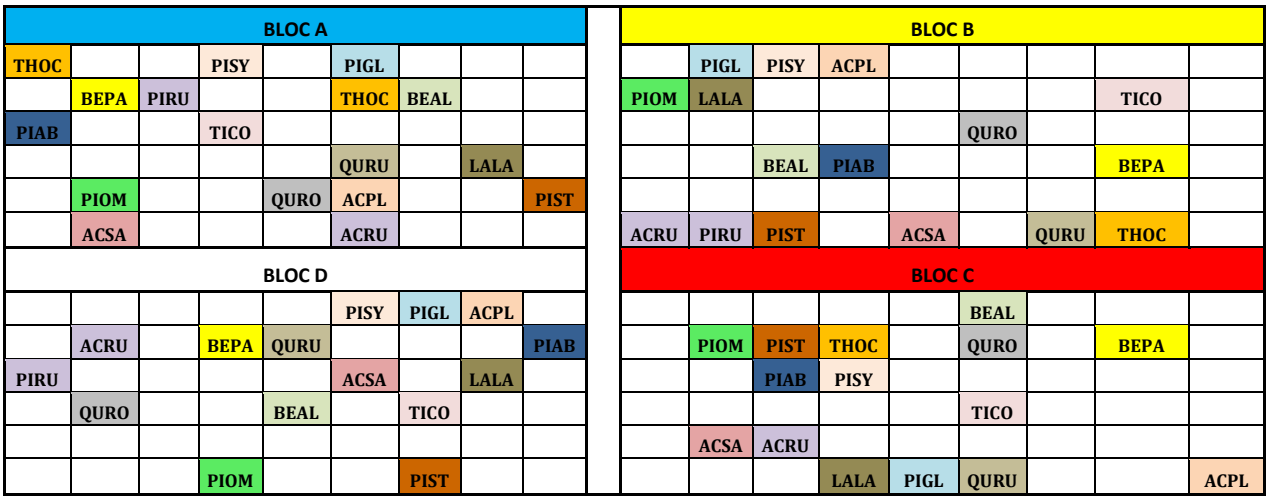


FIGURE 1-Experimental design for IDENT: 4 blocks, each with tree species monoculture plots denoted in colour

TABLE 1 – Species name and IDENT plot legend

Plot code	Common Name	Scientific name	Type
ACPL	Norway maple	*Acer plantanoides* L.	Hardwood
ACRU	red maple	*Acer rubrum* L.	Hardwood
ACSA	sugar maple	*Acer saccharum* Marsh.	Hardwood
BEAL	yellow birch	*Betula alleghaniensis* Britt.	Hardwood
BEPA	paper birch	*Betula papyrifera* Marsh.	Hardwood
QURO	English oak	*Quercus robur* L.	Hardwood
QURU	red oak	*Quercus rubra* L.	Hardwood
TICO	little-leaf linden	*Tilia cordata* Mill.	Hardwood
LALA	tamarack	*Larix laricina (Du Roi)* K. Koch	Conifer
PIAB	Norway spruce	*Picea abies* (L.) Karst.	Conifer
PIOM	Serbian spruce	*Picea omorika*	Conifer
PIGL	white spruce	*Picea glauca* (Moench) Voss	Conifer
PIRU	red spruce	*Picea rubens* Sarg.	Conifer
PIST	white pine	*Pinus strobus* L.	Conifer
PISY	Scots pine	*Pinus sylvestris* L.	Conifer
THOC	cedar	*Thuja occidentalis* L.	Conifer

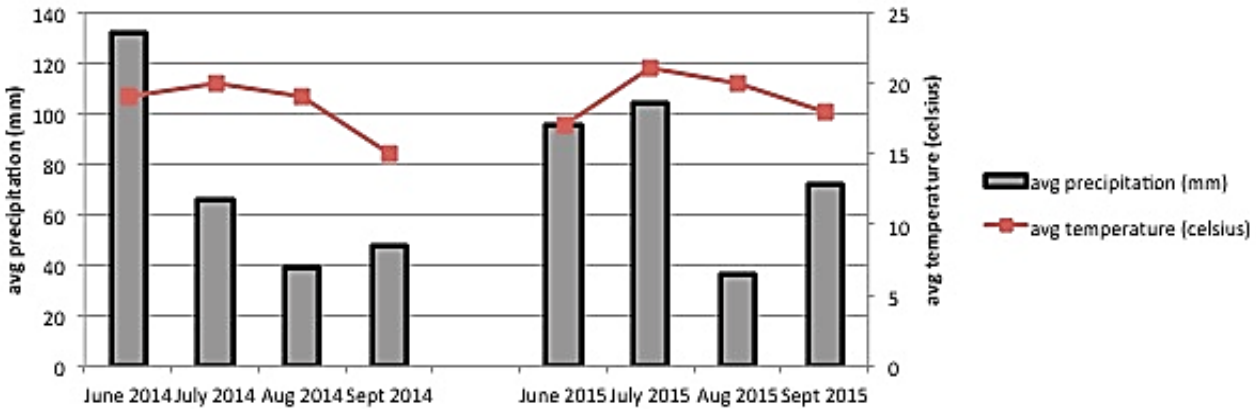


FIGURE 2 -Precipitation and temperature for 2014-2015 growing season, Montreal Quebec [source: Wunderground.com]

3.2 Sampling

Leaves of the hardwood trees were collected once a month from June to September 2015 whereas the needles of conifer trees were collected in August 2014 by Peter Tikasz. The species sampled are shown in Table 1. All species were sampled at the IDENT plantation, with the exception of American beech (*Fagus grandifolia* Ehrh.). American beech trees were sampled in a natural stand less than a km away (Figure 2). This was deemed necessary due to absence of American beech in the IDENT plantation, one of a few species that is likely to be an active accumulator of Si. Trees were sampled from monoculture plots in order to remove the effect of interspecific competition on Si uptake. Three trees within each plot that had direct sunlight and were not along the outer limits of the plot were selected. To minimize contamination from dry deposition, trees were sampled on a day that followed a significant rain episode. One or two branches were cut with a 25-foot pruner and about 20-25 leaves per tree were handpicked. Only healthy trees with leaves free of insect damage were sampled. Leaf samples were similarly collected from ten American beech trees (Figure 3). The samples were stored in paper bags, air dried at room temperature until constant weight and then with the petioles removed, the leaves were mechanically ground into a fine powder using a coffee grinder. The dry samples were stored in plastic bottles and kept at room temperature.

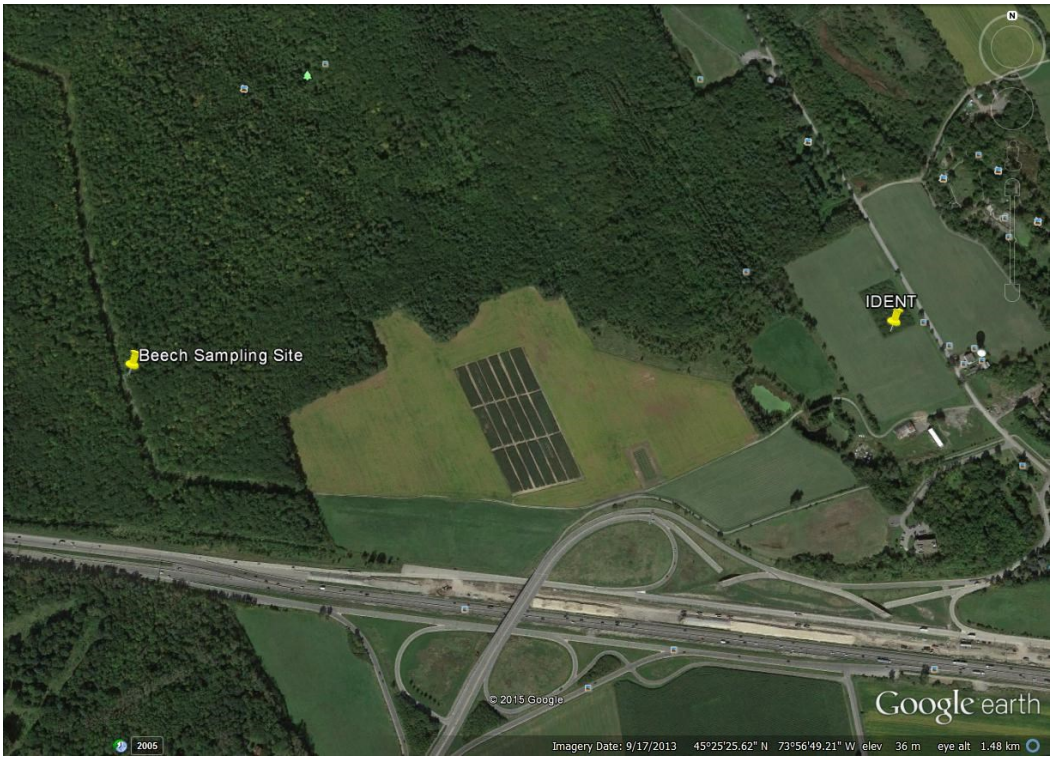


FIGURE 3 -Location of the beech stand relative to the IDENT plantation in the Morgan Arboretum. (scale: 1:8000)

3.3 Foliage analyses

3.3.1 Silicon concentration

3.3.1.1 Oven-induced digestion

Si concentration was determined following an oven-induced digestion (Kraska 2009). The dry, ground tissue (0.1 g) was transferred into 50-mL polyethylene tubes with 5 drops of octyl-alcohol to reduce foaming. Two ml of hydrogen peroxide (30%) were added to the tubes containing the above mixtures, and then placed in a convection oven at 95 ^{o}C. After 30 minutes, 4 mL of 50% sodium hydroxide (12.5 M) was added to the solution and gently vortexed. Once the reaction has subsided, tubes were returned to the oven (95^{o}C) for 4 hours. To ensure a complete reaction, samples were vortexed again within the first hour. After completion of the oven process, ammonium fluoride (1 mL of 5 mM NH_4F) was added to the solution to facilitate the formation of monosilic acid (H_4SiO_4). The solution was diluted to final volume (50 mL) with nanopure water (Milli-Q Water, by Millipure Corp). With 106 samples collected for each month, there were a total of 424 samples analyzed. Each oven digestion batch of samples (sets of 24) was prepared with a blank, a replicated sample and a quality control (sugar maple leaf sample) that were within 10%.

3.3.1.2 Colorimetry method

The colorimetric method of the Inland Water Directorate (1979) was used to quantify Si in the digest. In a 1 L container, stock ANSA, 1-amino-2-naphtol-4-sulfonic acid, was made by mixing 120 g of sodium bisulfate with 4.0 g anhydrous sodium sulphite and 2.0 g 1-amino-2-naphtol-4-sulfonic acid. The solution was completed to 1 L with nanopure water. With 0.5 mL of digest, 2

mL of nanopure water was dispensed in 15 mL polyethylene tubes together with diluted sulfuric acid 0.502 M (1.0 mL), ammonium molybdate 0.03 M (1.0 mL), oxalic acid (1.25 mL), and ANSA working solution (2.0 mL). Working ANSA, a mixture of 50 mL stock ANSA with 250 mL distilled water, was prepared fresh every morning. To end the reaction, 2.0 mL of nanopure water was added and mixed. After letting the mixture stand for 30 mins to 1 hr, this procedure was followed by colorimetry analysis on a LKB Biochrom 4050 Ultrospec II UV/VISIBLE Spectrophotometer at 660 nm to determine the concentrations of Si in the samples. Si standards (0.0, 2.0, 4.0, 8.0, 10.0, 20.0, 30.0, 40.0 mg/L in NaOH) were measured at the same time. Si concentration was determined by interpolation.

3.3.2 Condensed tannin concentrations

3.3.2.1 Extraction

There are a variety of approaches that can be pursued for extraction of tannins. Prior to using the samples from this study, I investigated several assays with different extractant volume, extraction time, and temperature in efforts to optimize the yield of phenolics extracted from this study's samples. With a survey of nine tree species to be made, Waterman and Mole (1994) suggested that it may not be possible to optimize for each species and that an arbitrary selection may have to be made. Aqueous methanol is a popular choice as solvent, while acetone-containing extracts are not recommended in direct proanthocyanidins assay for condensed tannins (Waterman and Mole 1994).

For my study, I therefore decided to use the aqueous methanol method adopted from Waterman and Mole (1994). Dried and ground samples (0.1 g) from early growing season (June)

and late growing season (September) were extracted with 50% methanol (5 mL) in a thick walled glass centrifuge tube. The tubes were placed in a water bath at 50 ^{o}C for 10 min and then sonicated (Cole-Palmer ultrasonic cleaner model 8845-4) for an additional 10 min. Samples were centrifuged (Damon IEC clinical centrifuge) for 15 min. After centrifugation, the supernatants were collected and another 5 mL of MeOH 50% was added to the pellet before centrifugation for 15 min. The supernatants from both extractions were combined and stored at room temperature. For each 24 sample batch, one blank and three samples were replicated and values measured and used had to be within 10%.

3.3.2.2 Analysis

Concentrations of condensed tannins were measured by the proanthocyanidin acid-butanol assay of Porter et al. (1986) with improvements described by Waterman and Mole (1994) and Hagerman (2002). In a glass vial, 6 mL BuOH-conc.HCL reagent (95:5, v/v) and 200 µL iron reagent (2% ferric ammonium sulphate in 2 N HCl) was added to a 1 mL aliquot of the sample supernatant prepared previously, capped loosely, vortexed and incubated in a hot water bath at 95 ^{o}C for 60 min. Absorbance of the extracts was read with a spectrophotometer (LKB Biochrom 4050 Ultrospec II UV/VISIBLE) at 550 nm and compared to a standard curve prepared from aqueous (+) catechin hydrate (Sigma-Aldrich C1788). Because the reactivity of condensed tannins varies by species, concentrations expressed in this study as catechin equivalents should only be used as an index of relative responses and not expressions of absolute amounts. Hence, all A_{550} tannin values are reported in mg/g catechin equivalents of the original sample.

