# DETERMINING THE LINK BETWEEN CERTAIN HYDRAULIC PROPERTIES AND POSTHARVEST NEEDLE ABSCISSION IN BALSAM FIR (*Abies balsamea* (L.) Mill.)

by

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## ABSTRACT

The goal of this project was to better understand the link between certain hydraulic properties and postharvest needle abscission (PNA) in balsam fir. Balsam fir branches were kept under various conditions to measure and manipulate stomatal conductance and water use over time. Following harvest, water use sharply declined, though a lack of significant xylem blockage progress over time suggests that xylem blockage may not be the cause for PNA. Stomatal conductance also declined postharvest, showing that the decrease in water use may be due to stomatal closure. Keeping branches under high light intensity and high humidity promoted stomatal conductance, while simulating root pressure in a postharvest situation resulted in a significant improvement in stomatal conductance and promoting needle retention. Results of these experiments suggest that lack of root pressure may be a biophysical trigger for needle abscission, possibly by signaling a closure of the stomata.

## LIST OF ABBREVIATIONS USED

| | |
|---|---|
| ABA | Abscisic Acid |
| AWU | Average Water Use |
| BW | Branch Weight |
| CWU | Cumulative Water Use |
| DW | Dry Weight |
| DNW | Dry Needle Weight |
| DWU | Daily Water Use |
| NA | Needle Abscission |
| NAR | Needle Abscission Resistance |
| NL | Needle Loss |
| NRD | Needle Retention Duration |
| PNA | Postharvest Needle Abscission |
| PNL | Percent Needle Loss |
| SRPS | Simulated Root Pressure System |

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# CHAPTER 1 INTRODUCTION

It has been a tradition to display an evergreen tree in one's home during the Christmas season for many centuries. One of the most popular Christmas trees in the Atlantic provinces is the balsam fir (*Abies balsamea* L.), well-known for its signature shape, color and fragrance (Burns and Honkala, 1990).

Unfortunately, the natural Christmas tree industry is threatened by the artificial Christmas tree market, whose popularity has been increasing in recent years (Statistics Canada, 2009). One major cause of this loss of ability to compete is postharvest needle abscission. The loss of needles postharvest at various stages, including during shipment, at the retail shops, and on display at home during the holiday season is a nuisance to consumers, who have zero tolerance. As a result, many choose to buy artificial trees, leading of loss of business for the Real Christmas tree industry (MacDonald et al., 2011; Davis, 1996).

Once a tree is harvested, and therefore detached from its root system, its hydraulic characteristics change from pre-harvest conditions. Postharvest procedures such as baling, storage and transportation can lead to constant dehydration due to: a) root detachment and b) a lack of water supply until it reaches consumer's home thus, leading to dehydration. Dehydration may lead to damage of the xylem conduits possibly through cavitation or embolism, which restricts or inhibits water flow (Sperry et al, 1994; Schultze and Matthews, 1988; Sperry and Pockman, 1993), further promoting dehydration and perhaps triggering needle abscission.

As well, with the removal of the root system, transpirational pull is the only driving force behind water flow. However, since the stomata close as a response to water stress or dehydration, transpiration may be reduced, thus resulting in decreased water uptake.

Studies by MacDonald et al. (2011) at the Christmas Tree Research Centre (CRC) in Bible Hill, Nova Scotia, have determined that xylem pressure potential (XPP) declines after harvest, which is a symptom of dehydration. Other works by the CRC have revealed decreased relative water content (RWC) of the needles as a result of root detachment,

postharvest. Another possible cause of water stress, and reduced hydraulic conductance, is xylem blockages at the cut end, since the cut given at the base of the stem heals over time possibly reducing water flow. Also, due to the secretion of sugars from the tree into the medium, microbial contamination is expected, which may block the xylem, thereby limiting water flux. Thus, it is possible that needle abscission (NA) in balsam fir may possibly be due to: i) xylem blockage and/or ii) reduction in stomatal conductance postharvest thus reducing water consumption.

This research project examined the link between NA and some of the hydraulic properties of postharvest balsam fir, for which there is currently little or no information. Investigations were made to achieve the following: i) determine whether a blockage at the cut end restricts water uptake and leads to PNA, ii) explore the link between stomatal conductance and water consumption postharvest, iii) determine whether needle retention duration can be explained by differences in stomatal behaviour between high and low NRD clones, iv) determine whether maintaining high stomatal conductance and water consumption over time will delay PNA and v) determine whether maintaining root pressure would maintain stomatal conductance thus, delaying PNA.

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# CHAPTER 2 LITERATURE REVIEW

## 2.1 THE CHRISTMAS TREE INDUSTRY AND THE ISSUE

### 2.1.1 The Christmas Tree Industry

The Christmas tree industry is a multi-million-dollar economy, with a market value of over $60 million as of 2008 (Stats Canada, 2009). Quebec is the largest centre of Christmas tree production in Canada, and also the principal exporter of Christmas trees out of all the Canadian provinces (Stats Canada, 2009). Nova Scotia is the second-largest producer of Christmas trees in Canada. This province has been exporting Christmas trees since the 1920s, and its industry peaked during the 1950s, with over 3.8 million trees shipped each year (Province of Nova Scotia, 2011).

The majority of Christmas trees produced in Canada are exported to other countries. The United States is by far the largest importer of Canada's Christmas trees. In 2008, about $32 million of the approximate $34 million value of Christmas trees was exported to the US. Other countries that make up the foreign export market of Canadian Christmas trees include Central and South American countries and the Caribbean Islands, as well as Japan and the United Arab Emirates (Stats Canada, 2009).

### 2.1.2 Benefits of Real Christmas Trees

The production of Christmas trees takes years of time and effort. Christmas trees are usually ready to harvest once they reach a height of 6-7 feet (National Christmas Tree Association, 2011), and on average, this can take about 10-15 years. Trees can also be harvested after 3 years to be exported and sold to consumers for tabletops (Christmas Tree Council of Nova Scotia, 2011). The process of growing natural Christmas trees can also be quite costly, and many of the profits gained through sales are offset by these production costs – including fertilizing, pest control, postharvest packaging, transport and equipment (CTCNS, 2011).

Fortunately, real Christmas trees provide many non-monetary benefits to the environment. Christmas trees provide habitats for birds and wildlife, increasing the species diversity of ecosystems (Christmas Tree Farmers of Ontario, 2011). They also improve air quality by removing pollutants and $CO_2$ from the atmosphere. Although real Christmas trees produce about 6.8 lbs of $CO_2$ per tree per year (Couillard et al., 2009), a typical tree can sequester about 48 lbs of $CO_2$ in a year, or 480 lbs in the 10 years it takes for a balsam fir to reach market size (McAliney, 1993). Real Christmas trees also replace this $CO_2$ with enough oxygen per acre of trees to meet the daily requirement of 19 people (Canadian Christmas Tree Growers Association, 2011; CTCNS, 2011; CTFO, 2011). Christmas trees are produced in sloppy terrains with very poor soils where nothing else can be grown, thus reducing soil erosion.

After a Christmas tree has served its primary purpose of being displayed in a consumer's home, it can be recycled into mulch for gardening. Christmas trees, as they decompose, supply nutrients such as carbon and nitrogen to the soil (NCTA, 2011). Post-display Christmas trees can also be converted into wood products or used as wildlife cover (CTFO, 2011).

### 2.1.3 The Threat of the Artificial Tree Market

Though the natural Christmas tree market is of considerable value, the artificial Christmas tree market is of competitive and increasing value. The better part of this market is centered outside of Canada - in 2008, approximately $47 million worth of artificial trees were imported into Canada, about $45 million of this amount from China alone (Stats Canada, 2009). The power this "mainly-foreign" market is gaining against the largely Canadian-based natural Christmas tree market is taking jobs out of the country. There are many jobs associated with the production of real Christmas trees, including growers, distributors and retailers. Keeping the Christmas tree market in Canada allows for the providing of jobs to Canadian citizens (CCTGA, 2011).

The artificial Christmas tree market is a threat not only in terms of competition with the real Christmas tree market, but also in the harmful effects is has on the environment. The

manufacturing of artificial Christmas trees consumes valuable natural resources, such as metals and petroleum, and results in a non-recyclable product that will remain in a landfill for centuries after it has served its purpose. Artificial trees often contain such harmful components as PVCs, lead and toxins (NCTA, 2011). It is also known that the carbon foot print for an artificial tree is higher than that of a real Christmas tree, based on the assumption that the average lifespan of an artificial tree is 6 years (Couillard et al., 2009).

## 2.2 NEEDLE ABSCISSION

Abscission can be defined as the shedding of various plant parts, such as fruit, leaves or flowers, throughout a plant's life cycle (Sexton, 2002; Gonzalez-Carranza et al, 1998). Abscission is an active developmental process linked to metabolic dysfunction, leading to the detachment of diseased, stressed or aging plant organs from the plant at specific sites known as abscission zones (AZ) (Sexton, 2002, Gonzalez-Carranza et al, 1998).

### 2.2.1 Balsam Fir and Needle Abscission

Some of the most popular Christmas trees are balsam fir, Douglas fir, Fraser fir, noble fir, Scotch pine, Virginia pine and white pine (NCTA, 2011). Balsam fir has many benefits that make it a desirable Christmas tree in the homes of consumers, including its color, shape and characteristic fragrance, which cannot be duplicated by any artificial tree (MacDonald et al., 2010; CCTGA, 2011).

Needle retention duration varies among genotypes of balsam fir. MacDonald and Lada (2008) discovered that abscission typically occurs between 6 and 60 days of harvest depending on the genotypes, and that needle retention can be improved by cold acclimation in some genotypes. Studies by Chastagner and Riley (2003) and Mitcham-Butler et al. (1988) also found that trees harvested earlier in the year, during warmer temperatures, display poorer needle retention duration. Unfortunately, since Christmas trees are generally harvested by mid-October to meet shipping demands (MacDonald et al., 2011b), the benefits of harvesting later in the year cannot often be achieved.

Postharvest needle abscission threatens balsam fir's status as the ideal Christmas tree choice in Atlantic Canada. In order to address this problem, which affects the Christmas tree industry and its ability to compete with the artificial tree market, it is necessary to determine the exact cause of needle abscission in postharvest balsam fir.

### 2.2.2 Abscission Process

Abscission of plant organs generally occurs at specialized, genetically-determined sites called abscission zones (AZs). These zones consist of anywhere from one to 20 rows of cells, which are morphologically different from the cells around them (Gonzalez-Carranza et al, 1998). The actual number of cells involved in postharvest needle abscission of balsam fir is not known. In most cases however, only a single layer of cells out of the entire zone is involved in the actual initation of the abscission process; this layer of cells is known as the separation layer (Sexton and Roberts, 1982). AZs can often be found at the base of leaf petioles, young fruit, petals and other plant organs – in other words, where these organs typically detach from the plant.

At the time of abscission, the cell walls in the separation layer enzymically degrade (Sexton, 2002; Gonzalez-Carranza et al, 1998), causing the AZ to weaken. Degradation of cell walls also allows the cells to expand, providing sufficient force to rupture the xylem and facilitate abscission (Sexton and Redshaw, 1981). In balsam fir, a tenfold increase in cellulase has been found to be related to needle abscission (MacDonald et al. 2011a). Mechanical forces, as well as differential plant growth at the abscission zone, may also play a part in the process (Sexton and Roberts, 1982; Wright and Osborne, 1974).

Following abscission of the plant organ, the remaining scar is suberized, and its xylem sealed with gums to protect the plant from disease at this site (Sexton and Roberts, 1982; Biggs and Northover, 1985). Peptides, such as β-1,3-glucanase and chitinase (Del Campillo and Lewis, 1992), play a further role at the site in preventing pathogenic attack (Gonzalez-Carranza et al, 1998).

Postharvest needle abscission in balsam fir is a topic that is under constant research (MacDonald and Lada, 2008; MacDonald et al., 2009, 2010, 2011a, 2011b). We know that the hormone content in the AZ, specifically that of ethylene, promotes needle abscission through increasing cellulase activity (MacDonald et al, 2012a). However, information is still required on whether the postharvest needle abscission is promoted by changes in hydraulic status.

### 2.2.3 Possible Causes

Though it is not known what exactly causes abscission to occur in plants, several studies have examined various factors that have been found to contribute to or inhibit the process (Sexton, 2002, Gonzalez-Carranza et al, 1998, MacDonald et al., 2009, 2010, 2011a, 2011b).

Abscission is known as a major part of a plant's senescence program, which is induced in temperate regions by a number of environmental factors, including photoperiod changes and low temperatures (Sexton, 2002) Factors such as drought and frost damage, which affect the leaf blade adversely, may cause foliage to be shed prematurely (Jordan et al., 1972).

It is generally accepted that the abscission process is influenced by hormonal control within the plant. The auxin gradient within the AZ is one of the major controlling factors of abscission (Sexton, 2002; Gonzalez-Carranza et al, 1998). A theory proposed by Addicott et al. (1955, 1970) explains that the direction of this gradient across the zone determines whether abscission is accelerated or prevented. Abscission can be inhibited if auxins approach the zone from the distal side, but once auxin levels drop, the AZ will weaken.

Ethylene is an additional potential regulator of abscission. Recent studies by MacDonald et al. (2009, 2010) have shown that ethylene can promote abscission at low

concentrations, and reduce the needle retention duration (NRD) in balsam fir. They also demonstrated inhibiting ethylene synthesis or masking the ethylene receptors using 1-MCP reduced postharvest needle abscission (MacDonald et al., 2010). Experiments performed early in the 20th century in other species support this possibility as well (Doubt, 1917).

Other possible regulators of the process include abscisic acid (ABA), auxins such as indoleacetic acid (IAA) and naphthalene acetic acid (NAA), gibberellic acid and cytokinins. ABA is an accelerator of senescence, and as abscission is a major part of this plant process, ABA can be linked to the acceleration of abscission (Sexton, 2002). IAA is known to prevent the effects of ethylene and delay abscission, while cytokinins may indirectly influence and delay senescence (Kuang et al., 1992). Gibberellic acid stimulates abscission, though it is unclear whether it does this on its own, or alongside ethylene (Chatterjee and Leopold, 1964; Morgan, 1976; Abeles, 1967; Marynick, 1977).

A study by MacDonald and Lada (2012) examined hormonal levels in both fresh and abscised needles. It was discovered that ABA and cytokinins increased at peak needle abscission, while auxins decreased. A recent study in balsam fir from Lada's research lab demonstrated that ABA in the shoots increase three fold and discovered GA44 declines postharvest (Thiagarajan et al., 2012). In addition, a significant decline in endogenous IAA has been found in postharvest balsam fir. These results suggest that a decline in auxin, an increase in ABA and an increase in ethylene can trigger needle abscission in balsam fir (MacDonald and Lada, 2011a, MacDonald et al., 2012b, Lada et al., 2014). However, exogenous application of any synthetic auxin at any concentration through any mode of application did not delay needle abscission significantly (Lada et al., 2014). It is possible that the hormonal changes associated with abscission may be triggered by a biophysical factor such as water status.

