Determining the Relationship Between Needle Nutrition and Post-harvest Needle Retention in Balsam Fir *(Abies balsamea* (L.) Mill.)

By

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DEDICATION PAGE

~ This thesis is dedicated to my grandparents.

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ABSTRACT

The Christmas tree and greenery trade is a multi-million dollar industry in Atlantic Canada. Christmas trees grown in Nova Scotia are shipped internationally especially, to the United States. This thesis was set out to answer the over-arching hypothesis: pre- and post- harvest needle nutrient content influences post-harvest needle retention. Through a series of experiments it was shown that the pre-harvest needle P, Ca, Mg, Mn, Zn and B contents do not appear to be directly linked to post-harvest needle retention. Alternatively, the pre-harvest needle N, K, Cu and Fe contents significantly, but negatively influenced post-harvest needle retention. By maintaining needle N, K, Cu and Fe concentrations below 1.5 %, 0.55 %, 3.7 ppm and 35 ppm, respectively may extend needle retention in balsam fir. Xylem-fed nutrients negatively influenced needle retention. As well, foliar applications of calcium and zinc citrate did not promote needle retention at the concentrations used.

LIST OF ABBREVIATIONS USED

Elements:

N- Nitrogen

P- Phosphorus

K- Potassium

Ca - Calcium

Mg - Magnesium

S - Sulphur

Fe - Iron

Zn - Zinc

Mn - Manganese

B - Boron

Cu - Copper

Chemical Compounds and Ions:

(Ca^{+2}) – calcium

(Cl^{-}) – chloride

(Cu^{+2}) - copper

(Fe^{+3}) - ferric iron

(Fe^{+2}) – ferrous iron

($H_2PO_4^{-}$) – dihydrogen phosphate

(HPO_4^{-2}) – hydrogen phosphate

(K^{+}) – potassium

(Mg^{+2}) – magnesium

(NO_3^-) - nitrate-N

-(NH_4^+) - ammonium

(SO_4^-) – sulphate

(Zn^{+2}) - zinc

Measurements:

AWU – average water use

CWU– cumulative water use

d – day

cm – centimetres

g – grams

mL – milliliters

m – meters

mm – millimetres

NRD –Needle retention duration

ppm – parts per million

% needle loss – percent needle loss

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1.0 INTRODUCTION

Christmas trees are important symbols, which have been used in the celebration of Christmas in North American and Western European cultures for over 170 years (Relf, 1997). The types of coniferous trees that are used as Christmas trees can vary from white pine, Fraser fir, Douglas fir, blue spruce, white spruce and the subject of this study, balsam fir, which is botanically known as *Abies balsamea* (L.). Balsam fir is one of the top choices as a Christmas tree because of its preferred fragrance, needle colour and architecture. Farmers across Canada export their Christmas tree products, such as wreaths and Christmas trees, to other countries. The main receiver of these products is the United States. Nova Scotia is one of the top five provinces in Canada growing Christmas trees and is the second largest exporter (Statistics Canada, 2008). Exports of Christmas trees provide annual revenue of 28.4 million dollars for Nova Scotia (Statistics Canada, 2010). In the past decade, consumers' preferences began to shift towards artificial trees. There are a couple of reasons for this shift, but the most common complaint against real trees is needle loss, for which consumers have zero tolerance.

Needle loss is expected, especially when consumers in the US prefer to have their Christmas trees in November for Thanksgiving, and would like to keep their trees until the end of January. To meet this demand, the producers need to cut their trees early in October for the November retail opportunity in the United States. Americans expect that their Christmas trees will retain their needles from November to early January. Previous studies have suggested that earlier harvests lead to earlier post-harvest needle loss. A study conducted by Mitcham-Butler et al. (1988) observed the effect of harvesting times on needle loss. Mitcham-Butler et al. (1988) reported that harvesting in October reduced Fraser fir's needle retention. It has been previously

speculated that cold acclimation could have a negative effect on needle retention duration (NRD). Although harvesting at an early date is an issue, this cannot be helped entirely due to the strict time schedule which must be met for retail. Due to the inflexible nature of when Christmas trees are harvested, there is a need to study other ways to improve post-harvest needle retention. There are several pre- and post-harvest factors proposed that promote needle loss.

Among the several pre-harvest factors that may affect needle retention post-harvest are soil fertility, environmental factors such as drought, flooding, salinity and acid rain. There are several nutritional factors that may influence post-harvest needle retention as well. Firstly, the demands for nutrients are not met post-harvest since the tree is severed from its roots and extracted from the soil. Secondly, needle nutrient content possibly could be changing post-harvest, which may influence needle retention. Lastly, there could be a variation in pre-harvest needle nutrient content among trees cut from different areas. This difference among trees may be due to differences in soil fertility, fertilizer application rates and methods that may lead to needle abscission.

Nutrients have a role in regulating abscission; for example, nitrogen is a component of chlorophyll, which is necessary in the absorption of light energy (Hopkins, 1999; Black, 1968). A lack of nitrogen could disrupt the plant's photosynthetic ability and this could lead to senescence, which may lead to abscission (Janick et al., 1969). Currently, there are no studies examining the relationship between pre and post-harvest nutritional composition and content of balsam fir to post-harvest needle retention duration (NRD). This research hopes to determine the link between the pre-harvest nutritional content of balsam fir to post-harvest NRD.

It is hypothesized that:

1) Nutrient composition and content in the needles differ among different tiers in a tree and this relates to the variation in post-harvest needle retention (Objective 1).
2) There is a difference in needle nutrient content between low and high NRD genotypes, which corresponds to the variation in their post-harvest needle retention (Objective 2).
3) There is a temporal change in needle nutrient content of balsam fir, which relates to the variation in monthly NRDs (Objective 3).
4) The soil and needle nutrient content of various balsam fir plantations reflects post-harvest NRD (Objective 4).
5) Increasing needle nutrient concentration enhances post-harvest needle retention duration in balsam fir branches (Objective 5 and 6).

This leads to the main objectives of this study, which are to:

1) Determine the relationship between pre-harvest needle nutrient content and NRD in different tiers of a known clone.
2) Determine the relationship between the pre-harvest nutrient status to post-harvest NRD in two contrasting clones (high and low NRD).
3) Determine the relationship between seasonal pre-harvest nutrient dynamics of balsam fir needles and post-harvest NRD.
4) Examine the relationship between nutrient status of soil and needles, and NRD.
5) Determine whether increasing needle nutrient concentrations pre-harvest would enhance post-harvest NRD in balsam fir branches.

6) Determine if the foliar application of zinc and calcium citrate increase post-harvest needle retention.

2.0 LITERATURE REVIEW

2.1 The Balsam Fir

There are about 40 species of true firs in the northern hemisphere and nine species that are native to North America, but only one is found in Nova Scotia, the balsam fir scientifically known as *Abies balsamea* (L.) (Zinck and Roland, 1998). Its name was given from the resin that it contains and the sweet smell that the resin produces. This resin is also commonly used as an adhesive in optical lenses and glass slides, called Canada Balsam (Sigma-Aldrich, 2011).

Figure 2.1.1: An example of the balsam fir tree. Adopted from: http://www.taylortrees.com/Christmas%20Tree%20Descriptions.html

2.1.1 The Botanical Description

The balsam fir tree is pyramid shaped and the branches are horizontal in regular whorls (Figure 2.1.1). The trunk is reddish without scales but has raised blisters filled with resin (Zinck and Roland, 1998). The staminate flowers are pendulous rising from the axils of the previous year's needles (Zinck and Roland, 1998). The cones are erect on the upper surface of branches. The needles are green with notched tips and are sessile (Zinck and Roland, 1998). The needles are arranged in two ranks on the sides of the light-coloured twigs. The lower surface of the needle has two white bands with a distinct midrib.

2.1.2 The Nature of the Balsam Fir's Needles

Needles have evolved to last for a long time through harsh conditions in poor soil. To survive harsh conditions the needles have a thick, waxy cuticle. This waxy cuticle prevents micro-organisms from being able to penetrate easily and prevents the leaching of nutrients and water loss. Conifer needles were shown to be 620-675% tougher than broadleaves and are lower in nutritional value (Hatcher, 1990). Toughness in this case is defined as leaf thickness, tissue density and fibre per volume (Hatcher, 1990). Higher levels of lignin were proven to have a strong, negative effect on the needle's decomposition rate (Berg et al., 1981). Consequently, the slow decomposition of conifer needles allows a thick organic layer to develop on the soil surface, where organically bound nitrogen and phosphorous remains until broken down (Miles, 1986).

2.2 Abscission

Abscission is a natural process that occurs in plants. During abscission leaves, flowers, flower parts or fruits separate from a plant/tree because of various physiological conditions. These physiological conditions can be caused by stress or terminating organs at the end of their growth

and/or function. Abscission is a natural developmental process because it allows the plant to strategically invest carbohydrates and other nutrients to functioning parts or sinks of the plant (Addicott, 1982). Abscission also allows the plant to successfully complete its life cycle and defend from natural enemies or pathogenic infections (Addicott, 1982). However, post-harvest needle abscission does not fit with any of the above natural abscission processes but may be due to a variety of stress factors including a limited supply of nutrients, water and other essential factors derived from its roots. This will be discussed further in Chapter 2.3.

2.2.1 The Abscission Zone

Abscission is carefully regulated in plants by pre-determined locations, and by the hormones ethylene and auxins (Addicott, 1982). The location of the separation area is called the "abscission zone" (AZ) and it is pre-determined physically at the base of the organ being shed (Sexton and Roberts, 1982). This area is usually comprised of a line of small, densely packed cytoplasmic cells that can vary in thickness (Addicott, 1982). Within the AZ, these cells have failed to become larger and vacuolate like other cells. But, when the right conditions occur, these cells will begin to enlarge and their middle lamella will weaken and dissolve between the cell walls. This allows the organ to break off of the parent plant (Sexton and Roberts, 1982). When the organ is shed, the remaining cells on the parent plant enlarge and differentiate, to produce a scar tissue that protects the main body from potential infection (Addicott, 1982).

2.2.2 Hormones Related to Abscission

Abscission in plants is regulated by the balance between ethylene (the promoter of abscission) and auxin (the inhibitor of abscission) (Gonzalez-Carranza et al., 1998). Ethylene is produced by the plant, but external production of ethylene may encourage senescence and abscission in

nearby plants (Hopkins, 1999). Typically, a young, productive organ has high concentrations of auxins within it just like the parent plant. Alternately, as this organ ages, the concentration of auxins decreases in the dying organ and thus begins the process of abscission (Hopkins, 1999). Previous research at the Christmas tree Research Centre (CRC) has showed that ethylene triggers needle abscission in balsam fir (MacDonald et al., 2010; MacDonald and Lada, 2011; MacDonald et al., 2011; MacDonald et al., 2012). Previously, it was also shown that when the ethylene receptors were blocked or the synthesis of ethylene was inhibited, needle retention increased (MacDonald et al., 2010). It is possible that several environmental, management (nutrients and water) and plant factors may influence needle abscission through ethylene mediation.

2.3 Factors that Influence Needle Abscission in Balsam Fir

There are several factors that may influence needle abscission, including: genetic variation, pre-harvest environmental conditions such as soil fertility; and environmental factors such as drought, flooding, salinity and acid rain. There are some proven post-harvest conditions that influence needle retention such as rough handling, temperature and dehydration.

2.3.1 Genetic Variation

There is great diversity in balsam fir, which makes each tree unique. Within these provenances, some clones are better at retaining needles than others (Lada and Veitch, 2009). For example, there are some clones that retain their needles for as long as two months, whereas others retain their needles for only ten days post-harvest (Lada and Veitch, 2009).

2.3.2 Rootstock

Another factor that can affect needle retention duration (NRD) is its unknown rootstock. Within the Debert Tree Improvement Centre there are a wide variety of balsam fir clones. These clones were grafted on pre-existing unknown rootstocks from seed-grown balsam fir. In an experiment to see if rootstocks would cause a variation in needle retention within the same clone, it was found that rootstocks can exert an influence on the needle retention. However, not all clones responded in a similar fashion (Lada and Adams, 2010). The roots of these rootstocks will obtain and/or transport nutrients and water differently and it would depend on how well the scion is compatable.

2.3.3 Post-harvest Factors

There are several post-harvest factors that may influence post-harvest needle retention. Some of these factors may be conditions that stress the tree and start the cycle of needle senescence and/or abscission. Firstly, the month of harvesting may influence the final NRD. If a tree was cut in October, before cold acclimation could take place, then it would be more likely to drop its needles prematurely, depending on the clone (Lada and Veitch, 2009). Alternatively, if balsam fir was cut after cold acclimation, it would then increase its NRD, again depending on the clone (Lada and Veitch, 2009). Due to this difference, the harvesting time can have a significant influence on needle retention. Secondly, the act of harvesting the tree may aggravate or promote needle drop. This is because the tree is separated from its roots and is unable to absorb nutrients, water and other root-derived factors such as, cytokinins. This separation is detrimental to normal cell or needle physiology and can cause needle abscission. Thirdly, rough post-harvest handling of trees increased needle loss by three times versus less traumatic handling **(**Lada and Adams, 2009**).** Rough post-harvest handling involves shaking, dragging and baling the trees before

transportation, which may damage the needles or branches, encouraging needle abscission (Lada and Adams, 2009).

Another factor that influences needle drop is temperature exposure post-harvest. Exposing freshly cut trees to high temperatures of 20°C or low temperatures of -20°C increases water uptake and decreases NRD (MacDonald and Lada, 2012). When balsam fir was exposed to 5°C, the post-harvest needle retention has been shown to improve (MacDonald and Lada, 2012). This research shows that Christmas tree producers could increase the quality of their trees if they attempted to minimize the temperature extremes to which root-detached trees were exposed.

2.3.4 Environmental Factors

There are several environmental factors that may influence needle abscission in balsam fir. The factors include drought or moisture stress, high or low temperatures, pH and the nutrient status of the soil. Prolonged drought can stress the plant and in response, a plant may close its stomata to decrease water loss and this will limit nutrient uptake (Cox and Bolasma, 1967). This response could be through reduced transpiration, which is involved in the passive uptake of water and, therefore, nutrients. Nutrient availability is very important for the health of the balsam fir and drought conditions can reduce the ability to transport nutrients effectively. The reduction in nutrient availability through drought could possibly promote needle abscission. Balsam fir tree plantations are cared for irregularly between growers and with little or no fertilization. By observing previous studies, the application of nutrients by fertilization of the soil does increase the plant's growth and health (Rodrigues et al., 2011; Bolland et al., 1997; Janick et al., 1969).

2.4 Macro and Micro-nutrients

Plants require different minerals that are separated into macro and micro-nutrients for their growth and developmental processes including abscission control (Miller and Donahu, 1990). The macro-nutrients are nitrogen (NO_3^-) and (NH_4^+), phosphorus ($H_2PO_4^-$) and (HPO_4^{-2}), potassium (K^+), calcium (Ca^{+2}), magnesium (Mg^{+2}) and sulphur (SO_4^-). The micro-nutrients are usually required in lesser amounts and have lower critical threshold values compared to macro-nutrients, they are: chloride (Cl^-), copper (Cu^{+2}), boron (H_3BO_3), iron (Fe^{+3}) and (Fe^{+2}), manganese (Mn^{+2}), (Mn^{+3}), (Mn^{+4}), molybdenum (MoO_4^-) and zinc (Zn^{+2}) (Hopkins, 1999).

Table 2.4.1: The plant available nutrients and their forms.

Macro-nutrient	Chemical Form	Micro-nutrient	Chemical Form
Nitrogen	(NO_3^-), (NH_4^+)	Iron	(Fe^{+3}) and (Fe^{+2})
Phosphorus	($H_2PO_4^-$), (HPO_4^{-2})	Zinc	(Zn^{+2})
Potassium	(K^+)	Manganese	(Mn^{+2}), (Mn^{+3}), (Mn^{+4})
Calcium	(Ca^{+2})	Copper	(Cu^{+2})
Magnesium	(Mg^{+2})	Molybdenum	(MoO_4^-)
Sulfur	(SO_4^-)	Boron	(H_3BO_3), ($B(OH)_4^-$)
		Chlorine	(Cl^-)

Source: Adopted from Pettipas, 2004

Photosynthesis allows plants to capture energy for growth and development. When photosynthesis is disrupted it interferes with the plant's ability to make, store and use its photosynthates. Therefore, the plant must switch to conserve its photosynthates and become more efficient in using carbohydrates; plants accomplish this by eliminating disposable parts such as leaves, flowers or fruits depending on what plant organ can be most expendable at that

time (Addicott, 1982). The major elements required by a plant will be discussed in more detail in the following sections as well as their physiological role within the plant.

2.4.1 Physiological Roles of Nutrients Within the Plant

2.4.1.1. Nitrogen is essential for a plant's growth, and it is seen as most often being the number one limiting nutrient in a plant's growth. Usually, soil nitrogen is available in the inorganic forms such as the nitrate ion or as an ammonium ion NH^4. When the plant absorbs nitrate it is reduced to (NH_4^+), before it is used for growth **(**Hopkins, 1999). The role nitrogen (N) has in a plant is complex. Nitrogen is part of many organic compounds such as amino acids, proteins and nucleic acids. Nitrogen is also the main component of chlorophyll, which makes nitrogen as one of the main components of photosynthesis (Hopkins, 1999; Black, 1968). Fifty to eighty percent of nitrogen is allocated to photosynthetic proteins, such as bisphosphate carboxylase-oxygenase (Rubisco) which is highly correlated to photosynthetic capacity (Livingston et al., 1998). When nitrogen is low, the plant is usually stunted and unable to grow to its full potential. The leaves of these plants usually become yellow (due to its inability to produce chlorophyll) and then abscise (Janick et al., 1969). Nitrogen is also a very mobile nutrient. This means that when there is nitrogen deficiency, nitrogen from the older leaves is transported through the plant to more recently produced organs (Salisbury and Ross, 1969). This mobilization also results in chlorosis in the older leaves first (Hopkins, 1999). Too much nitrogen is also detrimental to plants because of excessive shoot growth, stunting root growth as well as the excess nitrogen locking out other important nutrients (Salisbury and Ross, 1969).

Excess nitrogen is used to make extra carbohydrates which produce more and larger cells, which in turn increases water volume but with thinner cell walls. The weakened cell wall is the result of

fewer carbohydrates being used to thicken the cell wall. These carbohydrates are being used instead to increase the cell's size but not for cell wall quality (Letham, 1961). Consequently, this allows the structures of the plant to become less stable and more prone to freezing injury and abscission (Black, 1968). Little or no information is available on the role of nitrogen in post-harvest needle retention duration.

2.4.1.2. Phosphorus ($H_2PO_4^-$ - dihydrogen phosphate) and (HPO_4^{-2} - hydrogen phosphate), is one of the major macro-nutrients that plants require. Phosphorus is essential in the formation of sugar phosphates, nucleotides, nucleic acids, and coenzymes (Salisbury and Ross, 1969). Phosphorus's role in creating coenzymes and sugar phosphates are important because phosphorus allows sugars to be metabolized by the plant properly, as well as phosphorus being used as an energy carrier (Salisbury and Ross, 1969). For *Pinus radiata* in New Zealand, phosphorus was the most common nutritional limitation (Will, 1973). This may be due to the fact that phosphorus is readily taken in by aluminum and iron, and by free limestone (Ca^{2+}) present in some alkaline soils. This results in about 80% of applied phosphorus (P_2O_5) as a fertilizer being lost or unavailable for root uptake (Raghothama, 1999). Increased root surface area and root length was correlated with more phosphorus intake (Pang et al., 2010). Some species of plants are adapted to low-P soils, such as certain genotypes of barley which have long root hairs to increase surface area and absorption abilities (Gahoonia and Nielsen, 2004). Phosphorus is a main component of chemical energy such as ATP and $NADPH^+$. With a lack of phosphorus, it can be predicted that the plant's ability to store chemical energy would be greatly hampered. This would prevent the plant from doing energy requiring tasks and force it to save energy through death and abscission of its disposable organs such as needles. It is not known however, whether or not phosphorus has any role in the post-harvest needle retention of balsam fir.

2.4.1.3. Potassium (K^+) is another macro-nutrient that is essential for use as a coenzyme or as an activator for other enzymes involved in photosynthesis and respiration within the plant (Hopkins, 1999). It is also important in the role of cell elongation, leaf movements, metabolic homeostasis, osmoregulation, and stomatal movements (Kang et al., 2004). A deficiency in potassium and high salinity caused a significant reduction in plant weight and chlorophyll contents in maize (Gong et al., 2011). However, introduction of potassium can partially alleviate these symptoms. Potassium deficiency was noted to cause oxidative stress on the plant (Gong et al., 2011). This could be because of impairment in a) stomatal regulation, b) conversion of light energy into chemical energy and or c) the ability of phloem exporting sugars from the leaves to other organs (Cakmak, 2005). In a study by Hafsi et al. (2011), it has been proven that a deficiency in potassium caused a decrease in shoot and root dry weight of *H. Maritimum L.* Hafsi et al. (2011) explain that this may be due to an osmotic effect (root-water status), a difference in K^+-use efficiency in biomass production and the substitution of potassium by other minerals. It can be predicted that by the impairment of stomata regulation that photosynthesis may be disrupted. When photosynthesis is disrupted, the plant's ability in converting light energy to chemical energy is limited causing chlorosis and abscission. Again, the role of K in post-harvest needle retention has not been studied in balsam fir.

2.4.1.4. Sulphur (SO_4^{-2} : sulphate ion) is metabolized and used by the roots only to the extent that is needed. Sulphur is then transferred to the growing locations of a plant. This is where it is transformed into amino acids and then into proteins to be used in structure and function (Salisbury and Ross, 1969). Sulphur is very important in the oxidative phosphorylation, which is a more effective way of creating ATP (Salisbury and Ross, 1969). Sulphur is also used within the electron transport system during respiration, which is essential for normal cell functions.

Disruption of these processes can have an impact on the production of these compounds, and to save resources the plant may drop disposable organs. The role of SO_4^{-2} in post-harvest needle retention is not known in balsam fir.

2.4.1.5. Magnesium (Mg^{+2}) has a structural role within chlorophyll and this prevents chlorosis. Therefore, Mg aids in the absorption of light energy to transfer into sugars and energy and helps maintain normal cell processes. The second, but just as important process that Mg^{+2} performs in, is the activation of enzymes that use ATP. Salisbury and Ross (1969) explain that Mg^{+2} activates all of the enzymes that are required to utilize the energy in ATP. Keeping Salisbury and Ross' (1969) study in mind, it can be hypothesized that without Mg^{+2} normal cell processes would be severely hampered. Magnesium may have a role in post-harvest needle retention and further research is needed.

2.4.1.6. Calcium (Ca^{+2}) is an important element used in the formation of the lamella and the integrity of the cell wall. A lack of Ca^{+2} can cause weakened cell walls and the inability to maintain stability between cells. Deficiency of Ca^{+2} can encourage senescence and abscission of organs since the lamella may be weakened when Ca^{+2} is removed from the abscission zone (Addicott, 1982). Fruit with low Ca^{+2} had poorer storage potential; this is because Ca^{+2} plays an important role in the ripening and senescence processes (Stow, 1993). The application of Ca^{+2} was shown to slow the aging and abscission process (Poovaiah and Leopold, 1973). However, it is not known whether or not Ca^{+2} has any role in the post-harvest needle retention.

2.4.1.7. Iron (Fe^{+3} : ferric) and (Fe^{+2} : ferrous) is used to form a pigment called cytochromes, which has an important role in photosynthesis and respiration as an electron transport carrier (Salisbury and Ross, 1969). Iron is also used as an enzyme activator within the plant, perhaps

involved in chlorophyll synthesis, since its deficiency causes chlorosis (Katyal and Sharma, 1980). For that reason, a deficiency in iron is detrimental to the main functions of the plant. Iron is not very mobile in plants, and deficiencies occur first in the younger leaves. The role of iron in post-harvest needle retention in balsam fir is yet to be uncovered.

2.4.1.8. Chloride (Cl^-) has an important role in stomatal regulation, which is mediated by K (Chen et al., 2010). This may explain why one of its visual deficiency symptoms is wilted leaves, even in the presence of water. Previously, Ball et al., (1984) proved that Cl^- is necessary for water splitting in photosynthesis, which may explain why the leaves become chlorotic without adequate amounts of chloride. Chlorosis prevents the plant to create photosynthates and limits the plant's ability to grow and survive and possibly resulting in abscission. This has not been studied in balsam fir as of yet and it should be examined.

2.4.1.9. Boron (B) plays an essential role in creating cell walls, cell division and sugar transportation and regulation of hormones as well the growth of plants (Marschner, 1995). Boron is also a co-factor in the development of chlorophyll. Boron deficiency is more widespread than any other micro-nutrient (Brown, et al., 1997). The deficiency of boron is also more prevalent in vegetables, fruit and nut trees since it is required in larger quantities for reproductive growth (Brown et al., 1997). Deficiency symptoms depend on the species, but where it does occur most commonly is within the root meristems and can cause stem death; perhaps because of its function in cell wall development (Salisbury and Ross, 1969). Boron deficiency also causes poorly developed leaves due to its role in elongation of cells and this causes the leaves to be smaller and darker than usual. With increasing deficiency, necrotic spots will form and eventually organ death (Dell and Huang, 1997). Since boron is a co-factor in the development of chlorophyll it can be seen that a lack of this nutrient may limit the chlorophyll formation in a plant. This

consequently would result in chlorosis because the plant's limited ability in chlorophyll synthesis. The lack in chlorophyll will result in low carbohydrate production for sustaining plant functions, which may lead to needle abscission; although this is yet to be studied in balsam fir.

2.4.1.10. Zinc (Zn^{+2}) deficiency causes needles to become stunted and chlorotic and trees can lose all but first or second year needles. The branches will also become stunted and can die. Zinc is a factor in the development of chlorophyll (Salisbury and Ross, 1969). Zinc also has a role in developing carbon skeletons and proteins which are responsible for the synthesis of chlorophyll (Imas and Imas, 2010). Another interesting effect of a Zn^{+2} deficiency is the increase of abscission of organs (Addicott, 1982). Increased abscission occurs in Zn^{+2} deficiencies because zinc is required in plants to maintain normal auxin levels. When zinc is removed, the auxin levels are lowered in the leaves allowing ethylene to be more prominent which signals for abscission to begin (Skoog, 1940).