3.3.3 Lignin chemical analysis

Dried leaf samples (0.5 g) from August sampling were pooled per block for each species and were analyzed for acid detergent fibre (ADF) and acid detergent lignin (ADL) according to the procedures of the Association of Official Analytical Chemists (AOAC 1990). In the first extraction, the ADF procedure used an acid detergent solution in an Ankom fiber Analyzer (Ankom Technology, Macedon, NY). The process removed hemicellulose and the resulting ADF residue consisted of cellulose and ADL. In the next extraction, sulfuric acid is used to isolate the lignin (AOAC 1990). The sulfuric acid dissolves the ADF residues and then ADL was calculated from the residues remaining after the acid analysis. The ADF-lignin content was calculated by subtracting the mass before and after, and dividing by the initial sample weight. The method also allowed the determination of cellulose contents. The amount of cellulose (%) was determined by subtracting ADL from the ADF.

3.3.4 Calcium analysis

Ca concentration in leaves sampled in August 2014 was determined following the acid tissue digestion method of Allen et al. (1974). For this procedure, a 160 mg dry leaf sample was put in 4.4 mL of a mixture of concentrated sulfuric acid and hydrogen peroxide, overnight, and then heated to 340 ^{o}C for two hours. Calcium was measured by flame atomic absorption spectrophotometry on a Varian 220 FS. Standards were done in the same matrix with the addition of lanthanum and caesium to reduce interference.

3.4 Data analysis

Prior to all analyses, data were examined for outliers. Values greater than two-standard deviations from the mean (species or month) were removed as outliers from the analysis. A probability level of 5 % was used for all statistical analyses. Figures were created on GraphPad Prism 5 Software, Inc and all statistics were carried out with Statistical Analysis System (SAS 9.4).

3.4.1 Classification of species according to mode of Si uptake

To classify species as Si-accumulators, intermediates, and excluders, one-way paired t-tests were carried out to determine if the average Si concentration and Si/Ca ratio of species were significantly different than the referenced criteria values. Similarly, Student's t-tests were performed to determine if significant changes occurred in each species condensed tannin concentration averages for the June and September samples, and lignin and Ca from the August sample averages. A one-way ANOVA and Tukey's multiple comparison tests were also performed to determine interspecific differences in leaf Si and Si/Ca ratios.

3.4.2 Relationships between leaf Si and other leaf parameters

The relationships between leaf Si and other leaf parameters were analyzed using different approaches: 1) on a species basis for the months of June and September, 2) across species using the average of each species for the month of August, and 3) across species using a multivariate approach (PCA and discriminant analysis). For the first two approaches, linear regressions were

used between leaf Si concentrations and leaf tannins, lignin, calcium and cellulose concentrations at the end of the growing season. For the third approach designed to assess the potential of leaf Si to drive the concentration of lignin, tannins, cellulose and Ca in leaves across a large spectrum of tree species, principle component analyses (PCA) were performed on all species together as well as for hardwoods and conifer species separately. The normality of data was tested using the Shapiro-Wilk statistic. All species were normally distributed except for a few singular exceptions: English oak ($p = 0.03$) for Si, red maple ($p= 0.003$) and white spruce ($p= 0.01$) for lignin data, and white spruce ($p=0.04$) for calcium. Data for condensed tannins was normally distributed. The assumption of homogeneity of variance was tested using the Bayesian information criterion (BIC) (PROC MIXED). Multivariate analyses included a PCA (PROC FACTOR) with a varimax rotation and a discriminant analysis (PROC PRINQUALL). Loading factor values greater than ±0.5 indicated a strong significance, and only the significant factors were tested.

4 Results

4.1 Interspecific differences in leaf chemistry

4.1.1 Silicon

4.1.1.1 Classifying species Si uptake modes: accumulators versus non-accumulators

Leaf Si concentrations of hardwoods (5.2 ± 1.4 mg/g) were on average almost five times higher than for conifers (1.2 ± 0.38 mg/g) and the lowest Si concentration in hardwoods almost matched the highest Si concentration in conifers (Fig. 4a). The high Si concentration of certain species (Si-accumulators) impacted the hardwoods Si average. Sugar maple and American beech had leaf Si concentration higher than 1% (Fig. 4a). Norway maple, red maple and English oak had concentrations in-between 0.5 and 1% Si, while red oak, yellow birch, paper birch, little-leaf linden and all the conifers were lower than 0.5% Si. Amongst conifers, Norway spruce (2.6 ± 0.2 mg/g), tamarack (2.5 mg /g ± 0.11) and white spruce (2.3 ± 0.20 mg/g) had the highest Si concentrations.

The Si/Ca ratio of American beech was the highest of the 17 species studied. American beech and sugar maple had Si/Ca ratios above 1.0, and Norway maple and red maple with values of 0.64 and 0.59 had intermediate values. English oak, red oak, yellow birch, paper birch, little leaf linden and all of the conifers had Si/Ca ratios less than 0.5 (Fig 4b).

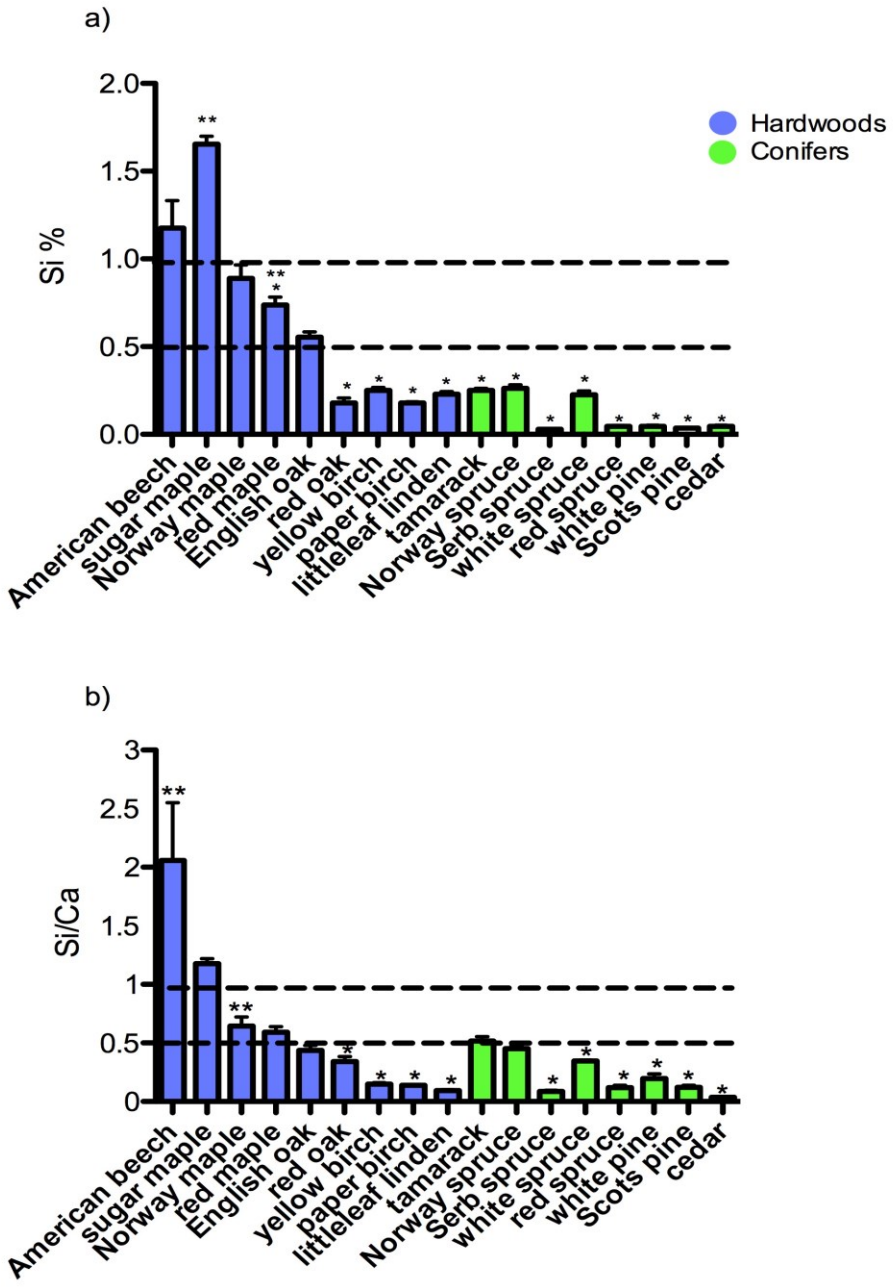


FIGURE 4 -Leaf Si concentration (a) and Si/Ca ratio (b) in August: hardwoods (2015) and conifers (2014). Accumulators are above 1% Si criteria (dotted line) and excluders below the 0.5% Si criteria (dotted line), with intermediates in-between. (**) signify significantly different ($p > 0.05$) value from Si-accumulator criteria (> 1.0); (*) signify significantly different ($p > 0.05$) value from Si-excluder criteria (< 0.5). Red oak Si/Ca ratio result based on Ca concentration from Hallett and Hornbeck, 1997.

4.1.1.2 Leaf Si and condensed tannins over the growing season in hardwood trees

The Si concentration in all species increased over the growing period from June to September (Fig. 5). Red oak was the only species where a minor decrease was observed from August to September. Pooling all species together resulted in a R^2 of 0.198 and a P value of 0.0065 indicating a significant increase in leaf Si from June to September (Figure 5). Thus expressing over the growing season an increase in Si levels (Figure 5). Linear regressions and the associated t-tests applied to each species Si concentration season are shown in Table 2.

For the June sampling, leaves of sugar maple and American beech had the highest Si concentration, with respective Si averages of 5.8 ± 0.38 mg g^{-1} and 4.1 ± 0.31 mg g^{-1} (Fig. 5). Both species also had the highest monthly leaf Si concentration with respective Si averages of 20.5 ±1.14 and 13.5mg/g ±1.0. Norway maple and red maple had intermediate values of 10.4 ± 0.79 mg/g and 10.6 ± 0.67 mg/g, respectively. English oak (6.0 mg/g ± 0.21), yellow birch (2.8 ± 0.20 mg/g), little-leaf linden (2.4 ± 0.13 mg/g), paper birch (2.1 ± 0.17 mg/g), and red oak (1.7 ± 0.20 mg/g) had low Si concentrations.

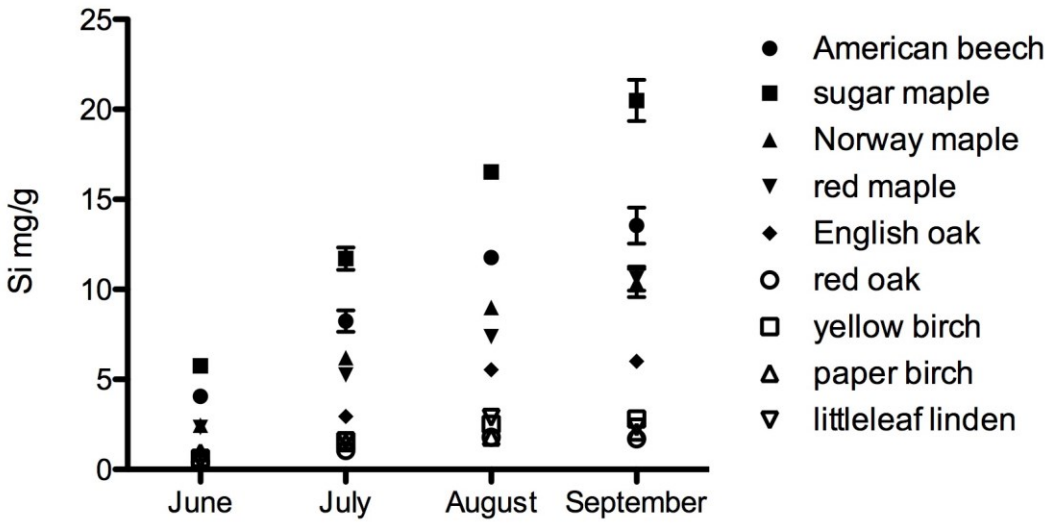


FIGURE 5 -Leaf Si concentrations for hardwood species over the growing season of 2015. Points represent averaged values of 12 trees for each month (10 trees for American beech), with error bars for SEM.

TABLE 2-Statistics for the linear regressions between leaf Si concentration over time (June to September) for the different species of hardwoods

	R^2	P value
American beech	0.97	**0.014**
sugar maple	0.99	**0.004**
Norway maple	0.96	**0.019**
red maple	0.99	**0.003**
yellow birch	0.96	**0.021**
paper birch	0.97	**0.015**
English oak	0.94	**0.029**
red oak	0.86	0.074
little-leaf linden	0.73	0.148

Data in bold represent significant values ($p < 0.05$)

Due to lack of sample materials, American beech was the only species analyzed for condensed tannins for each month of the growing season. Its leaf condensed tannins did not show a significant temporal trend ($R^2 = 0.28$ and $P = 0.47$) (Fig. 6). Averaged month concentrations of condensed tannins were analyzed in the other species for June and September (Fig.7). A two-sided t-test found that both yellow birch ($P = 0.002$) and paper birch ($P = 0.01$) have significant differences in condensed tannin averages for June versus September ($p < 0.05$). Yellow birch was the only species that observed significantly high condensed tannins in June relative to September ($p<0.05$) (Fig.7). While, paper birch had the opposite association in condensed tannin concentrations with the September average greater than June.

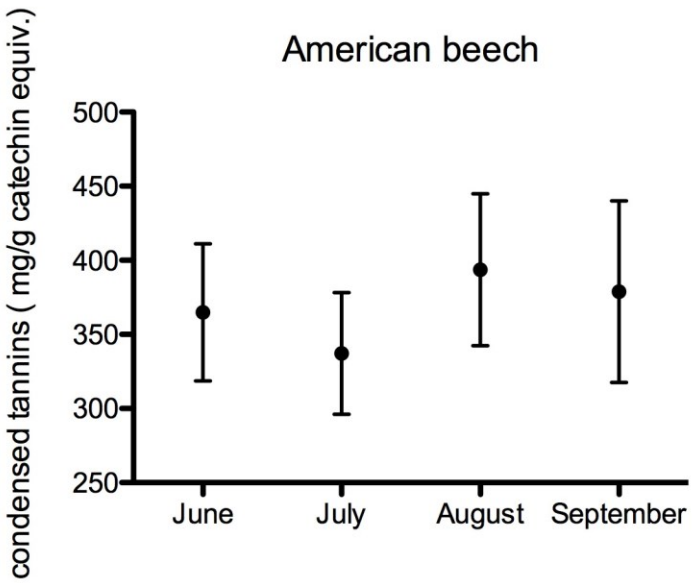


FIGURE 6- Leaf condensed tannins concentrations for American beech over the 2015 growing season. Points represent averaged values of 10 trees, with error bars for SEM.