### 2.2.4 The Link Between Water Relations and Needle Abscission

The direct link between hydraulic properties and needle abscission in postharvest balsam fir has not been established, and requires more extensive research. However, some

evidence has been found to support this potential link. According to Sexton (2002), the levels of free auxin in the abscission zone often decline when the plant is under water stress. Water deficit also causes an increase in the levels of ethylene receptors, increasing the tree's sensitivity to ethylene and therefore, accelerating the hormone's effects; this is further emphasized by the decrease in auxins, which normally reduce a plant's sensitivity to ethylene. Experiments on Scots pine by Zythkowiak et al. (2005) have also shown that premature shedding of needles can be caused by winter drought followed by a spring season with high temperatures and low precipitation.

Studies focusing on balsam fir have provided some evidence for a connection between water relations and needle abscission. MacDonald et al. (2011b) discovered that the xylem pressure potential (XPP) decreases (becomes more negative) in abscising branches and there has been a negative relationship between needle abscission and decline in xylem water potential, Relative Water Content (RWC) and water uptake (MacDonald and Lada, 2012, MacDonald et al., 2014). In general, both xylem pressure potential and relative water content decline postharvest, and both are linked to postharvest needle abscission. However, the average water use does not appear to change. It has also been discovered that when the balsam fir branches are kept under low vapour pressure deficit (VPD), needle retention exceeded 150 days with little change in XPP (MacDonald et al., 2012a). However, in the presence of ethylene, the benefits of low VPD disappear. Surprisingly, there has been no difference between high and low NAR clones in XPP despite significant variations in postharvest needle abscission. An investigation into the effects of dehydration on needle abscission by Adams et al. (2013) found distinct differences between genotypes in their ability to rehydrate after being allowed to dehydrate by 17.5%, with low NRD clones generally being more sensitive to dehydration.

In a study conducted at Lada's lab, it was found that giving a fresh cut to the base of the stem does not improve needle retention suggesting that xylem blockage may not be a factor in postharvest needle abscission (Adams and Lada, 2011). It has also been found that the needle abscission is generally high in branches that take up more water

(MacDonald et al., 2012a). It is postulated that a blockage in xylem reduces water flux, reducing xylem pressure potential, leading to dehydration of the needles resulting in needle abscission. There is however no evidence on the link between water consumption, stomatal conductance or xylem blockage and PNA.

## 2.3 Hydraulic Properties

### 2.3.1 Hydraulic Status

Water relations can be said to have an effect, be it direct or indirect, on most plant processes. Water serves several important functions – as a constituent of a large part of a plant's biomass; as a solvent for the transportation of solutes from one plant organ to another; as a reactant in major chemical reactions such as photosynthesis; and as a major component in maintaining turgidity, which is essential for plant growth (Kramer and Boyer, 1995).

Water is distributed throughout the plant by the tracheary elements of the xylem, connected by lateral pits (Esau, 1965). Most woody plants contain both tracheids and vessels. However, coniferous trees such as balsam fir lack the latter, so the transportation of water is primarily handled by tracheids alone (Tyree and Ewers, 1991).

The hydrogen bonds within water provide the surface tension required for its uptake in the tracheids. It is this factor that has assisted in the development of the cohesion-tension theory used to explain the method in which trees draw water from the soil to their uppermost branches (Dixon and Joly, 1895; Pickard, 1981). However, another factor that must be taken into consideration when discussing methods of water transport is the water potential gradient.

Water potential, a force which, along with transpirational pull, directs the movement of water in plants, is the amount by which the chemical potential of water in plants is below that of pure water, and is typically reduced by the presence of solutes and increased by the addition of pressure (Kramer and Boyer, 1995). Water follows a potential gradient

when traveling from one part of the plant to another, always moving towards the more negative potential (Sperry et al., 1994). The highest water potential (least negative) is found in the soil where, under optimum conditions, liquid water is readily available; in the atmosphere, the water potential is usually much more negative (Ewers and Cruiziat, 1990). In this way, the gradient directs water from the soil into the roots and stems, to the leaves, and into the atmosphere.

Transpiration from the leaves is controlled by the stomata, the connections between the atmosphere and the air space within the plant. Generally, stomata open during the daylight hours and close at night, but the turgidity of the stomatal guard cells also has an effect on their opening and closing. The guard cells open when they are turgid, and close to prevent water loss through transpiration when their turgidity decreases (Kramer and Boyer, 1995).

In root-detached balsam fir trees, however, the water pressure provided by the root system is eliminated. MacDonald et al. (2011b) have also revealed that xylem pressure decreases postharvest. Thus, in root-detached trees transpirational is the only biophysical force that is expected to move water from the source to the atmosphere. If this is so, high VPD could dehydrate the root-less trees faster under conditions of limited water supply. Alternatively, if the stomates respond to dehydration by closure of stomata, this would lead to a reduction in water suppy thus, further promoting dehydration. This situation may further be amplified by the fact that stresses caused by postharvest procedures such as baling, storage and transport can expose the tree to dehydration, which causes damage to the xylem conduits and reduces hydraulic flow, thus triggering PNA (Adams and Lada, 2011).

### 2.3.2 Hydraulic Pressure and the Link with Abscission

The hydraulic architecture of woody plants can be thought of as a sort of “pipe model”. Each pipe in the system is responsible for supporting a unit of leaves, and as the tree grows, new pipes are added, each with a new unit of leaves to support in terms of water supply (Shinozaki et al., 1964). However, the picture of a tree’s hydraulic architecture is

not complete with the pipe model alone – it must also include the environment in which the tree grows; this includes both the atmosphere and the soil environment (Kramer and Boyer, 1995). Each part of this picture contributes to the water potential gradient, and therefore, the flow of water within the tree.

The hydraulic efficiency of the pipe system as a whole is not consistent – Zimmermann (1978) discovered that the hydraulic conductance tends to drop off from the trunk outwards, with the trunk itself displaying the highest efficiency, and the branches it supports having less efficiency. As well, it has been found that hydraulic "bottlenecks", or constrictions, occur at the base of branches. Studies by Rust and Roloff (2002), have also found evidence in old oak trees that constrictions in water transport occur at abscission sites. Additional evidence can be found in the experiments involving the conifer *Wollemia nobilis* by Burrows et al. (2007). Each xylem "pipe" supports a large unit of leaves for the diameter of its xylem, and constrictions at the base of branches have been seen to contribute to branch abscission.

### 2.3.3 Hydraulic Conductance and Root Pressure

Hydraulic conductance can be defined as the quotient of flow rate against the pressure gradient (Sperry et al., 1988). In a typical growing situation, where the roots are still attached, the root system plays a major part in generating root pressure, maintaining the water potential gradient. A survey of 101 species of tropical vines and eight species of woody plants by Fisher et al. (1997) showed that normal root pressure can vary widely by species, with this particular study giving a range of 2 to 148 kPa (0.3 to 21.5 psi).

When balsam fir is harvested for distribution to consumers, it is detached from its root system, and therefore, a significant part of the water potential gradient is possibly eliminated. Furthermore, when roots are detached, the stomata in the leaves close as a defense mechanism to reduce water loss to the atmosphere. As the transpirational pull of water from the needles into the atmosphere is what drives the water potential gradient, removal of the root system severely reduces hydraulic flow throughout the plant. This

reduction in hydraulic conductance may possibly be a causal factor triggering needle drop in postharvest Christmas trees.

### 2.3.4 Xylem Blockages

#### 2.3.4.1 Structure of Tracheids

Tracheids in coniferous trees are approximately 3 mm in length, and are connected by pit membranes consisting of a thickened torus surrounded by a porous margo. The torus acts as a seal against embolism by air seeding (Section 2.3.4.2), while the margo minimizes the hydraulic resistance between cells (Sperry and Tyree, 1990).

#### 2.3.4.2 Blockages

Obstructions in tracheids reduce xylem diameter and restrict hydraulic flow (Sperry et al., 1994). Blockages may occur for various reasons – for example, as a mechanism of defense to prevent the spread of infection throughout the vascular system to other plant organs. Tyloses, which are outgrowths of living parenchyma cells into the lumen of the xylem, often form within the vascular cells. However, the benefits provided by this defense mechanism are often costly in the form of reduced water uptake by the plant (Van Ieperen et al., 2002).

Another major cause of xylem obstruction is cavitation, which is the formation of water-free pockets or air bubbles within the xylem, caused by the abrupt transition of xylem water from a liquid state to vapour (Tyree et al., 1994). The pockets, called embolisms, are formed when air enters a functional xylem conduit through its pit membranes (Sperry and Tyree, 1988, 1990). Cavitation by "air-seeding", or the aspiration of air from embolized tracheids into functional ones, occurs at inter-tracheid pit membranes. When the torus region of the membrane becomes displaced from the pit aperture, tracheids become vulnerable to air seeding, with vulnerability more prominent in conifers with more flexible pit membranes, due to the torus being more easily displaced. Water is transported throughout a plant under negative pressures, which makes the xylem highly susceptible to cavitation (Sperry et al, 1994), especially under conditions where water is

not readily available. Cavitation, especially in larger conduits (Ewers and Zimmermann, 1984) disrupts the water column within the xylem conduits and results in a decline in water uptake, hydraulic conductance, and rate of transpiration (Schultze and Matthews, 1988, Sperry and Pockman, 1993, Sperry et al. 1993, Alder et al. 1996).

Other common causes of xylem blockage include the deposition of gums, mucilage and other such substances within the conduits, as well as microbial growth (Van Ieperen et al., 2002). Studies by Sperry et al. (1988), which examine the correlation between microbial contamination and hydraulic conductance, suggest that long-term declines in conductance are a result of microbial growth within xylem.

It is unclear what causes blockages at the cut end of harvested balsam fir trees. It is possible that resin may accumulate in the xylem as a defense mechanism to prevent the spread of infection from the now-vulnerable cut end into other parts of the tree. Research on cut flowers by Van Ieperen et al. (2002) shows that pockets of air form in the xylem at the cut end upon harvest, and additional studies by Van Doorn and Cruz (2000) revealed that this issue may even be a prerequisite for more serious and permanent damage to xylem.

### 2.3.5 Stomatal Regulation

As discussed in Section 2.3.4, cavitation is one of the major sources of blockage in the xylem conduits, resulting from the pressure within the xylem becoming critically negative. Within and below the range of xylem pressures that cause cavitation, the plant's water conduction will decrease to the point of elimination, ceasing plant function (Sperry et al. 1993). In order to prevent the xylem pressure potential from becoming negative enough to cause cavitation, transpiration must be regulated by the stomata (Alder et al., 1996).

Stomatal conductance is influenced by light intensity, water status of the plant, humidity of the atmosphere, atmospheric carbon dioxide ($CO_2$) content, and to some extent, hormones. Under normal conditions, a plant's stomata will remain open under conditions

of high light intensity in order to assimilate $CO_2$, and close during periods of low light intensity to prevent water loss (Section 2.3.1). However, when water supplies are limited, such as during drought conditions, or after a tree has been harvested from its root system, the plant's priority changes from $CO_2$ assimilation to restricting water loss through transpiration, while still maintaining as much $CO_2$ assimilation as possible (Mansfield et al., 1990).

As discussed in Section 2.3.1, the atmosphere is part of a plant's water potential gradient, and the atmospheric humidity influences the rate of water loss by transpiration. When the humidity is low, the plant will lose more water through its stomata as a result of the atmospheric water potential being more negative. When it is high, the plant loses less water through transpiration because of a less negative atmospheric water potential.

$CO_2$ in the atmosphere has been shown to inhibit stomatal opening in many species of plants, according to studies by Morison (1985, 1987). Studies by Düring (1988) and Düring and Stoll (1996), have found that stomata close if the ambient $CO_2$ concentration exceeds a certain threshold, though it depends on the $CO_2$ sensitivity of the leaf. Normally, the response of photosynthesis to $CO_2$ greatly outweighs the inhibiting action of $CO_2$ on stomatal guard cells (Mansfield et al., 1990). However, in a postharvest situation, where the plant's priority becomes water conservation rather than $CO_2$ assimilation, the reverse may occur, causing the stomata to become more sensitive to the inhibiting action of $CO_2$. Thus, manipulating the environment, one could understand the link between stomatal behaviour, water consumption, and needle abscission, postharvest.

ABA has been shown to cause stomata to become sensitive to $CO_2$, either through external supply or endogenous production (Dubbe et al., 1978; Raschke, 1975), though the sensitivity varies depending on the plant's water status, with negligible water stress resulting in zero sensitivity. A study by Snaith and Mansfield (1982) showed that high concentrations of IAA in a plant's incubation medium can reduce or eliminate $CO_2$'s inhibitory effect on stomata. Cytokinins, in the presence of inhibitory agents such as

ABA or $CO_2$, can restore full stomatal opening, though they are not very effective on their own (Davies and Mansfield, 1987).

Harvesting a balsam fir tree removes the positive water pressure the root system provides, and causes the stomata to close to prevent further water loss through transpiration (Section 2.3.3). This results in a sharp decline in water consumption following harvest, leading to negative xylem pressures, the risk of cavitation, and possible dehydration and abscission of the needles. It is possible, therefore, that creating environmental conditions, which favor stomatal opening could result in a postharvest balsam fir tree maintaining a higher stomatal conductance and water consumption for a longer period of time. Furthermore, providing a positive water pressure to the cut end could restore that portion of the water potential gradient, maintaining the hydraulic status of the tree and possible delaying needle abscission.

## 2.4 Goals and Hypotheses

### 2.4.1 Overall Purpose of the Project

The objective of this project was to understand the link between water consumption and postharvest needle abscission; to identify the limiations for water flow; to determine whether manipulating stomatal function can improve water flow and thus, needle retention; and to test whether compensating root pressure would improve postharvest needle retention. Various components of hydraulic status, particularly xylem blockage, daily and cumulative water use, and stomatal conductance were examined under various situations for their possible link with postharvest needle abscission.

### 2.4.2 Hypotheses

[1] Blockages at the cut end of the stem limit water consumption and reduce Needle Retention Duration (NRD) (Experiment 1).
[2] Giving a fresh cut to the end of the stem improves stomatal conductance and water consumption, and promotes NRD (Experiment 2).

[3] High-NRD balsam fir clones are able to maintain high stomatal conductance and water consumption for a longer period of time, thus resulting in improved NRD (Experiment 3).
[4] Increasing light intensity will keep stomatal conductance and water consumption higher (Experiment 4).
[5] Increasing humidity will increase stomatal conductance and decrease water consumption (Experiment 4).
[6] Applying a positive water pressure, as root pressure compensation (simulating root pressure), to the cut end of the stem will improve water uptake and stomatal conductance, improving NRD (Experiment 5).

### 2.4.3 Objectives

[1] To establish whether blockages at the cut end of postharvest balsam fir reduce water consumption thus promote needle abscission. (Experiment #1)
[2] To examine the link if any of stomatal conductance, water consumption and postharvest needle abscission. (Experiment #2)
[3] To identify whether the variation in needle retention duration between clones of high and low NRD can be explained by variations in postharvest stomatal conductance and water consumption. (Experiment #3)
[4] To investigate factors that influence stomatal conductance and water consumption postharvest. (Experiment #4)
[5] To determine if increasing hydraulic pressure by simulating root pressure would help to maintain high stomatal conductance thus, improve needle retention. (Experiment #5)

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# CHAPTER 3 GENERAL METHODOLOGY

## 3.1 Preparation of the Plant Material

The protocol for sampling and postharvest set-up was based on previous studies by MacDonald et al. (2011). Samples for all experiments were taken from the balsam fir clonal orchard at the Tree Breeding Center in Debert, Nova Scotia (45° 25' 9" N, 63° 30' 0" W). Two-year-old branches from known clones were cut from the mother tree, placed in distilled water and transported to the lab.