2.4.1.11. Copper (Cu^{+2}) participates in respiration and photosynthesis through electron transport and therefore plays a direct role in obtaining, storing and using energy (Salisbury and Ross, 1969). Disruption of these processes would encourage death and abscission in the needles. Also, Cu^{+2} performs as a secondary influence on auxin levels in the plants, but usually only after the plant has been weakened by disease, pests, dehydration or other mineral deficiencies (Addicott, 1982).

2.4.1.12. Molybdenum (Mo) deficiency can be seen more commonly in acidic soils (Ide et al, 2011). Typically, molybdenum is associated with nitrate absorption from the soil. Without enough molybdenum, nitrates cannot be altered into useable forms and consequently they become stored within the leaves. Without the nitrates being transformed into useable forms, the

plant cannot create chlorophyll and so it is unable to absorb light. This can cause the plant to become stunted in growth, and the leaves to become scorched from the over-storage of nitrates (Weir, 1984).

2.5 Nutrient Uptake

2.5.1 Mechanisms

Plants take up nutrients in three main ways 1) simple diffusion 2) facilitated diffusion and 3) active transport; these are all linked to water uptake (Campbell, 2005). Simple diffusion is when the ions move randomly across the cell wall and membrane from high to low concentration. Facilitated diffusion requires transport proteins to assist with insoluble ions. This can be accomplished by carrier-active binding sites that make a complex to help transport the ion across the membrane or by channel proteins which provide an entrance to specific ions (Campbell, 2005). The last mechanism is active transport which requires energy or ATP to move ions across the cell membrane; energy is required since this process occurs against the electrochemical gradients (Campbell, 2005).

2.5.2 Factors that Influence Nutrient Uptake

Soil texture and structure affect nutrient uptake for plants (Miller and Donahu, 1990). Soil texture is determined by the amount and combination of silt, clay and sand in the soil (Brown, 2003). Soil structure is the arrangement of soil particles into aggregates; these aggregates are characterized by their shape (granular, wedge, blocky, etc.), distinctness (weak, moderate and strong) and by size (fine, medium and coarse) (Kramer and Boyer, 1995). Soil structure relates to water movement, aeration, organic matter and root penetration (Kramer and Boyer, 1995).

Soil acidity influences nutrient uptake for plants as well. Acidity negatively influences nutrient availability by three ways. Firstly, acidity reduces growth in the roots that was caused by Al toxicity and this limits the root's ability to absorb nutrients and water. Secondly, the competitive theory prevents other cations from being absorbed. Briefly, the competitive theory is when the H^+ ions compete for the individual bases at the selective sites on the root, preventing other cations from being absorbed (Black, 1968). Thirdly, some nutrients lose their availability because of soil acidity. For example, iron availability is related to the pH of the soil, where the higher the pH the less available iron is for the plant to absorb. This is because within a high pH the iron forms insoluble iron hydroxides and calcium complexes, whereas in acidic solutions it reacts with aluminum and is easily precipitated out of the soil (Hopkins, 1999). Phosphorus and molybdenum both decrease in solubility as well (Marschner, 1995). Since there are so many factors that can influence nutrient uptake, it is crucial to understand how these factors may influence needle retention post-harvest.

2.6 Fertilization

2.6.1 Balsam Fir's Impact on the Soil

Coniferous forests largely consist of conifer trees, such as spruce, fir and pines. Typically these forests have soils with a lower pH, which is seen as undesirable in other high-nutrient demanding crops. Plantations of commercially grown trees, such as the balsam fir for Christmas trees mimic this coniferous forest.

Acidic soils affect the plant in three main ways: 1) toxins 2) soil micro-organisms 3) and nutrient availability (as explained in Chapter 2.5.2) (Black, 1968); the development of podzolization may also occur in severe cases. Conifers are very well adapted for these scenarios because of their

shallow roots and their symbiotic relationship with ectotrophic-mycorrhizae. This symbiotic relationship increases their ability to absorb the limited nitrogen, phosphorus, other nutrients and water in their environment as well as increasing their root's surface area (Malloch et al., 1980). For this reason, plantations can be grown on many different types of soil, including poorer soil areas and on old farm fields. Even though conifers are well adapted to poor soils, it does not mean they are immune to nutrient or water deficiencies and there have been no past studies on this topic and its relationship to post-harvest needle retention.

2.6.2 Current Fertilization Regimes

Currently, there is little regulation in fertilization. Fertilization varies from location to location and from grower to grower. The Christmas Tree Grower's Manual (2002) gives rates and recommendations for nitrogen and when to apply (mid-May to June). But this was in contrast to the lack of information that was given for every other nutrient. The rates given for potassium and phosphorous are based on other studies (exact species is not given) and so the recommendation was not entirely applicable to balsam fir. Besides the little knowledge centring on fertilization, there is still no knowledge on nutrient deficiencies and its link to needle retention.

3.0 GENERAL METHODOLOGY

3.1 Clones, Source and Collection of Branches and Preparation

3.1.1 Clones

The clones that were used was a low NRD (clone 18) with an average post-harvest needle retention duration (NRD) pre-cold acclimation of 14 days and an average post-cold accumulation in post-harvest of 18 days. The high NRD clone was clone 8 with an average pre- cold accumulation of 45 days and an average post-cold accumulation of 42 days. However, these NRDs of clones were determined under dehydrated conditions.

3.1.2 Source

These clones were gathered from the Tree Breeding Center, Department of Natural Resources, Debert, Nova Scotia, Canada (long. 45º25' N, lat. 63º28' W.)

3.1.3 Collection

The branches were collected from about 1.5 meters above the ground; the branches were second year growth (Figure 3.1.1). They were taken on the same side of the tree to help eliminate possible environmental influences and cut during the same time of the day. For experiments requiring pre-harvest nutrient status to post-harvest NRD sister branches were collected (Figure 3.1.1). After cutting, they were immediately placed into a bucket of reverse osmotic water and transported back to the Christmas tree Research Centre (CRC) (MacDonald et al., 2010).

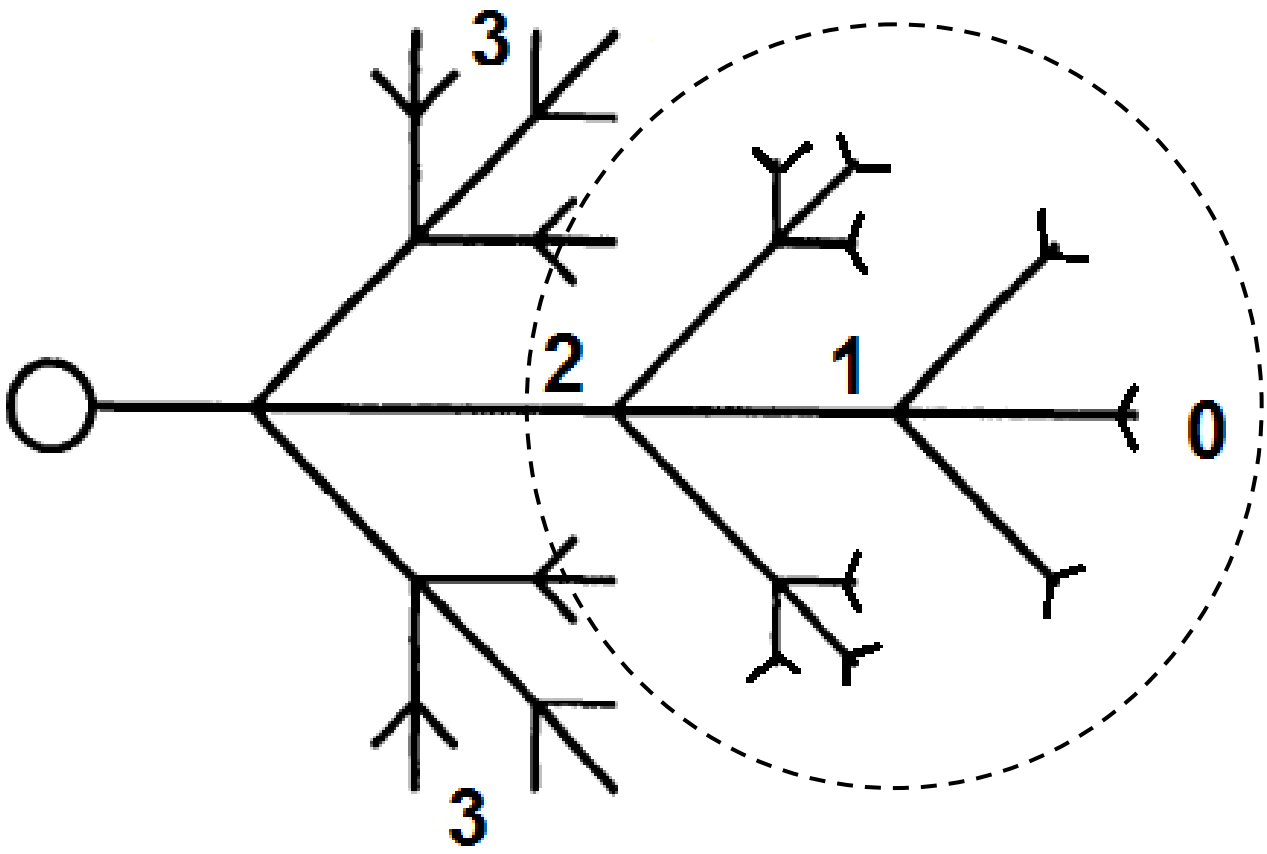

Figure 3.1.1: A diagram representing a branch of balsam fir. The dotted circle surrounds the branch sample size collected for the response variables. The dotted circle includes current year growth (0), first year growth (1) and second year growth (2). Sister branches are indicated by (3).

3.1.4 Preparation

Branches from each tree were freshly cut (about 2 cm) under water to prevent embolism before setting them up in 250 millilitres (mL), dark coloured flasks which helped inhibit algal growth. The branches were similar in weight after cutting. The flask was filled with 100 mL of reverse osmotic water (RO).

In natural osmosis, water travels over a semi-permeable membrane from the lowest concentration to the highest concentration to create equilibrium of solutes; the membrane only allows water through because of water's small molecular size. This osmotic process though, does not remove ions or other containments in the water. In RO, water is forced over this semi-permeable membrane from the highest concentration to the lowest concentration to remove these ions.

Next, the mouth of the flask was stuffed with cotton gauze to reduce evaporation of the water, while providing support to the branch. The experiment was set at 21ºC to mimic household conditions in fluorescent lighting for 24 hours (MacDonald et al., 2010). The individual light rack levels are 32 cm tall (from light to bench). Light intensity was measured at three different heights: 11.5 cm from the bottom up (represented where the bottle neck ends and the branch begins), the second point was at 18.5 cm which is about mid-branch height and then the final point was at 27 cm from the bottom. Twenty-seven centimeters would be the worst case scenario for branches. The mean (n = 9) light intensity at 11. 5 cm was 120 µmol $m^{-2}s^{-1}$ (± 15 µmol $m^{-2}s^{-1}$), at 18.5 cm it was 199 µmol $m^{-2}s^{-1}$ (± 26 µmol $m^{-2}s^{-1}$) and at 27 cm it was 358 µmol $m^{-2}s^{-1}$ (± 27 µmol $m^{-2}s^{-}$1).

3.1.5 Collection of Needles for Nutrient Analysis Protocol

To gain an understanding of the post-harvest nutrient status of the branches used in particular experiments; there were 5 branch replicates obtained from the same location on the tree where the experimental branches were taken. The needles from these branches were collected, labelled and then sent to the analytical facility at Harlow Institute, Truro for analysis.

3.1.6 Nutrient Analysis Protocol for Plant Tissues

The nutrient analysis was carried out as described by Pettipas (2004). Briefly, the needles were dried and the samples were ground through a 1 mm steel screen. One gram of this sample will be placed in a 250 mL digestion tube with 15 mL of HNO_3(nitric acid). Samples were placed on a digestion block at 90°C for 45 minutes and then the temperature was increased to 140°C until it is clear and 1 mL is remaining. 1% of HNO_3 was added and the samples were filtered and put into a volumetric flask. Total N was measured using a LECO model FP 528 analyser. Analysis of P, K, Ca, Mg, S, Fe, Zn, Mn, B and Cu were analysed with inductively coupled plasma atomic emission spectroscopy (ICP-OES).

3.1.7 Soil Sample Analysis

The soil was air-dried ground and sieved through a 2 mm stainless steel sieve. The Mehlich 3 (M3) soil extractant was used to obtain the plant available P, K, Ca, Mg, S, Fe. Zn, Mn, B and Cu in the soil (Pettipas, 2004). Ten grams of soil was placed in a plastic container with 100 mL of M3 and shaken for 15 minutes at 200 oscillations per minute. The supernatant was filtered through Whitman No. 5 paper and analyzed using a Jarell-Ash Inductively Coupled Argon Plasma Emissions Spectrometer (ICAP).

3.2 Response Variables

3.2.1 Average Water Use (AWU)

On the first day, the branch and bottle were weighed separately from the entire apparatus (flask and branch) to get an initial weight. The apparatus was then weighed daily to determine average water use in grams per day. With the fallen needles taken into account, the only change in weight can be assumed to be the water loss through transpiration and evaporation. The apparatus'

weight was subtracted from the initial weight. The subtracted number would be equal to the water loss for the whole plant (mL). This number was then to be divided by the weight of the branch (g) which was then divided again by the day it was measured (d). This represents the daily change in weight to describe the AWU in mL/g/d (MacDonald and Lada, 2012).

3.2.2 Cumulative Water Use (CWU)

This measurement was the overall water loss that occurred throughout the duration of the experiment by adding up the AWU (MacDonald et al., 2010; MacDonald et al., 2010; MacDonald et al., 2011; MacDonald et al., 2012).

3.2.3 Needle Retention Duration (NRD)

Needle retention duration was measured by the amount of time (days) it took for all of the needles to drop from the branch (MacDonald et al., 2010; MacDonald et al., 2010; MacDonald and Lada, 2011; MacDonald et al., 2011; MacDonald et al., 2012; Veitch et al., 2012).

3.2.4 Cumulative Needle Loss and % Needle Loss

Individual branches were weighed before it was inserted into the bottles. Then the branch and bottle with the water was weighed to give the total initial apparatus weight. Every second day measurements were taken from the fallen needles as fresh weight then collected and stored to determine needle loss and needle retention duration (NRD). A finger run test was initiated using rubber gloves to stimulate mechanical fall that may occur. Cumulative needle loss was the daily needle dropped weight added up over time until the final weight was reached.

Percent needle loss was determined by calculating the weight of needles dropped each day divided by the final total needle weight x 100.

4.0 EXPERIMENTS

4.1 The Relationship Between Pre-harvest Needle Nutrition of Different Tiers and Post-harvest Needle Retention Duration (NRD)

Abstract

Nutritional status alters various physiological and metabolic processes including senescence and abscission. Understanding the link between a specific nutrient element and post-harvest needle abscission would allow for the understanding of the mechanism of abscission itself. The objective was to determine if there was a link between pre-harvest nutrient content and post-harvest needle retention within a tree. Seven different tiers on one clone (clone 18) were examined for pre-harvest nutritional content and post-harvest needle loss dynamics. Needle N, P, K, Ca, Mn, Cu and Zn concentrations all differed significantly among tiers. Despite a significant variation in the needle nutrient content; days for commencement of needle abscission, days for 5, 10, 20, 40, 60, 80 % needle loss and NRD did not differ among various tiers. This suggests that needle nutrient content pre- harvest may not regulate post-harvest needle loss.

4.1.1 Introduction

One of the main goals of the Christmas tree growers is to increase the post-harvest needle retention of their Christmas trees. Post-harvest needle retention can be controlled and modulated by differences in the genetics of clones or phenotypes (Lada and Veitch, 2009), root stocks (Lada and Adams, 2010), handling of the tree for shipping and transportation (Lada and Adams, 2009), the temperature that a tree is exposed to after being cut (MacDonald and Lada, 2012) and hormones, such as ethylene, ABA and auxins (MacDonald et al., 2010; MacDonald and Lada, 2011; MacDonald et al., 2011; MacDonald et al., 2012). Nutrients play a key role in the growth and development of plants including senescence and abscission; however, the role or the physiological significance of needle nutrients on post-harvest needle retention is not fully understood. Currently, it is not known whether or not the needle drop in different tiers of a tree is due to the differences in needle nutrient content. Also, it is not known whether the differences

between clones in NRD and nutrient concentrations are brought about by different rootstocks (Lada and Adams, 2010). To eliminate the influence of rootstocks and to understand the role of nutrients, one approach was to examine the responses within one clone and within a tree in different tiers.

Calcium is considered to have intermediate mobility within a plant. Intermediate mobility is described to be when an element, like calcium, can move only short distances inside plant tissues. For example, in and out of the abscission zone during abscission – but not from older to newer growth (Fife et al., 2008). Iron, boron and zinc are all considered immobile whereas nitrogen, phosphorus and potassium are all mobile within a plant (Fife et al., 2008). Nutrient immobility can result in visual deficiencies in a plant. If the nutrient is immobile then the deficiency symptoms will occur in the newer growth before the older growth and *vice versa* for the mobile nutrients (Sprague, 1941). If there is a deficiency in the plant of these immobile nutrients, then the plant will be less likely to compensate for these deficiencies through translocation – causing localized deficiencies, which may cause abscission in the needles.

The process of nutrients moving from one organ to another within a plant is called, translocation. Translocation is an adaptive measure that plants use in times of deficiency or when a particular organ, such as fruit, requires more nutrients than normal. Translocation of the mobile elements may occur among the tiers of balsam fir. For example, the mobile elements such as nitrogen and magnesium may be transported from one tier to other tiers. Such a translocation may be influenced by sun-light for example; in branches with more exposure to the sun, chlorophyll synthesis is expected to increase. Shading, which occurs in lower tiers, may result in lower chlorophyll synthesis, which may result in browning and the abscission of needles in lower or covered tiers.

Nitrogen is a mobile nutrient and its movement within a plant is determined upon its need. If a plant is deficient in nitrogen, it will mobilize nitrogen to either newer growth or photosynthetically active regions (Schmidt and Stewart, 1997). In several conifers it was seen that there were different gradients of PPF (photosynthetic photon flux) from the top to the bottom of the tree as well as from the older to newer needles (Miyazawa et al., 2004). Nitrogen is translocated to the light harvesting apparatus of the photosynthetic process; this means that with decreasing PPF, decreasing nitrogen is seen as well (Miyazawa et al., 2004). This correlation was strongly linked, showing that nitrogen distribution is affected more by the PPF rather than by the leaf's age (Miyazawa et al., 2004). For this particular element it can be said that light may influence the amount of nitrogen in the leaves. If this occurs in balsam fir at different tiers, then it is expected that nitrogen levels in needles at different tiers would be affected, which may be reflected in needle retention. Identifying the relationships between the nutrient content and needle retention at different tiers may be helpful in understanding the underlying link between various nutrients and needle retention independent of the influence of external factors such as the rootstock effects and the variation in soil nutrition, which may be seen between different trees.

To date, no research has examined such a relationship between pre-harvest nutrient content in various tiers of a clone and its possible relationship with post-harvest needle retention in balsam fir.

4.1.2 Objective

The objective of this experiment was to determine the relationship between pre-harvest needle nutrient concentrations and its post-harvest needle retention in different tiers of clone 18.

4.1.3 Materials and Methods

This experiment was set up as a completely randomized design (CRD). Typically, the nutrient samples for nutrient analysis were collected on the same main branch where the samples for the post-harvest needle abscission study were obtained. Therefore, the assumption was made that both branches within the same tier were very similar in nutrient content and NRD. The samples were collected on September 28th, 2011 from the south side of the tree to promote consistency. Samples were collected from only one tree of clone 18 to eliminate any genetic and rootstock influence that may be seen between multiple trees. The tree's base trunk diameter was 11.7 cm and its height was at 470 cm. Samples were collected from seven tiers of a height from the ground at; T1: 60-80, T2: 100-120, T3: 140-160, T4: 180-200, T5: 240-260, T6: 300-320, T7: 360-380 cm. From each tier, 10 branches (terminal and lateral branches – Figure 3.1.1) were collected from the same side of the tree. Each branch was a two-year old growth and prepared in the laboratory as described in Chapter 3.1.4. The five branches were five replicates, which were submitted to the post-harvest study. Data on NRD and % needle loss was collected as described in Chapter 3.2. Needles from the other five replicate samples were used for the pre-harvest nutrient analysis as described in chapter 3.1.6. This experiment took about 90 days to complete.

Statistical Analysis

Measurements of the study were subjected to ANOVA. Tukey's test was used to differentiate between the means, if significant. As well if ANOVA was significant, a regression analysis was performed to identify the relationship between the nutrient concentration and NRD among different tiers.

4.1.4 Results

Needle abscission dynamics

Needle loss (%) was measured in 7 increments. The days for needle loss commencement, which was when the branch lost 1% of its needles, and at 5, 10, 20, 40, 80 % needle loss and final needle retention duration (NRD) were also measured. The date when these percentages occurred were noted and then analyzed using ANOVA (Table 4.1.1).

Table 4.1.1: The needle loss increments post-harvest in balsam fir. There were 7 tiers with 5 replications per tier. ANOVA analysed % needle loss among tiers at different loss increments to determine significance at $\alpha = 0.05$.

Increments	1 %	5 %	20 %	40 %	60 %	80 %	NRD
Tiers	0.295	0.515	0.208	0.103	0.112	0.074	0.124

Days for 1, 5, 20, 40, 60 and 80 % needle loss did not differ significantly among various tiers suggesting that the needle abscission dynamics of different tiers remained similar (Table 4.1.1). The final NRD of different tiers were also non-significant (Table 4.1.1).

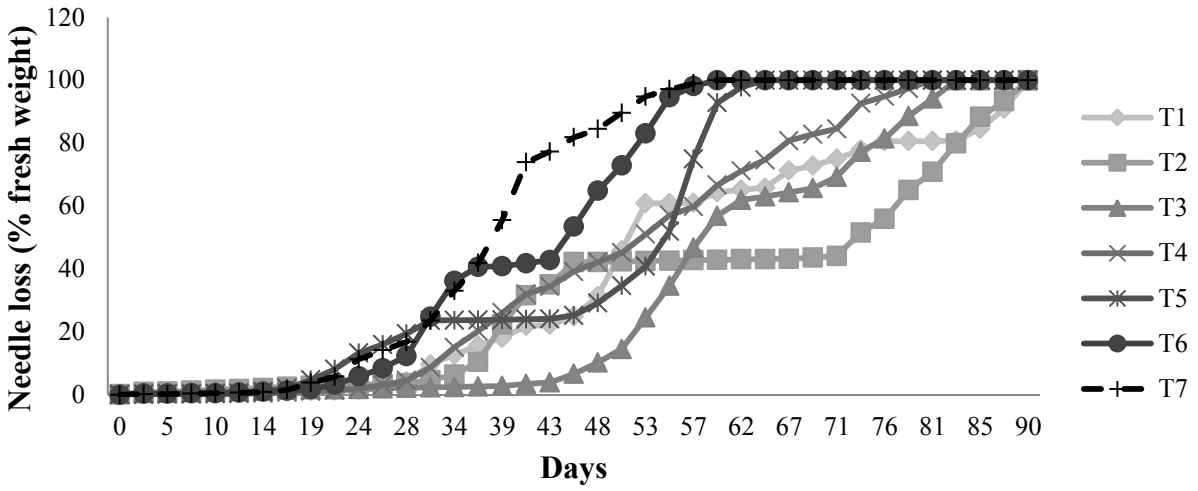

Figure 4.1.1: Cumulative percent needle loss in 7 tiers of a balsam fir clone 18. Each point represented a mean of 5 replications.

The cumulative needle loss (%) versus time was graphed for each tier to show their pattern of needle loss (Figure 4.1.1). All of the tiers had generally a similar pattern in cumulative needle loss except tier 5, 6 and 7. Tiers 5, 6 and 7 began to lose their needles sooner and reached 100 % of needle loss faster on average. The typical needle loss pattern was seen as first, there was little needle loss; second, there was a gradual increase in needle loss; third, there was a levelling off in needle abscission until finally there was total abscission.

Needle retention duration (NRD)

Needle retention did not differ between tiers significantly ($p= 0.0738$); even though there was a tendency for the higher tiers to abscise their needles at a higher percentage in a shorter period of time (Figure 4.1.2). Although it was not statistically significant, tier 2 had the highest NRD average of 70 days, whereas tier 7 had the lowest NRD of 45 days (Table 4.1.2).

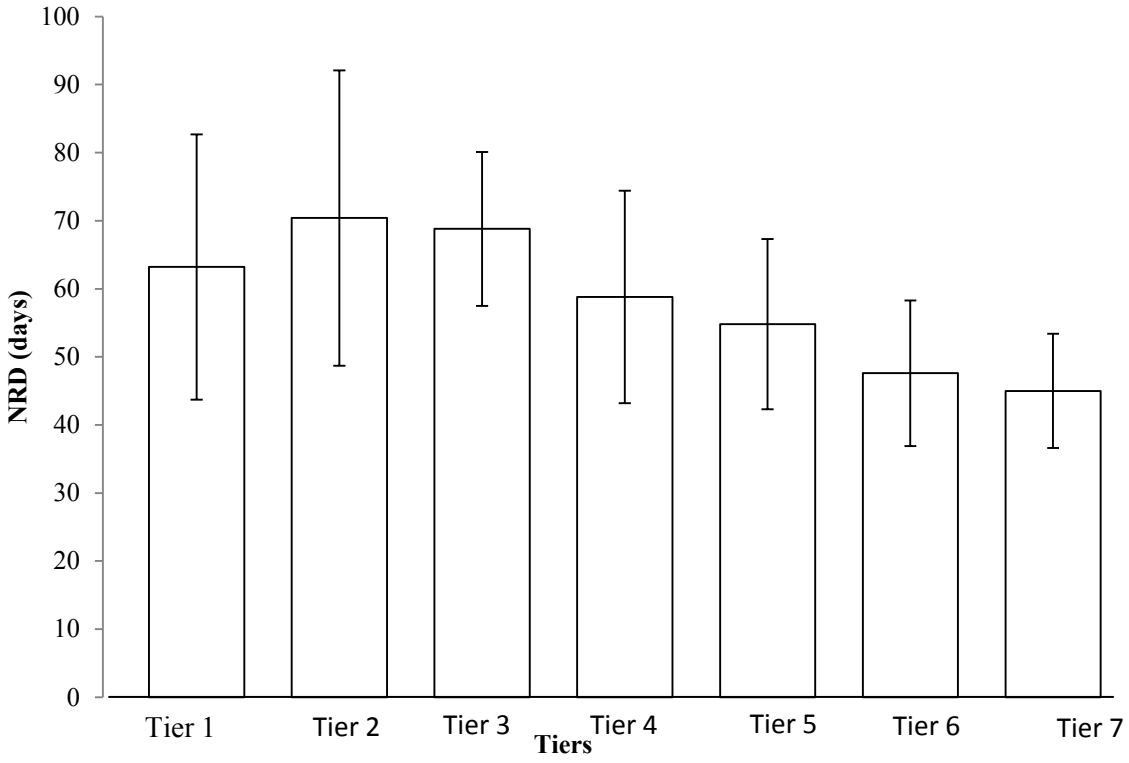

Figure 4.1.2: Needle retention duration for seven different tiers (p=0.0738).