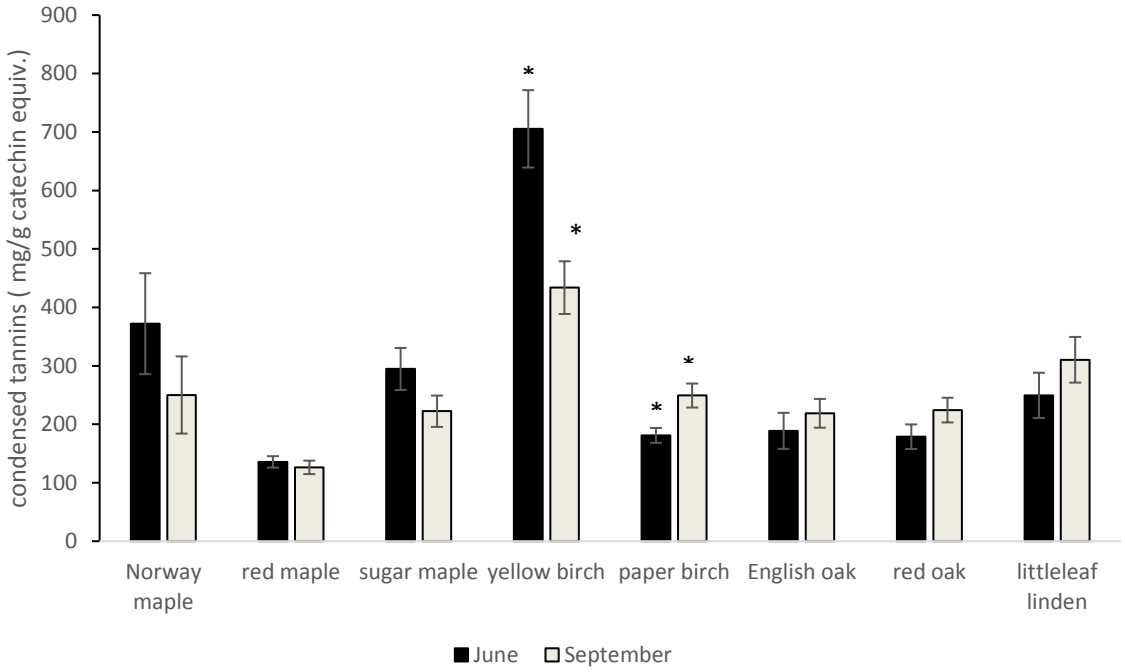


FIGURE 7- Leaf condensed tannins concentrations for hardwoods in 2015 June and September months. Bars represent averaged values of 12 trees, with error bars for SEM. (*) signify significant difference ($p > 0.05$) values.

4.1.2 Leaf condensed tannins

Seven out of the thirteen Si excluder species had low condensed tannins concentrations (≤ 200 mg/g) together with sugar maple and red maple (Fig. 8a). Yellow birch, white spruce and red spruce were highest in condensed tannin concentrations (≥ 400 mg/g) followed closely by American beech (394 mg/g). These aforementioned three species were significantly higher than sugar maple ($p < 0.05$) whereas American beech was significantly higher than red maple, English oak, Norway spruce, Serbian spruce, and white pine and Scots pine ($p < 0.05$). Student's t-tests were performed to produce these statistics.

4.1.3 Leaf lignin

Leaf lignin concentration was relatively low in Serbian spruce, American beech and all maples (Fig. 8b). Species with high lignin included yellow birch, tamarack and white pine. American beech was found to be significantly lower than red oak, yellow birch, tamarack and white pine ($p < 0.05$), while sugar maple was only significantly lower than red oak ($p < 0.05$).

4.1.4 Leaf cellulose

Except for eastern white cedar and tamarack, conifers tended to have a higher leaf cellulose concentration than hardwoods. The conifer Scots pine had a high leaf cellulose concentration relative to all other species. The hardwoods American beech had a cellulose concentration high relative to red maple, paper birch, tamarack, scots pine and cedar (Fig. 8c). Leaf cellulose concentration of sugar maple was not significantly different from any hardwood species or tamarack.

4.1.5 Leaf calcium

Leaf Ca was highest in little-leaf linden, while sugar maple leaf Ca was about average among hardwoods. Overall conifers had lower leaf Ca concentrations compared to hardwood species with the exception of cedar which was highest amongst conifers and higher than American beech ($p < 0.05$) (Fig. 8d).

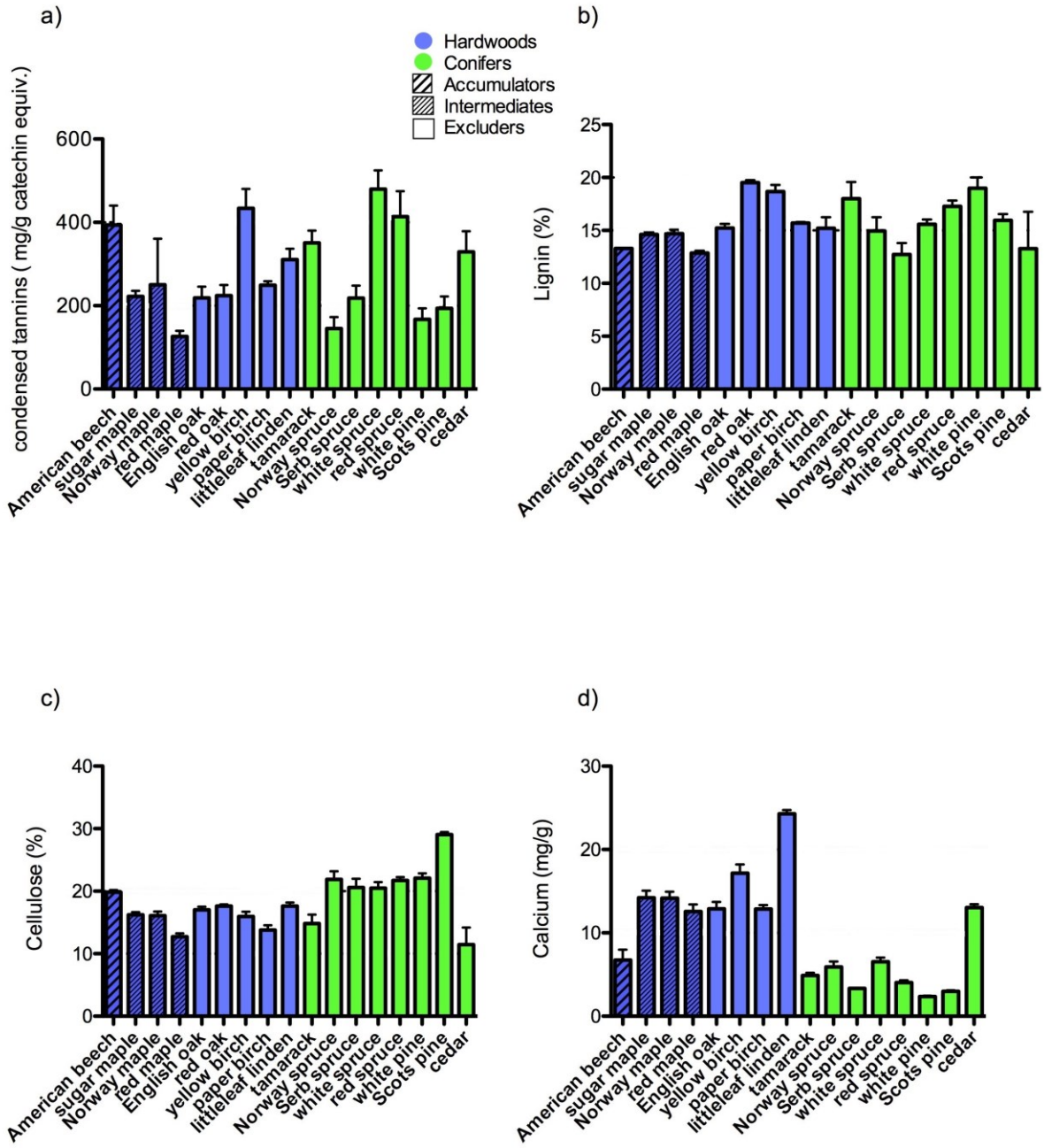


FIGURE 8 -Leaf condensed tannins (a), lignin (b), cellulose (c), and calcium (d) concentrations in August 2015 (hardwoods) and 2014 (conifers). Bars represent averaged values of 12 trees, with error bars for SEM.

4.2 Leaf Si vs other leaf components

4.2.1 Within species relationships between Si and condensed tannins

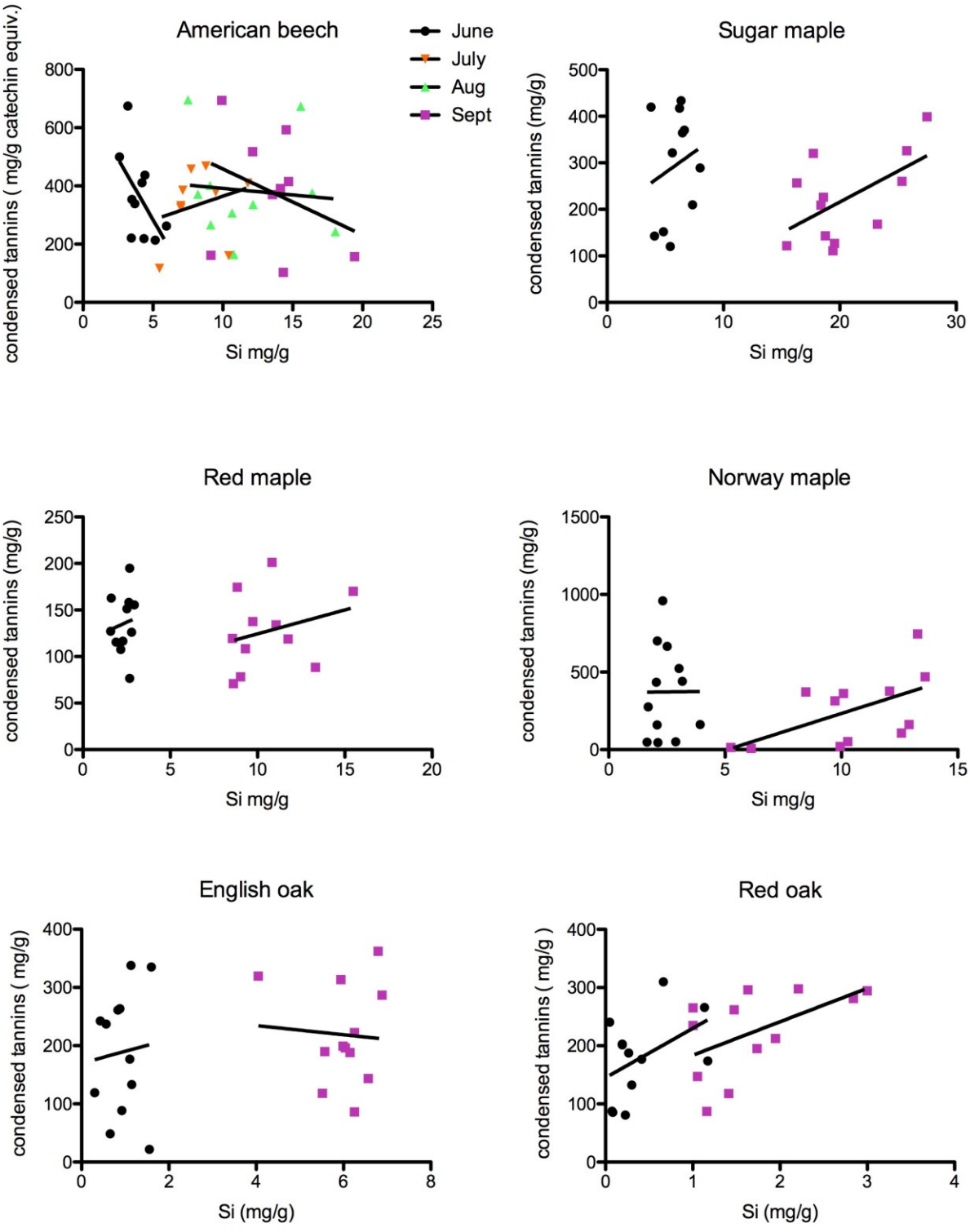
When considering the whole growing season (4 months pooled) for American beech, no significant relationship was detected between leaf Si concentration and condensed tannins concentration (R^2= 0.0017 p= 0.80). When analyzed on a monthly basis, no significant relationships were detected for June (R^2=0.31 p=0.09), July (R^2 =0.07 p=0.49), August (R^2=0.009 p=0.79) and September (R^2= 0.11 p=0.38) (Fig. 9).

For the other species for which analyses were restricted to June and September samples, yellow birch was the only species with a significant increase in condensed tannins concentration with an increase in Si concentration (Table 3). Leaf tannins concentration tended to increase with Si concentration in sugar maple, Norway maple and red oak in September ($p < 0.1$) whereas all other species did not show any trend on both sampling dates (Table 3 and Fig. 9).