Once brought to the lab, branches were acclimated for forty-eight hours to 10°C, which was the average temperature for the month of October 2012. They were then allowed to acclimate to lab temperature for an additional 24 hours. Following acclimation, they were re-cut under water and placed in amber bottles containing 100mL of distilled water, with the exception of treated branches in Experiment 5 (Chapter 8), which were installed into the pump system used for that particular experiment.

The same low-NRD clone, Clone 236, was used for all experiments. Experiment #3 (Chapter 6) used an additional clone of high NRD, Clone 506. Clones are classified as low or high-NRD based on the number of days taken for a dehydrated branch to shed all of its needles. A study by Adams and Lada (2011) of 206 New Brunswick clones found that NRD ranged from 7 to 19 days in the lowest-NRD clones, and 33 to 43 days in the highest-NRD clones, based on data taken prior to cold acclimation.

## 3.2 Needle Loss and Retention Duration

All experiments in this study required the measurement of needle loss. On each date of sampling, a finger-run test was performed on each branch, which consisted of gently brushing each branch between the thumb and fingers 3 to 4 times to simulate mechanical stress. This test was performed at approximately the same time of day with each sampling. Needles fallen were collected in trays and weighed, along with any natural needle fallen that may have occurred between samplings. In Experiments 2 to 5, needles collected on each sampling date were dried in an isotemp oven (Fisher Scientific,

Pittsburgh, PA, USA) for 24 hours (MacDonald et al., 2012) at 80°C. At the conclusion of Experiments 2 to 5, all branches were dried for 48 hours at 80°C. Any remaining needles were removed from the dried branches and weighed. Needles were weighed immediately after drying.

According to experiments by MacDonald and Lada (2008) and MacDonald et al. (2009, 2010), needle retention duration (NRD) can be defined as the length of time for a branch of balsam fir to lose half of its fresh weight through needle abscission. This is due to the fact that approximately 50% of the fresh weight of balsam fir branches is made up by the mass of its needles. The measurement of NRD can be achieved by recording the weight of each branch before the experiment begins, and monitoring the total needle loss for each branch until it reaches the target of approximately half of the branch's fresh weight. This method was used for the first experiment. In Experiments 3 and 5, NRD was measured by plotting cumulative percentage needle loss against time, and determining at which day there was no further needle loss. This would have ideally been at 100% needle loss.

### 3.2.1 Calculating Daily and Cumulative Percentage Needle Loss

In the first experiment, daily percentage needle loss was determined using a protocol suggested by MacDonald et al. (2011).

$$\%\ Needle\ Loss = \frac{Daily\ Needle\ Mass\ Lost}{Total\ Branch\ Fresh\ Weight}\ x\ 100$$

In following experiments, a modified protocol was used, substituting dry needle weight for fresh needle weight, and calculating a ratio of daily needle loss to total branch needle weight:

$$\%\ Needle\ Loss = \frac{Daily\ Dry\ Weight\ Needle\ Mass\ Lost}{Total\ Needle\ Dry\ Weight}\ x\ 100$$

This method was used to achieve greater accuracy and less error. Since branches are expected to lose some moisture over time as water use decreases, measuring percentage needle loss as a ratio of daily fresh weight needle loss to initial total branch weight may produce false results. Drier needles at the conclusion of an experiment will contribute less weight than fresher needles at the beginning of an experiment, causing a discrepancy in the results due to moisture loss. Calculating percentage needle loss on a dry weight basis eliminates any potential error that may have been caused by moisture loss in attached needles. Using total needle dry weight as opposed to total branch weight ensures only the needles, rather than both the needles and stem, are involved in the calculation.

Cumulative needle loss was calculated using the following equation:

$$Cumulative\ \%\ Needle\ Loss = NL_n + NL_{n-1} + \cdots + NL_1$$

where NL (Needle Loss) was the percent dry needle loss for the $n^{th}$ sampling date.

## 3.3 Water Consumption

Water consumption was measured in terms of weight of water lost over the duration of the experiment. Branches were placed in brown 100mL glass bottles containing distilled water, and weighed with each sampling. At the end of each experiment, daily, total, cumulative and average water use were calculated, using methods suggested by MacDonald et al. (2011).

Prior to the start of the experiment, the bottle, the water contained within the bottle, and the branch were weighed as a unit. On each sampling date, this unit was re-weighed. As well, any needles lost through natural needle fall and the finger run test (Section 3.1) were weighed. For each branch-bottle unit, the difference between the current day's weight and the weight from the previous sampling date was calculated to determine the combined loss of weight through needle abscission and water loss. The weight of needles lost was then subtracted from this difference to determine the weight of water lost for that sampling date.

### 3.3.1 Calculating Total, Daily, Cumulative and Average Water Consumption

Total water consumption (TWC) was determined using the following equation:

$$TWC = Initial\ mass - (Final\ mass + Needle\ mass)$$

Daily water consumption (DWC) was determined using the following equation:

$$DWC = (UW_{n-1} - UW_n) - FNW_n$$

where UW (Unit Weight) is the weight of the branch-bottle unit, n is the sampling date, and FNW (Fresh Needle Weight) is the fresh weight of any needles lost on the $n^{th}$ sampling date.

Cumulative water consumption (CWC) was determined using the following equation:

$$CWC = WL_n + WL_{n-1} + \cdots + WL_1$$

where, WL (Water Loss) is weight of water lost on the $n^{th}$ sampling date.

Average water use (AWU) was determined using the following equation:

$$AWU = \frac{Initial\ mass - Final\ mass + Needle\ mass}{Time}$$

(MacDonald et al., 2011)

## 3.4 Evaluation of Xylem Blockage

Using a microtome, four cross-sections were taken from the first 1cm of the base of each branch. The cross-sections were mounted on slides and stained with methylene blue dye. The slides were examined under a microscope, and pictures were taken of each individual cross-section. Using the rating scale shown in Figure 3.1, each cross-section was given a rating corresponding to degree of xylem blockage.

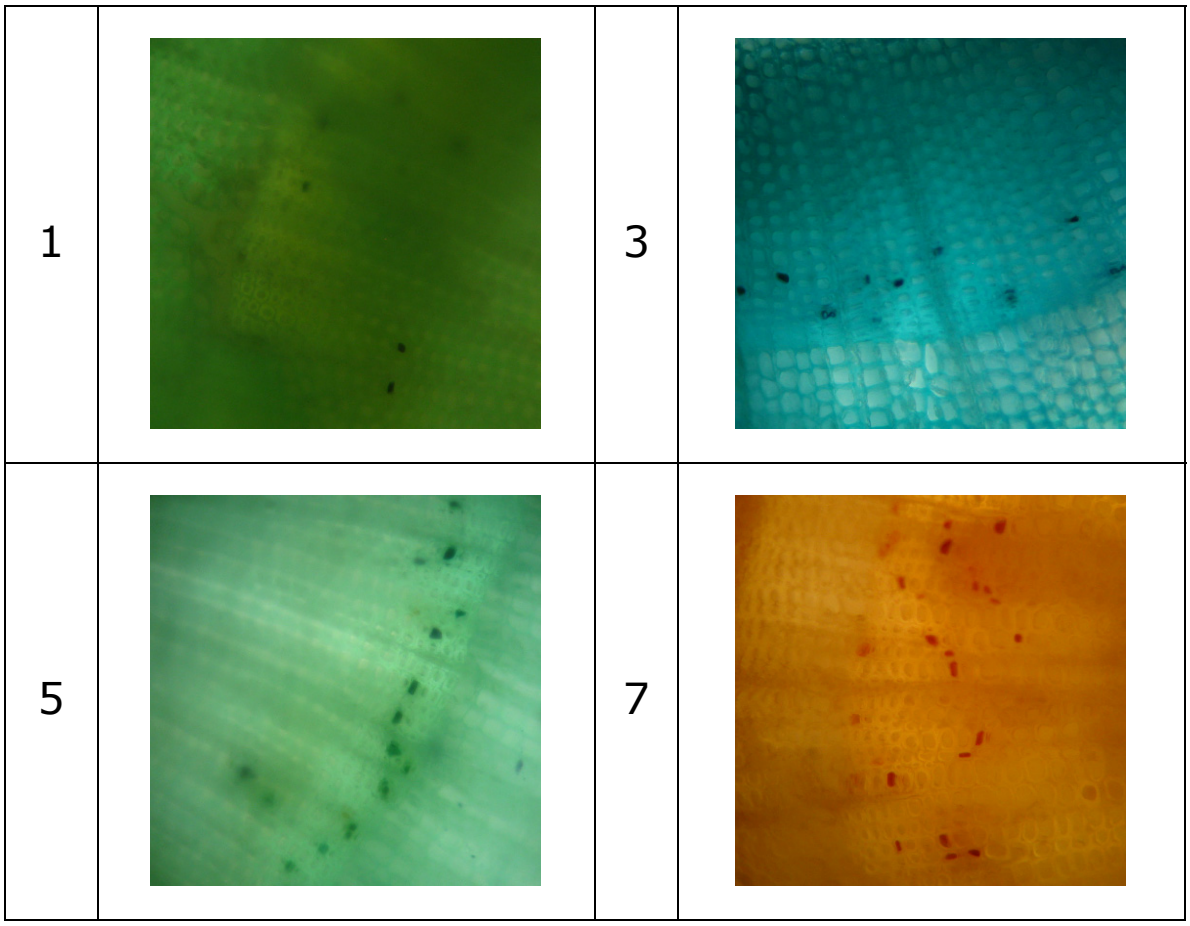


Figure 3.1 Examples from the rating scale used for designating degree of xylem blockage. Sections were given a score from 0 (no blockage) to 9 (severe blockage).

## 3.5 Stomatal Conductance

Measurement of stomatal conductance used a steady state diffusion leaf porometer (Model SC-1, Decagon Devices, Pullman, WA, USA), following protocol outlined in the User's Manual.

All experiments involving the measuring of stomatal conductance were taken on attached needles. This method is recommended for long-term experiments, since it does not require the removal of needles with each sampling. The sensor was attached to the needles of the branch, with the lower surfaces (stomata side) of the needles facing and completely covering the aperture (Figure 3.2). Conductance readings were then taken and

expressed as mmol $m^{-2}$ $s^{-1}$. Due to the fact that there must be enough needles to cover the aperture, readings could not be taken once a branch had lost a certain amount of its needles. Readings were taken at the same time of day and from the same section of the branch on each sampling date, and only on needles that were at least one-year old. Experimental room conditions varied, and are outlined in individual experimental chapters.


Figure 3.2 Taking a conductance reading from attached needles.

## 3.6 References

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# CHAPTER 4 EFFECTS OF XYLEM BLOCKAGE AT THE CUT END OF THE STEM ON WATER USE AND NEEDLE ABSCISSION


## ABSTRACT

Branches of two-year growth from a low-NRD balsam fir clone, Clone 236, were examined for patterns of water use, needle loss and xylem blockage over a two-month time period. Measurements were taken on 10 sampling dates until the conclusion of the experiment, and stem cross-sections were prepared and rated for degree of xylem blockage. It was found that mean xylem blockage had no significant relationship with date of sampling, but daily water use, cumulative water use and percent needle loss appeared to have definite nonlinear relationships with date of sampling. The fitted models for daily and cumulative water use revealed that harvested branches experienced a sharp drop in water uptake approximately 5 days after being placed in water, and that significant needle loss did not occur until approximately 50 days following placement in water. With this particular clone, xylem blockage did not appear to be the reason for postharvest needle loss. It is recommended that further studies be conducted in this field, comparing clones of varying NRD for differences in xylem blockage, and exploring other methods of image capture and section preparation.


## 4.1 INTRODUCTION

Studies by MacDonald et al. (2011) have shown evidence of a postharvest decline in the XPP, RWC and water use of balsam fir. Noted in particular were water consumption, xylem pressure potential and needle relative water content, all of which are indicators of dehydration when in a state of decline. As dehydration leads to eventual needle abscission, this suggests a possible link between the hydraulic status of the tree and its ability to retain needles, postharvest.

One possible cause of the decline in water uptake is xylem blockage at the cut end. Xylem tracheids function by distributing water throughout the plant under negative pressures. Water moves through the lateral pits connecting individual tracheids, following a water potential gradient toward the more negative potential (Kramer and Boyer, 1995).

However, in a situation of water stress, xylem becomes vulnerable to cavitation, or the formation of water-free pockets in the xylem conduits. Xylem blockage may also occur from outgrowths of parenchyma cells into the xylem conduits as a defense mechanism against infection, from the deposition of gums and mucilage in the conduits, or from microbial growth at the cut end (Van Ieperen et al., 2002; Tyree et al., 1994; Sperry et al., 1994). There is currently no evidence of a link between xylem blockage, water use and needle abscission in postharvest balsam fir, but it is speculated that obstructions in the xylem of balsam fir reduce hydraulic flow (Sperry et al., 1994), resulting in a closure of the stomata to prevent water loss, and further leading to a decline in water uptake. Since stomata, along with the water potential gradient, are the driving forces of water movement throughout the tree (Kramer and Boyer, 1995; Sperry et al., 1994), blockage of the xylem conduits may be expected to reduce water consumption thus leading to dehydration, promoting needle abscission in root detached trees postharvest.

This study investigates the possible link between xylem blockage and needle loss in postharvest balsam fir, and models relationships of xylem blockage, water use and needle loss over time and against each other. A clone of low needle retention duration (NRD) was used for this experiment.

## 4.2 Materials and Methods

### 4.2.1 Variables Studied

The parameters investigated in this experiment were xylem blockage, percent needle loss, daily water use and cumulative water use. These parameters were measured over a 63-day time period.

### 4.2.2 Preparation of the Sampling Units

Eighty branches of a known low-NRD clone, Clone 236, were harvested from three trees at the Debert Clonal Orchard in Debert, Nova Scotia, and prepared using methods described in Section 3.1. Initial measurements were taken for the total weight of each branch-bottle unit. Using four extra branches harvested at the same time as the 80

branches collected for the study, cross-sections and slides were prepared in order to take initial measurements for xylem blockage (the methods used are described in Section 3.4.)

### 4.2.3 Needle Loss and Water Use Measurements

Measurements for needle loss and water use calculations were taken at approximate 4-day intervals near the beginning of the experiment, and approximate weekly intervals nearing the conclusion of the experiment. These measurements were taken for all experimental units remaining on the table, using methods described in Sections 3.2 and 3.3. Cumulative percent needle loss, daily water use and cumulative water use were calculated using the methods described in Section 3.2.1.

### 4.2.4 Microscopy

Four branches were sacrificed for microscopy on each sampling date, from the eight replicates assigned to that date. Cross-sections of each stem were taken and examined using methods described in Section 3.4.