Nutrient Needle Content

Nitrogen, phosphorus, potassium, calcium, manganese, copper and zinc concentrations all differed significantly (p-values of 0.0165, 0.0006, 0.0425, < 0.0001, <0.0001, 0.0243 and < 0.001, respectively) among various tiers (Table 4.1.2). However, there was no significant difference in magnesium and boron concentrations among different tiers.

Table 4.1.2: The pre-harvest needle nutrient content of 7 tiers of a balsam fir tree, with 5 replications per tier. T1: 60-80 cm, T2: 100 120, T3: 140-160, T4:180-200, T5:240-260, T6:300-320, T7:360-380 cm. A Tukey's test was used to separate the means of significant nutrients. Values with the same lettering were not significantly different.

Nutrients	Tiers 1	2	3	4	5	6	7	p-value
N (%)	0.99^{AB}	0.91^{B}	0.97^{AB}	0.97^{AB}	1.05^{A}	0.96^{AB}	0.98^{AB}	0.0165*
P (%)	0.12^{B}	0.12^{B}	0.13^{AB}	0.13^{AB}	0.14^{A}	0.14^{A}	0.12^{AB}	0.0006*
K (%)	0.45^{A}	0.38^{A}	0.41^{A}	0.46^{A}	0.46^{A}	0.46^{A}	0.47^{A}	0.0425**
Ca (%)	0.73^{B}	1.11^{A}	1.00^{A}	0.73^{B}	0.69^{B}	0.60^{B}	0.60^{B}	<0.0001*
Mg (%)	0.08^{A}	0.08^{A}	0.08^{A}	0.07^{A}	0.08^{A}	0.07^{A}	0.07^{A}	0.4525
Mn (ppm)	808.66^{CD}	1145.38^{A}	1054.37^{AB}	886.75^{BC}	723.95^{CD}	663.64^{D}	828.00^{CD}	<0.0001*
Cu (ppm)	2.55^{AB}	2.17^{B}	2.23^{B}	2.44^{AB}	3.11^{A}	2.48^{AB}	2.47^{AB}	0.0243*
Zn (ppm)	48.78^{B}	61.36^{A}	43.78^{B}	30.25^{CD}	39.95^{BC}	24.23^{D}	26.78^{D}	<0.001*
B (ppm)	0.12^{A}	0.12^{A}	0.12^{A}	0.12^{A}	0.12^{A}	0.12^{A}	0.12^{A}	0.0675
NRD (days)	63.20^{A}	70.40^{A}	68.80^{A}	58.80^{A}	54.80^{A}	47.60^{A}	45.00^{A}	0.0738

* denotes significance at $\alpha = 0.05$

** Tukey's test could not separate means even though there was a significant difference seen in K.

A Tukey's test was used to separate the means which showed that nitrogen content was the highest in the 5th tier and lowest in the 2nd tier (Table 4.1.2). Over all, phosphorus was the highest in the upper tiers and lower in the bottom tiers. Whereas manganese was higher in tier 2 and had lower levels in the upper tiers. Calcium, copper and zinc concentrations were higher in the lower tiers and had lower levels in the higher tiers.

The significant elements among tiers (nitrogen, phosphorus, potassium, calcium, manganese, copper and zinc) were pooled against the pooled NRD and regressions were preformed. Only calcium (Figure 4.1.3), manganese (Figure 4.1.4) and zinc (Figure 4.1.5) proved to have a significant, positive relationship on needle retention (p = 0.007, 0.007 and 0.004, respectively).

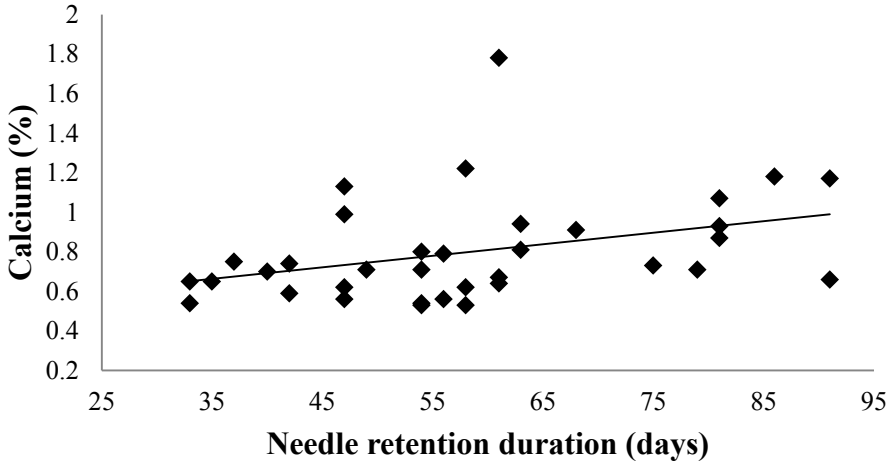

Figure 4.1.3: A linear regression between calcium (%) and NRD (days) with the pooled data of all 7 tiers (n=35) with a p-value of 0.007. The relationship between NRD and Ca content was significant, showing a positive relationship with a R^2 value of 0.208*.

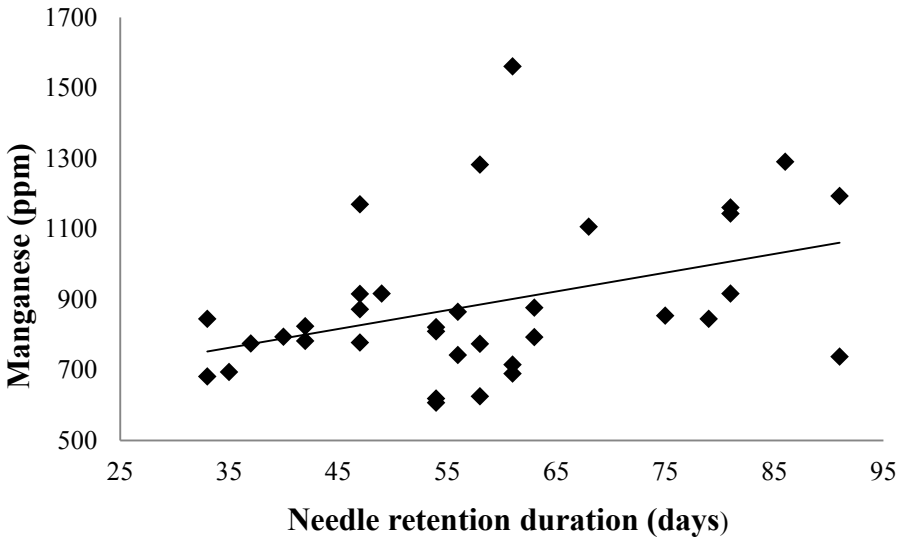

Figure 4.1.4: A linear regression between manganese (ppm) and NRD (days) with the pooled data of all 7 tiers (n=35) with a p-value of 0.007. The relationship between NRD and Mn content was significant, showing a positive relationship with a R^2 value of 0.205*.

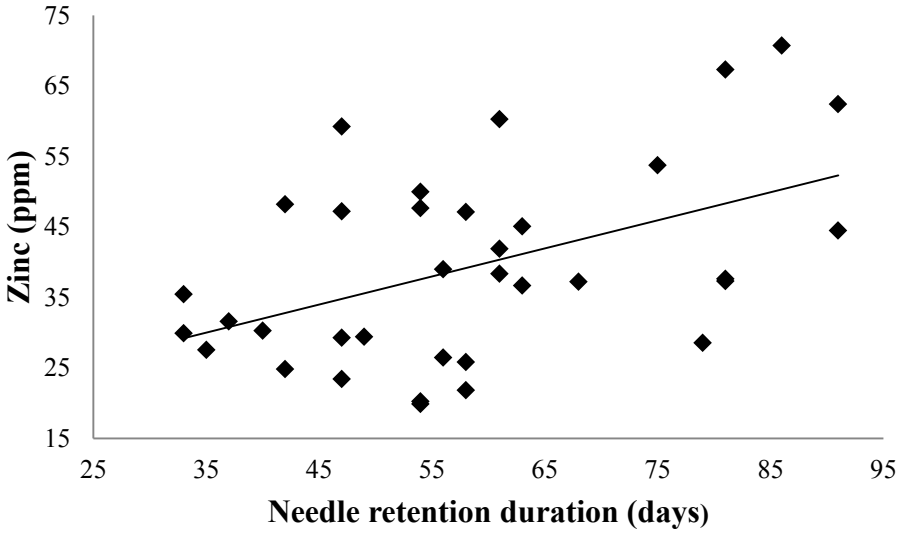

Figure 4.1.5: A linear regression between zinc (ppm) and NRD (days) with the pooled data of all 7 tiers (n=35) with a p-value of 0.004. Zn content and NRD was significant, showing a positive relationship, and a R^2 value of 0.228*.

4.1.5 Discussion

This study demonstrated that despite significant differences in various nutrient element concentrations (except for boron and magnesium) in various tiers, there were no significant differences in NRD. There were also no significant differences in the days for commencement of needle drop or days taken for 5, 10, 20, 40, and 80 % needle loss (Table 4.1.1). This demonstrated a weak link between needle nutrient content and needle retention. The lack of significant differences in needle retention among different tiers cannot be due to genetic variation or rootstock as the samples were collected from one single clone (clone 18). Neither can this variation be attributed to differences in the age of the branches, as all of the samples were two-year old branches collected from the same side on the same day. The weak relationship between needle loss and nutrient content questions the roles of the tested nutrients may have in post-harvest needle retention. Alternatively, post-harvest needle retention may be differentially regulated and not directly through the nutrients that were studied. The non-significant final NRD results could also be due to the wide variation of needle retention among replications and the number of branch replicates. Recently it was proven at CRC, that it would be beneficial to have 8 replicates to reduce variation (Lada and Veitch, 2012). When collecting the second year growth, both lateral and terminal branches were collected. Presently, it is now known that there is a wide variability between lateral and terminal branches in final NRD (Lada and Veitch, 2012). The variation among branches may have resulted in the lack of significance among tiers. Even though NRD was non-significant, it was noted that the different tiers appeared to be behaving differently overall throughout the experiment; the higher tiers appeared to lose needles sooner on average, as seen in Figure 4.1.2 and 4.1.3. In addition, when the higher tiered branches died, the needles

would drop off more quickly (higher percentage of needle loss in less time) in comparison to the lower tiered branches which, on average, abscised slower.

The needle's nitrogen, phosphorus, potassium, calcium, manganese, copper and zinc content all proved to be significantly different among tiers (Table 4.1.2). There has been previous research on nutrient concentration differences within tree height of conifers. Morrison (1972) noted that there was a wide variability amongst species in needle nutrient concentrations within height and so a comparison among species may be difficult to establish. Past studies on *Pinus sylvestris* L. (Leyton and Armson, 1955; Wright and Will, 1958) and *P. nigra* (Wright and Will, 1958) proved that nitrogen was higher in the uppermost tiers and decreased with lower tiers. Table 4.1.2 demonstrated how nitrogen levels were higher in tier 5 compared to tier 2. Nitrogen was seen to increase in concentration with increasing height in *Pinus radiate* (Livingston et al., 1998). However, this positive, linear relationship seen by Livingston et al. (1998) between nitrogen and height was not related to increased light availability as reported by Miyazawa et al. (2004); but related to the increased proximity of needles to the top leader branch. Unfortunately, this relationship was found to be weaker in this study with balsam fir. Such a weak relationship may be due perhaps to the type of material used in the study. In this study two year growth was collected (including first and current year growth) whereas in Livingston et al. (1998), only current year growth was collected and subjected to nutrient analysis.

Phosphorus and potassium had the highest concentrations in the upper tiers, which is also congruent to Morrison's (1972) work. Needles from higher tiers would have more direct access to sunlight and are closer to the main leader branch (Miyazawa et al., 2004; Livingston et al., 1998). These mobile elements would have been transported to higher tiers to help in the production of carbohydrates and/or new plant growth. Regressions of phosphorus and potassium

versus needle retention however were non-significant ($p = 0.548$ and $p = 0.060$, respectively) and had low R^2 values of 0.38 and 0.10. Even though differences in P and K among tiers were significant they had little influence on needle retention amongst tiers.

In Table 4.1.2 it can be seen that calcium and manganese all had higher concentrations in the lower tiers which corresponds with Morrison's (1972) findings in *Pinus banksiana* and with Turner's et al.'s in conifers (1977). Calcium plays a regulatory role in maintaining and controlling the membrane structures of plants (Hepler, 2005). Calcium maintains cell stability, but also it was shown to slow the loss of chlorophyll and protein – all necessary in the function and expression of DNA in conifer needles (Poovaiah and Leopold, 1973). Figure 4.1.3 shows a significant, positive regression between calcium and NRD; with a R^2 value of 0.208, meaning it was not the only factor influencing needle retention post-harvest. Zinc was also higher in the lower tiers than in the upper tiers of balsam fir (Table 4.1.2); *C. pinus pinus* also followed this trend in a previous study done by Wallin (1998). Zinc is known for its role in the formation of tryptophan, a precursor to indole-3-acetic acid (IAA) (Tekale et al., 2009). Higher levels of zinc may discourage abscission because of its role in creating IAA, which indirectly controls sensitivity of needles to ethylene, a hormone signal to cause needle abscission (Tekale et al., 2009). Ethylene is also known to cause needle loss in balsam fir (MacDonald et al., 2010; MacDonald and Lada, 2011; MacDonald et al., 2011; MacDonald et al., 2012). Figure 4.1.5 shows a significant regression ($p = 0.004$) between needle zinc and needle retention with a R^2 value of 0.228. This demonstrates that zinc does appear to have a significant but small influence on needle retention. Importantly, it was seen that on an average, the branches from the lower tiers (specifically in tier 2 and 3), had longer NRD averages and this trend may perhaps be due to their higher calcium and zinc contents.

4.1.6 Conclusion

Although, pre-harvest needle nitrogen, phosphorus, potassium, calcium, manganese, copper and zinc contents differed significantly among tiers, neither days for commencement of needle loss, days for 5, 10, 20, 40, 60, 80 % needle loss nor NRD were significantly affected in any tier. Regardless, in this study, the post-harvest needle loss may not be directly related to the pre-harvest needle nutrients content.

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4.2 The Physiological Significance of Pre-harvest Needle Nutrients on Post-harvest Needle Abscission and Post-harvest Needle Nutrient Dynamics in Balsam Fir

Abstract

Needle retention can be influenced by a variety of pre- and post-harvest factors such as genetic variability, rootstock differences, and environmental factors such as the weather, soil conditions, pests, cold acclimation and hormonal influences. Little research has been done on the physiological significance of needle nutrients and needle retention post-harvest in balsam fir. Two experiments in this chapter examined the pre-harvest nutritional differences in clones 8 and 18, the post-harvest nutrient dynamics in clone 18 on post-harvest needle retention. It was found that there was a significant difference in needle N, Ca, Mg, Mn and Zn contents between clones but not in post-harvest needle retention. These findings suggest that the pre-harvest needle N, Ca, Mg, Mn and Zn contents may not influence the post-harvest needle retention. Phosphorous, iron and copper were shown to declined significantly post-harvest. More research is needed however, to confirm where and how these elements are being re-translocated or used for.

4.2.1 Introduction to experiments 4.3 and 4.4

Post-harvest needle retention is affected by genetics (Lada and Veitch, 2009). Needle retention was proven to be higher in clone 8 compared to clone 18 (Lada and Veitch, 2009). These differences in NRDs could be due to the needle nutrient content variations between clones. In Chapter 4.1 it showed a possible positive, relationship between calcium, manganese and zinc to post-harvest NRD. Calcium, manganese and zinc are expected to be different between clone 8 and 18, explaining their differences in post-harvest NRD.

Calcium and boron are key components in preserving and increasing the storability of fruits (Sen and Karacali, 2010). Calcium and boron are applied as a foliar spray to enhance the post-harvest quality of several types of fruits and decrease the incidence of rot (Moor et al., 2006; Casero et al., 2010; Gupta et al., 2011). Boron is applied along with calcium because in boron deficient plants, the application of boron also has been proven to increase the mobility of calcium within the plant (Shear and Faust, 1970).

The role of calcium in a plant is complex. Calcium is important in maintaining the integrity of the cell wall and within the abscission zone (Addicott, 1982); and high levels of calcium may delay needle abscission. Genotypic differences in needle nutrient content may be one of the reasons for the variation in balsam fir's needle retention behaviour. In apples, N/Ca levels have been reported to be influenced mostly by cultivar (Drake et al., 1991); meaning the cultivar that had lower levels of N/Ca had a lower storage potential post-harvest. Apple cultivars with higher calcium content were firmer, and cultivars with higher nitrogen content were greener (Drake et al., 1991). The difference seen in NRDs between clones is possibly linked to differences in pre-harvest nutrient content. The link between pre-harvest needle nutrients to the post-harvest NRD between clones is important to understand. There is currently little or no knowledge on the needle nutrient differences and post-harvest needle retention between clones in balsam fir.

Post-harvest changes in nutrient content of branches may reveal translocation of important nutrients and/or a link between abscission and nutrients. Nutrients are essential for the maintenance of cellular metabolism, growth and the regulation of plant health, including the synthesis of key hormones such as, auxins and ethylene. For example, zinc has a role in the synthesis of auxins and in stomatal opening (Addicott, 1982; Sharma et al., 1995). Due to this fact, it is predicted that zinc may play an important role in the maintenance of normal auxin levels in a plant, which would prolong the onset of abscission (Addicott, 1982; Sdiri et al., 2013). Copper and iron have a role in regulating ethylene production (Ben-Yehoshua and Biggs, 1970). Currently, it is unknown if the role that copper and iron have is by inhibiting the production of auxins or by producing ethylene. Ethylene by itself promotes abscission in plants and when abscission occurs, calcium is removed from the abscission zone. The removal of calcium from the AZ weakens the cell wall, allowing the organ to break off of the plant (Poovaiah and

Leopold, 1973). If there were any nutrients related to the plant's abscission processes, this study may reveal their possible relationship.

4.3 The Link Between Pre-harvest Needle Nutritions and Post-harvest Needle Abscission in Two Clones of Balsam Fir

4.3.1 Objective

The objective of this experiment was to determine the relationship, if any, between the pre-harvest needle nutrient content and post-harvest NRD in two contrasting clones (high and low NRD).

4.3.2 Materials and Methods

Randomly, ten terminal and lateral branches were collected from each clone 18 (low NRD clone) and from clone 8 (high NRD clone) as described in Chapter 3.1.3. These branches were taken from about 1.5 m above ground on December 1, 2011. They were taken back to the Christmas tree Research Centre and prepared for the post-harvest study as described in Chapter 3, and monitored for AWU, % needle loss and NRD as described in 3.2. Five among the ten branches collected from each clone were sent for nutrient analysis as described in Chapter 3. The laboratory test environment had a consistent temperature of 20-22ºC, humidity of about 16-18% and 24 hour lighting as described in 3.1.4. The experimental setup of experiment 4.3 can be seen in Figure 4.3.1.

Figure 4.3.1: Clone 8 (left) and clone 18 (right) setup; plates are used to collect fallen needles.

Statistical Analysis

This experiment was set up as a completely randomized design (CRD). ANOVA was used to analyze the data and a Tukey's test was used to separate the means.

4.3.3 Results

Needle abscission

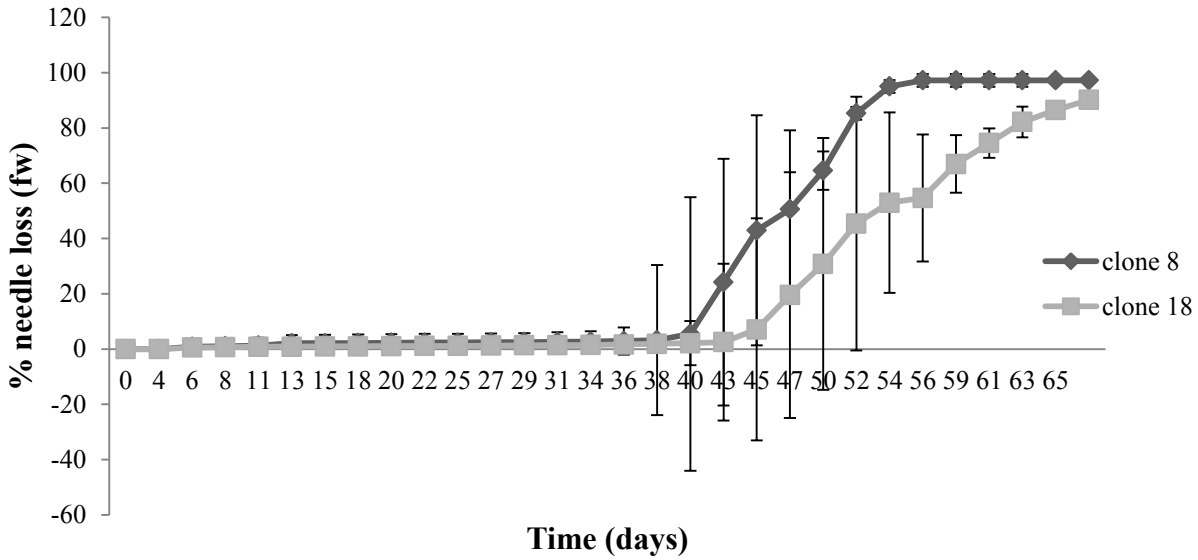

Figure 4.3.2: Cumulative % needle loss of fresh weight (fw) between contrasting clone 8 (5 replications) and clone 18 (5 replications).

Figure 4.3.2 shows the dynamics of needle loss post-harvest. Both clone 8 and clone 18 did not show any significant changes in needle loss dynamics until day 56 onwards and at this point clone 8 has lost all of its needles. Clone 8 was also fast to lose needles compared to clone 18. Figure 4.3.2 shows the difference in needle loss rates.

Needle retention duration (NRD)

There was no statistically signifcant differences in NRD between clones in this study. The high NRD clone (clone 8) lasted for 48 days whereas the low NRD clone (clone 18) lasted for 56.2

days (Table 4.3.1). The day for needle loss commencement (DNLC) was when the branch lost at least 1 % fresh weight of needles. There was no significant difference between clones 8 and 18 at any of the needle loss increments including NRD (Table 4.3.1).

Table 4.3.1: Days taken for needle loss at various abscission intensites, with 7 increments between clones using 5 replications per clone at α=0.05.

% needle loss	Clone 8 (high NRD)	Clone 18 (low NRD)	p-value
NRD	48.0	56.2	0.096
80	47.0	55.4	0.056
40	45.4	52.2	0.114
20	44.4	50.6	0.128
10	44.0	50.0	0.124
5	36.8	49.2	0.128
1	16.8	18.2	0.874

Needle nutrient content

Among all of the pre-harvest foliar nutrients analyzed only nitrogen, calcium, magnesium, manganese and zinc contents were significantly different between clones (Table 4.3.2). Table 4.3.3 shows these significant nutrients with their means separated by Tukey's.

Table 4.3.2: The pre-harvest elements between genotypes and their p-values.

Needle nutrients	N	P	K	Ca	Mg	Fe	Mn	Cu	Zn	B
Genotype	0.036*	0.397	0.785	0.039*	0.03*	0.792	0.04*	0.064	0.045*	0.451

* denotes significance at $\alpha = 0.05$

Table 4.3.3: Variation in needle nutrient content in clone 8 and 18 at α=0.05 and their mean separation using Tukey's test.

Nutrient Elements	Clone 8	Clone 18	p-value
N (%)	1.05^{B}	1.14^{A}	0.036*
Ca (%)	0.58^{B}	0.70^{A}	0.039*
Mg (%)	0.09^{A}	0.07^{B}	0.030*
Mn (ppm)	1073.5^{A}	945.8^{B}	0.040*
Zn (ppm)	32.31^{A}	28.29^{B}	0.045*

* denotes significance at α = 0.05

Pre-harvest needle nutrient analysis for 10 nutrients showed that only nitrogen, calcium, magnesium, manganese, and zinc contents were significantly different between clone 8 and 18. clone 8 had significantly higher magnesium, manganese and zinc contents than clone 18 while clone 18 had significantly higher nitrogen and calcium contents (Table 4.3.3). Despite such variation, there was no significant difference in NRD or days for various needle loss percentages between clones.

Regressions were performed on the pooled data all of the significant nutrients and pooled NRDs of clones. Only calcium had a positive, linear relationship with NRD (p = 0.023) as seen in Figure 4.3.3. Regressions of nitrogen, zinc, manganese and magnesium were also performed to determine their relationship with needle retention. Nitrogen, zinc, magnesium and manganese all had non-significant relationships with NRD (p- value of 0.429, 0.964, 0.900 and 0.063, respectively).