TABLE 3-Statistics for the linear regressions between leaf Si concentration and condensed tannins in June and September 2015 for the different species of hardwoods

Species	June 2015		September 2015	
	R^2	P value	R^2	P value
American beech	0.31	0.095	0.11	0.38
sugar maple	0.38	0.56	0.32	0.06
Norway maple	0.00005	0.90	0.32	0.06
red maple	0.02	0.72	0.07	0.42
English oak	0.006	0.81	0.005	0.83
red oak	0.21	0.14	0.29	0.07
paper birch	0.03	0.60	0.07	0.46
yellow birch	0.20	0.15	0.70	**0.0007**
little-leaf linden	0.02	0.65	0.005	0.82

Data in bold represents a significant value ($p < 0.05$)


American beech
June
July
Aug
Sept
condensed tannins (mg/g catechin equiv.)
Si mg/g
Sugar maple
condensed tannins (mg/g)
Si mg/g
Red maple
condensed tannins (mg/g)
Si mg/g
Norway maple
condensed tannins (mg/g)
Si mg/g
English oak
condensed tannins (mg/g)
Si (mg/g)
Red oak
condensed tannins (mg/g)
Si (mg/g)

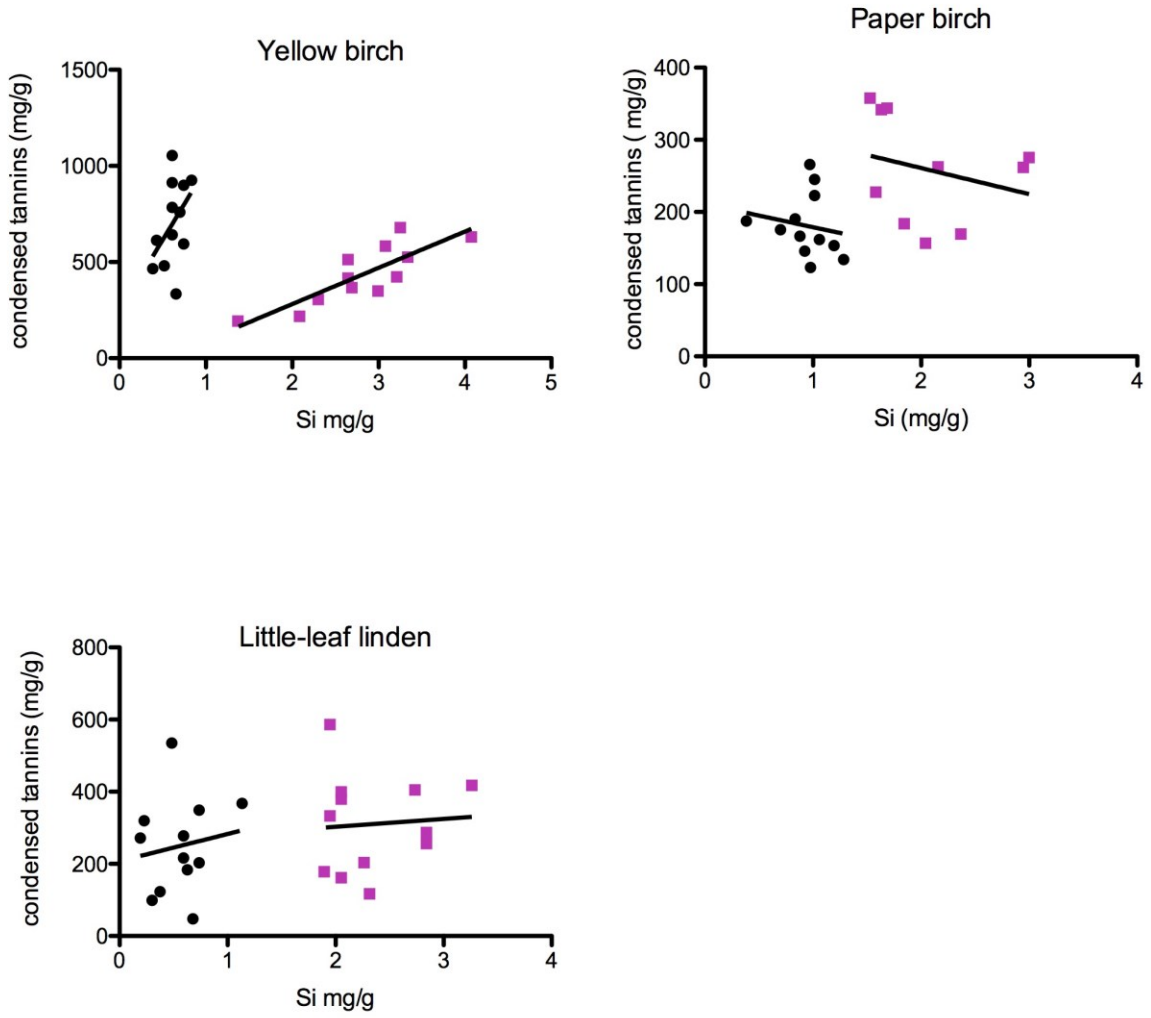


FIGURE 9 -Linear relationships between leaf Si and condensed tannins concentration (mg/g) in June and September 2015 for hardwood species. Points represent each tree sampled, outliers removed.

4.2.2 Across species relationships between Si and other leaf components

4.2.2.1 Univariate analysis

To detect a potential association between leaf Si and the other leaf parameters (lignin, condensed tannins, cellulose and calcium), I performed linear regression analyses for hardwood and conifer species separately and then together. Conclusions to be drawn from the three sets of regression were not different, therefore results are reported for all species only. Of the four parameters tested, the linear regression for Si and lignin was the only one significant (R^2= 0.29 p=0.025) (Fig. 10b). Large variation in all four dependant variables was generally observed for low Si concentrations with intermediate and Si-accumulating species sugar maple and American beech contributing for most of the explained variation by the models.

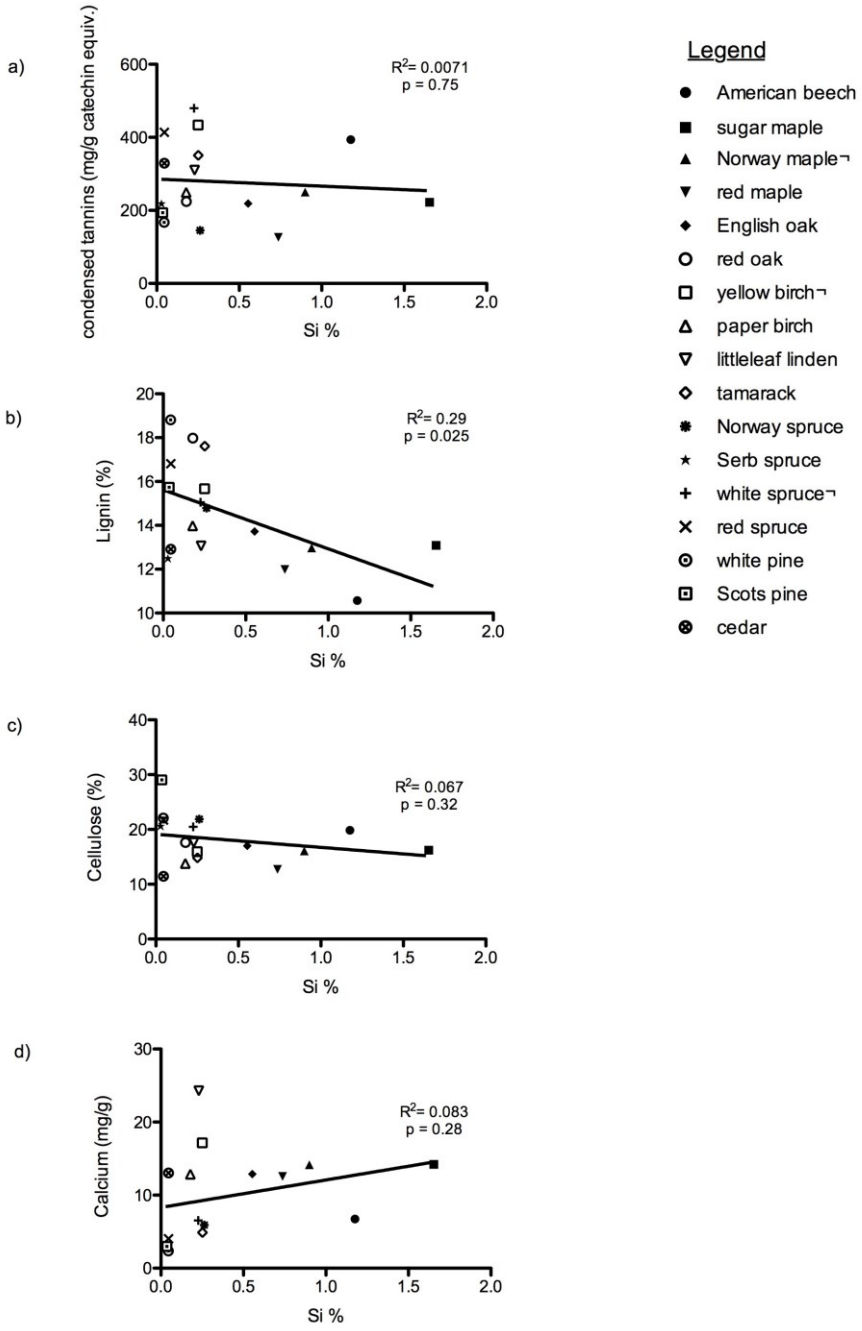


FIGURE 10 -Linear regressions between leaf Si concentrations and other leaf parameters using species average: lignin (a), condensed tannins (b), cellulose (c), and calcium (d). Points represent the average from 12 trees (10 trees for beech) sampled in the month of August.

4.2.2.2 Multivariate analysis

For both hardwoods and conifers, the first factor of the principle component analysis had cellulose as the primary contributor with condensed tannins for hardwoods and lignin for conifers being positively associated with cellulose, respectively (Table 4). Silicon was a significant component of the second factor for both hardwoods and conifers. It explained 33.5% of the common variance in hardwoods, and 24.7% in conifers. For hardwoods, Si was negatively related to lignin and Ca, whereas it was positively associated with condensed tannins for conifers (Table 4).

TABLE 4-Loading matrix of factor solution after Varimax rotation for leaf parameters of hardwoods and conifers. Significant loading values are printed in bold.

Variable	Hardwoods		Conifers	
	(F1)	**(F2)**	**(F1)**	**(F2)**
Si	0.08	**-0.84**	0.04	**0.79**
Lignin	0.49	**0.72**	**0.76**	0.35
Cellulose	**0.82**	0.36	**0.83**	-0.36
Condensed Tannins	**0.73**	0.20	-0.29	**0.71**
Calcium	0.03	**0.77**	**-0.73**	0.31
Variance	0.390	0.335	0.475	0.247

Using the significant variables from the PCA for hardwood species in a discriminant analysis results in the partitioning of Si, lignin and Ca to the upper left quadrat, upper right quadrat and lower right quadrat, respectively. Beech and to a lesser extent sugar maple were found in the upper left quadrat whereas yellow birch and to a lesser extent, paper birch and red oak lined up in the upper right quadrat. Little leaf linden was the only species found in the lower right quadrat (Fig.11). Small intraspecific variation was observed for all species.

Using a similar approach for the conifers, the discriminant analysis shows the upper and lower right quadrat associated with high Si and condensed tannins, respectively. Both tamarack and Norway spruce were found in the upper right quadrat whereas white spruce was the main species found in the lower right quadrat (Fig. 12). All other species were found in the left quadrats and therefore associated with low condensed tannins and Si. Like hardwood species, conifers showed tight species grouping. Although all conifers are Si-excluders, white spruce (PIGL), tamarack (LALA) and Norway spruce (PIAB) tended to have higher Si than other conifers. The Si variable provided a clear differentiation of species into two groups relatively left and right of the (0, 0) mark. The second variable of condensed tannins allowed further differentiation with tamarack and white spruce having high leaf condensed tannins while Norway spruce was low.

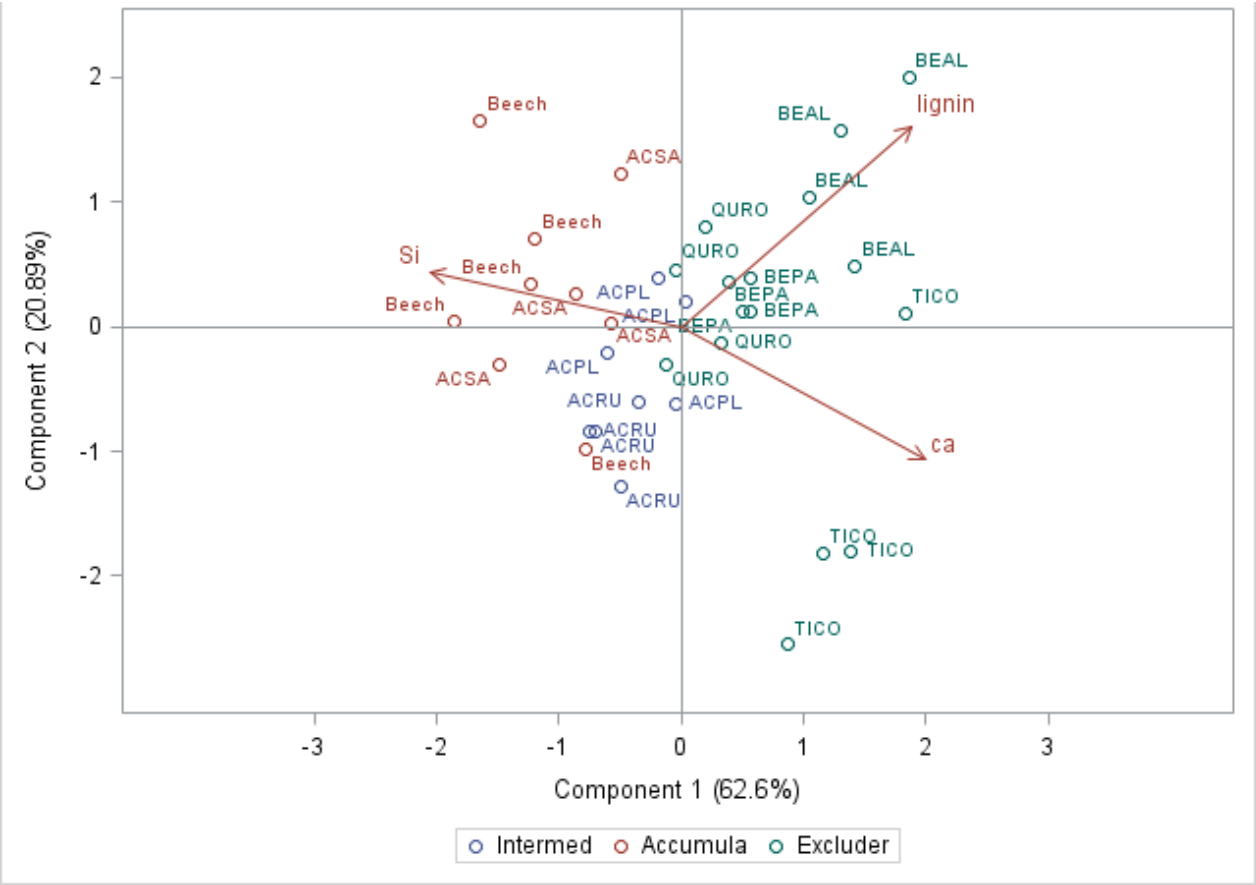


FIGURE 11 -Contribution of each hardwood species to the first two principal components using Si, lignin and calcium concentrations as input variables. Biplot made with PRINQUAL procedure in SAS (SAS 9.4).