### 4.2.5 Statistical Methods

Relationships for percent needle loss, cumulative water use, daily water use and xylem blockage against time were determined using correlation and regression analysis (SAS Version 9.1, SAS Institute Inc.; Minitab Version 16, Minitab Inc.). Xylem blockage was also plotted against percent needle loss, cumulative water use and daily water use for possible relationships. Due to the sampling occurring on fixed dates, it was decided that mean values for each date would be used for the regression analysis.

The plot of percent needle loss against time (Figure 4.1) had two outliers that were removed for the purpose of constructing a regression model. It is thought that these outliers occurred due to the fact that four branches were removed from the table each week, and it is possible that the removal of some of these branches resulted in the reduction of the mean cumulative needle loss.

It was determined that percent needle loss, daily water use and cumulative water use could be described by a nonlinear regression model. The plot for cumulative water loss (Figure 4.2) showed evidence of a long-term asymptote, so the Michaelis-Mention model, listed below, was chosen as a base for these data. Both percent needle loss and daily water use were found to best fit a modified version of the asymptotic regression model, listed below, while percent needle loss versus daily water use was modelled using a power regression model. Analysis was run using the NLIN procedure in SAS (version 9.3, SAS Institute Inc.), and convergence criteria were met for all variables involved.

The Michaelis-Mention Model:

$$y_i = \frac{\theta_1 x_i}{(\theta_2 + x_i)} + \varepsilon_i$$

The Modified Asymptotic Regression Model:

$$y_i = \theta_1 e^{(\theta_2 x_i)} + \varepsilon_i$$

The Power Regression Model:

$$y_i = \theta_1 (x_i^{\theta_2}) + \varepsilon_i$$

## 4.3 Results

Percent needle loss, daily water use and cumulative water use over time can be described using nonlinear models.

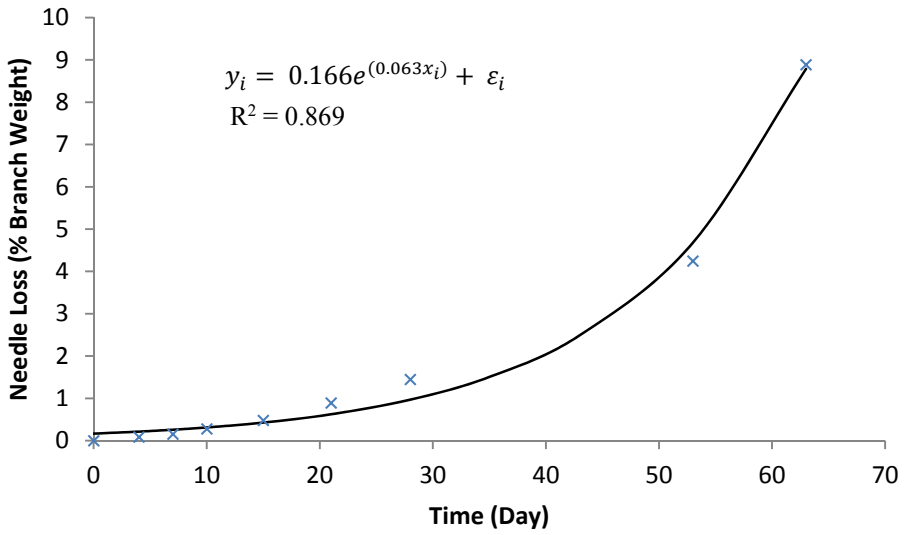


Figure 4.1 Dynamics of needle loss (% on a whole-branch basis) over a 63-day time period. Two outliers were removed. Regression coefficients can be found in Table 4.1. n = 9, 44-80 replicates.

Percent needle loss was significant over the entire experimental period. The dynamics of needle loss reflected a positive nonlinear phenomenon, with needle loss increasing progressively from Day 4 and reaching its peak on Day 63 (Figure 4.1). This experiment was terminated after 63 days; it is expected that percent needle loss will follow a logistic growth curve rather than an exponential growth curve if the branches are left for a longer period of time.

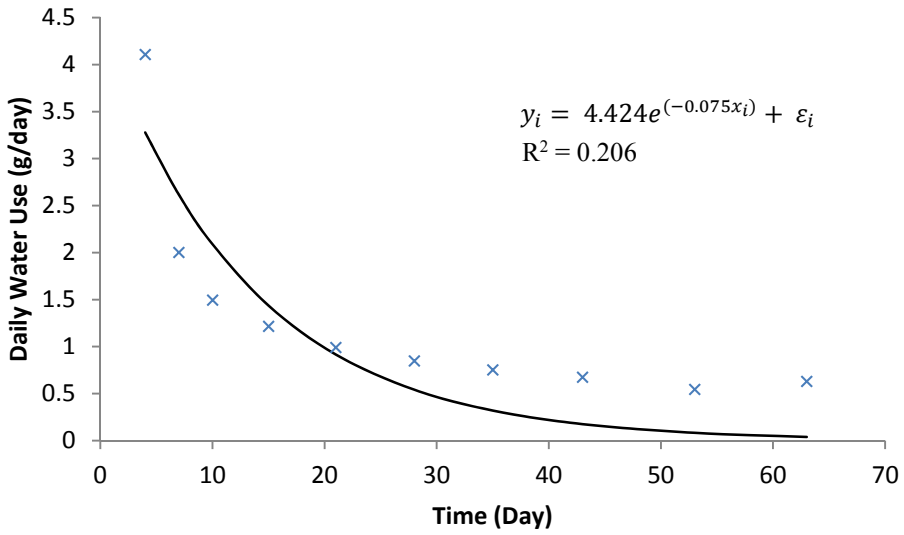


Figure 4.2 Dynamics of daily water use over a 63-day time period. Regression coefficients can be found in Table 4.1. n = 11, 44-80 replicates.

Dynamics of daily water use (Figure 4.2) reflected a negative nonlinear relationship, with the sharpest decline between the 5 and 10 day mark. Following Day 10, daily water use decreased progressively until the termination of the experiment, indicating that as time passed, less water became available.

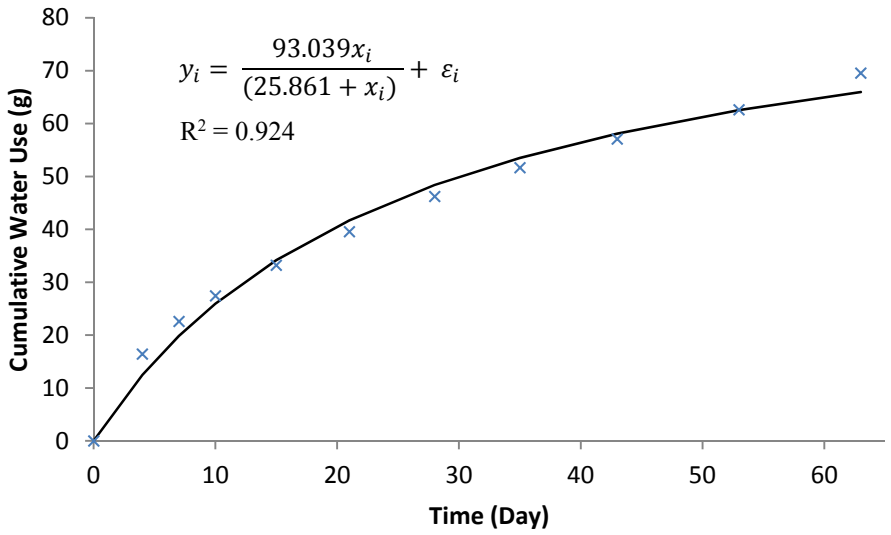


Figure 4.3 Dynamics of cumulative water use over a 63-day time period. Regression coefficients can be found in Table 4.1. n = 11, 44-80 replicates.

Cumulative water use dynamics followed a positive nonlinear trend (Figure 4.3). Dynamics indicate the most water was used within the first 5 to 10 days, but total cumulative water use continued to increase at a slower rate over the next 50 days. It is expected that cumulative water use would have continued increasing at a progressively slower rate until the point in time when branches no longer consumed water.

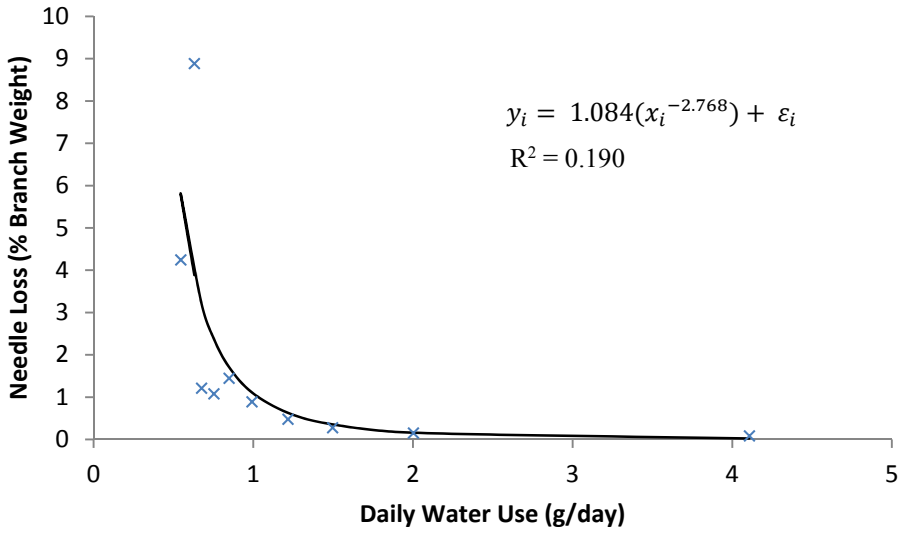


Figure 4.4 Dynamics of needle loss against daily water use. n = 11, 44-80 replicates.

The dynamics of daily water use against percent needle loss (Figure 4.4) can be described using a nonlinear model. High daily water use can be associated with low percent needle loss, while high percent needle loss occurs at a time when daily water use is low. A daily water use of 1 g/day appeared to be the point at which needle loss sharply increased, as the highest percent needle losses occurred beyond this threshold, while needle losses below this water use threshold were less than 1% of the total branch weight.

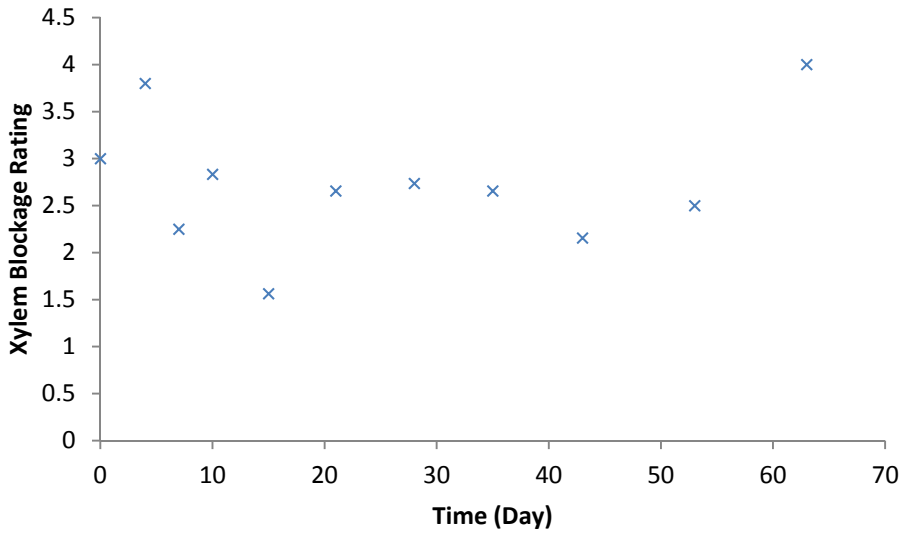


Figure 4.5 Dynamics of xylem blockage over a 63-day time period. n = 11, 44-80 replicates.

Figure 4.5 shows no definite pattern in the dynamics of xylem blockage over the experimental time frame. Correlation results for all relationships involving xylem blockage (Table 4.1) showed that none of the other parameters in the experiment have a significant relationship with xylem blockage at the 5% level.

Table 4.1 Correlation results describing the relationships between xylem blockage rating and time, daily water use, cumulative water use and percent needle loss.

| | Time (Day) | DWU (g/day) | CWU (g) | PNL (% BW) |
|---|---|---|---|---|
| **Correlation** | 0.141 | 0.287 | 0.006 | 0.474 |
| **P-Value** | 0.679 | 0.392 | 0.987 | 0.141 |

$\alpha = 0.05$

## 4.4 Discussion

These initial results indicate that xylem blockage may not be linked to water use and needle abscission in Clone 236 of balsam fir. However, even though the blockage was not related to needle loss, there was a decline in daily water use with dynamics that significantly related to needle loss (Figure 4.4). This could possibly be due to a closure of the stomata in a postharvest, root-detached system. Since ABA, which is known to

influence stomatal behaviour (Dubbe et al., 1978; Raschke, 1975), has been shown to increase postharvest, it is possible that this increase in ABA in postharvest needles promotes stomatal closure. With roots absent, there is no contribution to water flow from root pressure, resulting in a reduced chance of compensating water loss due to dehydration. Both the lack of root pressure and the closure of the stomata could be responsible for the decline in water use postharvest seen in this study.

Due to the fact that only the blockages visible in the pictures taken were accounted for in this experiment, it is possible that there may be other types of blockages, such as embolisms or microbial growth at the cut end, that are contributing to decreased water consumption and increased needle loss, and may not be visible in these images. In order to account for types of blockages that may not have been visible in the images used for this experiment, it is recommended that other types of image capture, such as electron microscopy, be explored before definite conclusions can be made. In addition, taking longitudinal sections, rather than cross-sections, of the first 1 cm from the cut end could possibly reveal other types of blockages not observed in this experiment. Also of note is that cross-sections were scored based on visible blockages in all xylem tissue. However, in the two-year-old branches used in this experiment, it is likely that only current-year xylem is functional. To improve results, cross-sections should be scored based on degree of blockage in current-year xylem tissue.

It is also possible that the majority of the blockage may occur closer to the cut end than the sections were taken (within the first 1 cm of the cut end), leading to false results. It is recommended that this field of study be investigated further, by comparing clones of varying NRD for differences in xylem blockage over time. As well, it is suggested that the method of taking cross-sections be improved, with sections being taken closer to the cut end of the stem in upcoming experiments.

Precautions were taken to ensure that xylem was not damaged during harvest and preparation of the plant material. The process of cutting down a Christmas tree in the field could expose current-year xylem to mechanical stress, particularly if it is struck

repeatedly with an axe. However, it was ensured that a perfect cut was made when harvesting branches from the mother tree for this experiment, thus eliminating this possible source of error.

To improve precision of the needle loss measurements, it is recommended that needle loss be measured on a dry weight basis rather than a fresh weight basis in upcoming experiments. As needles that are left on the trays between sampling dates are subject to moisture loss, drying needles before each weighing would reduce error. Needle loss results in upcoming experiments will be taken on a dry weight basis, using protocol suggested by MacDonald et al. (2014) and following procedures detailed in Section 3.2.1.