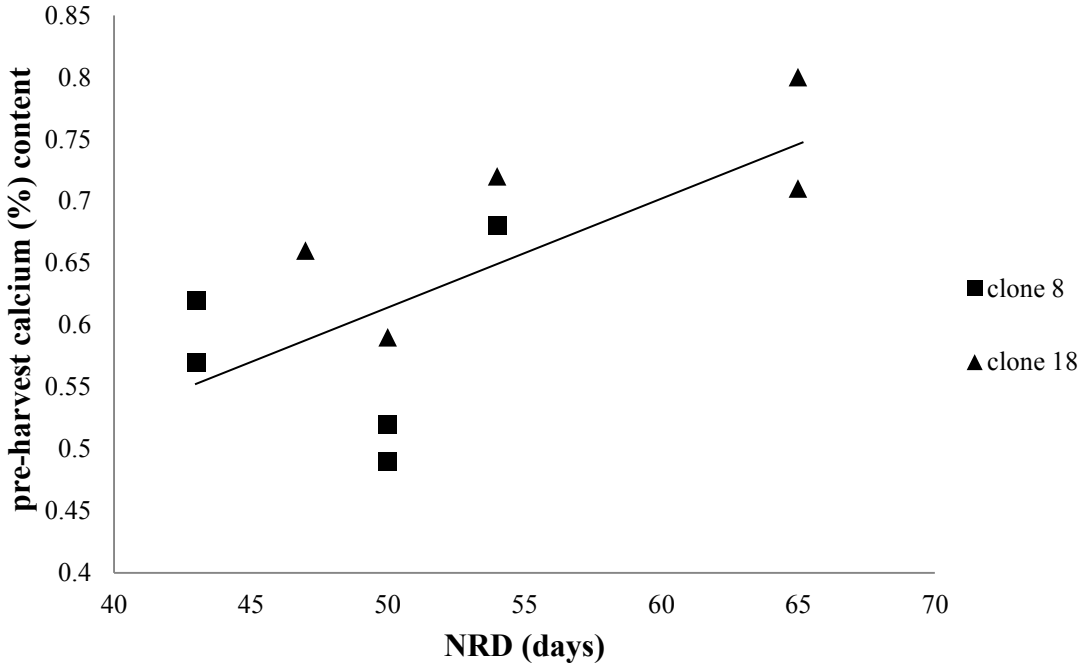

Figure 4.3.3: Calcium content was significantly different between clones (p = 0.039). Clone 18 and clone 8 were pooled to make the total of 10 replication points, showing the pre-harvest calcium content against NRD (days); with a p-value of 0.023 and a R^2 value = 0.66*.

Water Use

Cumulative water use between clones followed a similar pattern; clone 8 consumed more water over time than clone 18 (Figure 4.3.4).

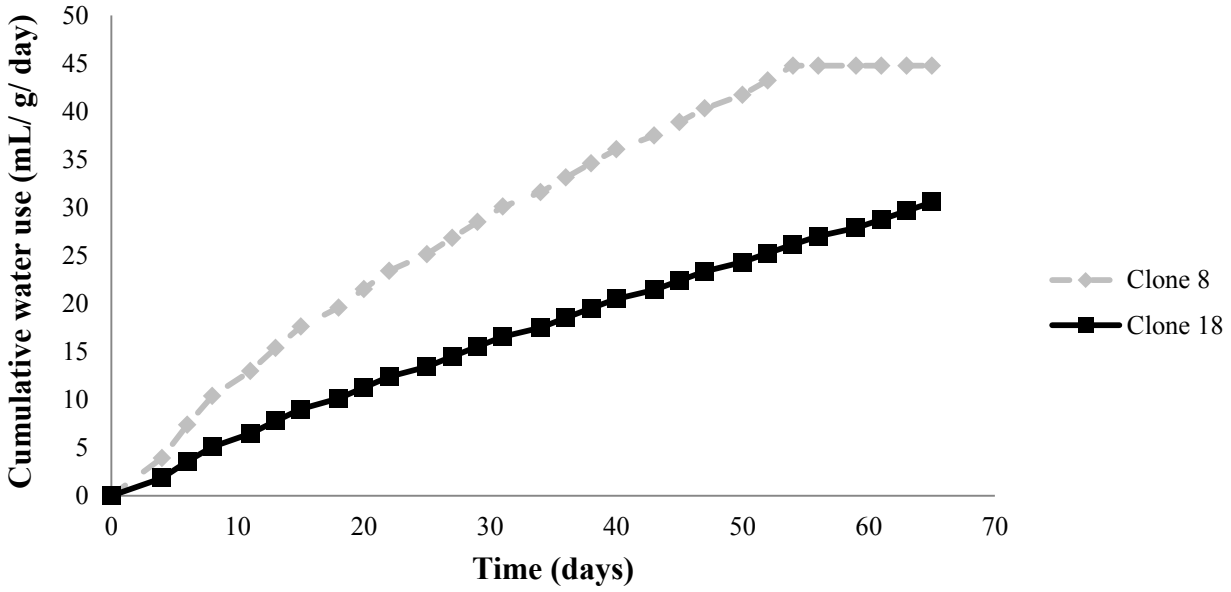

Figure 4.3.4: The cumulative average water use (mL/g/day) of clone 8 and 18 over time.

Daily water use was analyzed between clones for each day, up to 43 days. Clone 8 consumed more water each day than clone 18 (Figure 4.3.5).

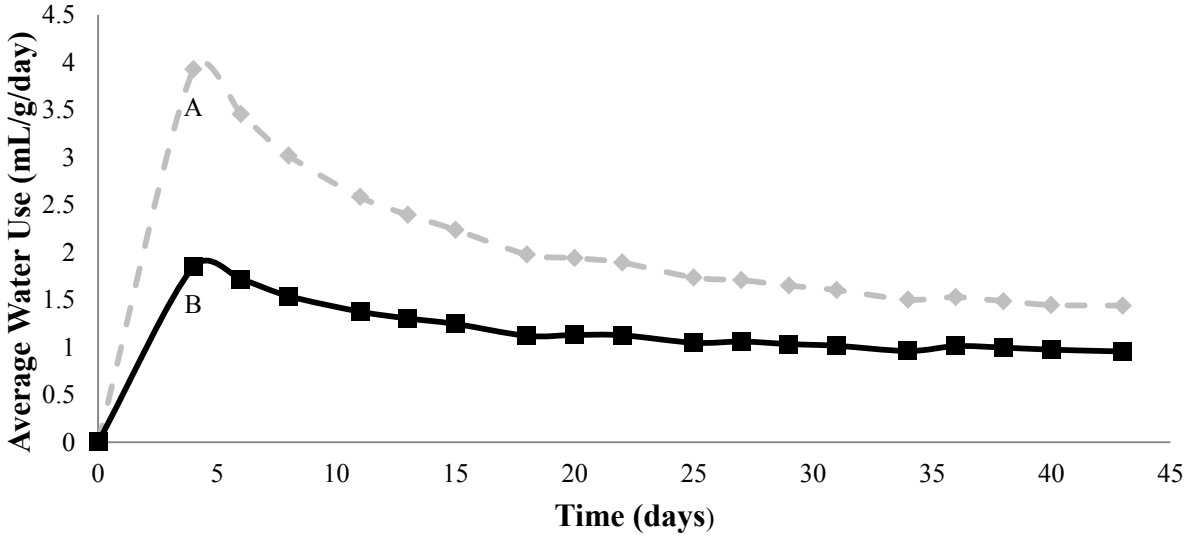

Figure 4.3.5: The daily water use between clones (clone 8 is grey; clone 18 is black); significance between each day started on day 5 to the last data point. There was significance at $\alpha = 0.05$.

4.4 Determining the Relationship Between Post-harvest Needle Nutrient Content and Post-harvest Needle Retention

4.4.1 Objective

The objective was to examine the relationship between post-harvest nutrient content and needle abscission.

4.4.2 Materials and Methods

Sixty branches from one tree (clone 18) were collected at 1.5 m above ground on January 7, 2012 from the Debert Orchard as described in Chapter 3.1.2. They were transported and prepared as described in Chapter 3; the nutrient analyses of 5 replicates were conducted as described in Chapter 3.1.6. This experiment was set up as a CRD with 5 treatment points (day of severance, 30, 37, 44 and 51 days) where nutrient analysis would take place. Ten branches were set up for each treatment time point. On the day of observation, 10 branches were stripped of their current, first-year and second-year needles and the branches were combined to make 5 replicates and sent for nutrient analysis at Harlow.

Statistical Analysis

This experiment was set up as a CRD with 5 time points as treatments. ANOVA was used to analyze nutrient changes over time. Tukey's test was used to separate the means. Regressions were used to determine the link between nutrient content and time where significant differences were found.

4.4.3 Results

Needle nutrient content

Sixty branches were submitted to five different time points. Upon arriving the designated day, the needles were harvested and needle nutrients were analyzed. Among all of the nutrients analyzed, it was seen that the concentrations of phosphorus iron and copper varied significantly during post-harvest (Table 4.4.1). While needle phosphorus and iron content declined significantly post-harvest beginning day 30 there was no significant change in needle copper concentrations up until 44 days in post-harvest.

Table 4.4.1: The nutrient content at various post-harvest time points 10 replications per treatment. Changes in phosphorus, iron and copper content were significant at 0.006, < 0.001 and 0.012 respectively.

		Post-harvest	Time Points	(days)		
Nutrients	0	30	37	44	51	p-value
N (%)	0.96^{A}	1.03^{A}	1.03^{A}	1.02^{A}	1.05^{A}	0.141
P (%)	0.17^{A}	0.16^{BC}	0.16^{B}	0.16^{AB}	0.15^{C}	0.006*
K (%)	0.38^{A}	0.34^{A}	0.36^{A}	0.36^{A}	0.36^{A}	0.503
Ca (%)	0.75^{A}	0.75^{A}	0.70^{A}	0.69^{A}	0.67^{A}	0.662
Mg (%)	0.002^{A}	0.17^{A}	0.17^{A}	0.17^{A}	0.17^{A}	0.241
Fe (ppm)	37.83^{A}	36.00^{B}	31.56^{C}	33.00^{C}	32.30^{C}	<0.001*
Mn (ppm)	1377.61^{A}	1265.23^{A}	1186.18^{A}	1146.52^{A}	1113.47^{A}	0.414
Cu (ppm)	2.01^{B}	2.76^{A}	2.71^{A}	2.17^{AB}	1.96^{B}	0.012*
Zn (ppm)	33.18^{A}	34.28^{A}	33.29^{A}	32.84^{A}	33.20^{A}	0.947
B (ppm)	13.52^{A}	15.83^{A}	16.18^{A}	16.37^{A}	17.49^{A}	0.211

* denotes significance at $\alpha = 0.05$

Phosphorus, iron and copper concentrations were used to establish relationship with needle retention. Phosphorus ($p = < 0.006$) and iron ($p = < 0.0001$) concentration in needles slowly declined over time in post-harvest with a moderately low R^2 of 0.285 and 0.493, respectively representing that there was an influence of time in post-harvest to phosphorus and iron contents in abscising needles (Figures 4.4.1 and 4.4.2). Copper also decreased over time in a reversed

parabola, but with a low R^2 of 0.33 (p = 0.006); meaning that time may not decrease Cu (Figure 4.4.3) consistently, as with P and Fe.

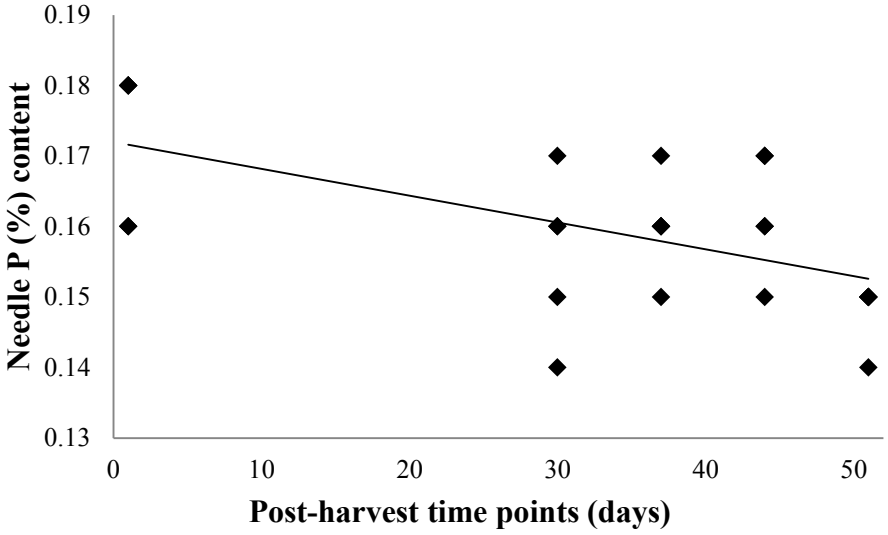

Figure 4.4.1: A linear regression between needle phosphorus content and post-harvest time points (days) of balsam fir. Significant at p = 0. 006 with a R^2 = 0.285*.

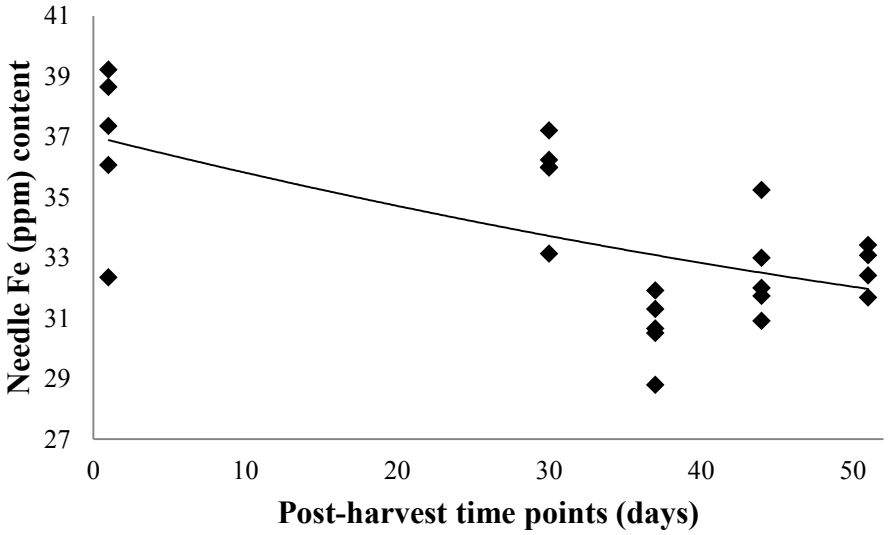

Figure 4.4.2: A linear regression between needle iron content and post-harvest time points (days) of balsam fir. Significant at a p = < 0.0001* with a R^2 value of 0.493*.

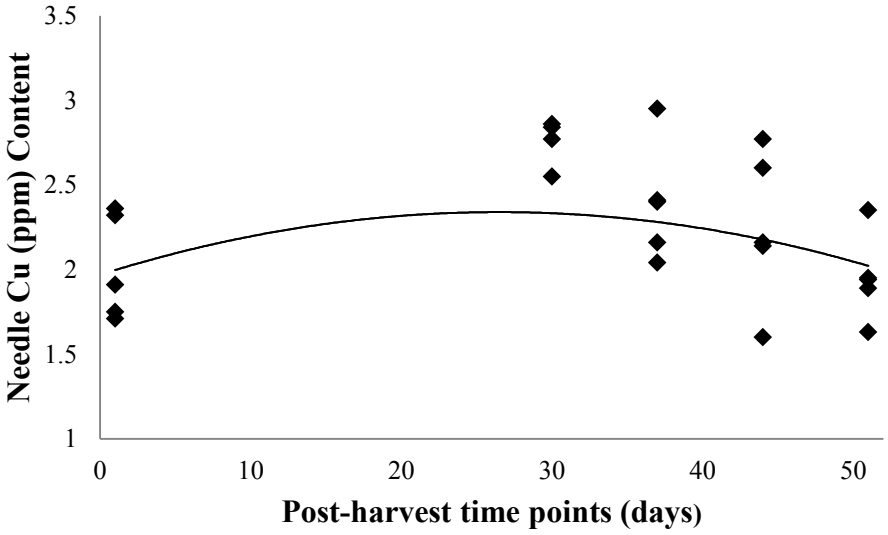

Figure 4.4.3: A quadratic regression between needle copper content and post-harvest time points (days) of balsam fir's needles. Significance at a p-value of 0.006* with a R^2 value of 0.33*.

4.4.4 Discussion for experiments 4.3, 4.4

Chapter 4.3 was set out to discover the link between pre and post-harvest nutrient content and its influence on NRD between clones. Despite the fact that there were a significant differences in pre-harvest needle nutrient content of nitrogen, calcium, magnesium, manganese and zinc (Table 4.3.3) between clone 8 and 18; there were no significant differences in the needle loss dynamics and needle retention duration (Table 4.3.1).

The lack of significance between clones and final NRDs could be explained by their collection date. The collection date of this experiment took place after cold acclimation on 1 December, 2011. Collecting branches after cold acclimation typically increases NRD especially, in the low NRD clones (Mitcham-Butler et al., 1988, Thiagarajan and Lada, 2010) by an increase of a 11

days on average (Lada and Veitch, 2009; Thiagarajan et al., 2012). In this study, clone 18 and 8 were chosen because of their consistency before and after cold acclimation in NRD (Lada and Veitch, 2009), so this reasoning does not entirely explain their non-significance. The non-significance between clones in Table 4.3.1 was most likely due to the number of replications and the collection of both terminal and lateral second year old branches. Now it is known that there is a significant difference in NRDs between terminal and lateral branches, and it is now recommended to only collect lateral branches (Lada and Veitch, 2012).

A reversal in NRDs can be seen in hydrated post-harvest conditions. High NRD clones retained their needles for shorter periods of time and low NRD clones retained their needles for longer periods of time in hydrated post-harvest conditions, post-cold acclimation (Thiagarajan and Lada, 2010). The initial screenings for needle retention on these clones were carried out under dehydrated conditions post-harvest (Lada and Veitch, 2009). The differences in NRD suggest that the low NRD clone (18) may retain needles longer under hydration whereas the high NRD clone (8) may retain their needles longer under dehydration. Regardless of this shift, there were no significant links between pre-harvest needle nutrient content and post-harvest needle retention.

Nitrogen was significant between clones and had the highest concentration in clone 18, which abscised needles slower and lasted a slightly longer than clone 8. When nitrogen was put into a lineal regression against NRD it was non-significant ($p = 0.429$); suggesting that nitrogen may not influence needle retention. A significant ($p = 0.023$) linear regression was observed for pre-harvest calcium concentrations in the pooled data of both clones, this suggested a trend that calcium may play a significant role delaying needle drop (R^2 value of 0.66). Higher pre-harvest nutrient content of calcium predicted longer post-harvest needle retention. Calcium is important

in the maintenance in cell walls and the middle lamella that protects the abscission zone from breaking down and causing abscission of the organ (Addicott, 1982).

Experiment in Chapter 4.4 was conducted to understand the nutrient dynamics post-harvest in abscising branches. This was important to uncover the roles that nutrients may play in post-harvest needle abscission of balsam fir. Sixty branches were submitted to five post-harvest time points where the needles were collected and analyzed, to observe nutrient dynamics post-harvest. This study revealed that needle phosphorus, iron and copper contents varied significantly during post-harvest. Phosphorus and iron contents declined significantly on day 30 in post-harvest from day 0. The needle content of phosphorus declined between day 37 and day 55 and the needle content of iron declined between day 30 and day 51 (Table 4.4.1). Previously, it was hypothesised that conifers succeeded in poor conditions because of their ability to conserve nutrients, even as they abscised their needles. This conservation of nutrients would have been done through the re-absorption of nutrients (Aerts and van der Peijl, 1990; Berendse, 1994).

A decline in the nutrient content post-harvest could not have been caused by growth induced dilution, as there was none during this time period. Translocation was possible for phosphorus and iron as they are mobile elements (Zhang et al., 1995; Abadia et al., 1996) but copper is relatively immobile. Possibly, these elements might have been transported to the branch or water (the medium) prior to abscission, but unfortunately this cannot be confirmed in this study, as analysis of the water and branch was not done.

Figure 4.4.3 shows the regression for copper content post-harvest, which showed a statistically significant decline. Copper is required in the production of lignin synthesis, which is needed in the cell wall and the prevention of wilting. Copper also is a co-factor for several enzymes (Yruela, 2005), in particular it mediates ethylene's binding as a transition metal cofactor (Burg

and Burg, 1967). As mentioned before, copper is reported as a relatively immobile element, although it has been seen in other studies of wheat, that copper can be mobile (Hill et al., 1979). Copper was predicted to be transported from the needles to the phloem as the needles abscised; which would be consistent with a study by Hill et al. (1979). More research needs to be completed to examine nutrient content of the branch and water to determine where the copper is being transported, if anywhere. Regardless, because of the reverse parabola shape of the regression, and the decline of copper content in needles during the last two time points, it can be predicted that copper has a limited role in needle abscission.

Phosphorus is considered as a mobile nutrient, transferable through the phloem of a plant. Figure 4.4.1 shows a significant decline of needle concentration post-harvest. This result was consistent with other studies examining nutrient movement during plant senescence (Hevia et al., 1999; Aerts, 1996). Phosphorus is important for sugar phosphates and energy transfer. Possibly, during senescence in the absence of roots, the plant may try to re-locate its limited supply of phosphorus back within its stems and/or newer plant growth. The reduction in needle phosphorus content post-harvest is predicted to be possibly due to re-translocation to the stem to prevent the loss of essential nutrients.

Needle iron content declined over time post-harvest, as seen in Figure 4.4.2. A study done by Shi et al. (2011) who wanted to re-examine Rissmuller's previous findings in 1874; stated that iron was mobile through the plant's phloem during senescence. More current studies also agree with Rissmuller's findings (Zhang et al., 1995; Abadia et al., 1996). Shi et al. (2011) found in their study that iron was not mobile. They acknowledged that their experiment was not entirely like Rissmuller's and that mobility was possible. They predicted that iron mobility depended on several factors: 1) a sink activity for the nutrient to go to (new buds, seeds) if it had no roots; 2)

mobilization of bound iron, as protein constituents, or from chloroplasts in the source organs; 3) only short distances to transport these compounds from the leaf to phloem and/or 4) adequate low-molecule-weight N compounds to bind iron for phloem transport, as shown for copper and zinc in wheat plants by Hill et al. (1979). Iron had a R^2 value of 0.493 and copper had a moderately, low R^2 value of 0.33, meaning that there is little change over time. Iron and copper both have important roles in photosynthesis, enzyme activation, respiration and within the electron chain (Datnoff et al., 2007) and may be transported to the phloem for its use elsewhere in the plant.

Currently, it is unknown whether or not the decline of these elements caused needle drop. Alternatively, it is known that copper and iron appear to have a role in regulating ethylene production (Ben-Yehoshua and Biggs, 1970). In this case, it is possible that these two elements may have participated in inducing abscission through the production of ethylene or inhibiting auxin synthesis in plants. Ethylene is known to increase needle drop in balsam fir (MacDonald et al., 2010; MacDonald and Lada, 2011; MacDonald et al., 2011; MacDonald et al., 2012; MacDonald and Lada, 2012).

4.4.5 Conclusions for 4.3, 4.4

Although pre-harvest needle nitrogen, calcium, magnesium, manganese and zinc differed significantly between clones, there were no significant differences in NRD or needle loss pattern between clone 8 and 18. The pooled data between clones showed that calcium had the highest R^2 of 0.66 with NRD. Among all of the nutrients studied, needle phosphorus, iron and copper contents declined significantly post-harvest. However, it is unclear whether if a decline in these nutrients had a direct link with needle abscission.

4.4.6 References for 4.3, 4.4

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4.5 The Relationship Between the Monthly Changes in Pre-harvest Needle Nutrient Content and Post-harvest Needle Retention

Abstract

Nutritional status alters various physiological and metabolic processes including senescence and abscission. Uncovering the link between the temporal changes in needle nutrient content and post-harvest needle retention would allow us to understand the interrelationships between needle nutrient status and retention *per se*. The objective of this study was to determine the relationship between temporal changes in pre-harvest nutrient content of balsam fir needles to post-harvest needle retention. Each month, 5 replications from Clone 18 were examined for pre-harvest nutritional content and for their post-harvest needle loss dynamics. Needle abscission dynamics and NRD were all significantly different among months, showing that branches harvested in February, August and September had significantly higher final NRD (59.4, 58.2 and 64.2 days, respectively) while NRD was the lowest in June's branches (22 days). All of the needle nutrients studied changed significantly among months, except manganese and copper. Regardless of significant changes in pre-harvest needle nutrient contents, there were no significant relationships between pre-harvest needle nutrient content and post-harvest needle retention, as none of the needle nutrients studied exhibited significant relationships with NRD.

4.5.1 Introduction

Needle loss in balsam fir post-harvest is a problem for producers, which reduces their sales to consumers. From research at the Christmas tree Research Centre, we know that needle loss fluctuates, depending on the month. Through previous laboratory experiments it was observed that there was an increase in needle loss and variability during the flushing of new buds (unpublished results). The flushing of new buds typically occurs during May and June. In previous seasonal experiments, it was proven that during the time of new growth, nutrient concentrations actually decreased with the temporary increase in volume (Jerome et al., 2002). Nutrients are re-located throughout the seasons depending on their need. For example, with the new growth of buds, excess nitrogen, magnesium and other key nutrients are needed and re-located to these areas of growth (Schmidt and Stewart, 1997).

The variation in post-harvest needle loss in balsam fir among various months may be due to the variation in needle nutrient contents. Previous research has proven that calcium, potassium, magnesium, nitrogen and phosphorus are all highly influenced by seasonal changes (Oliveira et al. 1996, Sabate et al., 1995, Escudero et al., 1992). Increased needle loss during certain months of the year may be attributed to the variances of needle nutrient content and fluctuations. By understanding the temporal changes in needle nutrient content and needle retention, one could confirm whether or not the needle nutrient content relates to needle retention and potentially explain the changes in needle retention as influenced by month. This information can be used to recommend times for Christmas tree harvest and times for nutrient analysis as well.

4.5.2 Objective

The objective of this study was to determine the relationship between temporal pre-harvest needle nutrient content and post-harvest needle retention in clone 18.