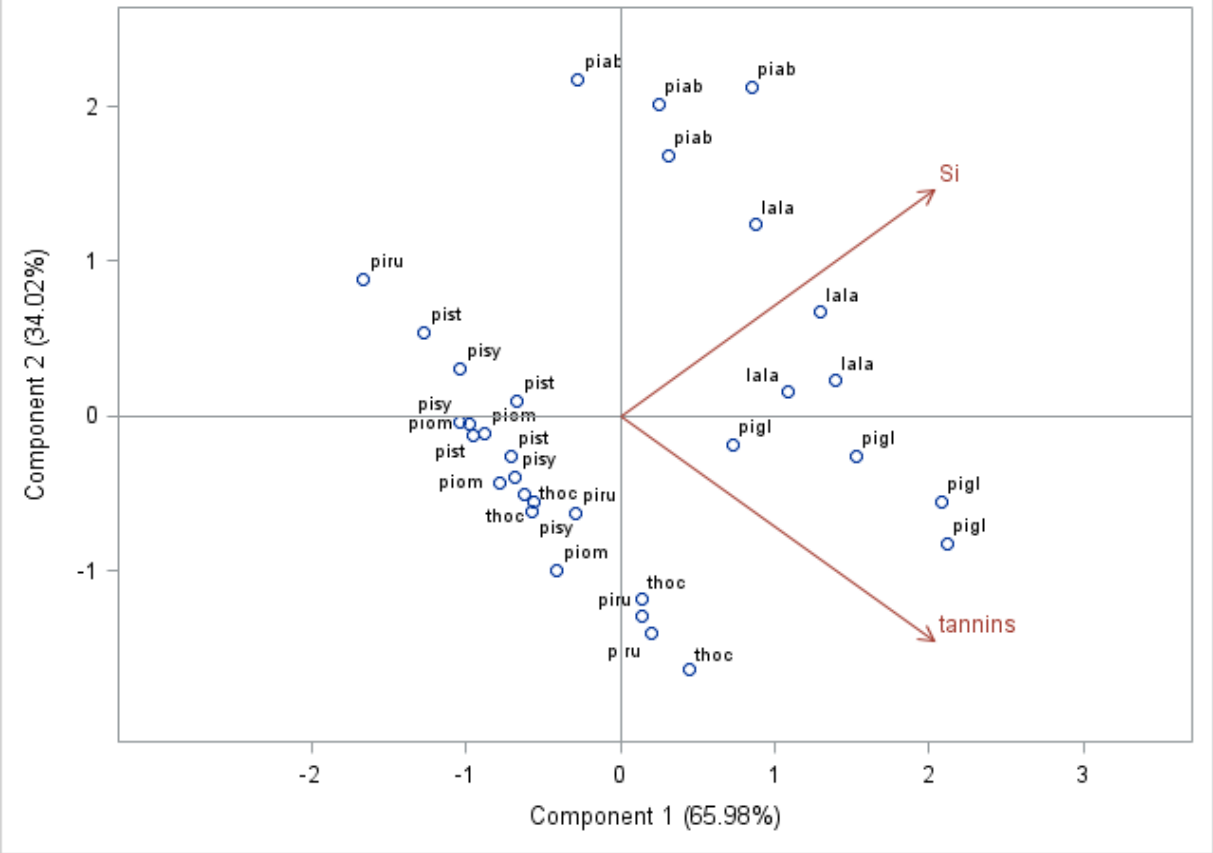


FIGURE 12 -Contribution of each conifer species to the first two principal components using leaf condensed tannins and Si concentrations as input variables. Biplot made with PRINQUAL procedure in SAS (SAS 9.4).

5 Discussion

5.1 Interspecific differences in leaf chemistry

5.1.1 Silicon

5.1.1.1 Classifying species Si uptake modes: accumulators versus non-accumulators

Three possible modes of Si uptake have been discussed based on the concentrations of Si in the aboveground plant tissue and the Si/Ca ratio: active accumulators (> 1% Si and > 1 Si/Ca ratio), passive accumulators (0.5 to 1% Si, 0.5 to 1 Si/Ca ratio), and excluders (< 0.5% Si, <0.5 Si/Ca ratio) (Takahashi and Miyake 1977; Takahashi et al. 1990). Based on these criteria, the hardwood species American beech is considered an active Si-accumulator, sugar maple, Norway maple and red maple are passive accumulators, and the oaks, birches and little-leaf linden and all the conifers are excluder species (Figure 4.2).

The amount of deposited silica in plant materials is partly determined by the concentration of silicic acid (H_4SiO_4) in the soil solution and it is important to note that the classification criteria of Takahashi et al. (1990) was based on the assumption that soil Si concentration is 10ppm and water requirement is 500 mm $year^{-1}$. If the assumptions are defined differently, such that the soil concentration is more or less than 10ppm, then the classification becomes more of a tentative definition. Cooke and Leishman (2011) challenged the Si uptake mode grouping of Takahashi and Ma (2002) and considered it somewhat arbitrary and instead suggested that leaf Si concentration should be considered as a continuous spectrum. Zhu et al. (2014) demonstrated that both passive and active Si uptake can coexist in plants. This could be one possible explanation for sugar maple, for which its high Si concentration matched the active accumulating criteria but its high Ca concentration resulted in a relatively low Si/Ca ratio that

does not match the criteria for an active Si accumulator. This points to the difficulty of classifying species, particularly those that border criteria thresholds because the boundaries cannot be clearly defined. I propose that based on the requirement that an active accumulator has both a concentration greater than 1% Si and a Si/Ca ratio larger than 1, sugar maple is an intermediate (passive) species. If also considering the criteria as a spectrum then sugar maple is a borderline active accumulator species.

Sugar maple, American beech, Norway maple, paper birch, yellow birch and red maple species in my study had slightly higher Si concentrations in comparison to a similar study done by Tikasz (2016). Both studies used the same IDENT plantation which provides a good basis for comparison. With the exception of sugar maple, the classification of species into the three Si uptake classes (Table 5) is the same. Other Si concentrations from the literature are also reported in Table 5 for comparison. The weather could explain some of the differences between the studies. High levels of precipitation were observed in June 2014 but low levels were observed in September, while the growing season of 2015 had average precipitation throughout (Fig.2). Additionally, the temperature was lower in September 2014 compared to 2015. High temperatures paired with wet conditions would enable higher transpiration rates and more Si accumulation, while high temperature with low precipitation would reduce transpiration.

Past studies have proposed that the Si amounts cycled in forest ecosystems may be species-dependent with angiosperms enhancing the weathering to a greater degree than gymnosperms (Moulton et al. 2000; Johnson-Maynard et al. 2005). Bartoli and Souchier (1978) demonstrated that tree species can have an impact on the biological turnover of Si in temperate ecosystems such that Si-uptake by a deciduous beech forest ranged from 36 to 46 kg ha^{-1} yr^{-1} in

contrast to about 7 kg ha^{-1} yr^{-1} uptake by a pine forest. Their study however can be considered an extreme case since they compared a forest dominated by a Si accumulating species versus a forest with rejective coniferous species, thus not an adequate representation of less Si accumulating tree species. Across species, Clymans et al. (2016) showed that the majority (65%) of biogenic Si in northern hardwood forests is stored in woody biomass (wood and bark) and 35% in foliage. Though the slow decomposition of wood resulted in only about 2% of annual biogenic Si dissolution coming from decayed wood, indicating leaf litter functions as the dominant source of Si to the forest floor. As such, the use of foliage samples in my study is a reasonable and effective way of measuring Si concentration present in the tree species and can contribute to estimations of Si returning to forest soil.

Relative to hardwood species, conifers generally accumulate low amounts of Si in their shoots (Geis 1973; Bartoli and Souchier 1978; Hodson et al. 2005). My results for conifers are in agreement with this observation with most species having Si concentration below 0.5%, and being classified as Si-excluder species using both distinguishing criteria of Si concentration and Si/Ca ratio. Possible exceptions may include the deciduous tamarack and exotic Norway spruce with a Si/Ca ratio that was higher than for the other conifers and as high as for many hardwoods. Its Si/Ca ratio was however not significantly different than the 0.5 Si/Ca ratio threshold given the error associated with the threshold, thus could be considered as passive accumulators. Similarly, English oak with Si concentration slightly above and Si/Ca ratio below the criteria thresholds is a borderline rejective/passive species. Further research with a controlled environment experiment like hydroponics could lead to better understanding of the uptake mechanisms involved in these species. Indeed, in a past hydroponic study, Si absorption in Douglas fir and black pine was

reported via mass balance to have been driven by passive Si transport at forest soil solutions (0.2mM Si) and was rejective at higher Si concentrations in nutrient solution (Cornelis et al. 2010b).

TABLE 5-Leaf Si concentrations from this and other studies. Data are averages for each species.

Species	Common name	Si (mg/g)		
Fagus grandifolia	American beech	**9.40**[1]	7.24[2]	6.65[3]
Acer saccharum	Sugar maple	**13.62**[1]	8.44[4]	5.60[2]
Acer platanoides	Norway maple	**7.00**[1]	3.45[4]	
Acer rubrum	Red maple	**6.38**[1]	5.87[4]	
Quercus robur	English oak	**3.85**[1]	4.24[4]	
Betula alleghaniensis	Yellow birch	**1.87**[1]	1.81[4]	1.40[2]
Betula papyrifera	Paper birch	**1.57**[1]	1.30[4]	1.65[3]

1 -This study (in bold); 2- Garvin (2006); 3- Hodson and Sangster (1999); 4- Peter Tikasz (2016)

5.1.1.2 Si over the growing season in hardwood trees

The Si concentrations in all hardwoods, except red oak and little-leaf linden species, were observed to significantly increase over the growing period of June to September, with the biggest increases seen in the Si-accumulating species and the smallest in the Si-excluding species (Fig.4 & Table 2). Bartoli and Beaucire (1976) noted that foliar Si continuously accumulate during the growing season with Si concentrations in the fall being five times that of spring. In my study, the largest increases were observed in sugar maple which had a four times fold increase whereas American beech had about a three times increase by the fall sampling.

Although sugar maple had a higher Si concentration than American beech, it was not classified as an active Si accumulator because of its relatively low Si/Ca ratio. How then did sugar maple have a higher average Si concentration and a greater rate of accumulation than the classified active accumulator American beech? Sugar maple had unusually high Si concentrations in this study and it was significantly higher than in the nearby natural forest site, while American beech and yellow birch did not have significant differences (Figure 13-appendix). Leaf Ca in sugar maple was also high, the likely result of past liming of the plantation site. These two unusual elemental concentrations may have resulted in a Si/Ca ratio that is not representative of the natural forest where nutrients are typically more limiting. In such an environment, sugar maple may have been able to demonstrate an active uptake of Si like American beech did in the nearby forest.

Sugar maple and American beech are both shade tolerant species that are common in temperate forests of Quebec. Sugar maple is considered better at exploiting gaps (Canham 1988) while in comparison American beech saplings have been found to have higher survival (Kobe et

al. 1995) and growth rate under low light conditions (Canham 1988). Shade tolerant species have the ability to grow successfully under the canopy without full sun and this necessitates the efficient use of nutrient and carbon resources. Trees with limited access to sunlight and shorter time frames for leaf growth may prefer utilizing leaf Si that can contribute to a more competitive carbon strategy. Raven (1983) calculated that 27 times less glucose was required to incorporate a mole of silicon into cell walls than a mole of carbon. Some studies have found evidence that plants with shorter leaf life spans have higher Si concentrations (Cooke and Leishman 2011), and higher photosynthetic capacity (Matsuki and Kioke 2006). The high Si concentrations of both American beech and sugar maple in my study does not support these claims for hardwoods. American beech is known to retain some of its leaves late in the fall whereas the timing of leaf senescence in sugar maple is not different than for most hardwoods. The generally lower Si concentrations observed in conifers compared to hardwoods in my study would however be consistent with the observation of Cooke and Leishman (2011). It is possible that energy saving associated with high Si accumulation may only be large enough to be detected when comparing plants with large functional differences, such as hardwood species higher in Si than conifers.

5.1.2 Leaf condensed tannins

American beech was the only species analyzed over four months. Based on the June to September data shown in Fig. 5, there was no temporal trend for condensed tannin concentrations in American beech. Since Si concentration increased steadily (Fig. 4), my hypothesis of increasing condensed tannins and Si concentrations in active Si uptake species over the growing season was rejected. A lack of association between Si and condensed tannins

may not be unreasonable considering that Si is accumulated mostly within the foliar epidermis (Piperno 2006) while condensed tannins are usually stored in cell vacuoles (Stafford 1988). As such, the physical distance and physiological barriers may prevent condensed tannins from potential interactions with Si in the structural matrix. Limited data is currently available concerning the effect of time over the growing season on condensed tannins for temperate forest trees. Early studies by Feeny and Bostock (1968) found a seasonal increase in condensed tannins of English oak, with appearance starting in May. This is supported by this study's findings for paper birch, however it is contradicted by the lack of any difference observed in the other species, with the exception of yellow birch (Fig. 7). Yellow birch was found with significantly higher condensed tannins in June relative to September ($p<0.05$) (Fig.7). A study in the Swedish boreal forest on dwarf-shrub species determined that certain phenolic metabolites, particularly phytotoxic batatasin-III, reached maximum concentrations in September (Nilsson et al. 1998). Howard et al. (2003) concluded that variation in total phenolics, total anthocyanins and total flavonols of cultivar blueberries was greater among genotypes than between growing seasons, and suggested that certain genotypes vary in their capacity to synthesize phenolics under different growing conditions. The results from Howard et al. (2003) however are less pertinent in making comparisons to my study because mine was based on a within growing season experiment.