Percent needle loss was calculated in this experiment as a ratio of the fresh needle weight taken at each sampling date to the total branch weight. While this is useful in showing the pattern of needle loss following harvest, it cannot be used to accurately predict the percentage of total needle weight lost, since the weight of the stem itself is included. Upcoming experiments use a different and more accurate method of measuring the progress of needle abscission, which calculates percent needle loss as a ratio of the dry needle weight taken at each sampling date to the total dry weight of needles only.

Examining the dynamics of daily water use against percent needle loss suggests that high percent needle loss may be linked to low daily water use. This is expected due to the fact that low water use leads to dehydration, which promotes needle abscission.

## 4.5 Conclusion

Though xylem blockage does not appear to have a link with any of the other parameters involved in this experiment, the rapid decline in water consumption within the first 10 days following harvest suggests that a barrier to water uptake exists, which presumably leads to dehydration, low xylem pressures, and eventually, needle abscission. Upcoming experiments investigate this further, by measuring patterns of stomatal conductance alongside water use patterns, and linking these trends with the initation and rate of needle abscission. In the following experiment, branches are given a fresh cut on set days as an

attempt to remove this barrier to water uptake, which will ideally improve water consumption and delay dehydration and needle abscission.

## 4.6 References

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# CHAPTER 5 EFFECTS OF STEM CUTTING ON STOMATAL CONDUCTANCE, WATER USE AND NEEDLE ABSCISSION


## ABSTRACT

Thirty branches of a low-NRD balsam fir clone, Clone 236, were examined for dynamics of water use, stomatal conductance and percent needle loss over an 80-day time period. Ten branches were given a 0.5mm fresh cut at the end of the stem every day, ten were given a fresh cut every two days, and ten control branches remained uncut. Data were analyzed using repeated measures and regression analysis in SAS (Version 9.3, SAS Institute Inc.). Giving a fresh cut to the end of the stem was found to significantly influence water use when compared to controls, though there was no significant difference in water use between branches cut daily and every two days. There was also no significant difference in stomatal conductance between levels of the treatment, including the control. Both stomatal conductance and water use declined sharply within the first eight days following harvest, with a continued decline through to Day 80. The two variables were found to have a positive linear relationship, indicating that high water use can be associated with high stomatal conductance. Needle loss initiation took place by Day 60, with branches cut every second day losing needles at a significantly slower rate than control branches and branches cut daily. Results of this experiment suggest a link between stomatal conductance, water use and needle loss, and that giving a fresh cut to branches promotes water use and reduces needle loss, but does not appear to significantly influence stomatal conductance.


## 5.1 INTRODUCTION

The results of the xylem blockage experiment (Chapter 4) suggest no apparent increase or decrease in xylem blockage during the 60 days following harvest, and that there is a great deal of variation in xylem blockage between branches sampled at the same time. Presence of xylem blockage appeared to be random and did not appear to have any link with water use or needle loss. However, it is possible that other types of xylem blockages are not apparent in cross-sections taken from the end of the stem. Embolisms, pockets of air

within the xylem formed by a disruption of the water column under water stress (Tyree et al., 1994), would not be visible in a cross-section due to the nature of the cutting.

However, the models for daily and cumulative water use (Section 4.3) showed a significant decline in water consumption within the first 10 days following harvest and the decline in daily water use is significantly related to needle loss (Chapter 4). Though there was no apparent link with degree of xylem blockage, these results indicate that there is a barrier restricting water uptake, though it is unknown what is causing this barrier.

This experiment investigates whether giving a fresh cut to the end of the stem, in order to eliminate any blockage at the cut end caused by healing processes, will delay any decline in water consumption. The hypothesis is that if this barrier does restrict water uptake, giving a fresh cut daily will allow the branches to maintain high water uptake for a longer period of time and delay dehydration, which will in turn delay needle abscission.

This experiment also investigates whether patterns of water use and needle loss following harvest can be linked to postharvest stomatal conductance. This is expected due to the fact that water loss is regulated by the opening and closing of the stomata (Kramer and Boyer, 1995). Previous experiments by MacDonald et al. (2011a, 2011b) have shown that stomata close as a postharvest defense mechanism to prevent excess water loss, and possibly from loss of turgidity of the stomatal guard cells (Kramer and Boyer, 1995). If a higher water uptake can be maintained by giving a fresh cut daily, it is hypothesized that the closing of the stomata will also be delayed.

## 5.2 Materials and Methods

### 5.2.1 Variables Studied

The parameters investigated in this experiment were stomatal conductance, water loss, and percent needle loss over an eighty-day time period. The treatment was timing of stem cutting (daily and every second day). Ten replicates were used for each level of the treatment.

### 5.2.2 Preparation of the Sampling Units

Thirty branches from balsam fir clone 236 were collected and prepared using methods described in Section 3.1. Ten branches were designated as controls (C), while ten each of the remaining twenty were assigned to the two cutting treatment levels (T1 and T2). The treatments and controls were randomized using Minitab (Version 16, Minitab Inc.) prior to setup. The light intensity in the lab was 11 μmol $m^{-2}$ $s^{-1}$, in order to simulate "home" display conditions.

### 5.2.3 Cutting

Branch-bottle units were weighed prior to cutting. The branch was removed from the bottle, and a 0.5mm section was taken from the end of the stem. The branch-bottle unit was weighed again to account for any loss in weight from the cutting procedure. T1 represents that branches were cut daily starting on Day 1, T2 represents that the branches were cut every second day starting on Day 2, and controls received no cutting.

### 5.2.4 Measurement of Needle Loss and Water Use

Measurements of needle loss were taken daily using methods described in Section 3.2, with percent needle loss calculated using formulas from Section 3.2.1. Using the methods outlined in Section 3.3, daily water consumption was measured and calculated using formulas from Section 3.3.1.

### 5.2.5 Measurement of Stomatal Conductance

Measurements of stomatal conductance were taken daily using methods described in Section 3.5.

### 5.2.6 Statistical Methods

Data were analyzed using repeated measures analysis in SAS (version 9.3, SAS Institute Inc.). Relationships between stomatal conductance, water use, percent needle loss and the factor of interest, level of cutting treatment, were investigated. Percent needle loss was

plotted against time, and relationships were investigated for each level of the treatment. Linear regression was used to calculate the slope of each plotted line following initation of needle loss, and these slopes were compared to determine the treatment level that produced the lowest rate of needle abscission. All response variables were plotted against each other and analyzed for relationships using correlation and regression. The asymptotic regression model, shown below, was used to describe all relationships aside from those which fit a linear model.

$$y_i = \theta_1 + \theta_2 e^{(\theta_3 x_i)} + \varepsilon_i$$

## 5.3 RESULTS

### 5.3.1 Stomatal Conductance

There was no significant difference in stomatal conductance between the two levels of the treatment, indicating that giving a fresh cut either daily or once in two days did not significantly alter stomatal conductance. Comparison of mean conductance readings over time (Table 5.1) showed a significant and a sharp decline in stomatal conductance occurring within the first eight days following harvest, with a continued decline through Day 80, however (Figure 5.1).

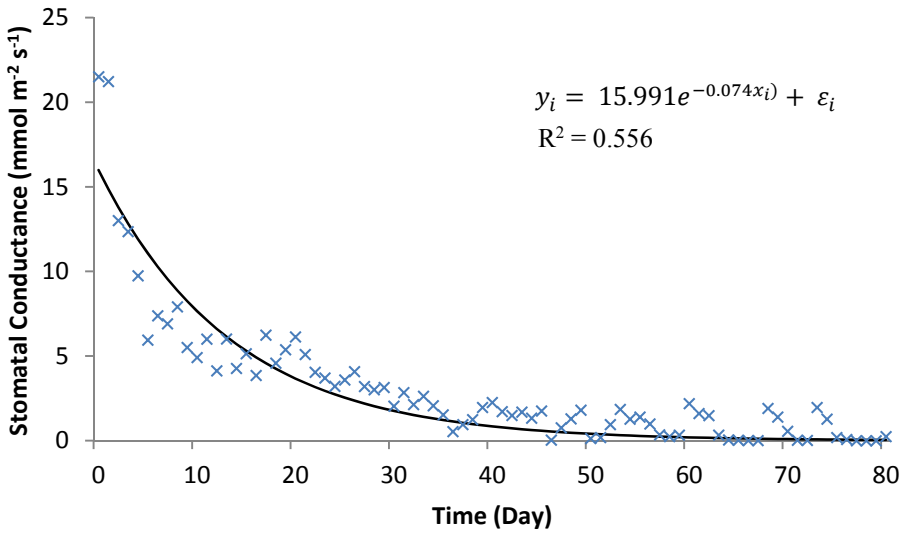



Figure 5.1 Dynamics of stomatal conductance for all branches over the 80 days following harvest.

Table 5.1 Comparison of stomatal conductance for all branches over each of the 80 days following harvest.

| Day | Conductance (mmol $m^{-2}$ $s^{-1}$) | Day | Conductance (mmol $m^{-2}$ $s^{-1}$) |
|---|---|---|---|
| 0 | $21.5^{a}$ | 41 | $1.7^{fg}$ |
| 1 | $21.2^{a}$ | 42 | $1.5^{fg}$ |
| 2 | $13.0^{ab}$ | 43 | $1.7^{fg}$ |
| 3 | $12.4^{ab}$ | 44 | $1.3^{fg}$ |
| 4 | $9.7^{b}$ | 45 | $1.8^{fg}$ |
| 5 | $5.9^{cd}$ | 46 | $0.0^{j}$ |
| 6 | $7.4^{bc}$ | 47 | $0.8^{g}$ |
| 7 | $6.9^{c}$ | 48 | $1.3^{fg}$ |
| 8 | $7.9^{bc}$ | 49 | $1.8^{fg}$ |
| 9 | $5.5^{cd}$ | 50 | $0.1^{i}$ |
| 10 | $4.9^{cd}$ | 51 | $0.2^{hi}$ |
| 11 | $6.0^{cd}$ | 52 | $1.0^{g}$ |
| 12 | $4.1^{de}$ | 53 | $1.8^{f}$ |
| 13 | $6.0^{cd}$ | 54 | $1.3^{fg}$ |
| 14 | $4.3^{de}$ | 55 | $1.4^{fg}$ |
| 15 | $5.1^{cd}$ | 56 | $1.0^{g}$ |
| 16 | $3.9^{de}$ | 57 | $0.3^{h}$ |
| 17 | $6.2^{cd}$ | 58 | $0.2^{hi}$ |
| 18 | $4.6^{d}$ | 59 | $0.3^{h}$ |
| 19 | $5.4^{cd}$ | 60 | $2.2^{ef}$ |
| 20 | $6.1^{cd}$ | 61 | $1.6^{fg}$ |
| 21 | $5.1^{cd}$ | 62 | $1.5^{fg}$ |
| 22 | $4.0^{de}$ | 63 | $0.3^{h}$ |
| 23 | $3.7^{de}$ | 64 | $0.0^{ij}$ |
| 24 | $3.2^{de}$ | 65 | $0.0^{j}$ |
| 25 | $3.6^{de}$ | 66 | $0.0^{k}$ |
| 26 | $4.1^{de}$ | 67 | $0.0^{jk}$ |
| 27 | $3.2^{de}$ | 68 | $1.9^{ef}$ |
| 28 | $3.0^{e}$ | 69 | $1.4^{fg}$ |
| 29 | $3.1^{de}$ | 70 | $0.5^{gh}$ |
| 30 | $2.0^{ef}$ | 71 | $0.0^{j}$ |
| 31 | $2.8^{ef}$ | 72 | $0.0^{j}$ |
| 32 | $2.1^{ef}$ | 73 | $2.0^{ef}$ |
| 33 | $2.6^{ef}$ | 74 | $1.3^{fg}$ |
| 34 | $2.1^{ef}$ | 75 | $0.2^{hi}$ |
| 35 | $1.5^{fg}$ | 76 | $0.1^{ij}$ |
| 36 | $0.5^{gh}$ | 77 | $0.0^{kl}$ |
| 37 | $1.0^{g}$ | 78 | $0.0^{l}$ |
| 38 | $1.2^{fg}$ | 79 | $0.0^{l}$ |
| 39 | $2.0^{ef}$ | 80 | $0.2^{hi}$ |
| 40 | $2.3^{ef}$ | | |

Means with the same letter are not significantly different. $\alpha = 0.05$, n= 30.

### 5.3.2 Water Use

Cutting the base of a branch significantly influenced water use. However, there was no significant difference between branches cut daily and every other day. Interestingly, cutting the base of a branch had a significantly higher water uptake than uncut branches. This suggests that the cutting treatment has some positive effect on increasing water uptake, though choosing to cut daily or every two days does not appear to make a difference (Figure 5.2; Table 5.2).

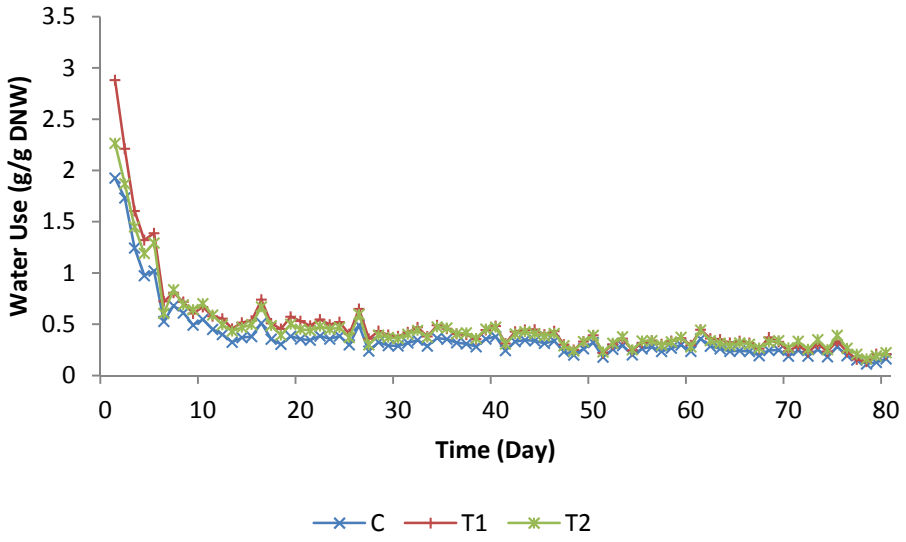



Figure 5.2 Dynamics of average water use over an 80-day time period for all levels of the cutting treatment. C = Control (no cutting), T1 = Cut daily, T2 = Cut every second day. n = 80, 10 replicates.

Table 5.2 Comparison of average water use over an 80-day time period for all levels of the cutting treatment. Control = No cutting, Treatment 1 = Cut daily, Treatment 2 = Cut every second day.

| Cutting Treatment Level | Water Use (g/g DNW) |
|---|---|
| Control | 0.311[b] |
| Treatment 1 | 0.407[a] |
| Treatment 2 | 0.395[a] |

Means with the same letter are not significantly different. α = 0.05, n = 80, 10 replicates

Water use followed a similar pattern as to that of stomatal conductance. Comparison of mean water use readings over time (Table 5.3) showed a sharp decline in water use within the first eight days following harvest (Figure 5.3), with a slower decline following until the conclusion of the experiment.