4.5.3 Materials and Methods

This experiment was set as a completely randomized design using clone 18 starting in October 2011 (Chapter 3.1.4). Treatments were the month of collection and for each treatment there were five replicates for the post-harvest study and 5 replicates for needle nutrient analysis. Soil samples were collected along the drip line of the tree and soil moisture was measured concurrently using a HH2 moisture meter (Delta-T Device Ltd, United Kingdom, Cambridge). Weather data was collected from Environment Canada for Debert to find the average monthly temperatures (high and low) and the monthly precipitation. Ten branches were cut at about 1.5 meters high on the tree, the first five branches were subjected to pre-harvest nutrient analysis

(Chapter 3.1.6) and the other five (replicates) were observed for % needle loss, AWU and NRD (Chapter 3.2).

Weather Conditions

Weather conditions were monitored by Environmental Canada and the data was extracted and put into graphs to view the monthly changes. Soil moisture was collected each month around the drip line of the tree when possible, as sometimes the ground was frozen**.**

Table 4.5.1: The average soil moisture conditions (4 replications around tree) for each month.

	Jan.	Feb.	Mar.	Apr.	May	Jun.	July	Aug.	Sept.	Oct.	Nov.
Soil Moisture (%)	n/a	n/a	n/a	43.9	12.6	14.4	13.6	n/a	31.3*	26.9	22.2

* Measurements occurred after heavy rainfall

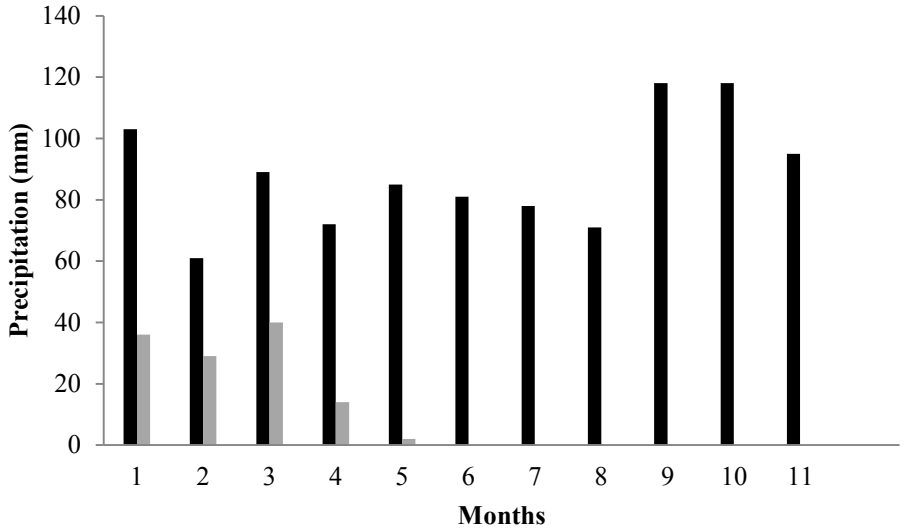

Figure 4.5.1: The monthly averages of precipitation (mm) for each month of year 2012, excluding months 10 and 11, which were 2011. 1 = Jan, 2 = Feb, etc. The black bars are for rainfall, the grey bars are for snowfall averages (cm).

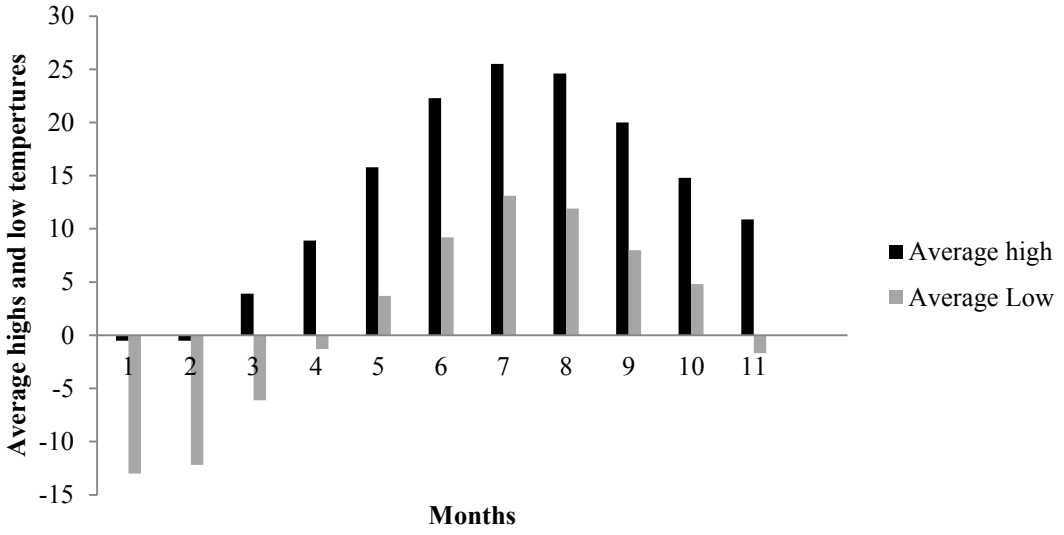

Figure 4.5.2: The monthly high and low temperatures (ºC) of year 2012, excluding months 10 and 11, which were 2011. 1 = Jan, 2 = Feb...12 = Dec, etc. The black bars are for the high temperature averages, the grey bars are for the low temperature averages.

Statistical analysis

Parameters under study were subjected to ANOVA, means were separated using Tukey's. Nitrogen, phosphorus and potassium were plotted in a scatter plot to view their fluctuations over the months and to determine which months would be the best for sampling for nutrient analysis.

4.5.4 Results

Needle abscission dynamics

The needle abscission dynamics were all significant among months (Table 4.5.2). The needles from the branches collected in September took the longest to commence needle loss (26.6 days), and achieve 5 % (40.4 days), 10 % (56.4 days), 20 % (57.6 days), 40 % (59.8 days), 80 % needle loss (63.6 days) and final NRD (64.2 days). Branches collected in April and June were evenly matched for being the fastest to lose their needles. For the most extreme example out of these

months, needle loss commenced after 2.8 days in branches collected in April while those harvested in September started to lose their needles at 26.6 days.

Table 4.5.2: Days for needle loss (NL) at various percent levels of all months studied. There were 5 replications per month.

	Days for NL						
Months	1%	5 %	10 %	20 %	40 %	80 %	NRD
Jan	9.0^{AB}	20.0^{AB}	28.8^{ABC}	36.4^{ABC}	38.4^{ABC}	40.0^{AB}	40.0^{AB}
Feb	8.8^{AB}	20.0^{AB}	28.0^{ABC}	34.0^{ABC}	42.4^{ABC}	58.0^{A}	59.4^{A}
Mar	7.6^{AB}	28.8^{AB}	33.4^{ABC}	44.8^{ABC}	50.2^{AB}	49.6^{AB}	46.9^{AB}
Apr	2.8^{B}	26.4^{B}	26.2^{ABC}	46.4^{AB}	49.4^{AB}	51.0^{AB}	51.8^{AB}
May	4.8^{B}	12.2^{AB}	21.4^{BC}	22.4^{BC}	22.4^{BC}	23.8^{B}	26.4^{B}
Jun	5.0^{B}	16.0^{B}	11.4^{C}	14.2^{C}	14.2^{C}	21.0^{B}	22.0^{B}
Jul	6.0^{B}	8.8^{AB}	28.8^{ABC}	33.0^{ABC}	34.0^{ABC}	35.6^{AB}	36.0^{AB}
Aug	22.0^{AB}	22.4^{AB}	44.6^{AB}	47.8^{AB}	54.0^{A}	56.2^{A}	58.2^{A}
Sept	26.6^{A}	40.4^{A}	56.4^{A}	57.6^{A}	59.8^{A}	63.6^{A}	64.2^{A}
Oct	11.6^{AB}	53.8^{AB}	36^{ABC}	39.0^{ABC}	40.8^{ABC}	43.6^{AB}	44.6^{AB}
Nov	16.0^{AB}	28.4^{AB}	29.8^{ABC}	32.6^{ABC}	34.8^{ABC}	36.4^{AB}	36.8^{AB}
p value	0.004*	0.004*	0.003*	0.002*	<0.0001*	<0.0001*	<0.0001*

* denotes significance at $\alpha = 0.05$

As previously mentioned, overall the branches collected in September took the longest to reach any of the needle loss percentages (Table 4.5.2), and those harvested in June took the shortest period of time (days) to reach any of the needle loss percent increments. For example, September lost 1 % of its needles, on average, at 26.6 days whereas for June, commencement took only 5 days.

Needle retention duration (NRD)

Needle retention duration differed significantly among months ($p = <0.0001$). The branches that were collected on September, August and February, all retained their needles for the longest post-harvest duration reaching 64.2, 58.2 and 59.4 days, respectively. While this is so, the branches that were collected in May and June retained their needles for the shortest period post-harvest for only 26.4 and 22 days, respectively (Table 4.5.2).

Nutrient content

Table 4.5.3: The pre-harvest needle nutrient content from each month in a balsam fir tree, with 5 replications per month. A Tukey's test was used to separate the means of significant nutrients. Values with the same lettering are not significantly different.

Nutrients	Months											
	Jan.	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	p value
N	1.03^{AB}	1.08^{AB}	0.98^{AB}	0.94^{B}	0.94^{B}	1.00^{AB}	0.97^{AB}	1.04^{AB}	1.14^{A}	1.34^{AB}	1.09^{AB}	0.009*
P	0.17^{AB}	0.19^{A}	0.14^{BC}	0.13^{C}	0.14^{BC}	0.14^{BC}	0.15^{ABC}	0.14^{BC}	0.16^{ABC}	0.17^{AB}	0.18^{A}	0.0001*
K	0.40^{AB}	0.44^{AB}	0.32^{B}	0.35^{AB}	0.41^{AB}	0.42^{AB}	0.36^{AB}	0.33^{AB}	0.44^{AB}	0.42^{AB}	0.45^{A}	0.03*
Ca	1.18^{A}	1.13^{A}	1.24^{A}	0.84^{A}	0.98^{A}	0.98^{A}	1.26^{A}	1.15^{A}	1.03^{A}	1.20^{A}	1.21^{A}	0.031*
**Mg	0.13^{AB}	0.14^{A}	0.12^{BCD}	0.12^{BCD}	0.11^{CDE}	0.11^{DE}	0.13^{ABCD}	0.11^{E}	0.13^{AB}	0.13^{ABCDE}	0.14^{A}	0.001*
Fe	27.90^{AB}	29.81^{AB}	28.97^{AB}	33.02^{AB}	32.29^{A}	27.63^{AB}	27.68^{AB}	25.19^{B}	25.60^{B}	26.96^{AB}	25.59^{B}	0.021*
Mn	1560.96^{A}	1461.04^{A}	1422.57^{A}	821.74^{A}	1005.09^{A}	1181.68^{A}	1255.05^{A}	1358.10^{A}	1166.89^{A}	1106.98^{A}	1449.19^{A}	0.09
Cu	2.21^{A}	2.88^{A}	2.09^{A}	2.22^{A}	2.24^{A}	2.59^{A}	2.38^{A}	2.40^{A}	2.77^{A}	2.41^{A}	2.50^{A}	0.232
Zn	47.54^{A}	54.93^{A}	45.17^{A}	29.87^{B}	38.88^{AB}	42.47^{AB}	37.82^{AB}	40.76^{AB}	40.61^{AB}	43.21^{AB}	39.38^{AB}	0.001*
B	10.06^{B}	9.05^{B}	10.50^{AB}	11.49^{AB}	8.49^{B}	9.88^{B}	15.60^{A}	8.27^{B}	10.28^{B}	11.61^{AB}	12.92^{AB}	0.001*

* denotes significance at $\alpha = 0.05$; **Kruskal-Wallis test

All of the nutrients, except manganese and copper, significantly differed among months (Table 4.5.3). The branches harvested in September had the highest levels of nitrogen compared to April and May, while November and February had the highest levels of phosphorus compared to April. Zinc was the highest during January to March and then the branches had significantly lower nutrient content of zinc in April. Calcium was significant, but Tukey's was unable to separate the means. When regressions between these statistically, significant nutrients among months and NRD were performed, none of the needle nutrient contents showed any significant relationships to the final NRD.

Water use (data not shown)

The branches collected in June consumed more water compared to the branches collected in other months. Even so, the branches collected in September consumed water more steadily. The average daily water use between June and September for the first 19 days were analyzed for significance. There was marginal significance ($p = 0.054$) in water use between June and September, which were the months with the highest and lowest NRDs.

Nutrient analysis for N: P: K

To predict the best time for nutrient analysis on balsam fir, scatter plots of nitrogen, phosphorus and potassium were produced to look at the nutrient dynamics over time (Figures 4.5.3 - 4.5.5). The elements nitrogen, phosphorus and potassium were chosen because these were the nutrients that are commonly applied to Christmas tree plantations.

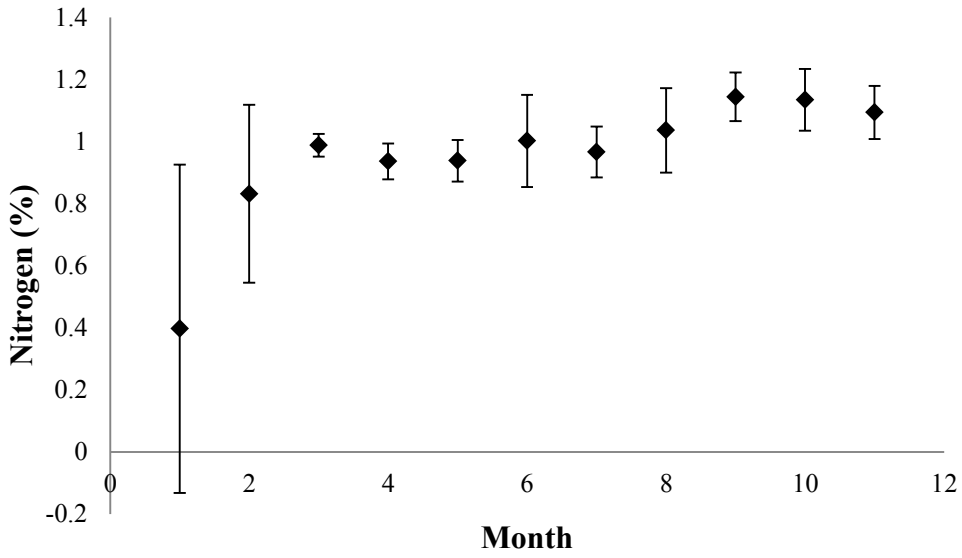

Figure 4.5.3: The average pre-harvest needle nitrogen content (%) from 5 replications per month (1-11 = Jan-Nov) with standard deviations.

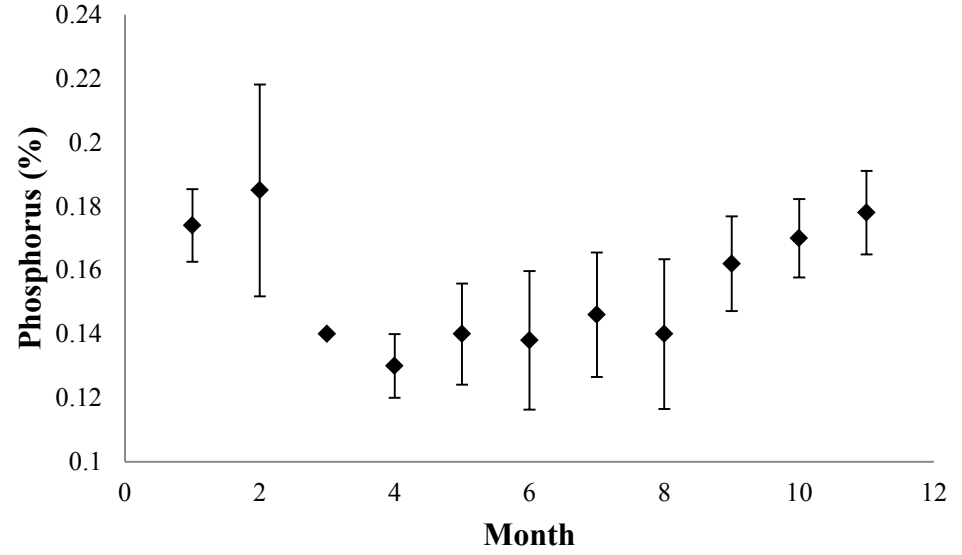

Figure 4.5.4: The average pre-harvest needle phosphorus content (%) from 5 replications per month (1-11 = Jan-Nov) with standard deviations.

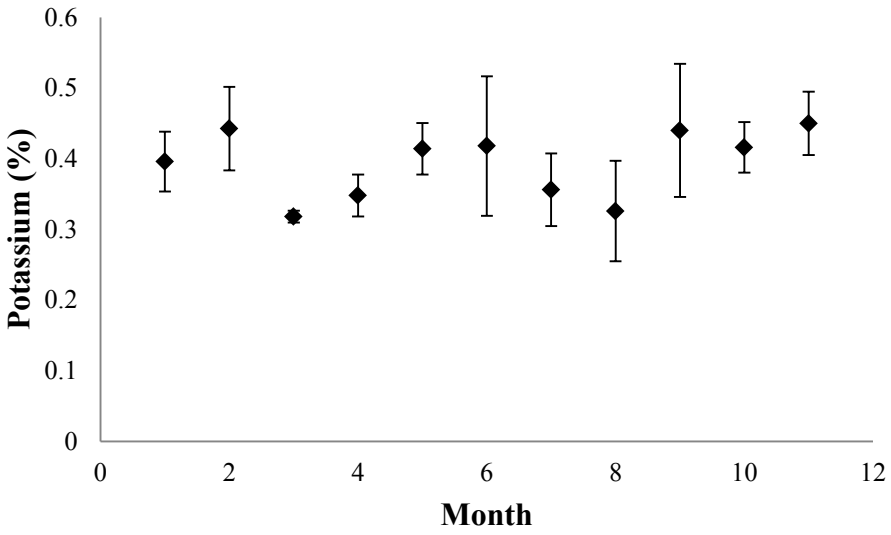

Figure 4.5.5: The average pre-harvest needle potassium content (%) from 5 replications per month (1-11 = Jan-Nov) with standard deviations.

Figures 4.5.3 - 4.5.5 provide information for the best time to conduct a nutrient analysis for nitrogen, phosphorus and potassium. According to these figures the best time for a nutrient analysis would be between October to November, this is because there is some stability during this time period and minimal variation.

4.5.5 Discussion

There was no direct research on the significance of temporal changes in needle nutrients and post-harvest needle retention in conifers. The needle abscission dynamics and the final NRD were statistically significant among months. The branches that were harvested in February, August and September had the highest final NRD (59.4, 58.2 and 64.2 days, respectively). The branches of September also had all of the highest days for needle loss at various needle loss percentages than any of the other months (Table 4.5.2). Regardless, there was no relationship between pre-harvest nutrient content and post-harvest needle retention. The branches collected in September also had the lowest cumulative water usage than any other month and the branches

collected in June had the highest cumulative water usage. There is one reason why there was a difference seen in cumulative water use between branches collected in September and June. The branches collected in June were producing new growth of needles. The needles were developing and therefore, may have had less regulatory control over their stomata, which would have increased transpiration. This new growth of needles seen in the spring may have reduced May and June's branches needle retentive abilities, which were seen in Table 4.5.2. When the average water usage (AWU) was calculated over 19 days it was seen that there was statistically no difference between June and September. Even though the branches collected in June consumed more water over all, the water uptake in branches was similar in both months during the first 19 days. The first 19 days were chosen because branches began to die after this point and it was necessary to prevent data from being skewed towards the living branches.

Nutrient content was significantly different among months. The needles from branches that were harvested in September had the highest nitrogen content in comparison to April and May's branches. This result was in contrast to what was seen in Chapter 4.4, which showed that with increasing nitrogen, NRD decreased. Other research has also shown that excess nitrogen is detrimental to needle retention in conifers (Black, 1968; Hinesley, 2000). Regardless, there must have been other influences increasing needle retention.

By looking at the weather data (Figures 4.5.1 and 4.5.2), it can be seen that there was increased precipitation and cooler temperatures in September after a dry, hot summer. Soil moisture over the summer months remained between 12 – 14 % and then in September increased to 31.3 % (Table 4.5.1). Water stress decreases net photosynthesis which also decreases growth in Douglas fir (Littell et al., 2008). Trees have two main mechanisms to cope with drought: (1) reducing transpiration - by closing stomata and/or reducing leaf area or (2) increasing root growth to

obtain more water (Littell et al., 2008). Consequently, increased needle loss during June, may have been the result of the tree trying to reduce its transpiration (a carryover effect even after harvested). Needle loss may perhaps be due to the sudden re-hydration of the branches that may have encouraged defoliation. After a period of drought, it is possible that increased rains during September may have re-opened the stomata. This increased transpiration could increase water uptake and nutrient uptake maintaining the demand for needle retention. Although without data on stomatal conductance, this cannot be confirmed.

Boron was shown to be significantly highest in July compared to January, February, May, June, August and September; which coincides with the formation of cones and seed maturation (Owens, 1984). Boron is required for the reproductive growth of plants and trees (Brown and Shelp, 1997) and explains its increased concentrations in the needles. Zinc was highest in the branches from January to March (Table 4.5.3). Zinc is essential in the production of auxins (Addicott, 1982); which is one of the plant hormones responsible for growth. This explains why there were increased levels during the months before bud emergence and the growth of new needles, which occur in May and June. This bud flushing could also be responsible for the increased needle loss during the month of June. Needle loss in June was during a time when new needles were forming with increased water volume, but with the same nutrient content (Jerome et al., 2002); decreasing the cell wall and abscission zone's strength (Addicott, 1982).

There was little research on the best times to collect needles for a nutrient analysis in Christmas trees. Currently, it is recommended to collect the needles during late summer and early fall (Christmas Tree Grower's Manual, 2011; Spectrum, 2012), but this is most likely based on forestry practises and not on actual data. A minimum of 2 months of little change in nutrient content is optimal for an accurate sampling (Hart et al., 2012). Hart et al. (2012) determined the

best time to collect needles in Douglas fir, Noble fir, Turkish fir, Grand fir and Nordmann fir, for a nitrogen analysis, was during February. Even though there is data for these Christmas tree species, there is still no data for balsam fir in eastern Canada. According to Figures 4.5.3 – 4.5.5, the best time for needle sampling and analysis would be between October and November, when there was little nutrient content fluctuations, especially in nitrogen, confirming past recommendations (Christmas Tree Grower's Manual, 2011). Finally, the best time for tree harvest would be in September (Table 4.5.2). Branches collected during the month of September took the longest to reach any of the needle loss percent increments and was the longest to last overall, reaching a final NRD of 62 days.

4.5.6 Conclusion

While the needle nutrient contents changed significantly over months, except manganese and copper, none of these pre-harvest needle nutrients had a significant relationship with NRD. The needle abscission dynamics and final NRD was significant among months. Branches collected in February, August or September all had the highest final NRD (59.4, 58.2 and 64.2 days, respectively) and days for 1 % needle loss. In contrast, harvesting June had the lowest NRD of 22 days and the earliest at all levels of needle loss. The best times for nutrient analysis sampling would be between October and November. For longer lasting Christmas trees (for export), harvest should occur late September where there was high needle retention.

4.5.7 References

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4.6 The Relationship Between Soil, Needle Nutrient Content and Post-harvest Needle Retention

Abstract

Currently, we know little about the relationship between soil and needle nutrient content and how it influences post harvest needle abscission in balsam fir. This study was conducted by obtaining branches from locations in Pictou, Colchester and Kings counties, Nova Scotia. Three trees were selected at each site with 5 branches that served as replications from each tree –a total of 15 branch replications per site. Soil samples were taken around each tree and soil moisture was taken with a TDR probe. All of the foliar nutrients were significantly different among locations except for boron. For the soil characteristics, significant relationship existed among locations for soil pH, CEC, calcium, magnesium, aluminum, iron and zinc contents. NRD differed among three locations. Needle retention duration was the highest with the branches that were collected from Kings county compared to Colchester or Pictou counties. Needle nutrient concentration revealed that higher nutrient content (N, P, K, Ca, Fe, Mn, Cu, Zn and B) in Pictou and Colchester counties correlated with low NRD, while low nutrient status may have contributed to higher NRD in Kings county. A Pearson's correlation was preformed to see the relationship between the soil characteristics and needle nutrient content and NRD. It was shown that none of the soil characteristics influenced the post-harvest NRD, although some of these characteristics did influence needle nutrient content. Four foliar nutrients had a significant correlation with NRD, copper, nitrogen, iron and phosphorus, which had a negative effect on NRD at higher concentrations.

4.6.1 Introduction

When balsam fir trees are harvested, they are severed from their roots. Such a practice may prevent or reduce nutrients and water uptake affecting post-harvest quality. Nutrients are essential for the maintenance of cellular metabolism, growth and the regulation of plant health (Campbell, 2005). Root severance can disrupt these physiological processes as decline in nutrient and water uptake can cause needle senescence and/or abscission. Previous research has shown possible links to needle calcium and zinc content to increasing needle retention (Chapter 4.1 and Chapter 4.3) but it was not consistent. There are also genetic influences and rootstock differences on needle retention (Lada and Adams, 2009) that can possibly be explained by variation in nutrient uptake differences. Other research has shown the importance of calcium for post-harvest storability of apples (Asgharzade, 2012); as well as the prevention of leaf and/or flower

abscission (Beyer and Qubedeaux, 1974). Currently, we know little about the linkage between soil and needle nutrient content and how it may influence needle abscission post-harvest.

Soil is important for maintaining plant health and it is the medium where plants obtain all of their nutrients and water. Soil quality is influenced by physical, chemical and biological properties (Karlen et al., 1997). Fertilization is used to improve soil nutritional content and as a result plants, which rely on the soil nutrients and contents for their growth. Fertilizing crop lands is a common practise in agriculture because it improves soil fertility and plant health, particularly in poorer soils. Balsam fir orchards receive irregular to little fertilization. There is little knowledge between soil fertility and needle nutrient content and needle retention. Previous fertilization studies on conifers have been shown to improve the appearance and general growth of the tree, not needle retention (Rothstein, 2005; Rideout and Overstreet, 2004). Needle nutrient content was found to be influenced by the soil's nutrient content and fertilization increases needle length, needle area and increased apical growth (Hinesley and Snelling, 2000). Rideout and Overstreet (2004) confirmed that it is best to base fertilization practices on soil and tissue nutrient analysis because it would give the most accurate picture of any potential nutrient deficiencies.