Species that demonstrate similar Si uptake mechanisms could possibly follow common mechanisms for defensive phenolic compounds like condensed tannins. However, the distribution of tannins within leaves is considered highly species specific (Krauss et al. 2003) and large genetic variation in defensive strategies (e.g. phenolics) have been demonstrated even

within a natural population of birch (*Betula pendula* Roth) (Yamaji et al. 2003) indicating the complexity of drawing conclusions from even a single species. Baldwin et al. (1987) found in sugar maple and yellow birch that leaf weight was positively correlated with condensed tannins while negatively correlated to protein binding capacity suggesting that tannin synthesis may be influenced by leaf expansion and growth. A similar result was reported by Zucker (1982), who showed that the largest *Populus angustifolia* leaves had the lowest total phenolic concentrations. Differences were acknowledged however between samples from forest trees and plantation grown trees. Furthermore, the leaf tannin measurements were found to vary substantially among individual trees potentially because elevated tannin contents in some trees were generated by previous damage or infection (Baldwin et al. 1987). In my study the sampling method specified that unhealthy leaves were not chosen in order to eliminate the effect of stress. To further knowledge about the regulation and structure of the phenylpropanoid pathway, Kosonen et al. (2015) suggested that inhibiting the expression of biosynthesis pathway precursor enzymes, such as anthocyanidin reductase, using the RNA interference method could be a valuable tool for investigating the relationship of condensed tannin accumulation with plant growth over time.

5.2 Leaf Si vs other leaf components

5.2.1 Within species relationships between Si and condensed tannins

The association between leaf Si and leaf condensed tannins was measured for all hardwood species in June and September, while American beech was analyzed over all four months of the growing season. September samples were chosen to represent maximum phenolic concentrations

along with maximum Si concentrations. No positive correlation was observed between concentrations of Si and condensed tannins in the active Si accumulating American beech. These results do not support my hypothesis and instead, the September results show a weakly negative association. Plants with high Si are debated to need less phenolic- and tannin-based defences because Si itself can be used as an effective herbivore defence (Cooke and Leishman 2012) and/or because a trade-off exists between foliar ability in production and maintenance referred to as the 'cost–benefit hypothesis' (Mooney and Gulmon 1982) that causes competition between plant growth and specialized metabolites (differentiation) for carbon resources (King et al. 2001). When carbon resources exceeds the requirements for plant growth, then the production of C-based specialized compounds will arguably be enhanced (Herms and Mattson 1992; Liu, King and Giardina 2005).

American beech, Norway maple and English oak had some observed seasonal variation in regressions (Fig. 9) such that the relationship early in the season was different than that of the relationship in September. Only American beech had results for condensed tannins and Si over the four sampling months. The increasing Si concentration in American beech over the growing season (Fig. 5) was paired with condensed tannin levels that stayed generally the same throughout the season (Fig. 6). Norway maple samples show a non-significant decrease in condensed tannin concentrations while observing an increase in Si concentrations for the months of June and September (Fig. 6 & 7).

Overall, high or low leaf condensed tannins concentrations could not be linked to any particular grouping of species whether it be by type of Si uptake (passive vs active) or growth form (conifers vs hardwoods) (Fig. 9). A weak positive association between foliar Si and

condensed tannins was observed in sugar maple, Norway maple and red maple but correlations were not significant. Yellow birch was the only species to have a significant association between Si and condensed tannins ($p<0.05$). The high condensed tannins results for yellow birch represents an outlier among the other Si excluder species but is consistent with my hypothesis of increasing condensed tannin concentration with increasing Si. However, the fact that Si was low in yellow birch suggest that other interactions are likely involved. Yellow birch's high condensed tannins could be representative of an interaction with a leaf component other than Si. For instance, manganese (Mn) is known to be in high concentrations in yellow birch. Mn is involved in the synthesis of phenols and lignin which can consequently impact metabolic functions (Graham et al. 1988). Mountain birches have been found to have increased leaf phenolics (+catechin and gallic acid) when in proximity to polluting sources which was proposed to be related to the effects of contamination on the shikimate and phenylypropanoid pathways (Loponen et al. 2001).

5.2.2 Across species relationships between Si and other leaf parameters

5.2.2.1 Univariate

To further investigate the potential function of Si in tree leaves, I analyzed the relationship between leaf Si and lignin, condensed tannins, cellulose and calcium concentrations of all species. These results provide insight on leaf Si relationships with other leaf parameters at a scale greater than individual species. Si and condensed tannins have arguably similar defensive functions (Cooke and Leishman 2012) that can provide plants with advantageous options against abiotic and biotic stresses, while cellulose (Agarie et al. 1996), lignin (Wagner et al. 2012;

Boudet 2007) and calcium (Maathuis 2009) have important structural roles for cellular support that could overlap with Si. My results show a significant and negative relationship between Si and lignin but no significant relationship between Si and condensed tannins, cellulose and Ca,

Of the four hypotheses postulated, only one passes the test. Results show that foliar lignin is the only leaf constituent measured that seem to respond to increasing levels of leaf Si. This is an interesting result because the premise for the hypothesis for higher condensed tannins was based on the assumption that Si adequately serves the structural role of lignin (Schoelynck et al. 2010) and would therefore free phenolic precursors for defensive chemicals production. In rice crops, Si treated plants had been observed to decrease lignin biosynthesis precursors (phenolic ferulic and p-coumaric acids) (Goto et al. 2003). Similarly, the leaf tissue concentrations of coniferyl alcohol and coumaric and ferulic acids showed a tendency to be lower in the Si-treated cucumber plants (Maksimovic et al. 2007). Certain challenges had previously been identified regarding the Si and lignin trade-off such as the possibility that the added density of silica in leaves requires the plant to reinforce, and then potentially silicifying the cells of structures beneath the leaves (Raven 1983). It remains unknown whether this strategy would then result being as expensive as simply using lignin. However the results demonstrate that high levels of Si are associated with low lignin concentrations across species indicating the occurrence of an energy trade-off in temperate forest trees.

I had hypothesized that leaf Si would be a useful resource for species, especially those that can accumulate high Si like American beech, due to the wide array of mechanical and biological benefits derived from Si. For some species however, the extra mass acquired by the denser Si may be a limiting factor that makes Si less of a favourable option (Piperno 2006). This

could be an explanation for some of the excluder species considered in this study. The high initial construction costs of herbivory defenses, like condensed tannins and lignin, are linked to long life span (Coley, Bryant & Chapin 1985; Coley 1988). Cooke and Leishman (2011) found a significant negative correlation between relative Si concentration and leaf longevity of 155 species ($p < 0.001$), indicating that plants (leaves) with shorter life spans contain higher concentrations of silicon. Hence, this is in accordance with the general Si grouping between hardwoods and conifers such that hardwoods with deciduous leaves would have shorter leaf life spans than conifer pine needles. Plants with high Si would have lower condensed tannins and lignin due to a short life span strategy that can utilize the defenses provided by Si, making condensed tannins less necessary, without being undermined by the extra weight (Matsuki et al 2006; Cooke and Leishman 2011). In contrast, long life leaf span plants would limit Si accumulation to prevent the extra weights, and prefer to utilize the lighter lignin and opt for defenses from condensed tannins. My results found eleven out of the thirteen excluder species had high lignin content paired with low Si as predicted, while seven excluders had low condensed tannin concentrations. The trade-off interaction between Si, and lignin and condensed tannins is in agreement with my initial hypothesis except for the fact that condensed tannins increases with leaf Si primarily at low leaf Si levels. .

The findings of this study suggest that condensed tannins are not the main phenolic compounds associated with Si nutrition and/or that Si nutrition influences differently the phenolic compounds of different plant species (Ma 2004). Condensed tannins are downstream phenlypropanoid pathway products that I believed would illustrate a response resulting from the decrease competition of precursors between growth and phenolic synthesis because of Si

substitution of lignin. The strategic choice would have been harder to identify in hydrolysable tannins because the precursor gallic acid originates from the shikimate acid pathways, as well as the phenylpropanoid pathway.

The lack of a relationship between Si and condensed tannins was contrary to studies on cucumber (Chérif et al. 1994), roses (Shetty et al. 2011) and rice (Dallagnol et al. 2011; Van Bockhaven et al. 2013) that reported increased defence related enzymes in response to Si. One possible explanation for the discrepancy is that these results are from crop plants, while forest trees could reasonably be influenced by Si nutrition differently and consequently the phenolic compounds are impacted in different ways (Ma 2004). The larger grouping of defence related phenolic compounds considered in that study may provide a more robust approach to detect significant associations, in comparison to specifically focusing on a single compound as was done in this study with condensed tannins.

Using the cost-benefit hypothesis, some have hypothesized that plants could exhibit a trade-off between silicon- and carbon-based herbivore defenses. This would go against my hypothesis of increasing condensed tannins with a reduction in lignin synthesis and increased uptake of Si. My results do not provide support for this hypothesis either. Similar results were obtained by Cooke and Leishman (2012) who studied a diverse arrangement of Australian plants and hypothesized that plants with higher silicon concentrations would have lower phenolic concentrations. They found leaf Si to be weakly associated to total phenols and condensed tannins. Similarly, a negative correlation was found between root Si and total phenolics in another Australian study that looked at sugarcane and insect root herbivory by greyback cane beetle larvae (canegrub) (Frew et al. 2016). Experimental work that focuses extensively on the

study of leaf condensed tannins, lignin and Si in temperate forest trees is limited. Given the diversity of phenolic compounds involved in anti-herbivory functions, it is clear that more research will be needed to elucidate the interactions between Si and phenolics in tree leaves.

5.2.2.2. Multivariate

The use of the multivariate approach was meant to provide a more robust approach to explore and better understand the relationships between all variables measured in my study (Si, condensed tannins, lignin, cellulose and Ca) and to characterize the species belonging to the three classes of Si uptake (active, passive and rejective). The PCA provided interesting results with loading factors suggesting different drivers for hardwoods and conifers. Si was negatively correlated with lignin and Ca in hardwoods and Si was positively correlated with condensed tannins in conifers (Table 4). The results for hardwoods are in line with our conclusions derived from the univariate analysis and support the idea of a complementarity between Si, lignin and Ca for providing strength and support to leaf tissues as hypothesized. Species contributing to this effect includes American beech with its high Si but low lignin and Ca, yellow birch and red oak with their high lignin but low Si, and little-leaf linden with its high Ca but low lignin. Both univariate and multivariate analyses also suggest a lack of relationship between Si and condensed tannins in hardwoods. The positive relationship between Si and condensed tannins in conifers was not expected since none of the conifers were considered active Si accumulators. The significant relation can be linked to the low and high condensed tannin concentration in Norway and white spruce. Conifers as a group tended to be low in leaf Si and Ca, and high in cellulose. This puts forward a very different strategy to achieve support and defense and could be connected to leaf longevity as discussed before.

The discriminant analysis shows small intraspecific and spatial variation for both hardwood and coniferous species (Figure 11 and 12). This indicates that the composition of the structural components (Si, lignin, cellulose and Ca) and the mixture of defensive chemicals of the leaf cells are very species specific. This is a good demonstration of functional diversity where each species can achieve the functions of strength and defense using basically the same metabolic building blocks available to all plants but in different proportion. For example, the Si excluder little-leaf linden was high in Ca but relatively low in lignin while other Si-excluding species such as red oak and yellow birch were high in lignin and lower in Ca. Both American beech and yellow birch demonstrated the predicted response linked to leaf structure to Si uptake with American beech being high in Si but low in lignin and Ca whereas yellow birch was low in Si but high in lignin and calcium concentrations.

The primary driving factor differentiating the 17 species was Si concentration with the three Si uptake classes for hardwoods and the passive and rejective coniferous species lining up quite well with the gradient of Si. Two subgroups were identified for hardwoods by the multivariate analyses with Ca and lignin as drivers. Interestingly the Ca relationship had not been observed in the univariate analyses. For hardwoods, American beech and sugar maple were both high in Si but the Ca was low in American beech and high in sugar maple. I classified sugar maple as an intermediate accumulating and a borderline active accumulating species because of its relatively low Si/Ca ratio associated with its high Ca concentration. The high Ca concentration of sugar maple is consistent with previous findings that report sugar maple as relatively Ca demanding in comparison to other species (Horsley et al. 2002; Fahey et al. 2006; St-Clair et al. 2008). Leaf Ca together with lignin were instrumental in isolating little-leaf linden

from other excluder species such as yellow birch which was higher in lignin than little-leaf linden. As expected intermediate species were found primarily in the center of the figure with intermediate values for Si, Ca and lignin. Condensed tannins were not selected as a discriminant variable for hardwoods. This suggests that the underlying strategies regarding plant phenolics or even specialized metabolite mechanisms in hardwood are quite complex and cannot be restricted to a single class of phenolics such as condensed tannins. As for conifers, only condensed tannins contributed to further discriminate the species within the two classes of Si uptake. All species of conifers in the high Si group (Norway spruce, larch and white spruce) are relatively fast growers and more nutrient demanding species. No particular trait could be linked to the gradient of condensed tannins.

6 Conclusion and summary

In this study, I compared foliar concentrations of Si, lignin, condensed tannins and calcium from 17 hardwood and coniferous species grown in monoculture plots at the IDENT plantation. Leaf samples collected in the summers of 2014 and 2015 provided information on possible links between Si accumulation and other leaf components associated with plant defenses (condensed tannins) and structure (lignin and Ca) for hardwood and coniferous species of differing Si uptake mechanisms under identical soil and climate conditions. I hypothesized that active Si accumulating species would have high levels of condensed tannins with low levels of lignin, cellulose and calcium.

Most hardwoods were found to be higher in leaf Si than conifers. Foliar Si concentrations increased over the growing season but the concentrations of condensed tannins did not in most species. American beech was the only conclusive active Si accumulating species, whereas all conifers were Si excluders. Most hardwoods including sugar maple were found to be passive Si accumulators. Although high in Si, sugar maple had low lignin concentrations but condensed tannins and calcium levels were not low. Its Si/Ca ratio was a major factor in distinguishing it as an intermediate type species. The assumption for the calculation of the reference criteria by Takahashi (1990) - soil concentration as more or less than 10ppm – can be challenged and leaf Si concentration would likely be more useful if considered as a continuous spectrum (Cooke and Leishman 2011). Species with Si/Ca ratio and Si concentration that borders criteria thresholds would otherwise have the likelihood of being improperly classified.

The lack of relationships observed between condensed tannins and Si in hardwoods illustrates the challenge of identifying the specific phenolic defense compounds potentially

involved with or affected by the biochemical Si uptake strategies. Condensed tannins arguably can be said to have a negative association with Si in hardwoods, in contrast to our initial hypothesis, due to 1) the trade-off between plant growth and specialized metabolites, and/or 2) because Si and condensed tannins provide similar defense roles in plants. In contrast, the positive relationship observed between leaf Si and condensed tannins in conifers revealed by the PCA is in agreement with my hypothesis although it could be linked to needle lifespan.