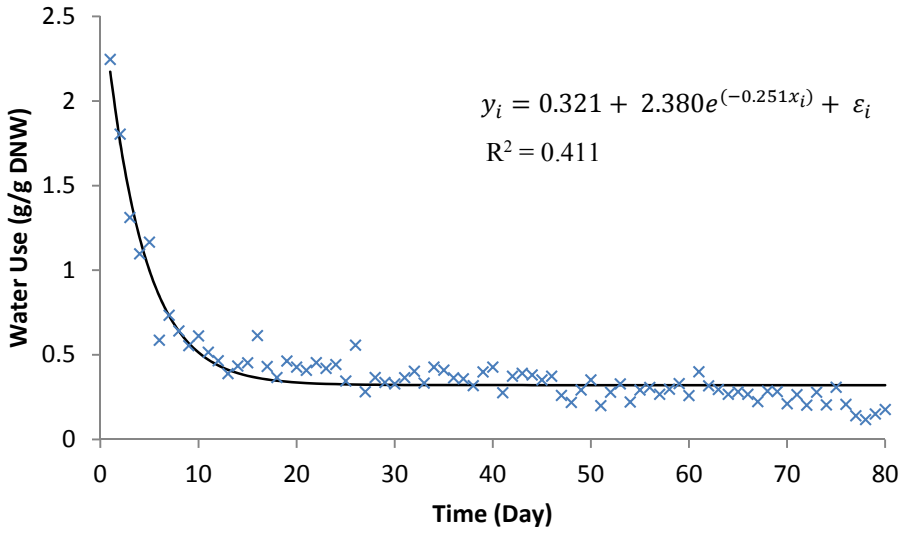



Figure 5.3 Dynamics of average water use for all branches over the 80 days following harvest.

Table 5.3 Comparison of average water use for all branches over each of the 80 days following harvest.

| Day | Water Use (g/g DNW) | Day | Water Use (g/g DNW) |
|---|---|---|---|
| 1 | 2.246$^{a}$ | 41 | 0.277$^{jk}$ |
| 2 | 1.804$^{b}$ | 42 | 0.373$^{i}$ |
| 3 | 1.312$^{c}$ | 43 | 0.391$^{hi}$ |
| 4 | 1.097$^{d}$ | 44 | 0.382$^{hi}$ |
| 5 | 1.166$^{cd}$ | 45 | 0.352$^{ij}$ |
| 6 | 0.586$^{fg}$ | 46 | 0.374$^{i}$ |
| 7 | 0.734$^{e}$ | 47 | 0.26$^{kl}$ |
| 8 | 0.642$^{ef}$ | 48 | 0.219$^{l}$ |
| 9 | 0.555$^{fg}$ | 49 | 0.292$^{jk}$ |
| 10 | 0.612$^{f}$ | 50 | 0.352$^{ij}$ |
| 11 | 0.516$^{g}$ | 51 | 0.2$^{lm}$ |
| 12 | 0.466$^{gh}$ | 52 | 0.279$^{jk}$ |
| 13 | 0.389$^{hi}$ | 53 | 0.328$^{ij}$ |
| 14 | 0.434$^{hi}$ | 54 | 0.221$^{l}$ |
| 15 | 0.453$^{gh}$ | 55 | 0.292$^{jk}$ |
| 16 | 0.614$^{f}$ | 56 | 0.307$^{jk}$ |
| 17 | 0.432$^{hi}$ | 57 | 0.267$^{k}$ |
| 18 | 0.367$^{ij}$ | 58 | 0.297$^{jk}$ |
| 19 | 0.464$^{gh}$ | 59 | 0.33$^{ij}$ |
| 20 | 0.427$^{hi}$ | 60 | 0.26$^{kl}$ |
| 21 | 0.409$^{hi}$ | 61 | 0.4$^{hi}$ |
| 22 | 0.454$^{gh}$ | 62 | 0.317$^{j}$ |
| 23 | 0.42$^{hi}$ | 63 | 0.295$^{jk}$ |
| 24 | 0.442$^{h}$ | 64 | 0.267$^{k}$ |
| 25 | 0.345$^{ij}$ | 65 | 0.282$^{jk}$ |
| 26 | 0.557$^{fg}$ | 66 | 0.267$^{k}$ |
| 27 | 0.282$^{jk}$ | 67 | 0.223$^{l}$ |
| 28 | 0.366$^{ij}$ | 68 | 0.286$^{jk}$ |
| 29 | 0.337$^{ij}$ | 69 | 0.283$^{jk}$ |
| 30 | 0.328$^{ij}$ | 70 | 0.211$^{l}$ |
| 31 | 0.365$^{ij}$ | 71 | 0.265$^{k}$ |
| 32 | 0.403$^{hi}$ | 72 | 0.203$^{lm}$ |
| 33 | 0.333$^{ij}$ | 73 | 0.28$^{jk}$ |
| 34 | 0.428$^{hi}$ | 74 | 0.204$^{lm}$ |
| 35 | 0.41$^{hi}$ | 75 | 0.308$^{jk}$ |
| 36 | 0.365$^{ij}$ | 76 | 0.208$^{lm}$ |
| 37 | 0.358$^{ij}$ | 77 | 0.14$^{n}$ |
| 38 | 0.319$^{j}$ | 78 | 0.117$^{o}$ |
| 39 | 0.399$^{hi}$ | 79 | 0.15$^{mn}$ |
| 40 | 0.427$^{hi}$ | 80 | 0.178$^{m}$ |

Means with the same letter are not significantly different. $\alpha = 0.05$, n= 30

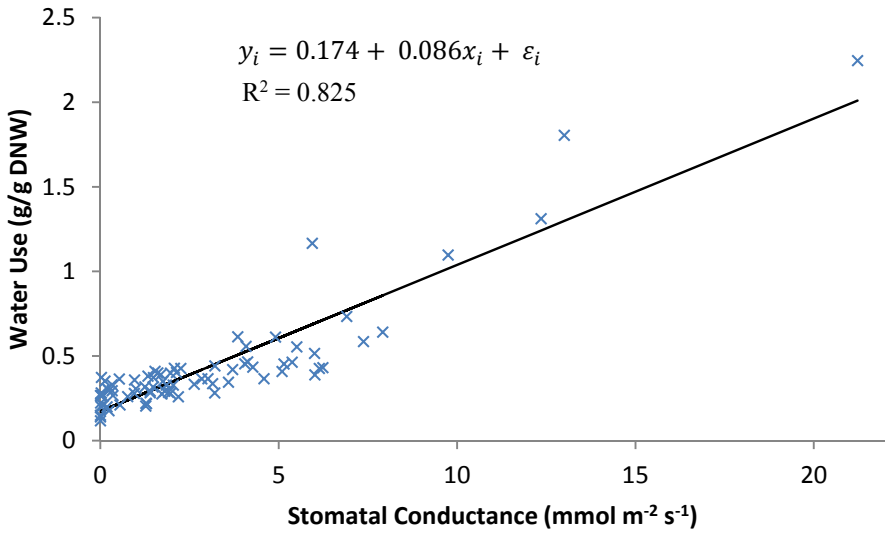



Figure 5.4 Dynamics of average water use against stomatal conductance for all branches over the 80 days following harvest.

As expected, water use and conductance were found to have a high correlation ($R^2$ = 0.825) (Figure 5.4). This strong positive linear relationship shows that higher water use is as a result of higher stomatal conductance, indicating that for water uptake to occur, stomata need to remain open.

### 5.3.3 Needle Loss

Both time and the cutting treatment had a significant influence on needle loss. Comparison of mean percent needle loss measurements and examination of the dynamics of needle loss over time (Table 5.4; Figure 5.5) showed a progressive increase in cumulative percent needle loss until approximately day 60, followed by a sharp increase until the end of the experimental period. Percent needle loss was significantly lower in branches cut every second day, when compared to uncut branches and branches cut daily (Table 5.5).

Table 5.4 Comparison of percent needle loss for all branches over each of the 80 days following harvest.

| Day | Needle Loss (% DW) | Day | Needle Loss (% DW) |
|---|---|---|---|
| 1 | $0.025^{k}$ | 41 | $3.512^{ef}$ |
| 2 | $0.079^{k}$ | 42 | $3.686^{ef}$ |
| 3 | $0.094^{j}$ | 43 | $3.726^{ef}$ |
| 4 | $0.211^{ij}$ | 44 | $3.773^{ef}$ |
| 5 | $0.249^{i}$ | 45 | $3.794^{ef}$ |
| 6 | $0.348^{hi}$ | 46 | $3.873^{ef}$ |
| 7 | $0.491^{hi}$ | 47 | $4.007^{ef}$ |
| 8 | $0.554^{h}$ | 48 | $4.064^{ef}$ |
| 9 | $0.780^{gh}$ | 49 | $4.104^{ef}$ |
| 10 | $0.811^{gh}$ | 50 | $4.136^{ef}$ |
| 11 | $0.963^{gh}$ | 51 | $4.173^{e}$ |
| 12 | $1.349^{g}$ | 52 | $4.240^{e}$ |
| 13 | $1.401^{fg}$ | 53 | $4.300^{e}$ |
| 14 | $1.493^{fg}$ | 54 | $4.375^{de}$ |
| 15 | $1.546^{fg}$ | 55 | $4.499^{de}$ |
| 16 | $1.644^{fg}$ | 56 | $4.553^{de}$ |
| 17 | $1.705^{fg}$ | 57 | $4.613^{de}$ |
| 18 | $1.723^{fg}$ | 58 | $4.693^{de}$ |
| 19 | $1.832^{fg}$ | 59 | $4.835^{de}$ |
| 20 | $1.934^{fg}$ | 60 | $5.032^{de}$ |
| 21 | $2.026^{fg}$ | 61 | $5.574^{de}$ |
| 22 | $2.141^{fg}$ | 62 | $5.931^{de}$ |
| 23 | $2.167^{fg}$ | 63 | $6.245^{de}$ |
| 24 | $2.202^{fg}$ | 64 | $6.824^{d}$ |
| 25 | $2.330^{fg}$ | 65 | $7.183^{cd}$ |
| 26 | $2.422^{f}$ | 66 | $7.836^{cd}$ |
| 27 | $2.475^{f}$ | 67 | $8.338^{cd}$ |
| 28 | $2.595^{ef}$ | 68 | $8.896^{cd}$ |
| 29 | $2.685^{ef}$ | 69 | $10.539^{c}$ |
| 30 | $2.713^{ef}$ | 70 | $11.290^{bc}$ |
| 31 | $2.815^{ef}$ | 71 | $12.021^{bc}$ |
| 32 | $2.883^{ef}$ | 72 | $12.552^{bc}$ |
| 33 | $2.941^{ef}$ | 73 | $13.564^{bc}$ |
| 34 | $3.003^{ef}$ | 74 | $14.212^{bc}$ |
| 35 | $3.061^{ef}$ | 75 | $15.388^{b}$ |
| 36 | $3.128^{ef}$ | 76 | $17.255^{ab}$ |
| 37 | $3.160^{ef}$ | 77 | $18.471^{ab}$ |
| 38 | $3.247^{ef}$ | 78 | $19.785^{ab}$ |
| 39 | $3.360^{ef}$ | 79 | $20.600^{ab}$ |
| 40 | $3.455^{ef}$ | 80 | $21.838^{a}$ |

Means with the same letter are not significantly different. $\alpha = 0.05$, n= 30

Table 5.5 Comparison of percent needle loss over an 80-day time period for all levels of the cutting treatment. Control = No cutting, Treatment 1 = Cut daily, Treatment 2 = Cut every second day.

| Cutting Treatment Level | Needle Loss (% DW) |
|---|---|
| Control | 3.894[a] |
| Treatment 1 | 3.828[a] |
| Treatment 2 | 2.669[b] |

Means with the same letter are not significantly different. $\alpha = 0.05$, $n = 80$, 10 replicates

Figures 5.5 to 5.8 showed an approximate 3-day difference in point of needle loss initiation between levels of the treatment. Point of needle loss initiation, for the purpose of this experiment, was defined as the point in time at which the rate of needle loss sharply increased, which for all levels of the treatment was when percent needle loss reached approximately 5%.

By the conclusion of the experiment, control branches had lost the greatest amount of needles by percent dry weight, with an average of 36% on the final day. Branches cut daily (T1) had lost less with an average of 32% of their dry needle weight by the final day, while branches cut every second day (T2) had lost only 19% of their dry needle weight by the final day. It is expected that if the experiment were to continue, needle loss patterns would eventually follow a logistic curve.

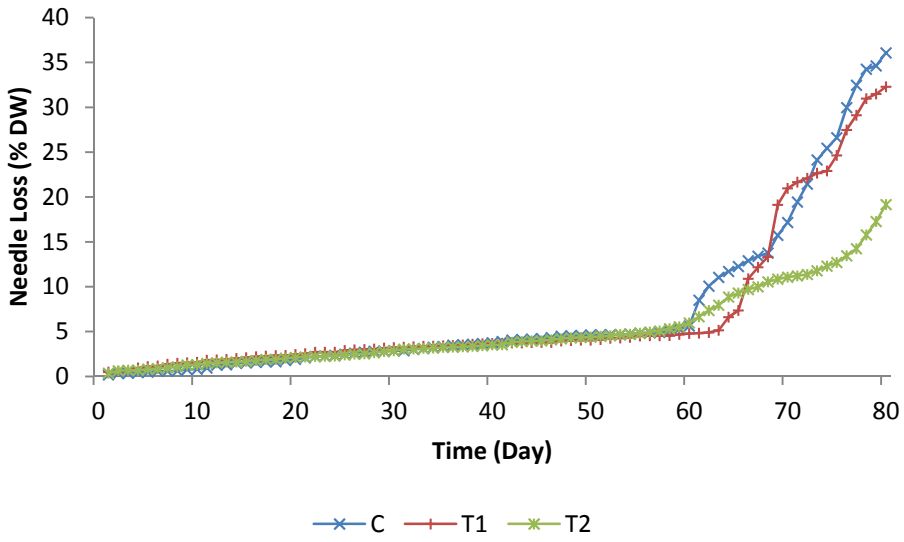



Figure 5.5 Dynamics of percent needle loss over an 80-day time period for all levels of the cutting treatment. C = Control (no cutting), T1 = Cut daily, T2 = Cut every second day. n = 80, 10 replicates.