While there has been research completed on tissue nutrient content and leaf and/or flower abscission in other plant species (Beyer and Quebedeaux, 1974; Osundina and Osonubi, 1988; Ruuhola et al., 2011) there was no direct evidence that needle nutrient content alters needle retention post-harvest in balsam fir. Therefore, it was important to understand the relationship between soil fertility, needle nutrient content and needle retention.

4.6.2 Objective

The objective of this experiment was to determine the relationship, if any, between soil, needle nutrient content and post-harvest needle abscission

4.6.3 Materials and Methods

Branches were collected from three counties in Nova Scotia, Canada, Colchester County **(**45.341732º N,-63.394053º W), Pictou County (45.487063 º N,-62.905054 º W) and Kings County **(**44.538334º N,-64.712477º W) and subjected to this experiment, which used a completely randomized design (CRD). Three trees were selected in one field from each county. The tree height (mean = 285.75 cm, +/- 28.45 cm) and the trunk diameter (mean = 5.23 cm, +/- 0.94 cm) were measured at breast height (~ 100 cm). This was done to ensure that each tree was similar in size and consequently, in age (Platt et al., 1988). From each tree, 10 branches were sampled on the eastern side of the tree. When these branches were sampled, it was ensured that they were sister branches (Figure 3.1.1). Five branches (replicates) were sent for nutrient analysis at Harlow Institute as described in Chapter 3.1.6; the other 5 replicates were prepared for the post-harvest examination as described in Chapter 3.1.4. The response variables studied were NRD, percent needle loss, soil and tissue nutrient content, and soil moisture which were described in more detail in Chapter 3.2.

Management practises and environmental influences

Soil management was similar among locations. All three locations had soil treatments of 25-5-5 (N: P: K) during the spring. The branches sampled from the trees in Pictou were originally seedlings obtained from Debert, Nova Scotia.

Table 4.6.1: Management practises, soil series and textural analysis of balsam fir plantations

Location	Soil Series	Texture	Soil Treatment	Tree Type
Colchester (45.341732º N,-63.394053º W)	Old Barns	Sandy loam	25-5-5 (N-P-K)	Natural stand
Pictou (45.487063º N,-62.905054º W)	Millbrook	Gravelly sandy loam	25-5-5 (N-P-K)	Seedling*
King's County (44.769888º N -64.801655º W)	New Ross	Coarse sandy loam-	25-5-5 (N-P-K)	Natural stand

* seedlings obtained from Debert, Nova Scotia

Statistical analysis

The variables were submitted to ANOVA and the means were separated by Tukey's test. Pearson's correlation was used to determine the relationship between soil characteristics, needle nutrient content and final NRD. Significant nutrients that influenced the final NRD in the Pearson's correlation's analysis were submitted to regressions.

4.6.4 Results

Needle loss dynamics

Needle loss was significant at all increments except at the commencement date (where 1% of the needles were lost). The samples collected from King's county had the highest NRD and the highest days of 5, 10, 20, 40, 80 % needle loss increments (Table 4.6.2).

Table 4.6.2: Needle loss dynamics post-harvest in balsam fir at 7 increments: commencement date (1%), 5%, 10%, 20%, 40%, 80% and 100 %. There were 15 branch replications per location.

	Increments (days)						
Location	1%	5%	10%	20%	40%	80%	NRD
Kings	15.7^{A}	42.8^{A}	45.9^{A}	46.5^{A}	48.7^{A}	52.3^{A}	53.5^{A}
Colchester	13.7^{A}	24.7^{B}	28.0^{B}	31.9^{B}	35.1^{B}	41.6^{AB}	41.6^{AB}
Pictou	10.3^{A}	20.8^{B}	24.9^{B}	26.9^{B}	29.5^{B}	33.7^{B}	34.5^{B}
p-value	0.379	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.001*	0.006*

* denotes significance at $\alpha = 0.05$

Needle retention duration (NRD)

Needle retention duration significantly differed among locations (Table 4.6.2). The NRD was the highest in branches collected from Kings county with 53.5 days while Pictou and Colchester county had statistically similar NRDs with 34.5 and 41.6 days, respectively.

Figure 4.6.1: A comparison of needle loss patterns among locations and/or branches.

There was a wide variability among the tree branches from three different counties in the pattern of needle fall. In total, there were three main ways that the needles could fall. 1) The Mummified Way (Figure 4.6.1 – right side) when the needles would dry out excessively and cling to the branch. The needles would then fall in off in clumps, and only through mechanical stimulation. 2) The Fresh Way (Figure 4.6.1 – left side) when the needles would fall off the branch green and

soft, just like the day that it was collected; and finally, 3) Senescence and Abscission Way: when the needles appear to go through senescence and brown, loosen at the abscission zone, and then fall off by mechanical and/or unaided stimulation.

In addition, the needles fall in two main quantities. Sometimes the needles fall all at once off of the branch, in one to three days, or the needles would take a couple of weeks to slowly fall off of the branches

Figure 4.6.2: An example of Pictou county's branches – they had long, thin, flat needles, which abscised quickly.

Figure 4.6.2 shows how the Pictou branches were different than the branches from Colchester and Kings counties. The Pictou branches had longer, flatter, thinner and softer needles than the needles from the other two locations. Colchester and Kings had needles that were shorter, thicker and tougher with needles configured a whorl around each twig. The needle arrangement

differences seen among locations could not be due to low light availability since all three locations had similar tree density. Differences seen in needle morphology could be due to the fact that Pictou's trees were seedlings and hand planted compared to Colchester and Kings county's trees, which are managed wild stands.

Needle and soil nutrient content

Eight of the nine needle nutrient contents, except manganese were significantly different among locations. Among all three locations, Pictou had the highest levels of the nutrients but disappointingly the lowest final NRD (34.53 days), over all. Kings on the other hand had the lowest needle nutrient content but had the highest NRD (Table 4.6.3 and 4.6.2).

Table 4.6.3: The p-values, means and lettering among locations in needle nutrient content and the post-harvest NRD of balsam fir from three different Counties (Pictou, Colchester and Kings). There were 15 branch replications for each site.

	Locations			p-value
Elements	Kings	Pictou	Colchester	
N	1.27^{C}	1.63^{A}	1.50^{B}	<0.0001*
P	0.36^{B}	0.42^{A}	0.35^{B}	<0.0001*
K	0.46^{C}	0.62^{A}	0.56^{B}	<0.0001*
Ca	0.66^{B}	0.83^{A}	0.64^{B}	0.0002*
Fe	27.76^{C}	41.16^{A}	35.08^{B}	<0.0001*
Mn	891.59^{B}	1149.52^{A}	778.71^{B}	0.0016*
Cu	3.05^{B}	3.93^{A}	3.89^{A}	<0.0001*
Zn	7.00^{C}	11.26^{A}	8.07^{B}	<0.0001*
B	15.36^{A}	14.11^{A}	12.96^{A}	0.1665
NRD	53.53^{A}	34.53^{B}	41.6^{B}	0.0016*

* denotes significance at $\alpha = 0.05$

The soil characteristics that were significant between locations were the pH, CEC, calcium, magnesium, aluminum, iron and zinc (Table 4.6.4). Colchester had the lowest pH (3.97) and Kings had the highest pH (4.9) of the three locations, which were generally acidic. Also it was

noted that Colchester had the highest organic matter (13.50) and the higher CEC (19.13). Over all, Colchester had the highest levels of soil nutrients, which is in contrast to its foliar nutrients content (Table 4.6.4).

Table 4.6.4: The p-values, means and lettering of soil characteristics among locations (Colchester, Pictou and Kings).

Soil characteristics	Locations			
	Colchester	Pictou	Kings	p-value
pH	3.97^{B}	4.47^{AB}	4.90^{A}	0.015*
Organic	13.50^{A}	7.6^{B}	9.13^{AB}	0.062
CEC	19.13^{A}	11.23^{B}	11.57^{B}	0.012*
K	137.33^{A}	228.33^{A}	214.67^{A}	0.341
Ca	803.67^{A}	391.00^{B}	369.00^{B}	0.019*
Mg	195.00^{A}	84.00^{B}	103.00^{B}	0.019*
Na	48.33^{A}	78.33^{A}	51.00^{A}	0.143
S	32.67^{A}	41.00^{A}	52.33^{A}	0.180
Al	1376.90^{B}	1535.11^{B}	1982.31^{A}	0.020*
Fe	410.00^{A}	366.33^{A}	215.67^{B}	0.019*
Mn	42.67^{A}	53.33^{A}	32.00^{A}	0.589
Cu	0.377^{A}	0.597^{A}	0.440^{A}	0.185
Zn	6.00^{A}	7.13^{A}	1.53^{B}	0.021*

* denotes significance at $\alpha = 0.05$

Table 4.6.5: The Pearson correlations and p-values between the soil nutrients and foliar nutrients, with all locations pooled (Colchester, Kings and Pictou).

Tissue	Soil														
	pH	OM	CEC	P	K	Ca	Mg	Na	S	Al	Fe	Mn	Cu	Zn	NRD
N (%)	-0.33 0.390	-0.16 0.678	-0.05 0.897	-0.24 0.534	0.12 0.757	0.036 0.926	-0.11 0.776	0.44 0.239	-0.43 0.246	-0.63 0.069	0.45 0.221	0.53 0.146	0.55 0.124	0.89 **0.001***	**-0.82** **0.006***
P (%)	0.21 0.597	-0.50 0.172	-0.57 0.106	0.51 0.161	0.64 0.061	-0.42 0.259	-0.43 0.249	0.64 0.064	-0.01 0.984	-0.23 0.556	0.13 0.746	0.22 0.576	0.62 0.075	0.57 0.113	-0.46 0.212
K (%)	-0.27 0.491	-0.28 0.472	-0.10 0.792	-0.13 0.736	0.04 0.913	0.14 0.721	-0.05 0.890	0.22 0.562	-0.48 0.196	-0.70 **0.037***	0.52 0.150	0.55 0.125	0.42 0.265	0.95 **0.000***	**-0.72** **0.028***
Ca (%)	0.19 0.629	-0.44 0.237	-0.47 0.202	0.53 0.146	0.55 0.124	-0.08 0.841	-0.18 0.646	0.28 0.459	-0.16 0.676	-0.39 0.294	0.23 0.558	0.19 0.630	0.36 0.348	0.60 0.086	-0.24 0.542
Mg (%)	0.30 0.441	-0.06 0.872	-0.30 0.436	0.65 0.059	0.69 **0.040***	0.01 0.973	0.20 0.606	0.27 0.488	-0.17 0.669	-0.20 0.601	0.04 0.915	0.03 0.949	0.41 0.271	0.21 0.594	0.22 0.568
Fe (%)	-0.27 0.478	-0.04 0.922	-0.06 0.879	0.18 0.638	0.52 0.155	-0.03 0.973	-0.05 0.902	0.55 0.125	-0.21 0.591	-0.53 0.145	0.32 0.409	0.09 0.817	0.35 0.349	0.71 **0.032***	-0.69 **0.039***
Mn (ppm)	0.12 0.759	-0.43 0.246	-0.44 0.239	0.06 0.876	0.23 0.546	-0.75 **0.020***	-0.72 **0.03***	0.72 **0.03***	0.30 0.440	0.23 0.553	-0.13 0.747	0.17 0.660	0.57 0.106	0.15 0.705	-0.58 0.101
Cu (ppm)	-0.50 0.167	0.03 0.931	0.18 0.648	-0.32 0.406	-0.08 0.846	0.17 0.661	0.06 0.877	0.35 0.353	-0.51 0.158	-0.68 **0.044***	0.57 0.110	0.43 0.251	.038 0.310	0.82 **0.007***	-0.80 **0.009***
Zn (ppm)	0.05 0.901	-0.48 0.194	-0.44 0.237	0.19 0.624	0.43 0.253	-0.18 0.649	-0.30 0.430	0.41 0.279	-0.17 0.667	-0.44 0.233	0.25 0.521	0.46 0.214	0.55 0.122	0.83 **0.006***	-0.63 0.072
B (ppm)	0.14 0.717	-0.08 0.829	-0.06 0.883	-0.33 0.386	-0.22 0.572	-0.38 0.310	-0.44 0.231	0.28 0.466	-0.02 0.953	0.41 0.270	-0.29 0.454	-0.03 0.933	0.33 0.381	-0.56 0.119	0.09 0.826
NRD (days)	0.46 0.211	-0.01 0.993	-0.12 0.761	0.36 0.342	0.07 0.868	0.08 0.830	0.30 0.426	-0.39 0.295	0.36 0.343	0.48 0.188	-0.30 0.435	-0.18 0.652	-0.27 0.485	-0.60 0.086	n/a

* denotes significance at $\alpha = 0.05$

There were no significant relationships between soil nutrients and NRD (Table 4.6.5). However, there were significant correlations between 10 soil nutrients to tissue nutrient content. Soil potassium with tissue magnesium (r^2 = 0.69, p-value = 0.040), soil calcium with tissue manganese (r^2 = -0.75, p-value= 0.020), soil magnesium with tissue manganese (r^2 = -0.72, p-value = 0.030), soil sodium with tissue manganese (r^2 = 0.72, p-value = 0.030), soil aluminum with tissue potassium (r^2 = -0.07, p-value = 0.037), soil aluminum with tissue copper (r^2 = -0.68, p-value = 0.044) and soil zinc with tissue potassium (r^2 = 0.95, p-value = 0.000), tissue iron (r^2 = 0.71, p-value = 0.32), tissue copper (r^2 = 0.82, p-value = 0.007) and with tissue zinc (r^2 = 0.83, p-value= 0.006). Some of these values are negative and others are positive. A negative relationship means that the soil nutrient would reduce the needle nutrient content that it had significance with. A positive relationship infers that the increase in that particular soil nutrient would increase the needle nutrient content that it had significance with. These correlations suggest that the soil nutrients analysed do not alter the final NRD directly, but they could influence the uptake of other elements. The positive correlation of soil nutrients and needle nutrient content seen is called synergism. An antagonist relationship refers to the opposite; this is when a soil nutrient competes with other nutrients at uptake sites on the root and therefore interfers with the plant's ability to uptake that nutrient. In this particular experiment, zinc has a synergistic relationship with N, K, Fe, Cu and Zn.

Among all, only four foliar nutrients had a significant correlation with NRD; copper, nitrogen, iron and phosphorus. All of these elements (nitrogen, phosphorus, iron and copper) had a negative relationship with NRD suggesting that these nutrients may be detrimental to needle retention. Regressions were preformed on these 4 tissue nutrients with NRD (Figures 4.6.3 - 4.6.6).

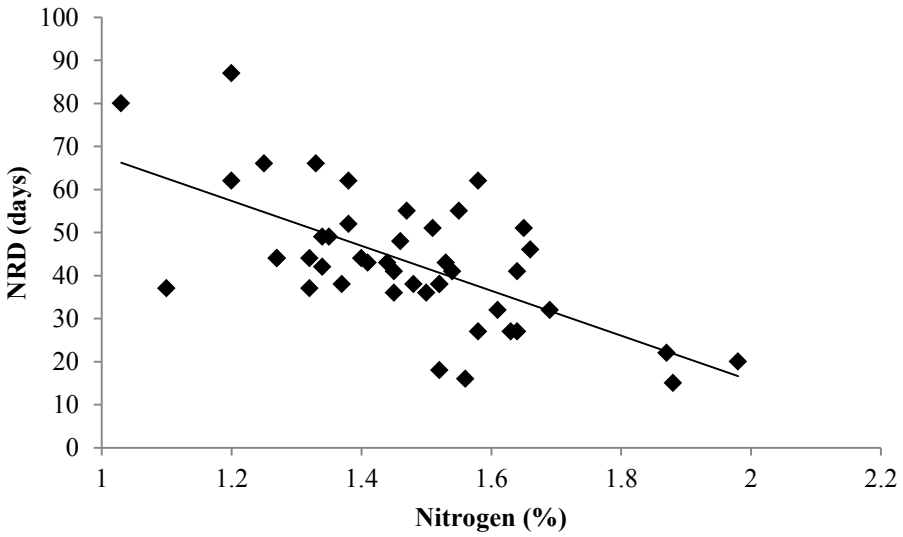

Figure 4.6.3: A linear regression between pre-harvest foliar nitrogen (%) and NRD was preformed to demonstrate the relationship between increasing levels of nitrogen and decreasing NRD; with a p-value of < 0.0001 at an $\alpha = 0.05$ and a R^2 value of 0.43*.

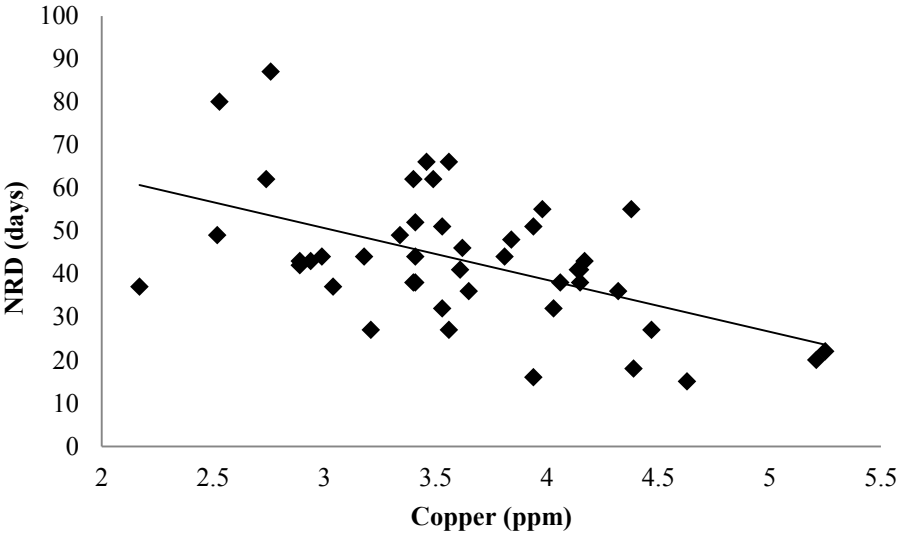

Figure 4.6.4: A linear regression between pre-harvest foliar copper levels (ppm) and NRD. Significant p-value of < 0.0001 at an $\alpha = 0.05$ and a R^2 of 0.271*.

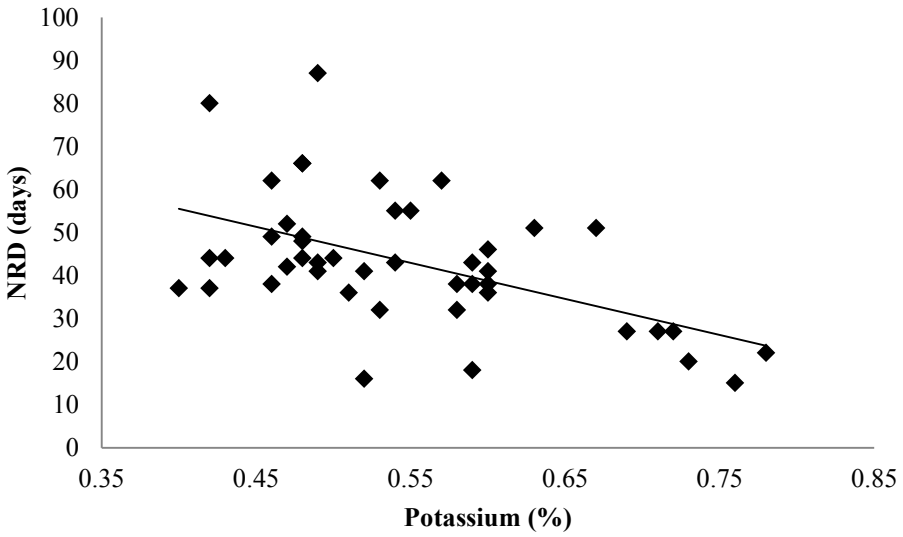

Figure 4.6.5: A linear regression between pre-harvest foliar potassium (%) content and NRD. A significant p-value of < 0.0001 at an $\alpha = 0.05$ and a R^2 value of 0.274*.

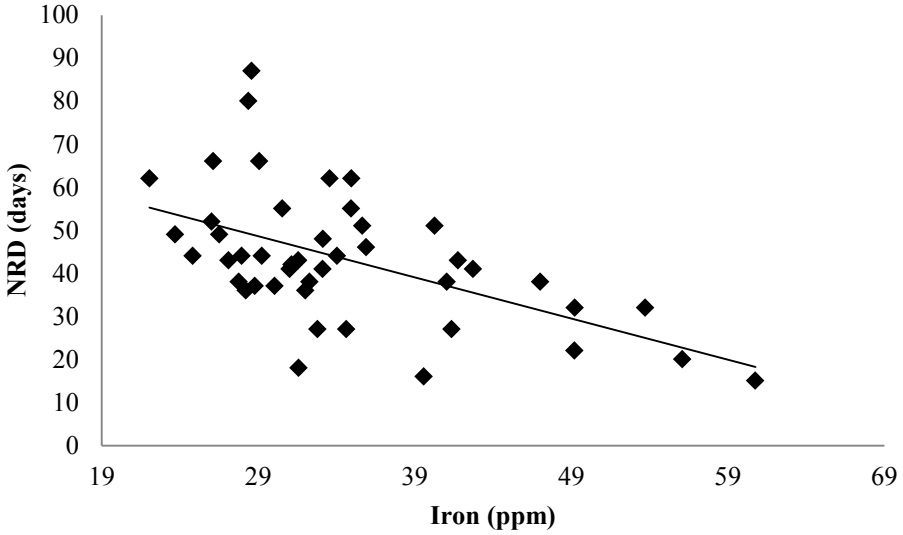

Figure 4.6.6: A linear regression between pre-harvest foliar iron (ppm) content and NRD. A significant p-value of < 0.0001 at an $\alpha = 0.05$ and a R^2 value of 0.297*.

Nitrogen ($R^2 = 0.422$), Copper ($R^2 = 0.271$), potassium ($R^2 = 0.274$) and iron ($R^2 = 0.297$) all had a high, negative relationship with needle retention. All of these elements had a high R^2 value above 0.5, meaning that they significantly influenced needle retention.

4.6.5 Discussion

The % needle loss dynamics and final needle retention duration was significant among locations. Needle loss was significant at all increments except at the commencement date (where 1% of the needles were lost). Kings county's branches took the longest to lose 5, 10, 20, 40, 80 % of its needles than Pictou county's branches. Needle retention was the highest in the branches collected from Kings county with 53.5 days than the branches from Pictou county which had a NRD of 34.5. The foliar nutrients were all significantly different among locations, which similar to previous location experiments on other tree species in other areas (Schomaker, 1973; Steinbeck, 1966). The branches from Pictou county proved to have, on average, the highest levels of all nutrients tested. Whereas the branches from Kings county had the lowest levels, on average, for the majority of the nutrients – which is in contrast to their final NRDs. Previous fertilization studies, which increase needle nutrient content, have been shown to reduce needle retention (Balster and Marshall, 1999). Conifers are known to grow in nutrient poor soils, to protect their nutrient resources, they have long foliage longevity. When conifers grow in nutrient rich soils, nutrient availability is not a limiting factor anymore and needle growth and abscission quickens (Balster and Marshell, 1999). They saw that fertilization increases above ground biomass, which causes self shading of lower and internal needles (which are closer to the trunk); this self shading promotes needle loss of these shaded needles (Balster and Marshell, 1999).

There were 6 different soil nutrients that influenced needle nutrient content (potassium, calcium, magnesium, sodium, aluminum and zinc); with a total of 10 correlations. Out of these 10 correlations, zinc had the most positive, correlations with needle nutrient content. Zinc was the only nutrient that had positive correlations with the needle potassium, iron, copper and zinc contents. This means that with increasing soil zinc content, it may increase needle potassium, iron, copper and zinc content.

Pearson's correlations were completed among all of the variables to determine which factor influenced NRD (Table 4.6.5). As seen from table 4.6.5, none of the soil nutrients influenced NRD directly. The correlations between foliar nitrogen, copper, potassium, iron and NRD were all significant, showing a p-value of 0.006, 0.009, 0.028 and 0.039, respectively. All of these elements had a negative correlation to NRD.

A high level of needle nitrogen content in balsam fir was not beneficial for needle retention. Extra nitrogen would encourage needle growth, it is predicted that less growth would occur around the abscission zone and cell wall which improves stability (Lethan, 1961). Typically, this would cause the needles to become longer, softer and more prone to needle loss (Black, 1968; Hinesley and Snelling, 2000). Interestingly, it was noted that the higher concentrations of iron and copper also reduced needle retention. Fry et al. (2002) looked at the role copper ions have in abscission more closely in suggesting a hypothesis on why copper may induce abscission. Fry et al. (2002) explains that Cu^{2+} can be reduced to Cu^{+} within the plant and undergoes a Fenton reaction with apoplastic hydrogen peroxide to generate hydroxyl radicals. This reaction would then cause a non-enzymatic scission of the wall's polysaccharides and the abscission of the cell wall. Ben-Yehoshua and Biggs (1970) believe that the acceleration of abscission by Cu and Fe chloride salts was related to the enhancement of ethylene or the inactivation of IAA as seen in

citrus species. More recent work by Maksymiec and Krupa (2007) confirmed that ethylene, jasmonate and NADPH oxidase activity may be involved in Cu^{2+} role of inhibition of root growth in dicotyledon species. Iron toxicity also increased ethylene production through the plant's stress responses in rice (Yamauchi and Peng, 1995). Yamauchi and Peng (1995) showed that when Fe^{2+} uptake increased in rice plants while the roots were damaged or severed, it caused toxic levels of iron to accumulate in the plant and therefore increase ethylene production. Ethylene is known to promote needle abscission in balsam fir trees (MacDonald et al., 2010).

4.6.6 Conclusions

Soil nutrient status had no significant influence on needle loss or retention. However, needle nitrogen, potassium, copper and iron concentrations had a significant, but negative relationship with NRD, implying that higher levels of these nutrients may reduce needle retention.