Leaf Si and lignin concentrations were found to be negatively related as hypothesized with American beech and Si-excluder conifers contributing most to this relationship. The reported negative association occurring between Si and lignin in this study indicates therefore that Si can be a potentially appropriate substitute for lignin in some species. Further research is necessary to determine the underlying mechanisms and functions of Si and how it is intertwined with phenol metabolism, calcium nutrition and plant cell wall components in different species.

7 Recommendations for Future Research

Our knowledge of silicon functions in individual plant species is developing but is still insufficient to understand the complex role of Si in plant adaptation to adverse environmental conditions. The research conducted in this study provides a general baseline for future research to address the association of foliar Si with condensed tannins and lignin in trees. Overall, limited studies have focused on Si uptake in temperate forest trees, leaving many species with inconclusive Si uptake mode classification. Although American beech responded as hypothesized with high Si being associated with high condensed tannins and low lignin and Ca, sugar maple did not. This is rather intriguing as it had the highest Si concentration in our study. Being likely an active Si accumulator at least part time, Si has to have an important role in sugar maple. Conducting experiments with sugar maple grown in a Si deprived environment would provide us with a hint on the role of Si in that species.

Future studies would benefit from incorporating site characteristics such as soil Si which was not measured in my study. Soil Si measurements would provide another dimension helpful in understanding the plant-soil relationships. Sampling species from more representative stands would also increase the significance of potential conclusions.

Furthermore, the generally poor relationship observed in this study between condensed tannins and Si in leaves of hardwood and conifer trees suggests that other classes of phenolics be included in future studies. More research in this domain would benefit our knowledge of the abundance and diversity of phenol compounds occurring in plants. Yellow birch would be of particular interest for research on the relationship between condensed tannins and Si.. While low

in Si, it was highest in condensed tannins which suggests a different strategy in terms of production of defense chemicals. This hypothesis could be tested by providing yellow birch with an ample supply of Si through the roots or via direct application on the foliage. Further research with a controlled environment experiment like hydroponics could lead to better understanding of the mechanisms involved in countless hardwood and conifer species.

APPENDIX

Comparison of American beech sample location and IDENT plantation

Due to the IDENT plantation not having American beech species present, the samples for this study were collected from a neighbouring site 1km away. We acknowledged that this might introduce a confounding variable because of potentially different physical site characteristics. In an attempt to justify the associations made in this research, my American beech samples were compared to previous samples collected at a natural forest research site located at St. Hippolyte in the Lower Laurentians north of Montreal, which had species of sugar maple and yellow birch also present. This allowed a comparison of the species Si content at the two sites, and verified that there was no statistical difference between the two American beech samples (Fig 13). Sugar maple Si content was higher at the IDENT site compared to the St. Hippolyte site.

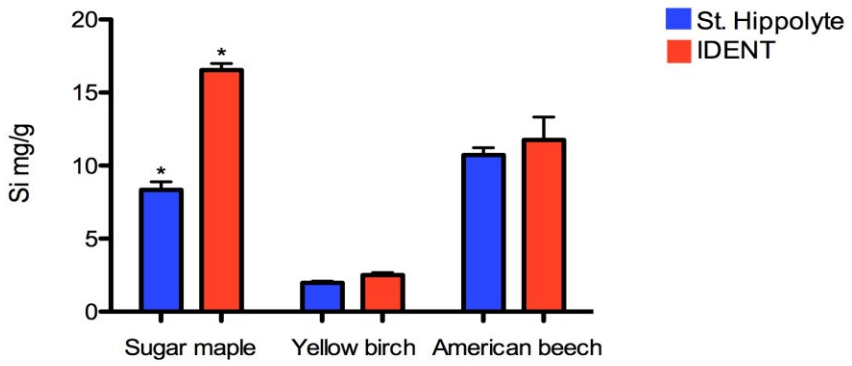


FIGURE 13--Site comparisons for American beech, sugar maple and yellow birch foliar Si concentrations. (*) signify statistically different value ($p < 0.05$)

BIBLIOGRAPHY

1. Allen S. E., Grimshaw H. M., Parkinson J. A., Quarmby C. 1974. Chemical analysis of ecological materials. Blackwell Scientific Publications.
2. Agarie S., Agata W., Uchida H., Kubota F., Kaufman P. 1996. Function of silica bodies in the epidermal system of rice (*Oryza sativa L.*): testing the window hypothesis. Journal of experimental botany, 47 (298): 655-660.
3. AOAC. 1990. Official Methods of Analysis. Association of Official Analytical Chemists, Washington, D.C.
4. Arnon D.I., Stout P.R. 1939. The essentiality of certain elements in minute quantity for plants with special reference to copper. Plant Physiology, 14:371-375.
5. Balakhnina T., Borkowska A. 2013. Effects of silicon on plant resistance to environmental stresses: review. International Agrophysics, 27: 225–232.
6. Baldwin I. T., Schultz J. C., Ward D. 1987. Patterns and sources of leaf tannin variation in yellow birch (*Betula alleghaniensis*) and sugar maple (*Acer saccharum*). Journal of Chemical Ecology, 13(5):1069-1078.
7. Bartoli F., Beaucire F. 1976. Accumulation du silicium dans les plantes vivantes en milieux pédogénétiques tempérés aérés. Comptes rendus de l'Académie des Sciences, Paris, 282D
8. Bartoli F., Souchier B. 1978. Cycle et rôle du silicium d'origine végétale dans les écosystèmes forestiers tempérés: Annales des Sciences Forestières, 35 :187-202.
9. Bartoli F. 1983. The biogeochemical cycle of silicon in two temperate forest ecosystems. Ecological Bulletins, 35: 469-476.
10. Bate-Smith E. C., Swain T. 1962. Flavonoid compounds. Comparative biochemistry, 3:755-809.
11. Bennett R. N., Wallsgrove R. M. 1994. Secondary metabolites in plant defence mechanisms. New Phytologist, 127(4): 617-633.
12. Boudet A. M. 2007. Evolution and current status of research in phenolic compounds. Phytochemistry, 68: 2722–2735.
13. Bourgaud F., Gravot A., Milesi S., Gontier E. 2001. Production of plant secondary metabolites: a historical perspective. Plant Science, 161(5), 839-851.
14. Cakmak I., Römheld V. 1997. Boron deficiency-induced impairments of cellular functions in plants. Plant Soil, 193:71–83.

15. Canham C.D. 1988. Growth and canopy architecture of shade-tolerant trees: response to canopy gaps. Ecology, 69:786–795.
16. Cannas A., Giner-Chavez B.I., Van Soest P.J. 2015. Tannins: fascinating but sometimes dangerous molecules. Department of Animal Science-Plants Poisonous to Livestock, Cornell University. Web. http://poisonousplants.ansci.cornell.edu/toxicagents/tannin.html
17. Carey J.C., Fulweiler R.W. 2014. Silica uptake by Spartina—evidence of multiple modes of accumulation from salt marshes around the world. Frontiers in Plant Science, 5: 186.
18. Chérif M., Asselin A., Bélanger R.R. 1994. Defense responses induced by soluble silicon in cucumber roots infected by *Pythium* spp. Phytopathology, 84:236–242.
19. Coley P.D.1988. Effects of plant growth rate and leaf lifetime on the amount and type of anti-herbivore defense. Oecologia, 74:531–536.
20. Coley P.D., Bryant J.P., Chapin F.S. III. 1985. Resource availability and plant antiherbivore defense. Science, 230:895–899.
21. Conley D.J. 2002. Terrestrial ecosystems and the global biogeochemical silica cycle. Global Biogeochemical Cycles, 16(4).
22. Conley D.J., Carey J.C. 2015. Biogeochemistry: Silica cycling over geologic time. Nature Geoscience, 8(6): 431-432.
23. Cooke J., Leishman M.R. 2011. Silicon concentration and leaf longevity: is silicon a player in the leaf dry mass spectrum? Functional Ecology, 25(6): 1181-1188.
24. Cooke J., Leishman M. R. 2012. Trade-offs between foliar silicon and carbon-based defences: evidence from vegetation communities of contrasting soil types. Oikos, 121(12): 2052-2060.
25. Cornelis J.T., Ranger J., Iserentant A., Delvaux B. 2010a. Tree species impact the terrestrial cycle of silicon through various uptakes. Biogeochemistry, 97:231-245.
26. Cornelis J.T., Delvaux B., Titeux H. 2010b. Contrasting silicon uptakes by coniferous trees: a hydroponic experiment on young seedlings. Plant Soil, 336:99-106.
27. Currie H.A., Perry C.C. 2007. Silica in plants: biological, biochemical and chemical studies. Annals of Botany, 100(7): 1383-1389.
28. Currie H.A., Perry C.C. 2009. Chemical evidence for intrinsic ‘Si’ within *Equisetum* cell walls. Phytochemistry, 70(17): 2089-2095.
29. Dallagnol L.J., Rodrigues F.A., DaMatta F.M., Mielli M.V.B., Pereira S.C. 2011. Deficiency in silicon uptake affects cytological, physiological, and biochemical events in the rice-*Bipolaris oryzae* interaction. Phytopathology, 101: 92–104.

30. De Bakker N.V.J., Hemmings M.A., Van Soelen J. 1999. The relationship between silicon availability, and growth and silicon concentration of the salt marsh halophyte *Spartina anglica.* Plant and Soil, 215(1): 19-27.
31. Demarty M., Morvan C., Thellier M. 1984. Calcium and the cell wall. Plant, Cell & Environment, 7: 441–448.
32. Epstein E. 1994. The Anomaly of Silicon in Plant Biology. Proceedings of the National Academy of Sciences of the United States of America, 91(1): 11–17.
33. Epstein E. 1999. Silicon. Annual Review of Plant Physiology and Plant Molecular Biology 50: 641 – 664.
34. Epstein E. 2001. Chapter 1 Silicon in plants: Facts vs. concepts. In G.H.S.L.E. Datnoff & G.H. Korndörfer (Eds.), Studies in Plant Science. 8: 1-15: Elsevier.
35. Epstein E., Bloom A.J. 2005. Mineral Nutrition of Plants: Principles and Perspectives, second ed. Sinauer, Sunderland, MA.
36. Fahey T.J., Siccama T. G., Driscoll C.T., Denny E.G., Eagar C., Cleavitt N.L., Richardson A.D. 2006. Response of sugar maple to calcium addition to northern hardwood forest. Ecology, 87(5):1267-1280.
37. Fang J.Y., Wang H., Chen Y., Zhang F.S. 2003. Silica nano-sphere formation induced by peroxidase-catalyzed phenol polymerization. Progress in Natural Science, 13: 501–504.
38. Fauteux F., Rémus-Borel W., Menzies, J.G., Bélanger R.R. 2005. Silicon and plant disease resistance against pathogenic fungi. FEMS Microbiology Letters, 249(1): 1-6.
39. Fawe A., Abou-Zaid M., Menzies J.G., Bélanger R.R. 1998 Silicon-mediated accumulation of flavonoid phytoalexins in cucumber. Phytopathology, 88(5):396-401.
40. Feeny P.P., Bostock H. 1968. Seasonal changes in the tannin content of oak leaves. Phytochemistry, 7:871-880.
41. Frew A., Powell J.R., Sallam N., Allsopp P.G., Johnson S.N. 2016. Trade-offs between silicon and phenolic defenses may explain enhanced performance of root herbivores on phenolic-rich plants. Journal of Chemical Ecology, 42(8): 768-771.
42. Geis J.W. 1973. Biogenic silica in selected species of deciduous angiosperms. Soil Science, 116(2):13-130.
43. Goto M., Ehara H., Karita S., Takabe K., Ogawa N., Yamada Y., Morita O. 2003. Protective effect of silicon on phenolic biosynthesis and ultraviolet spectral stress in rice crop. Plant Science, 164(3):349-356.

44. Grib J., Lanning F.C.1970. Absorption of Silicon by Sorghum Plants. Transactions of the Kansas Academy of Science, 73(3): 399–403.
45. Guerriero G., Hausman J.F., Legay S. 2016. Silicon and the Plant Extracellular Matrix. Frontiers in Plant Science, 7(463).
46. Hagerman A. E., L. G. Butler. 1991. Chapter 10 - Tannins and Lignins: their Interactions with Secondary Plant Metabolites (Second Edition). San Diego, Academic Press: 355-388.
47. Hagerman A.E. 2002. The Tannin Handbook. Acid Butanol Assay for Proanthocyanidins.
48. Hallett R. A., Hornbeck J. W. 1997. Foliar and soil nutrient relationships in red oak and white pine forests. Canadian Journal of Forest Research, 27(8): 1233-1244.
49. Halman J.M., Schaberg P.G., Hawley G.J., Eager C. 2008. Calcium addition at the Hubbard Brook Experimental Forest increases sugar storage, antioxidant activity, and cold tolerance in native red spruce (*Picea rubens*). Tree Physiology, 28(6): 855–862.
50. Harborne A.J. 1988. Phytochemical Methods A Guide to Modern Techniques of Plant Analysis. Chapman. London. GB.
51. Harris P. J. 2006. Primary and secondary plant cell walls: A comparative overview. New Zealand Journal of Forestry Science, 36:36–53.
52. Hassanpour S., Maherisis N., Eshratkhah B. 2011. Plants and secondary metabolites (Tannins): A Review. International Journal of Forest, Soil and Erosion, 1(1):47-53.
53. Haslam E. 1989. - Plant polyphenols. Cambridge University Press, Cambridge, UK. Chemistry and significance of condensed tannins. Ed. by Hemingway R.W. and Karchesy J.J. Plenum Press, New York.
54. He C., Wang L., Liu J., Liu X., Li X., Ma J., et al. 2013. Evidence for “silicon” within the cell walls of suspension-cultured rice cells. New Phytologist. 200:700–709.
55. Herms D.A., Mattson W.J. 1992. The Dilemma of Plants: To Grow or Defend. The Quarterly Review of Biology, 67(3): 283–335.
56. Hoagland D.R., Arnon DI. 1938. The water-culture method for growing plants without soil. Circular 347, University of California, College of Agriculture, Berkeley.
57. Hodson M.J., White P.J., Mead A., Broadley M.R. 2005. Phylogenetic variation in the silicon composition of plants. Annals of Botany, 96: 1027–1046. London.
58. Hodson M. J., Sangster A.G. 1999 Aluminium/silicon interactions in conifers. Journal of Inorganic Biochemistry.76: 89–98.