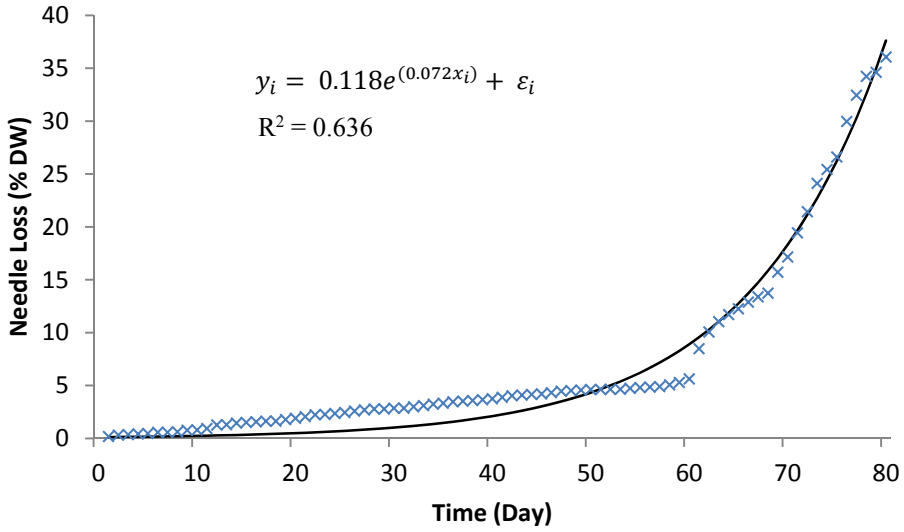



Figure 5.6 Dynamics of percent needle loss over the 80 days following harvest in uncut branches. Regression coefficients for the fitted line can be found in Table 5.6. n = 80, 10 replicates.

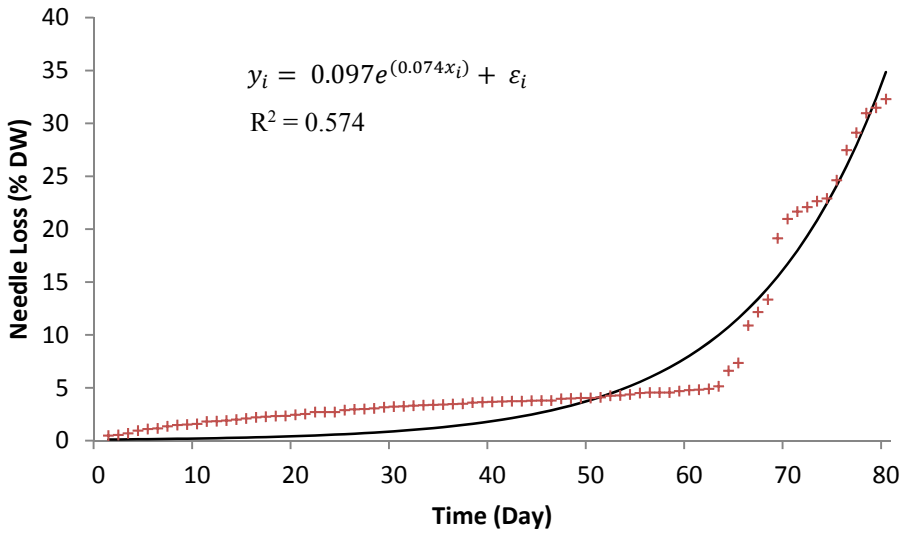



Figure 5.7 Dynamics of water use over the 80 days following harvest in branches cut daily. Regression coefficients for the fitted line can be found in Table 5.6. n = 80, 10 replicates.

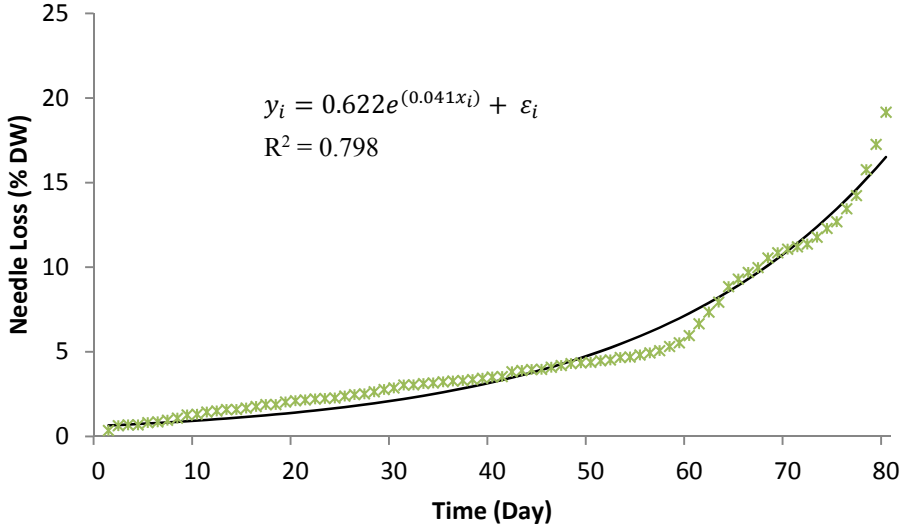



Figure 5.8 Dynamics of percent needle loss over the 80 days following harvest in branches cut every second day. Regression coefficients for the fitted line can be found in Table 5.6. n = 80, 10 replicates.

Table 5.6 Regression coefficients for the fitted lines describing the relationship between time and percent needle loss for each level of the cutting treatment. Control = No cutting, Treatment 1 = Cut daily, Treatment 2 = Cut every second day.

| Cutting Treatment Level | $\theta_1$ | $\theta_2$ |
|---|---|---|
| Control | 0.118 | 0.072 |
| Treatment 1 | 0.097 | 0.074 |
| Treatment 2 | 0.622 | 0.041 |

n = 80, 10 replicates

As shown in Figures 5.9 to 5.11, mean percent needle loss values following the date of needle loss initiation were fitted to a linear equation in order to compare rate of needle loss between levels of the treatment. Using linear regression, the slope of each fitted line was calculated, and these values were compared; results can be found in Table 5.7.

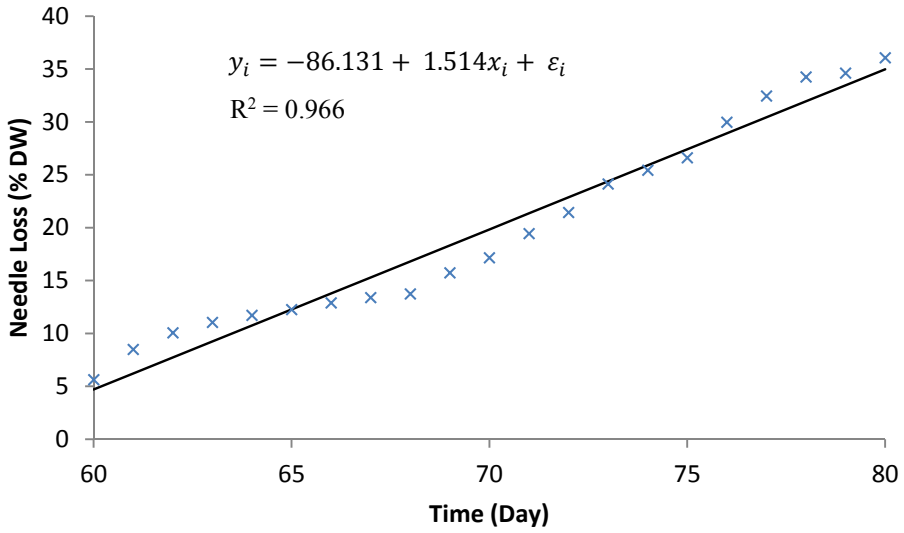



Figure 5.9 Dynamics of percent needle loss in uncut branches. Only values from the point of needle loss initiation were included. n = 21, 10 replicates.

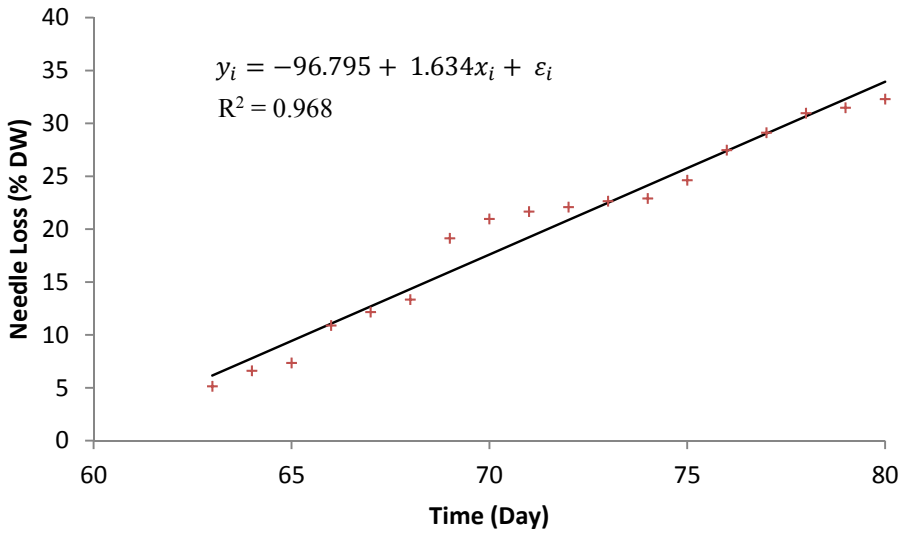



Figure 5.10 Dynamics of percent needle loss in branches cut daily. Only values from the point of needle loss initiation were included. n = 18, 10 replicates.

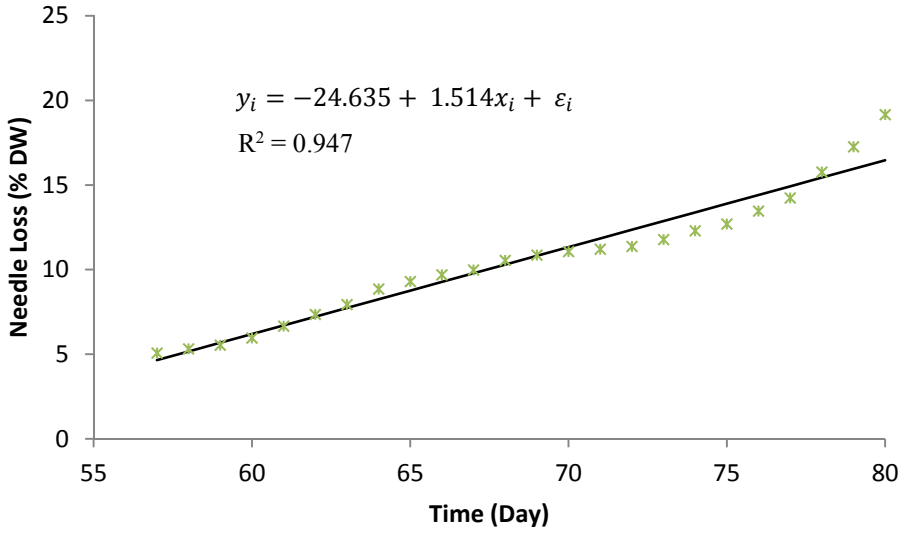



Figure 5.11 Dynamics of percent needle loss in branches cut every second day. Only values from the point of needle loss initiation were included. n = 24, 10 replicates.

Table 5.7 Comparison of the fitted rates of needle loss between levels of the treatment. Control = No cutting, Treatment 1 = Cut daily, Treatment 2 = Cut every second day.

| Treatment | Fitted Rate of Needle Loss (%/Day) |
|---|---|
| Control | 1.514[a] |
| Treatment 1 | 1.634[a] |
| Treatment 2 | 0.514[b] |

Means with the same letter are not significantly different. α = 0.05; n = 21 (Control), 18 (Treatment 1), 24 (Treatment 2); 10 replicates.

Figures 5.12 and 5.13 showed the relationships between percent needle loss and the other two factors of interest, stomatal conductance and daily water use. As expected due to the positive linear relationship between stomatal conductance and water use, both of these relationships follow a similar trend. Both relationships suggest that lower percent needle loss can be associated with high stomatal conductance and high water use, and that higher percent needle loss can be associated with low stomatal conductance and low water use. However, these relationships are non-linear, and showed that a wide range of percent needle loss values can be associated with low stomatal conductance and low needle loss. This could be explained by the fact that in all levels of the treatment, needle loss did not begin until approximately 50 days after the initial drop in stomatal conductance and water use (as shown in Figures 5.5 to 5.8).

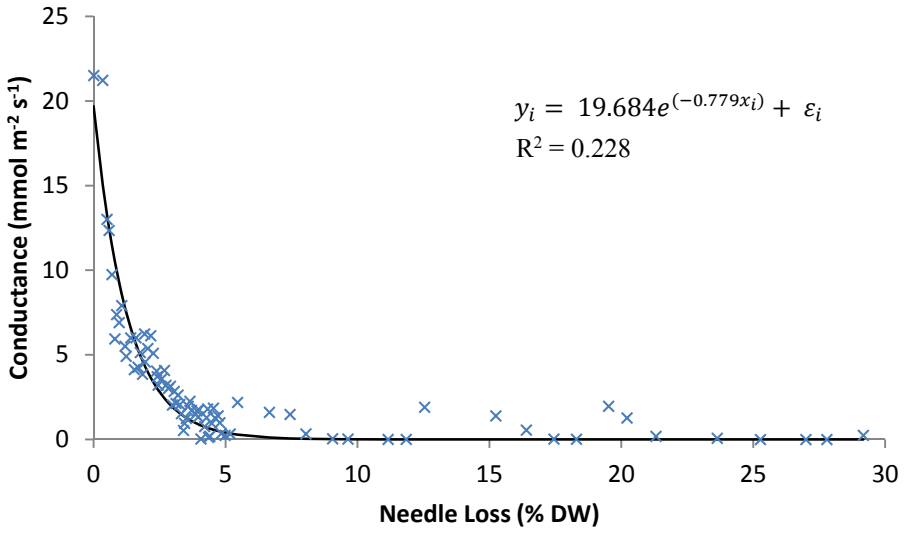



Figure 5.12 Dynamics of stomatal conductance against percent needle loss for all branches over the 80 days following harvest.

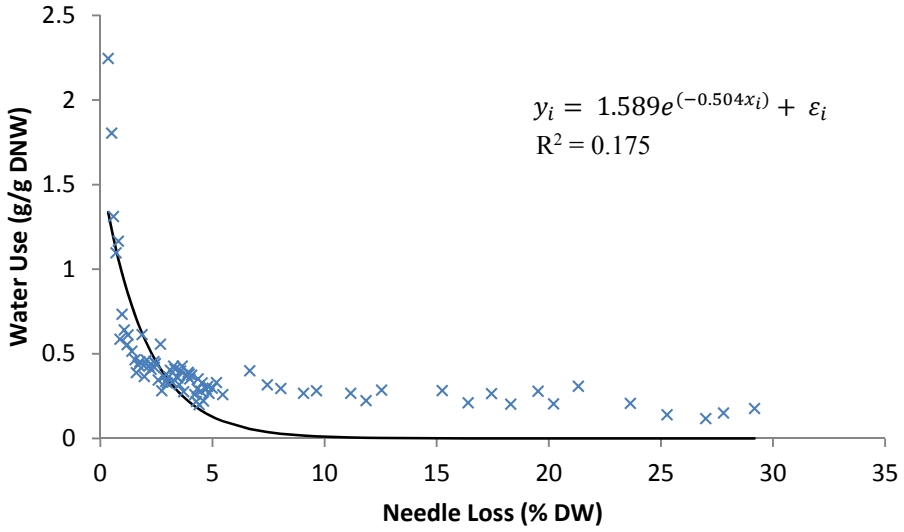



Figure 5.13 Dynamics of average water use against percent needle loss for all branches over the 80 days following harvest.