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4.7 The Effect of Certain Applied Nutrients on Post-Harvest Needle Retention

Abstract

Little research has been done on the physiological significance of nutrients and needle retention post-harvest in balsam fir. Two experiments in this chapter examined the influence of nutrient application on clone 18 and 8 to test whether or not nutrient feeding post-harvest will increase needle nutrient content. Post-harvest nutrient feeding through the xylem decreased needle retention in both clones. It was also seen that there was no significant effect due to pre-loading of calcium and zinc citrate. Increasing concentration of these nutrients did not result in a significant increase in needle Ca or Zn. These experiments suggest that xylem-fed nutrients can reduce needle retention.

4.7.1 Introduction to experiments 4.8 and 4.9

One of the main goals of the Christmas tree growers is to increase the post-harvest needle retention of their Christmas trees; which may be possible through nutrient application post-harvest. The role of calcium in a plant is complex; it is important in maintaining the integrity of the cell wall and within the abscission zone as discussed in the Literature Review. Chapter 4.1 and Chapter 4.3 indicated that calcium may have a positive, significant role in post-harvest needle retention, encouraging the hypothesis that calcium may reduce needle abscission. For that reason applications of calcium may increase needle retention post-harvest.

Christmas trees are cut from their roots for their use in the home during the Christmas season. When a tree is cut from its roots, there is a cascade of stressful events influencing hormone production and/or transport, which may be influenced through pre- and/or post-harvest nutrient levels. Root separation also occurs with other plants organs, such as flowers. Florists apply water containing sugars, anti-microbial additives, buffers to lower the pH and/or anti-ethylene solutions to increase the post-harvest shelf life of flowers (Lazar et al., 2010). Consumers have realised in the past that trees, like cut flowers may need nutrients post-harvest. This was

extended to Christmas trees where consumers believed that the application of water soluble products might help increase needle retention. In previous studies it was seen that the application of Clorox (sodium hypochlorite) caused 99 % needle loss, Aspirin (salicyclic acid) caused 72 % needle loss and needle loss was 5-6 % with sucrose, 7-Up and distilled water (Hinesley and Blankenship, 1991) – all negative, but significant results. There were no studies found on the influence of water soluble fertilizer on post-harvest needle retention in balsam fir. Nutrients, if applied post-harvest may increase post-harvest needle retention.

The study of nutrient application to balsam fir has not yet been examined to see if it increases needle retention. The next two experiments in this Chapter will examine the effects of xylem-fed fertilizer on post-harvest needle retention and the effect of xylem-fed zinc and calcium citrate treatments on post-harvest zinc and calcium levels in the needles.

4.8 The Effect of Applied Nutrients on Post-Harvest NRD in Two Contrasting Clones

4.8.1 Objective

To determine if applying nutrients post-harvest will influence needle retention in two contrasting clones of balsam fir.

4.8.2 Materials and Methods

Ninety-six branches were collected from clones 18 (low) and 8 (high) on January 13th, 2012 from Debert as described in 3.1.2. Five branch replicates from each clone were used for pre-harvest nutrient analysis as described in 3.1.6. These branches were collected, transported and prepared as described in Chapter 3.1.4. There were 7 concentrations of a 20:20:20 fertilizer of Plant-prod Ultimate by Plant-prod. This fertilizer contained N: P: K and micro-nutrients. These elements were present in the following percentages: N 20 %, P_2O_5 (phosphoric acid) 20 %, K_2O (potash) 20 %, boron 0.02 % chelated copper 0.05 %, chelated iron 0.10 %, chelated manganese 0.05 %, molybdenum 0.0005 %, chelated zinc 0.05 % and EDTA (ethylene diamine tetra-actate a chelating agent) 1 %. This fertilizer was applied to 100 mL de-ionized water. These concentrations were 0 ppm (control, no added fertilizer), 1, 5, 10, 20, 50, and 100 ppm of water soluble fertilizer. The branches were observed for AWU, % needle loss and NRD as mentioned in Chapter 3.2. The post-harvest environment had a consistent temperature of 20-22ºC, humidity of about 16-18% and 24 hour lighting.

Statistical analysis

This experiment was a factorial with 2 clones (clone 8 and 18) and 7 treatments. ANOVA was used to analyze the data and a Tukey's test was used to separate the means. Regressions were used to observe the relationship between treatments and NRD.

4.8.3 Results

Needle retention duration (NRD)

The NRD between clones were non-significant with a p-value of 0.094 (Figures 4.8.1 and 4.8.2). The NRD was significant between concentrations with a p-value of < 0.0001 (Table 4.8.1).

Needle loss dynamics

Interestingly, in both clones needle loss followed a similar trend under all treatments (Figures 4.8.1 and 4.8.2). The branches at first lost a lot of needles, but then levelled off for a period of time until they peaked in needle loss again until complete abscission.

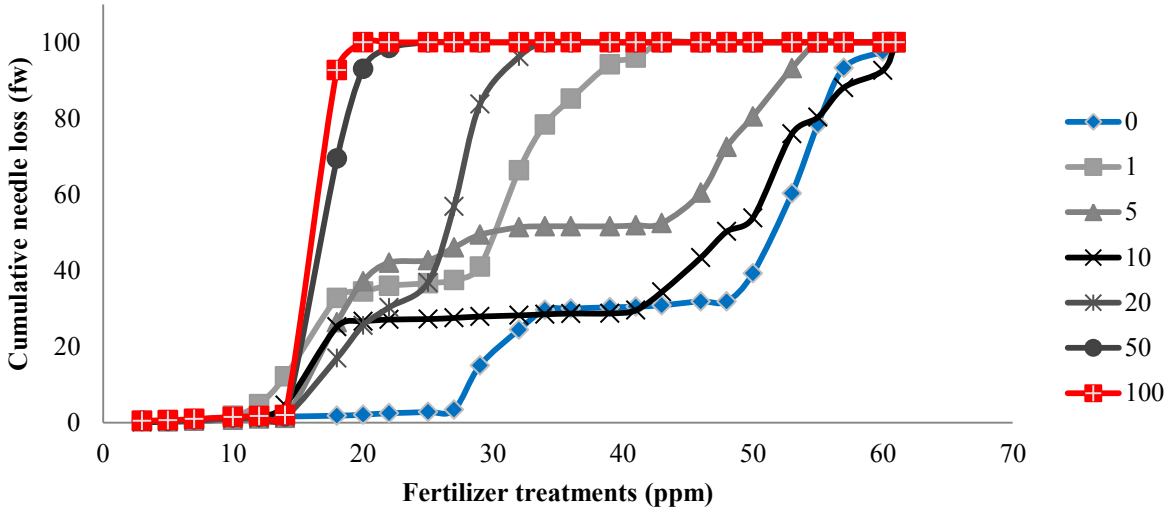

Figure 4.8.1: The cumulative % needle loss (fresh weight) of clone 18 with 7 nutrient treatments.

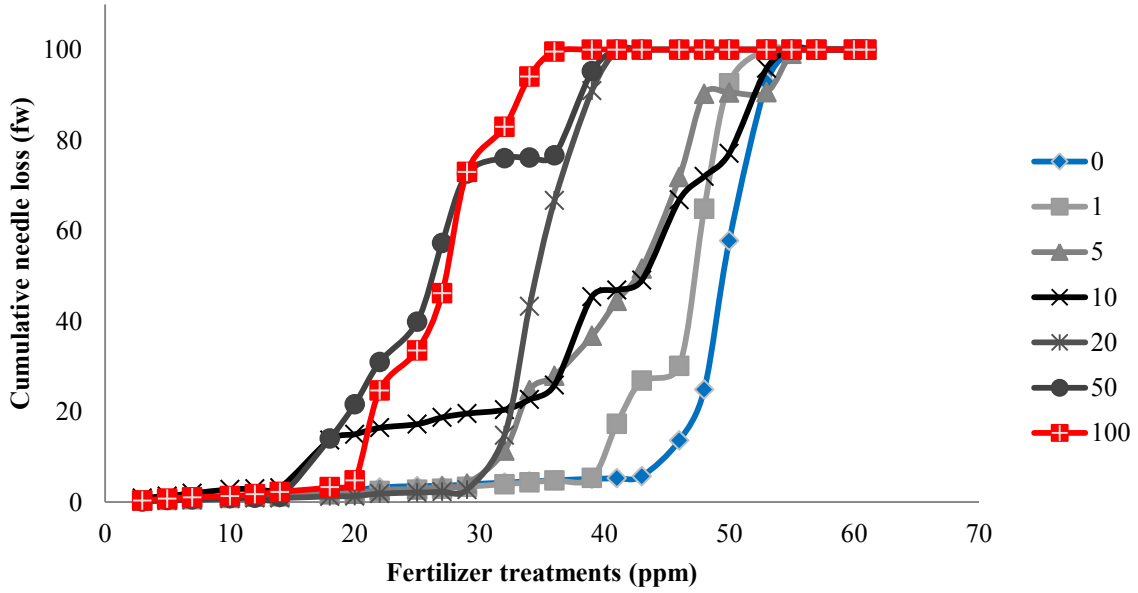

Figure 4.8.2: The cumulative % needle loss (fresh weight) of clone 8 with 7 nutrient treatments.

Treatment influences

Fertilizer application post-harvest significantly influenced post-harvest needle loss. Needle retention declined significantly as the concentrations of fertilizer increased even at the lowest level of 1 ppm. The treatments significantly influenced needle loss in both clones ($p = < 0.0001$). The main effect of nutrient concentration on needle retention (Table 4.8.1) showed that the control and 10 ppm were statistically similar. The application of fertilizer had no significant benefit of increasing needle retention. The higher nutrient concentrations significantly decreased needle retention.

Table 4.8.1: Concentration effect of a 20: 20: 20 fertilizer (Plant-Prod Ultimate) on needle retention duration with pooled clones (8 and 18), the means were separated with Tukey's test.

Treatment (ppm)	0	1	5	10	25	50	100
NRD	53^{A}	41^{BC}	45^{B}	49^{AB}	34^{CD}	25^{D}	25^{D}

A regression was used to see if the relationship between treatments and needle retention was significant (Figure 4.8.4). There was a high R^2 value of 0.73 for the high NRD clone and 0.58 for the low NRD clone. These high R^2 values represent the significant negative influence that the fertilizer concentrations had on needle retention on both clones of balsam fir. Increasing fertilizer concentration proportionally decreased NRD with increasing concentrations (Figure 4.8.3 and Figure 4.8.4). At the highest concentration, the NRD declined by 46 % compared to the non-fertilized control.

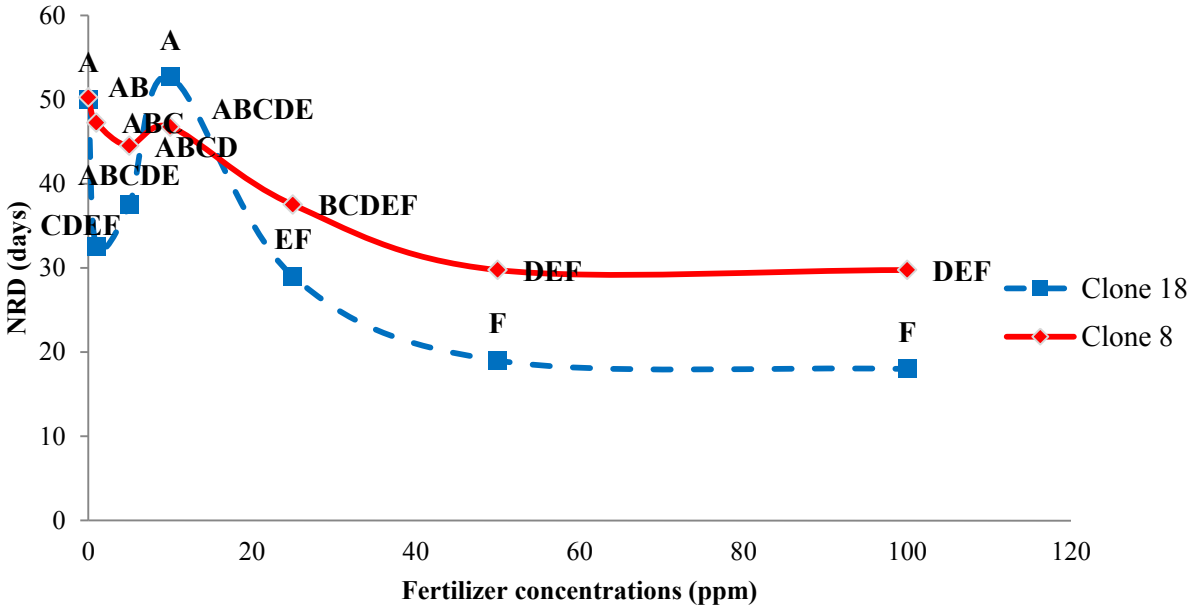

Figure 4.8.3: The effect between nutrient concentrations and NRD in two contrasting clones. Concentrations were significant at <0.0001 at α = 0.05.

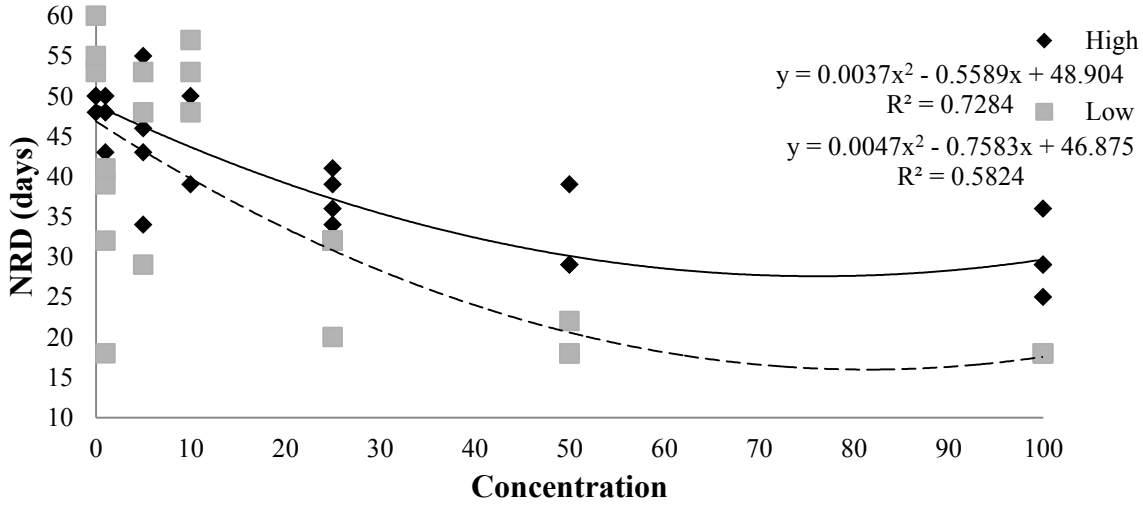

Figure 4.8.4: A polynomial regression showing the applied 20: 20: 20 + micro nutrient fertilizer in two contrasting clones with a p-value of <0.0001 among concentrations and a p-value of 0.005 between concentrations and genotype at α = 0.05

Interaction effects

There was an interaction effect between clones and nutrient concentrations (p-value = 0.005).

Table 4.8.2: The interaction effect of clones and nutrient concentrations on post-harvest NRD was significant (p = 0.005) and the means were separated by a Tukey's test.

Clone	Treatment (ppm)	Mean
18	0	56^{A}
18	1	34^{CDEF}
18	5	44^{ABCDE}
18	10	52^{A}
18	25	29^{EF}
18	50	19^{F}
18	100	18^{F}
8	0	50^{AB}
8	1	47^{ABC}
8	5	45^{ABCD}
8	10	45^{ABCDE}
8	25	37^{BCDEF}
8	50	30^{DEF}
8	100	30^{DEF}

* denotes significance at $\alpha = 0.05$.

Water use

Average water use was calculated over the first 18 days until the first branch died. This was done to prevent the skewing of results towards the treatments that prevailed. Figure 4.8.5 shows the AWU over the course of 18 days for each treatment. Clone 8 consistently had a higher average water use than clone 18 and there was significance between clones (p value = 0.0001). On the other hand there was no significance among treatments (p value = 0.093) and there was no interaction effect (p-value = 0.179).

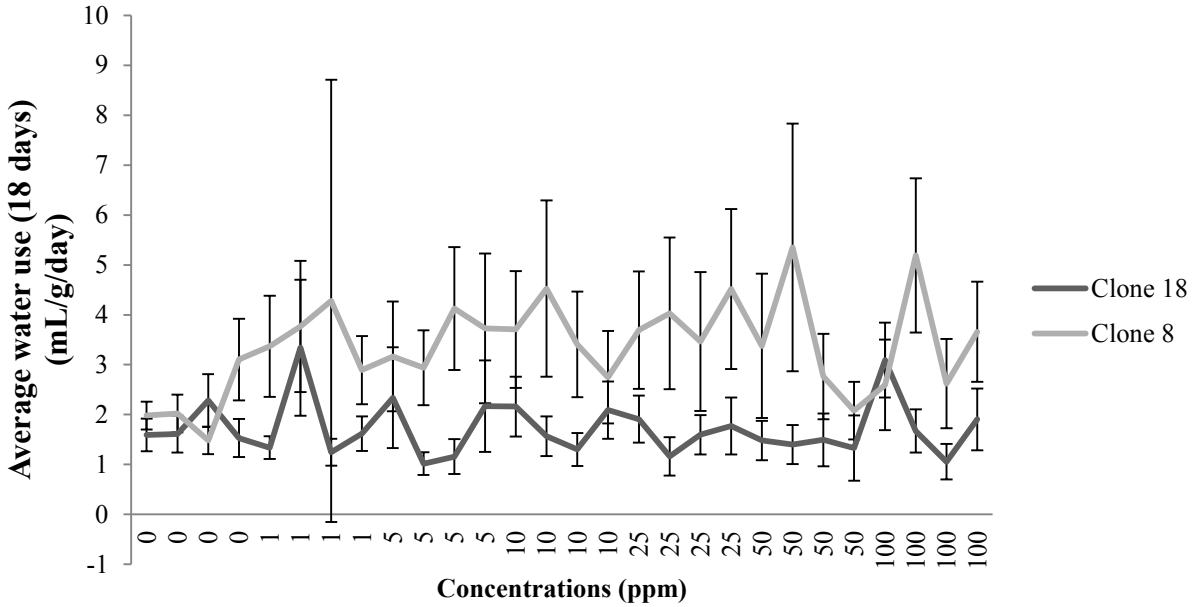

Figure 4.8.5: This diagram represents the AWU (18 days) among treatments with standard deviations of clone 8 and 18. Clone 8 had a statistically higher average water use than clone 18 ($p = 0.0001$).

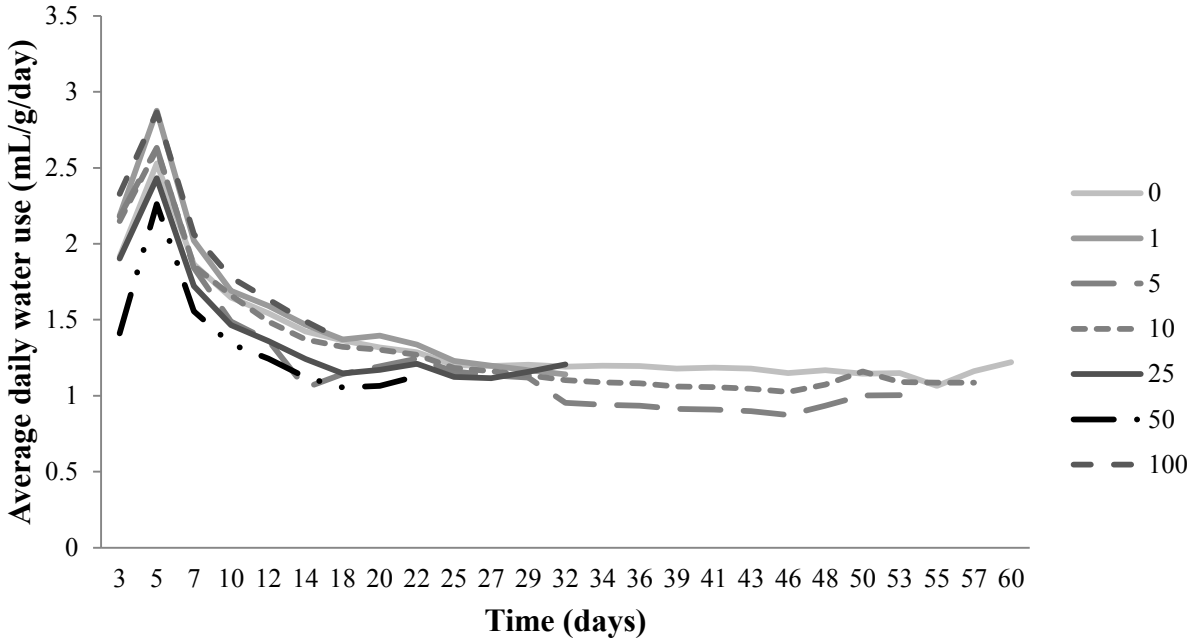

Figure 4.8.6: The average daily water use of clone 18 (low NRD) and its 7 nutrient concentrations (4 replications per concentration) over the duration of the experiment.

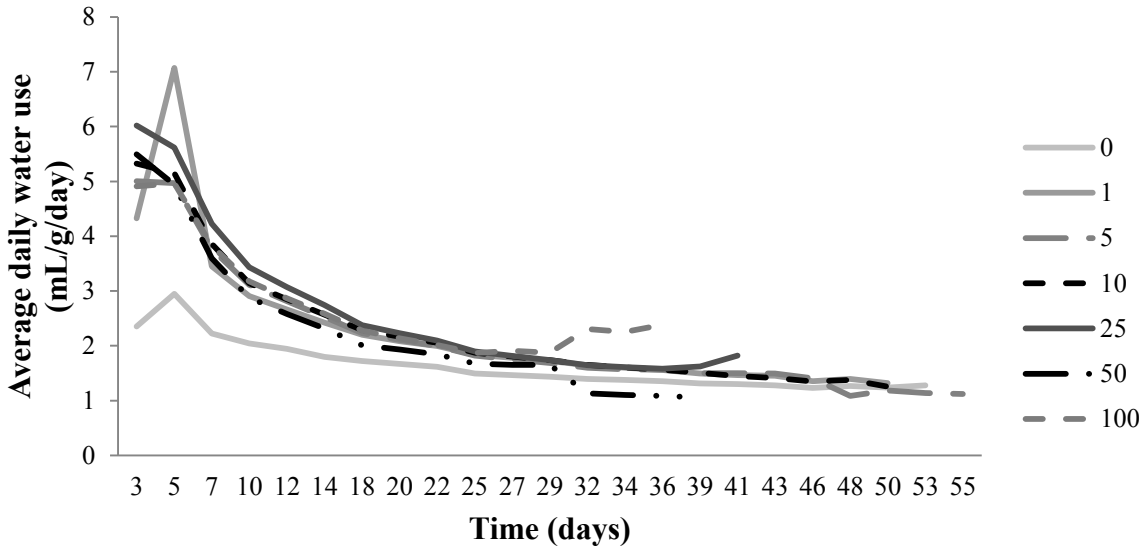

Figure 4.8.7: The average daily water use of clone 8 (high NRD) and its nutrient concentrations (4 replications per concentration) over the duration of the experiment.

Both clones did decrease in their daily water use (mL/ g / day) over time (Figure 4.8.6 and Figure 4.8.7); but water use continued throughout the experiment indicating that there were no embolisms preventing nutreint and water uptake.

4.9 Calcium and Zinc Citrate Pre-loading to a Low NRD Clone

4.9.1 Objectives

1) To determine the effect of xylem-fed calcium citrate on the needle calcium content and loading;
2) To determine the effect of xylem-fed zinc citrate on the needle zinc content and loading.

4.9.2 Materials and Methods

The zinc citrate trial had a total of 28 branches (4 replicates) from the same clone (18). Seven concentrations of zinc citrate were applied (0, 10, 20, 40, 60, 80 and 100 ppm). Pre-harvest samples were sent for nutrient analysis of each tree. The branches were then allowed to sit under constant light (24 hours), temperature (20-24 ºC) and humidity (19-21 %) for 3 days. The needles of the branches were then removed, dried and analyzed for zinc, to see if pre-loading occurred.

For the calcium citrate experiment a total of 16 branches from 4 replicates of the same clone 18 were collected. Four concentrations of calcium citrate were applied (0, 1, 10, and 100 ppm). Pre-harvest samples were analyzed for needle calcium concentration (4 replicates). The branches were allowed to set under constant light (24 hours), monitored temperature (20-24 ºC) and humidity (19-21 %) for 3 days. The needles (current, first and second year) of the branches were then removed, dried and analyzed for calcium content.

Statistical analysis

The targeted nutrient under each study was subjected to ANOVA to determine which concentration proved significant.

4.9.3 Results

The normal range of the targeted nutrients was researched for balsam fir (Table 4.9.1) and the pre-harvest nutrient levels of calcium and zinc in the needles were recorded (Table 4.9.2). Water nutrient levels were also analysed (Table 4.9.3).

Table 4.9.1: The acceptable ranges of needle nutrient levels in balsam fir

	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Zn (ppm)	Cu (ppm)	Fe (ppm)
balsam fir	0.91-1.5	0.1-0.2	0.48-0.61	0.30-1.39	0.08-0.16	46.5-50	2.3-7.5	35-120

*Adopted from Blinn and Bucker (1988)

Table 4.9.2: The pre-harvest needle nutrient levels of calcium and zinc and their standard deviations.

Targeted elements	Calcium (%)	Zinc (ppm)
Pre-harvest levels	0.70 % +/- 0.17	30.23 +/- 4.96

Table 4.9.3: Water analysis report of the reverse osmosis (RO) water used in the foliar application experiment.

	pH	Nitrate +Nitrate -N	Cond	Alk.	Cl	Hd	Ca	Cu	Fe	Mg	Mn	Na	Zn	K
mg/L	5.13	< 1.00	10	< 5.5	< 10	0.04	< 0.1	< 0.01	< 0.01	< 0.1	< 0.01	< 0.1	< 0.01	< 1.00

*Cond. = conductivity (mmhos); Alk. = alkaline; Hd = hardness

Needle Ca and Zn Concentrations

Pre-loading citrates at various concentrations did not show any significant change in needle calcium or zinc concentrations (Table 4.9.4). Throughout the experiment it appears that there was no uptake occurring in the needles.