59. Horsley S.B., Long R.P., Bailey S.W., Hallett R.A., Wargo P.M. 2002. Health of eastern North American sugar maple forests and factors affecting decline. Northern Journal of Applied Forestry, 19(1): 34-44.
60. Howard L.R., Clark J.R., Brownmiller C. 2003. Antioxidant capacity and phenolic content in blueberries as affected by genotype and growing season. Journal of the Science of Food and Agriculture, 83(12): 1238-1247.
61. Hümmer W., Schreier P. 2008. Analysis of proanthocyanidins. Molecular Nutrition & Food Research, 52(12): 1381-1398.
62. Ignea C., Athanasakoglou A., Andreadelli A., Apostolak M., Iakovides M., Stephanou E.G., Kampranis S.C. 2017. Overcoming the plasticity of plant specialized metabolism for selective diterpene production in yeast. Scientific Reports, 7(1), 8855.
63. Intergovernmental Panel on Climate Change. 2007. Fourth Assessment Report: Working Report Group III: Mitigation of Climate Change. Web. http://www.ipcc.ch/publications_and_data/ar4/wg3/en/ch9s9-2.html
64. Ishizuka Y.1971. Physiology of the rice plant. Advances in Agronomy, 23, 241-315.
65. Jenny H. 1980. The Soil Resource: Origin and Behavior. Springer-Verlag, New York, 377.
66. Johnson-Maynard J.L., Graham R.C., Shouse P.J., Quideau S.A. 2005. Base cation and silicon biogeochemistry under pine and scrub oak monocultures: implications for weathering rates. Geoderma, 126:353–365.
67. Jung H-JG., Varel V.H., Weimer P.J., Ralph J. 1999. Accuracy of klason lignin and acid detergent lignin methods as assessed by bomb calorimetry. Journal of Agricultural and Food Chemistry, 47: 2005–2008.
68. Kaufman P.B., Dayanandan P., Takeoka Y., Bigelow W.C., Jones J.D., Iler R. 1981. Silica in shoots of higher plants. In: Simpson TL, Valcani BE, eds. Silicon and siliceous structures in biological systems. Springer-Verlag, 409-99.
69. Kraska J. E. 2009. Assessing the silicon status of rice (*Oryza sativa*). Louisiana State University.
70. Kraus T.E.C., Dahlgren R.A., Zasoski R.J. 2003. Tannins in nutrient dynamics of forest ecosystems - a review. Plant Soil, 256: 41–66.
71. King J.S., Pregitzer K.S., Zak D.R., Kubiske M.E., Holmes W.E. 2001. Correlation of foliage and litter chemistry of sugar maple, *Acer saccharum*, as affected by elevated CO_2 and varying N availability, and effects on decomposition. Oikos, 94:403-416.
72. Kobe R.K., Pacala S.W., Silander J.A. Jr., Canham C.D. 1995. Juvenile tree survivorship as a component of shade tolerance. Ecological Applications, 5: 517–532.

73. Kosonen M., Lännenpää M., Ratilainen M., Kontunen Soppela S., Julkunen Tiitto R. 2015. Decreased anthocyanidin reductase expression strongly decreases silver birch (*Betula pendula)* growth and alters accumulation of phenolics. Physiologia Plantarum, 155(4):384-399.
74. Lewin J., Reimann BEF. 1969. Silicon and plant growth. Annual Review of Plant Physiology, 20: 289–304.
75. Liu L., King J.S., Giardina C.P. 2005. Effects of elevated concentrations of atmospheric CO_2 and tropospheric O_3 on leaf litter production and chemistry in trembling aspen and paper birch communities. Tree Physiology, 25: 1511-1522.
76. Loponen J., Lempa K., Ossipov V., Kozlov M.V., Girs A., Hangasmaa K., Pihlaja K. 2001. Patterns in content of phenolic compounds in leaves of mountain birches along a strong pollution gradient. Chemosphere, 45(3): 291-301.
77. Ma J.F., Takahashi E. 2002. Soil, Fertilizer, and Plant Silicon Research in Japan, Elsevier Science
78. Ma J.F. 2003. Functions of silicon in higher plants. Silicon Biomineralization, 127-147. Springer, Berlin, Heidelberg.
79. Ma J.F. 2004. Role of silicon in enhancing the resistance of plants to biotic and abiotic stress. Soil Science and Plant Nutrition, 50 (1):11-18.
80. Ma J.F., Yamaji N. 2006. Silicon uptake and accumulation in higher plants. Trends in Plant Science, 11 : 392–397
81. Ma J.F., Yamaji N., Mitani N., Tamai K., Konishi S., Fujiwara T., Katsuhara M., Yano M. 2007. An efflux transporter of silicon in rice. Nature, 448(7150): 209-212.
82. Ma J.F., Yamaji N. 2008. Functions and transport of silicon in plants. Cellular and Molecular Life Sciences, 65(19), 3049-3057.
83. Ma J.F. 2010. Silicon transporters in higher plants. In MIPs and their Role in the Exchange of Metalloids (pp. 99-109). Springer, New York, NY.
84. Ma J.F., Yamaji N. 2015. A cooperative system of silicon transport in plants. Trends in Plant Science, 20(7): 435-442.
85. Maksimović D.J., Bogdanović J., Maksimović V., Nikolic M. 2007. Silicon modulates the metabolism and utilization of phenolic compounds in cucumber (*Cucumis sativus* L.) grown at excess manganese. Journal of Plant Nutrition and Soil Science, 170(6): 739-744.
86. Massey F.P. Ennos A.R., Hartley S.E. 2007. Grasses and the resource availability hypothesis: the importance of silica-based defences. Journal of Ecology. 95: 414 – 424.
87. Matichenkov V.V., Ammosova J.M. 1996.Effect of amorphous silica on soil properties of a sod-podzolic soil. Eurasian Soil Science, 28(10): 87-99

88. Matsuki S., Koike T. 2006. Comparison of leaf life span, photosynthesis and defensive traits across seven species of deciduous broad-leaf tree seedlings. Annals of Botany, 97, 813–817.
89. Meharg C., Meharg A.A. 2015. Silicon, the silver bullet for mitigating biotic and abiotic stress, and improving grain quality, in rice? Environmental and Experimental Botany, 120:8-17.
90. Mitani N., Ma J.F. 2005. Uptake system of silicon in different plant species. Journal of Experimental Botany, 56(414): 1255-1261.
91. Mooney H.A., Gulmon S.L. 1982. Constraints on leaf structure and function in reference to herbivory. BioScience, 32: 198–206
92. Morikawa C. K., Saigusa M. 2003. Mineral composition and accumulation of silicon in tissues of blueberry (*Vaccinum corymbosus* cv. Bluecrop) cuttings. Plant and Soil, 258(1): 1-8.
93. Moura J.C., Bonine C.A., De Oliveira Fernandes Viana J., Dornelas M.C., Mazzafera P. 2010. Abiotic and biotic stresses and changes in the lignin content and composition in plants. Journal of Integrative Plant Biology, 52(4): 360-376.
94. Moulton K.L., West J., Berner R.A. 2000. Solute flux and mineral mass balance approaches to the quantification of plant effects on silicate weathering. American Journal of Science, 300:539–570.
95. Muir S. 2001. Plant-available silicon (Si) as a protectant against fungal diseases in soil-less potting media. Final Report. Campbelltown: Horticultural Research and Development Corporation. Horticulture Australia.
96. Nilsson M. C., Gallet C., Wallstedt A. 1998. Temporal variability of phenolics and batatasin-III in *Empetrum hermaphroditum* leaves over an eight-year period: interpretations of ecological function. Oikos, 6-16.
97. Parry D.W., Kelso M. 1975.The distribution of silicon deposits in the root of *Molina caerulea* (L.) Moench and *Sorghum bicolor* (L.) Moench. Annals of Botany, 39: 995-1001.
98. Parry D.W., Winslow A. 1977. Electron-Probe Microanalysis of Silicon Accumulation in the Leaves and Tendrils of *Pisum sativum* (L.) Following Root Severance. Annals of Botany, 41:275-78.
99. Piperno Dolores R. 2006. Phytoliths: a comprehensive guide for archaeologists and paleoecologists. Lanham, Maryland: AltaMira Press.
100. Prychid C.J., Rudall P.J., Gregory M. 2004. Systematics and biology of silica bodies in monocotyledons. The Botanical Review, 69: 377 – 440.

101. Ranganathan S., Suvarchala V., Rajesh Y.B.R.D., Prasad M.S., Padmakumari A.P., Voleti S.R. 2006. Effects of silicon sources on its deposition, chlorophyll content, and disease and pest resistance in rice. Biologia Plantarium, 50: 713-716.
102. Raven J.A. 1983. The transport and function of silicon in plants. Biological Reviews, 58: 179–207.
103. Royer D.L. 2014. Atmospheric CO_2 and O_2 During the Phanerozoic: Tools, Patterns, and Impacts A2 - Holland, Heinrich D. Treatise on Geochemistry (Second Edition). K. K. Turekian. Oxford, Elsevier: 251-267.
104. Sahebi M., Hanafi M.M., Siti Nor Akmar A., Rafii M.Y., Azizi P., Tengoua F.F., Nurul Mayzaitul Azwa J., Shabanimofrad M. 2015. Importance of Silicon and Mechanisms of Biosilica Formation in Plants. BioMed Research International 2015:1-16.
105. Sangster A.G., Hodson M.J. 1986. Silica in higher plants. Silicon Biochemistry, 90-107.
106. Sangster A. G., Hodson M.J., Tubb H.J. 2001. Silicon deposition in higher plants. Studies in Plant Science, 8:85-113.
107. Schoelynck J., Bal K., Backx H., Okruszko T., Meire P., Struyf E. 2010. Silica uptake in aquatic and wetland macrophytes: a strategic choice between silica, lignin and cellulose? New Phytologist, 186: 385–391.
108. Shetty R., Frette X., Jensen B., Shetty N.P., Jensen J.D., Jorgensen H.J.L., Newman M.A., Christensen L.P. 2011. Silicon-induced changes in antifungal phenolic acids, flavonoids, and key phenylpropanoid pathway genes during the interaction between miniature roses and the biotrophic pathogen *Podosphaera pannosa*. Plant Physiology, 157: 2194–2205.
109. Singer M. J., Munns D. N. 1999. Soils: An Introduction, 4 ed. Prentice Hall, Upper Saddle River, New Jersey, 527.
110. Sivanesan I., Park S.W. 2014. The role of silicon in plant tissue culture. Frontiers in Plant Science, 5: 571.
111. Stafford H. A. 1988. Proanthocyanidins—and the lignin connection. Phytochemistry 27, 1–5.
112. Statistics Canada. 2018. Human Activity and the Environment-Forests in Canada, 2017. No. 16-201-X. Ottawa. Version updated March 2018.
113. Saint. Clair S.B., Sharpe W.E., Lynch J.P. 2008. Key interactions between nutrient limitation and climatic factors in temperate forests: a synthesis of the sugar maple literature. Canadian Journal of Forest Research, 38(3): 401-414.
114. Struyf E., Conley D.J. 2012. Emerging understanding of the ecosystem silica filter. Biogeochemistry, 107(1-3): 9-18.

115. Takahashi E., Miyake Y.1977. Silicon and plant growth. Proceedings of the International Seminar on Soil Environment and Fertility Management in Intensive Agriculture.
116. Takahashi E., Ma J.F., Miyake Y. 1990. The possibility of silicon as an essential element for higher plants. Comments Agricultural Food Chemistry, 2:99–10.
117. Takijima Y., Shiojima M., Kanno K.1949. Studies on soil of peaty paddy fields. Effect of silica on the growth of the rice plant and its nutrient absorption. Journal of Soil Science 30:181-6.
118. Tobner C.M., Paquette A., Reich P.B., Gravel D., Messier C. 2013. Advancing biodiversity-ecosystem functioning science using high-density tree-based experiments over functional diversity gradients. Oecologia, 174(3): 609-621.
119. Tikasz P. 2016. Silicate dynamics in common hardwoods of southern Quebec with a focus on its role on Al and Mn toxicity. M.Sc.Thesis, McGill University.
120. Trembath-Reichert E., Wilson J.P., McGlynn S.E., Fischer W.W. 2015. Four hundred million years of silica biomineralization in land plants. Proceedings of the National Academy of Sciences, 112(17): 5449-5454.
121. Treguer P., Nelson D.M., Van Bennekom A.J., De Master D.J., Leynaert A., Queguiner B. 1995. The silica balance in the world ocean: a re-estimate. Science, 268:375–379.
122. Van Bockhaven J., DeVleesschauwer D., Höfte M. 2013. Towards establishing broad-spectrum disease resistance in plants: silicon leads the way. Journal of Experimental Botany, 64:1281–1293.
123. Vanholme R., Demedts B., Morreel K., Ralph J., Boerjan W. 2010. Lignin biosynthesis and structure. Plant Physiology, 153(3): 895-905.
124. Wagner A., Donaldson L., Ralph J. 2012. Lignification and lignin manipulations in conifers. In Advances in Botanical Research, 61:37-76. Academic Press
125. Waterman P.G., Mole S. 1994. Analysis of phenolic plant metabolites. Blackwell Scientific Publications, Oxford, UK.
126. White T. 1956. The scope of vegetable tannin chemistry. The chemistry of vegetable tannins. Annual Symposium. *Geo. Marshal & Co., Ltd.,* London SE, 1, 22.
127. Wink M. 1988. Plant breeding: importance of plant secondary metabolites for protection against pathogens and herbivores. Theoretical and Applied Genetics, 75(2): 225-233.
128. Yamaji K., Julkunen Tiitto R., Rousi M., Freiwald V., Oksanen E. 2003. Ozone exposure over two growing seasons alters root to shoot ratio and chemical composition of birch (*Betula pendula* Roth). Global Change Biology, 9(10): 1363-1377.

129. Yang X., Song Z., Liu H., Bolan N. S., Wang H., Li Z. 2014. Plant silicon content in forests of north China and its implications for phytolith carbon sequestration. Ecological Research, 30(2): 347-355.
130. Yoshida S.1975.The physiology of silicon in rice. Technical Bulletin. 25:24-27.
131. Zhu Y., Gong H. 2014. Beneficial effects of silicon on salt and drought tolerance in plants. Agronomy for Sustainable Development, 34: 455–472.
132. Zucker W.V. 1982. How aphids choose leaves: the roles of phenolics in host selection by a galling aphid. Ecology, 63:977-981.