Table 4.9.4: The p-values for each compound between treatments post-harvest in balsam fir.

	Calcium Citrate	Zinc Citrate
Treatments	0.955	0.995

A scatter plot was created for the two compounds and different treatments for an illustrative effect on how the calcium and zinc citrate applications to the branches did not alter the internal needle calcium and zinc concentrations.

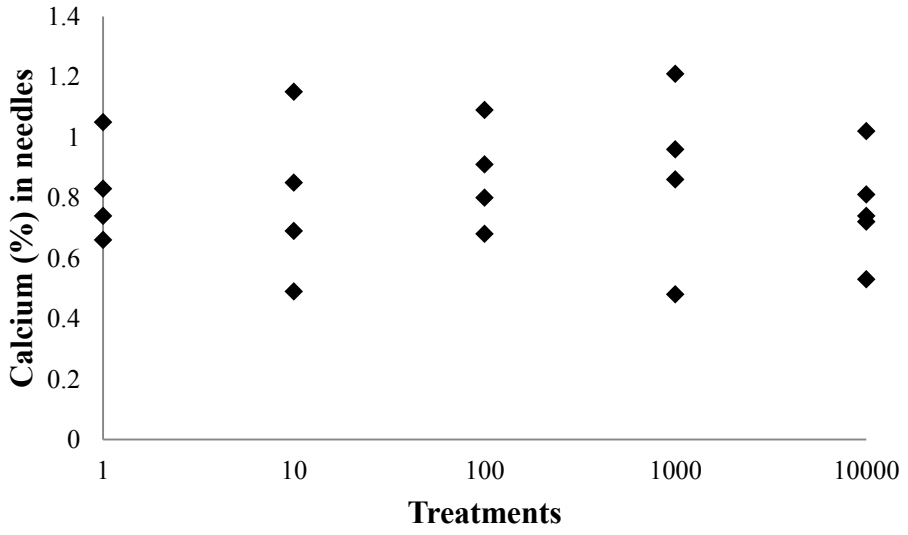

Figure 4.9.1: Xylem feeding of clone 18 with 4 treatments (1= pre-harvest, 10 = control, 100 = 1 ppm and 1000 = 10 ppm and 10000 = 100 ppm of calcium citrate) to determine changes pre- and post treatment needle nutrient content. Treatments were non-significant with a p-value of 0.955*

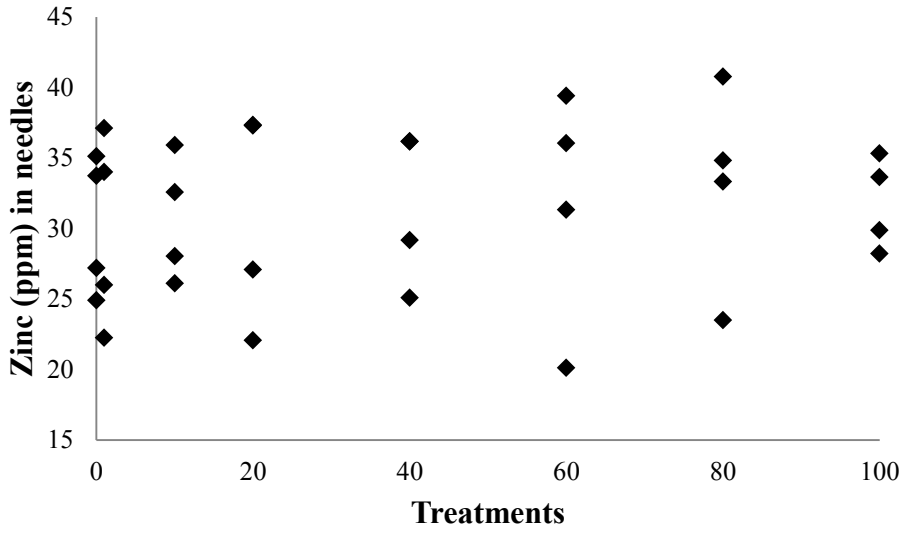

Figure 4.9.2: Xylem feeding of clone 18 with 4 treatments (0 = pre-harvest, 1 = control, 10 ppm, 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 pppm of zinc citrate) to determine changes pre- and post treatment needle nutrient content. Treatments were non-significant with a p-value of 0.995*

4.9.4 Discussion for 4.8 and 4.9

The addition of soluble nutrients to water decreased needle retention as seen in other Christmas tree species like Fraser fir (Hinesley and Blankenship, 1991). Hinesley and Blankenship (1991) have also shown that aspirin (salicylic acid) and 7-Up increased needle loss post-harvest compared to distilled water in Fraser fir. These results were consistent with other experiments trying to increase needle retention by adding compounds to the tree's water (Van Wagner, 1963; Athrens and Stephens, 1975; Montano and Proebsting 1985).

By examining the water use data, it can be seen that there was a consistent decrease in water uptake (Figures 4.8.6 and 4.8.7). This could be attributed to a couple of facts: one, there were fewer needles as time went on because of needle loss and thus less transpiration is occurring to

pull water up into the xylem. Secondly, as time goes on the xylem may have been blocked off by the plant's own natural healing processes, interfering with the uptake of water. As time went on it is possible that the needle nutrient content was increasing as the branch dried out; but more research is needed to confirm if nutrients are even being up taken into the needle (Chapter 4.9). Figure 4.8.5 represents the average water use over 18 days between clones and treatments. Clone 8 consistently had a higher AWU than clone 18 indicating increased transpiration or less xylem blockage was occurring. Future examination of the xylem and stomata functions will help explain genetic differences between clones and its potential influence in the differences in water uptake.

In response to Chapter 4.8, it was necessary to see if its significant, negative results were due to excessive pre-loading (over satisfactory levels) in the needles. These elements were chosen because of their previous positive relationships seen in Chapter 4.3 and 4.1. Xylem feeding calcium and zinc as citrates at various concentrations did not result in a proportional increase in needle calcium or zinc concentrations post-harvest. In Chapter 4.8, in the fertilizer application experiment, it was also shown that there was a negative effect on needle retention to increasing nutrient concentrations. This negative effect was not shown in these zinc and calcium citrate pre-loading experiments, where it was shown to be non-significant. This lack of a response to nutrient application is in contrast to other experiments using water additives to increase needle retention (Van Wagner, 1963; Athrens and Stephens, 1975; Montano and Proebsting 1985); which showed only negative, significant results, such as Chapter 4.8.

The lack of response to increasing calcium and zinc concentrations cannot be attributed to the calcium and zinc content in the RO (reverse osmotic) water in the control, as the concentration of calcium and zinc were very low in the RO water (Table 4.9.3). The branches in the calcium

citrate experiment had a pre-harvest calcium nutrient content of 0.70 % ± 0.17 (Table 4.9.2). The adequate range for calcium in balsam fir is 0.30 % -1.39 %, as shown in Table 4.9.1 (Blinn, 1988). Possibly, the lack of response by feeding calcium through the xylem may be due to the fact that the needles contained sufficient levels of calcium already. Due to this fact a homeostasis may have already achieved within the needle for calcium content. However, this does not explain the lack of response to zinc application, since the branches in the zinc citrate experiment had a pre-harvest zinc content of 30.23 ± 4.96 ppm(Table 4.9.2). The adequate range for zinc in balsam fir was determined to be 46.5-50 ppm, as shown in Table 4.9.1 (Blinn and Bucker, 1988).

The lack of response to the application of zinc citrate may be due to the limitation for zinc to be transported to the needle from the stem of the branch. Calcium and zinc citrate are expected to be mobile; as these nutrients were applied as an organic salt (citrates). Citrates are able to be transported into the cells and so it was expected that zinc and calcium would be co-transported into the cell as well (Zhao et al., 2007). Alternatively, immobility may be contributed to the lack of roots which regulate the uptake of nutrients and transport as seen in the Chapter 4.8 (Wang et al., 2006). Without root regulation of the nutrients, nutrients may be directly taken through the xylem and build up within the stem (Wang et al., 2006). In the future, nutrient analysis of the stem should be completed as well, to determine where zinc and calcium may be transported to.

4.9.5 Conclusions for 4.8 and 4.9

Application of water soluble fertilizer post-harvest at any of the 7 concentrations tested reduced needle retention in the two clones chosen. Post-harvest water soluble fertilizer application is not recommended for needle retention in balsam fir. As well, the pre-loading of calcium and zinc did not result in significant changes in needle calcium and zinc contents post-harvest. This implies that elements fed by water may not be transported to the needle over the abscission zone. Since

no pre-loading occurred with these compounds, it is expected that there would be no benefit to applying these compounds post-harvest, by water, for needle retention.

4.9.6 References for 4.8, 4.9

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4.10 Foliar Applications of Calcium and Zinc Citrate on Post-harvest Needle Retention

Abstract

This experiment was designed to determine the influence of foliar applications of calcium citrate (CC) and zinc citrate (ZC) on clone 18. For each compound there were 4 treatments with 8 replications per treatment. For CC there was a treatment where nothing was applied, a control, where RO water was applied, 1 ppm and 10 ppm of CC. In the ZC experiment there was a treatment with nothing applied to the branches, a control (RO water), 2 ppm and 4 ppm of ZC. It was shown to be non-significant among treatments in both compounds (CC and ZC) and NRD. However, more research is needed with foliar applications with different compounds, concentrations and frequencies.

4.10.1 Introduction

In Chapter 4.8 it was seen that fertilizer application *via* the xylem post-harvest had significant, negative results on post-harvest needle retention. Chapter 4.9 also showed that there was no apparent pre-loading effect to calcium and zinc citrate. This leads one to believe that water-based nutrients may not be effective to promote post-harvest needle retention, based upon the plant's limitations on regulating its uptake of these compounds, by its lack of roots (Wang et al., 2006). Another alternative method of nutrient uptake is through foliar feeding.

Foliar feeding is when nutrients are directly applied to a whole plant covering all plant organs including stem, leaves, and flowering parts and this practice was first documented in 1844 (Pace, 1982). Scientific research on foliar feeding was first recorded in the 1940's (Fageria et al., 2009); and continued on throughout the years on selected crops such as apples, tomato and cereal plants. Fageria et al. (2009) stated that there was an increased interest in foliar application because of the more frequent use of machinery in irrigating fields, applying pesticides, herbicides and fungicides. This increased interest was because growers wanted to reduce the application costs by using the same machinery used in pesticide application for fertilization. Recently, there is an

increased concern for reducing crop production costs as well as improving the soil's quality; the application of soluble fertilizers makes these two needs possible.

While leaves are the key photosynthetic organ of a plant, research shows that leaves can also absorb nutrients. Franke (1967) suggests that nutrient ions are absorbed in a series of events; first the ions must pass through the outermost, waxy cuticle (a thicker waxy cuticle may slow this process down) by limited/free diffusion. In the second stage, nutrient ions are absorbed to the surface of the plasma membrane by some form of binding, principally due to their charge. In the third stage, the nutrient ions are transported into the cytoplasm by a metabolically derived energy. Nutrient ions can pass through these layers in sequence or in between these layers; they can also be absorbed through the stomates (Eichert et al., 2001). Mobilization of nutrients needs to be taken into consideration while spraying. An immobile nutrient cannot be transported from a sprayed plant part to another organ within the plant. In this case re-application of the nutrient will be needed in the newer and older growths of the plant, if deficiencies were suspected. Typically, most macro-nutrients appear to have some mobility throughout the plant (except sulfur and mostly calcium) and most micro-nutrients have poor mobility (Fageria et al., 2009).

Foliar uptake of nutrients has been researched in other plants especially soybean and wheat. Foliar applications of calcium are routinely done in apples to prevent post-harvest rot, cork spot and bitter pit (Asgharzade, 2012). An advantage of foliar application of nitrogen to cereals is the reduced nitrogen losses through de-nitrification and leaching compared to a soil treatment (Gooding and Davis, 1992). Foliar applications allow quick utilization of nutrients and allow the correction of observed nutrient deficiencies in less time compared to soil treatments (Fageria et al., 2009). There was previous research on nitrogen in a foliar application to Christmas trees;

which increased the greenness of needles significantly compared to soil only applied nitrogen (Huxster, 1992).

Foliar applications of macro and micro-nutrients have been applied to Christmas trees in the past; nitrogen, phosphorus and potassium have been researched in particular. Miller (1979) investigated the possibility of fertilizing a stand of Douglas fir with concentrated nitrogen by a foliar spray with two main questions. First, can Douglas fir absorb nitrogen efficiently through its needles; and second, whether or not conifers can tolerate dosages of 20-32% of nitrogen solutions. In conclusion conifer needles can absorb the nitrogen effectively; just like other crop plant. Applications improved the greenness of their needles quicker than soil applications and it was found that foliar burning did occur in solutions above 30% (Miller, 1979). Foliar applications of nutrients is efficient at absorbing nutrients because it is believed that when applied directly to the leaf the elements are absorbed by the stomata (for one example) which allow the nutrients to flow through the plant unhampered. This means that fewer nutrients are lost through leaching, competition and fixation as it would in the soil.

Foliar applications of calcium and zinc citrate may have an influence on post-harvest needle retention. Previous research by Veitch and Lada (2012) concluded that xylem-fed calcium and zinc citrates and nitrates improved needle retention post-harvest slightly. However, as seen in Chapter 4.9 that xylem feeding of calcium and zinc citrate had no positive influence on nutrient loading in needles. Therefore, the citrates were used in this experiment to see they influenced post-harvest needle retention through foliar feeding. It is expected that balsam fir will have a similar response as apples and other plants with a foliar spray of calcium by increasing post-harvest life and decreasing abscission (Asgharzade, 2012). By increasing the balsam fir's calcium levels in the abscission zone, needle abscission may be delayed, which would prolong

needle retention. By increasing zinc citrate it is predicted that auxin levels may be maintained which may promote needle retention (Addicott, 1982).

4.10.2 Objective

To determine the effects of foliar applications of calcium and zinc citrate on post-harvest needle retention in balsam fir.

4.10.3 Materials and Methods

This experiment was set as a completely randomized design (CRD) using clone 18. The experiment started on July 16, 2012. Four trees of the same clone were used as replicates, 2 branches from each tree, with the total of 8 branches per treatment was used in this experiment. The branches were set up in Lab 22 of CRC on light racks as described in Chapter 3.1.4. Temperature was monitored throughout this time period and was consistently between 20-23 degrees Celsius; humidity was consistent between 16-18%. The controls consisted of i) no foliar application, ii) foliar application of RO water. Zinc citrate was applied at 2 concentrations at 2 and 4 ppm. Calcium citrate was applied at 1 and 10 ppm. Three sprays were administered to each branch each week since commencement of the experiment, which was about 3 mL of solution each time. Needle loss measurements involved finger run tests, which involved touching the needles. In order to prevent any nutrient removal from the surface of the needles, applications were done on the 6^{th} day of each week (Friday), so that it was expected that there was an uninterrupted supply of nutrients as no finger run tests were done during weekends.

Needle retention duration and needle loss increments were measured three times a week as describe in Chapter 3.2. When a finger run test was administered to the branches different gloves were used between treatments to prevent cross contamination.

Table 4.10.1: Water analysis report of the reverse osmotic water used in the foliar application experiment.

	pH	Nitrate +Nitrate -N	Cond.	Alk.	Cl	Hd	Ca	Cu	Fe	Mg	Mn	Na	Zn	K
mg/L	5.13	< 1.00	10	<5.5	< 10	0.04	<0.1	<0.01	< 0.01	<0.1	<0.01	<0.1	<0.01	<1.00

*Cond. = conductivity (mmhos); Alk. = alkaline; Hd = hardness

Table 4.10.2: The pre-harvest foliar levels of targeted nutrients in balsam fir and their standard deviations.

Targeted elements	Calcium (%)	Zinc (ppm)
balsam fir pre-harvest element levels	0.70 % +/- 0.21	27.67/- 4.70

Statistical Analysis

The parameters under study were subjected to ANOVA. Tukey's test separated the means.

4.10.4 Results

Needle abscission dynamics and needle retention duration (NRD)

The applications of calcium citrate and zinc citrate at any concentration tested did not affect needle loss commencement, days for 5, 20, 40, 60 or 80% needle loss. There were also no significant differences in NRD between treatments of the calcium and zinc citrate experiments (Table 4.10.3). While ANOVA showed a significant influence of application of zinc citrate on days for commencement of needle abscission, it was unable to be separated by Tukey's (4.10.3). Calcium citrate and zinc citrate application did not significantly promote needle retention.

Table 4.10.3: The needle loss increments post-harvest in balsam fir. There were 4 zinc citrate (ZC) and calcium citrate (CC) treatments with 8 replications per treatment. ANOVA analysed % needle loss among treatments at different needle loss increments to determine significance.

Treatments	Increments						
	1 %	5%	20%	40%	60 %	80%	NRD
CC	0.998	0.651	0.355	0.164	0.133	0.118	0.130
ZC	0.032*	0.209	0.146	0.139	0.191	0.187	0.221

Figure 4.10.1: A picture of the calcium citrate experiment which was taken on August 15th, 2012- 30 days into the experiment. The branches were randomly selected. The left 3 branches were the absolute control (nothing was applied to the branches); the middle three were the 1 ppm treatments and the furthest right were the 10 ppm treatments.

Figure 4.10.2: A picture of the calcium citrate experiment which was taken on September 19th, 2012 - 63 days into the experiment. The branches were randomly selected. The left 2 branches are the absolute control (nothing was applied to the branches); the next 2 branches were the di-ionized water applied, the next 2 were the 1 ppm treatments and the furthest right were the 10 ppm treatments.

Visual changes (Figure 4.10.1) between treatments were visible on day 30 with the calcium citrate experiment. On average it was noted that the controls were losing needles faster than the treatments. It also appeared that the calcium treated needles were softer feeling than the untreated branches. This distinction diminished at day 60 (Figure 4.10.2). The zinc treatments appeared to have the opposite trend, the controls lasting longer than the branches that received treatments of zinc citrate.

4.10.5 Discussion

There was little research completed in conifers for needle retention using foliar applications of nutrients; and so there was little information to draw from. Foliar applications of calcium or zinc did not significantly promote needle retention at any point in post-harvest at any concentrations tested. In the zinc and calcium citrate experiments, both were non-significant in their final NRDs.

Both experiments were also non-significant at their commencement date and at 5, 10, 20, 40, 80% needle loss (Table 4.10.3). With little past research to draw from, these nutrients were selected from our previous xylem feeding experiments (Veitch and Lada, 2012). The nutrient compounds that promoted needle retention significantly in these experiments by Veitch and Lada (2012) were chosen for this foliar spray experiment.

Calcium and zinc can be transported through the plant's stomates, which can be seen in previous research (Eckhoff et al., 2009; Aref, 2012; Pandey and Gupta, 2012); therefore it is unlikely that the non-significance was the result of the elements inability to be absorbed. The nutrient levels in the water were very low (Table 4.10.1) and would have been unlikely to contribute a significant amount of nutrients to the branch. As well, all of the treatments had the same amount of water and theoretically should have the same mixture of water-based elements.

The pre-harvest nutrients of the branches could have caused the non-significance in the calcium citrate treatments. In Table 4.10.2, the needle's calcium levels of the 4 trees were 0.70 % ± 0.21, which is an acceptable range for balsam fir (Table 4.9.1). In this case the needles already had sufficient quantities and did not require or benefit from the extra calcium citrate applications. Conversely, the pre-harvest nutrient levels of zinc were 27.67 ± 4.70 ppm, which were actually below the acceptable range for balsam fir (Table 4.9.1), yet there was no significant effect of zinc on needle retention. The lack of significant effect may perhaps be due to the nature of the compound used, concentrations and/or the foliar feeding frequencies.

It would be interesting to test the same compounds at different concentrations and/or at different feeding frequencies. For the calcium citrate experiment higher concentrations could be used, such as, 10, 20 and 40 ppm at the same frequencies. Since the needles appeared to be yellowing

in the zinc citrate experiment, the same concentrations could be used, but at different spraying frequencies or at a lower concentration with the same spraying frequency. Other compounds could be used such as calcium nitrate or calcium chloride as a foliar spray on balsam fir. These compounds were commonly used in other crops, such as apples (Spectrum Analytic, 2012). It was noted that although these compounds are effective in apples, they have high salt content which may damage young conifer needles (Spectrum Analytic, 2012). To accommodate this damaging effect on needles, different rates would have to be calculated and used.

4.10.6Conclusion

Foliar application of calcium and zinc citrate was ineffective in promoting needle retention at these concentrations and application frequencies.

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5.0 DISCUSSION

In order to determine the role nutrients have on post-harvest needle retention it is necessary to examine the post-harvest needle abscission responses in a systematic and rationale way. Preparing these experiments in a systematic way will help establish the link between pre- and post-harvest nutrient content and post-harvest needle retention. First, to prevent any variation between clones, environment and rootstocks, a study was performed to quantify needle nutrient contents (N P, K, Ca, Mg, Fe, Mn, Cu, Zn and B) within a tree (clone 18) and its relationship to needle abscission. A study was also conducted to understand the temporal changes in needle nutrient contents and post-harvest needle loss as well. The next phase of experiments was to observe the pre-harvest needle nutrient status between contrasting clones and observe their post-harvest needle abscission relationship. Another experiment was also conducted to understand the soil and needle nutritional link and needle retention. Finally, experiments were conducted to confirm the role of potential nutrient elements on post-harvest needle retention by nutrient application to the water supply and through the needles, post-harvest.

Relationships between pre-harvest needle nutrient status and post-harvest needle retention:

There were significant differences in pre-harvest nitrogen, phosphorus, potassium, calcium, manganese, copper and zinc content among tiers. However, post-harvest needle retention, was not significantly different among tiers proving that the post-harvest needle retention may not be directly influenced by the pre-harvest needle nutrient status. There were a significant differences in pre-harvest needle nutrient concentrations (nitrogen, calcium, magnesium, manganese and zinc) between clones; however there were no significant influence on post-harvest needle

retention. Again, suggesting that needle nutrient statFus may not have a significant role in post-harvest needle retention.

Interestingly, it was seen that needle phosphorus, iron and copper contents were all significant among collection points (day 0, 30, 37, 44, 51) in clone 18 and over time these nutrient concentrations decreased (Chapter 4.4). Declining copper and iron may perhaps be linked to increased needle abscission through their potential role in ethylene synthesis (Mapson and Wardale, 1967; Ben-Yehoshua, 1970).

Despite a significant temporal change in needle retention during the months, such variations did not correlate with any pre-harvest nutrients in the needles, again suggesting a weak link between needle nutrients and post-harvest needle retention.

In the study conducted to tease out any possible relationships among soil and pre-harvest needle nutrients and needle retention. None of the soil characteristics (CEC, pH and % organic matter) and soil nutrients had a direct influence on needle retention. However, some soil nutrients (zinc, potassium, calcium, magnesium, sodium and aluminum) did directly influence the needle nutrients of magnesium, manganese, zinc, copper, iron, potassium and nitrogen significantly. The needle nitrogen, potassium, copper and iron contents were all significant, but negatively affected final NRD.

<u>The link between post-harvest needle nutrient dynamics and post-harvest needle retention:</u>

While none of the pre-harvest needle nutrient contents appeared to increase post-harvest needle retention, there was evidence that pre-harvest needle nutrient status of nitrogen, potassium, copper and iron decreased needle retention. The needle nitrogen, potassium, copper and iron contents were found to be significantly but negatively correlated with needle retention. As the

concentrations of these elements increased, needle retention declined. Needle nitrogen, potassium, copper and iron concentrations remained within normal ranges for balsam fir (Blinn and Bucker, 1988) and so toxicity levels were not the cause of the decline of needle retention. Copper has been reported to have a role in ethylene signalling in plants (Mapson and Wardale, 1968; Burg and Burg, 1967; Ben-Yehoshua, 1970). Ethylene binding is mediated by a transition metal cofactor, which was previously confirmed to be copper by Burg and Burg (1967). This may explain the increase in needle abscission with the increase in copper content. Although, the role of iron is not clear, it seems to have a role in ethylene production. A study by Ben-Yehoshua (1970) showed that the applications of Fe^{3+} in aqueous solutions of chloride salts increased fruit abscission. Iron's role has also been proposed to be part of a protein essential in an important enzyme that purifies methional for ethylene production (Mapson and Wardale 1968).

All of these experiments conducted have confirmed that post-harvest needle retention may not be regulated by pre-harvest needle nutrient content and needle abscission may be differentially regulated. A lack of response may also be due to the fact that balsam fir may be efficient in regulating nutrients and perhaps capable of maintaining adequate levels on its own. This argument however does not seem to hold as needle nitrogen, potassium, copper and iron content exhibited a significant, negative correlation to NRD, suggesting a negative role for nitrogen, potassium, copper and iron in needle retention.

Influence of nutrient supply on post-harvest needle retention:

It was also seen that xylem-fed complete liquid fertilizer had a significant but negative effect on needle retention, suggesting that xylem-fed nutrients reduce post-harvest needle retention. Foliar fed nutrients (calcium and zinc citrate) on the other hand, had a non-significant response,

proposing that these or other compounds can be studied at stronger concentrations through foliar applications at various application frequencies.

Future research:

1) More knowledge is needed on inter and intra-cellular changes in nutrient concentrations and their effect on needle abscission.
2) Nutrient concentration dynamics at the abscission zone.
3) Understanding the dynamics of ethylene evolution along with nutrient concentrations as well as nutrient application to see if nutrients either inhibit or promote needle retention post-harvest.

6.0 CONCLUSION

Pre-harvest needle nutrients phosphorus, calcium, magnesium, manganese, zinc and boron do not appear to be directly linked to post-harvest needle retention. However, pre-harvest needle nutrients of nitrogen, potassium, copper and iron appear to have significantly, but negatively influenced post-harvest needle retention. Maintaining needle nitrogen, potassium, copper and iron concentrations below 1.5 %, 0.55 %, 3.7 ppm and 35 ppm, respectively may extend needle retention in balsam fir. Xylem-fed nutrients negatively influenced needle retention and the application of soluble and/or liquid nutrients post-harvest is discouraged as it may cause needle loss. As well, foliar applications of calcium and zinc citrate do not promote needle retention at those specific concentration and application frequencies.

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