## 5.4 Discussion

When comparing needle loss trends following the date of initiation for each level of the treatment, branches cut every second day (T2) were found to lose needles at a significantly slower rate than branches cut every day (T1) and control branches. T2 branches were also found to have the lowest mean cumulative percent needle loss at the conclusion of the experiment. However, several of the T2 replicates had not started losing needles by the final day of the experiment, while the majority of T1 and control branches had lost a great amount of needles. Since only mean percent needle loss values were used in determining the rate of needle loss for each treatment, the T2 branches that had failed to reach needle loss initiation by Day 80, which would have contributed to lower mean needle loss values for this level of the treatment. While this suggests that cutting branches every second day may be effective in delaying needle abscission, the great degree of variation between replicates indicates that regardless of treatment, date of initiation of needle loss may be independently regulated by each branch.

The results of this experiment suggest a possible link between stomatal conductance, water use and percent needle loss. Low needle loss can be associated with high stomatal conductance and water use, with high needle loss associated with low stomatal conductance and water use. The nonlinear nature of the relationship suggests a possible mechanism that triggers after the initial decline in stomatal conductance, which leads to eventual needle abscission. However, this relationship is not entirely conclusive, due to the fact that there was an approximate 50-day difference between the conclusion of the initial decline in water use and conductance (approximately Day 10) and the onset of needle abscission (approximately Day 60). This would indicate that in all levels of the treatment, most major needle loss takes place at a point in time where stomatal conductance and water use are at or near their minimum, making it difficult to determine whether there is in fact a link. The fact that T2 branches experienced both significantly higher average water use and a significantly lower rate of needle abscission than control branches supports this possible link, though the lack of significant difference in stomatal conductance between levels of the treatment shows that further investigation is required to make a definite conclusion. It is possible that giving branches a fresh cut daily clears

any blockage at the immediate end of the stem, which could explain why cut (T1 and T2) branches were able to consume more water per gram of dry needle weight than uncut branches, but this treatment alone is not enough to keep stomata open and allow branches to maintain high stomatal conductance for a longer period of time.

## 5.5 Conclusion

The results of this experiment suggest that giving a fresh cut to branches every second day may be more beneficial to reducing needle loss than cutting branches daily, and that cutting branches (daily or every second day) results in higher water uptake when compared to uncut branches. However, there is not enough information from this experiment alone to determine why it is preferable to cut every second day rather than daily. Due to the fact that there is so much variation between replicates, it is also difficult to say whether it is the treatment itself that is delaying needle loss, or whether it is another factor unrelated to the cutting treatment. This area of study could be investigated further, possibly by taking sections from the end of the stem at each date of sampling, and examining them using microscopy for degree of xylem blockage.

This experiment was terminated on the 80$^{th}$ day following harvest, at the point where there was insufficient branch length remaining in T1 branches for further cuttings. It is recommended that if this experiment is to be repeated, samples of greater branch length be used, and the experiment be run until complete needle loss occurs in all treatments for best results.

In the following experiment, the possible link between stomatal conductance, water use and needle abscission is examined further by comparing a clone of low NRD and one of high NRD. If a link exists between these components of water relations and needle abscission, it is expected that there will be a difference in the maintenance of high stomatal conductance and water use between a low-NRD clone and a high-NRD clone, which can be associated with better needle retention in high-NRD clones.

## 5.6 References

[1] Kramer, P.J. and Boyer, J.S. 1995. Water relations of plants and soils. Academic Press, San Diego, California

[2] MacDonald, M.T., Lada, R.R., Dorais, M. and Pépin, S. 2011a. Endogenous and exogenous ethylene induces needle abscission and cellulase activity in postharvest balsam fir (*Abies balsamea* L.). Trees 25: 947

[3] MacDonald, M.T., Lada, R.R., Martynenko, A.I., Dorais, M., Pépin, S. and Desjardins, Y. 2011b. Ethylene exposure duration affects postharvest needle abscission in balsam fir (*Abies balsamea* L.) HortScience 46: 260

[4] Tyree, M.T., Davis, S.D. and Cochard, H. 1994. Biophysical perspectives of xylem evolution: is there a tradeoff of hydraulic efficiency for vulnerability to dysfunction? International Assocation of Wood Anatomists 15: 335

# CHAPTER 6 COMPARISON OF LOW-NRD AND HIGH-NRD CLONES FOR DIFFERENCES IN STOMATAL CONDUCTANCE, WATER USE AND NEEDLE ABSCISSION


## ABSTRACT

Branches of two contrasting balsam fir genotypes, one classified as a low-NRD clone, Clone 236, and one as a high-NRD clone, Clone 506, were compared to understand the dynamics of change in stomatal conductance, water use and needle loss over a 5-month period. The low-NRD clone showed significantly lower stomatal conductance throughout the experiment, but consumed more water on a daily basis than the high-NRD clone. These results suggest that high-NRD clones may be able to more efficiently conserve water in a postharvest water stress situation, possibly delaying needle abscission. Differences in stomatal conductance between clones could be explained by differences in physiological characteristics, such as stomatal size and density, xylem diameter and number of tracheids, and cuticle thickness of the needles. There may also be a clone-specific response in stomatal conductance that is unrelated to water use. Needle loss dynamics between clones could not be directly compared due to the time factor having a significant interaction with the treatment, but the relationships between needle loss, stomatal conductance and water use suggest that in both clones, needle abscission began after both stomatal conductance and water use had reached a certain point and increased with little to no change in either conductance or water use. However, since needle abscission in the low-NRD clone did not appear to begin until Day 43, more than 20 days after the initial decline in both conductance and water use, and most of the high-NRD branches dried rather than losing needles, it cannot be concluded based on these results that this decline directly promoted needle abscission.


## 6.1 INTRODUCTION

Results of the stem cutting experiment (Chapter 5) showed an evidence for a possible link between stomatal conductance, water use, and needle abscission. Stomatal conductance and water use were shown to have a high correlation, as both were found to experience a sharp decline within the first eight days following harvest (Chapter 5). Even though

giving a fresh cut to the end of the stem on set days did not appear to significantly affect stomatal conductance, it had some effect on water uptake, with cut branches having a higher average water consumption than uncut branches. Furthermore, branches that had been cut ever second day were found to lose needles at a slower rate than uncut branches, with many of these branches failing to reach peak abscission rate by the date of termination, suggesting that improving water uptake can delay needle abscission.

The link between stomatal conductance, water use and needle abscission is investigated further in this experiment, which compares two balsam fir genotypes for differences in these components of water relations. Due to the high variation in needle abscission between genotypes, but the low variation within the same genotype, balsam fir genotypes can be classified according to their needle abscission resistance (NAR), which is the length of time a tree resists abscission after it has been harvested, or by their needle retention duration (NRD), which is the length of time taken for a tree to shed all of its needles (MacDonald et al., 2014, Adams and Lada, 2011). MacDonald et al. (2010) classified genotypes as low-NAR if they resisted abscission for less than 20 days, moderate-NAR for 21 to 40 days, and high-NAR for greater than 41 days.

A study by MacDonald et al. (2012) found that low-NAR genotypes release ethylene, a hormone found to promote needle abscission (MacDonald et al., 2010, 2011a, 2011b) at a 50% higher rate than high-NAR trees, and that low-NAR genotypes are more sensitive to low (< 10 ppm) concentrations of ethylene than high-NAR varieties. More recently, MacDonald et al. (2014) discovered that high and low-NAR genotypes differ signficantly in branch diameter, initial mass, needle break strength and needle density. An investigation into the effects of dehydration on needle abscission by Adams et al. (2013) showed that low-NRD clones are more sensitive to dehydration than high-NRD clones. However, there is currently no information available on whether there is a link between NRD, postharvest water consumption, and stomatal conductance among contrasting clones. This experiment investigates whether differences in the initiation and rate of needle abscission between high-NRD and low-NRD clones can be explained by differences in stomatal conductance and water use postharvest.

## 6.2 MATERIALS AND METHODS

### 6.2.1 Variables Studied

The parameters investigated in this experiment were stomatal conductance, water loss, and percent needle loss over a five-month time period. Two clones, the low-NRD Clone 236 and the high-NRD Clone 506, were used as the treatment. Ten replicates were used for each level of the treatment.

### 6.2.2 Preparation of the Sampling Units

Twenty branches, ten of a low-NRD clone (C1) and ten of a high-NRD clone (C2), were collected and prepared using methods described in Section 3.1. The treatments were randomized using Minitab (version 16) prior to setup.

### 6.2.3 Measurement of Needle Loss and Water Use

Measurements of needle loss were taken daily using methods described in Section 3.2, with percent needle loss calculated using formulas from Section 3.2.1. Using the methods outlined in Section 3.3, daily water consumption was measured and calculated using formulas from Section 3.3.1.

### 6.2.4 Measurement of Stomatal Conductance

Measurements of stomatal conductance were taken daily using methods described in Section 3.5.

### 6.2.5 Statistical Methods

Data were analyzed using repeated measures analysis in SAS (version 9.3, SAS Institute Inc.). Relationships between stomatal conductance, water use, percent needle loss and the factor of interest, clone, were investigated. Correlation analysis was used to investigate the link between stomatal conductance and needle loss for each clone.

## 6.3 RESULTS

### 6.3.1 Stomatal Conductance

Results from the first 76 days only were used, due to the inability of the data to converge beyond this point. There was a significant difference in stomatal conductance between the low-NRD and high-NRD clones, with higher stomatal conductance being seen in the high-NRD clone (Figure 6.2). Stomatal conductance in both clones declined sharply within the first 10 days following harvest, with its minimum being reached by Day 80 (Figure 6.1). By Day 113, conductance readings had reached at its lowest level to record on low-NRD branches, due to lack of remaining needles on all branches of this clone.

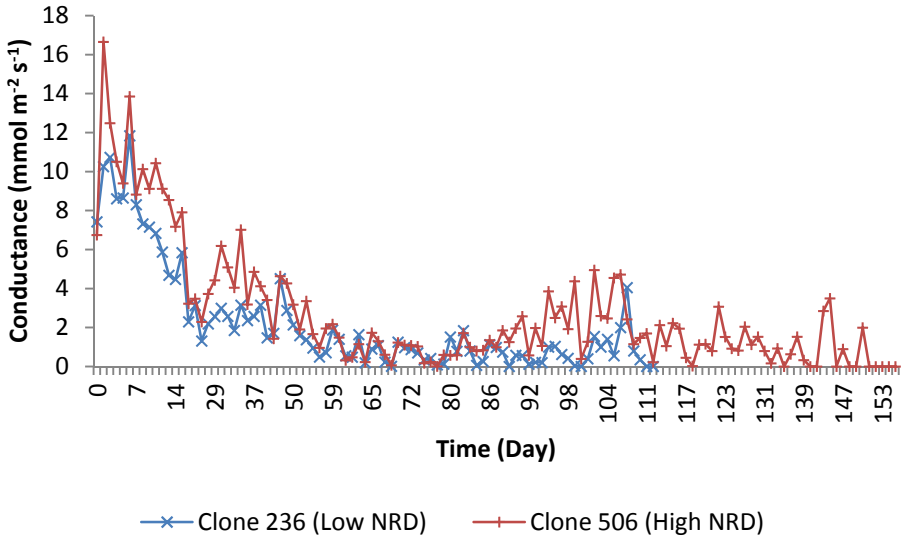



Figure 6.1 Dynamics of stomatal conductance for a low-NRD and high-NRD clone over the 5 months following harvest. n = 10

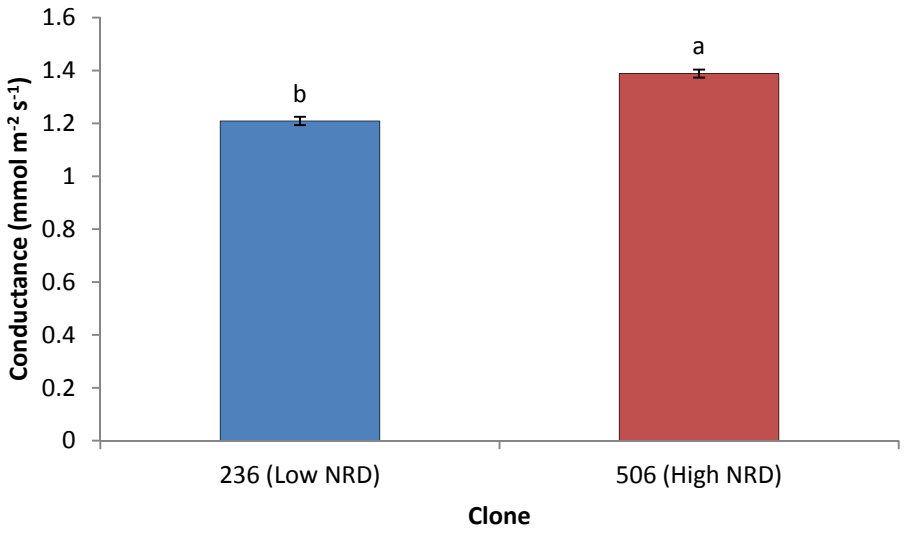



Figure 6.2 Comparison of stomatal conductance between a low-NRD and a high-NRD clone. Results are an average over the first 76 days following harvest. Means with the same letter are not significantly different. n = 10

### 6.3.2 Water Use

There was a significant difference in average water use between the low-NRD and high-NRD clones, with higher water use being seen in the low-NRD clone (Figure 6.4). Similar to the trend shown by stomatal conductance, average water use in both clones was shown to sharply decline within the first 10 days following harvest (Figure 6.3).

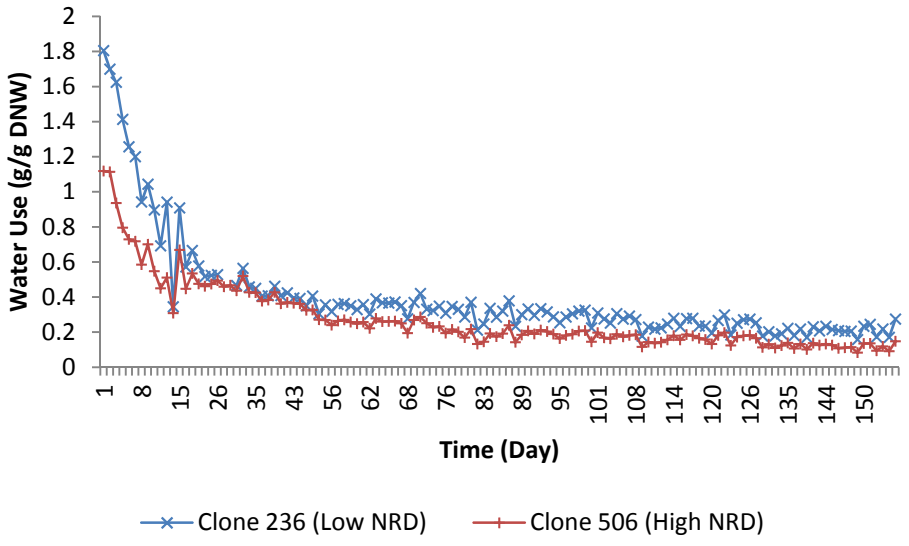



Figure 6.3 Dynamics of average water use for a low-NRD and high-NRD clone over the 5 months following harvest. n = 10

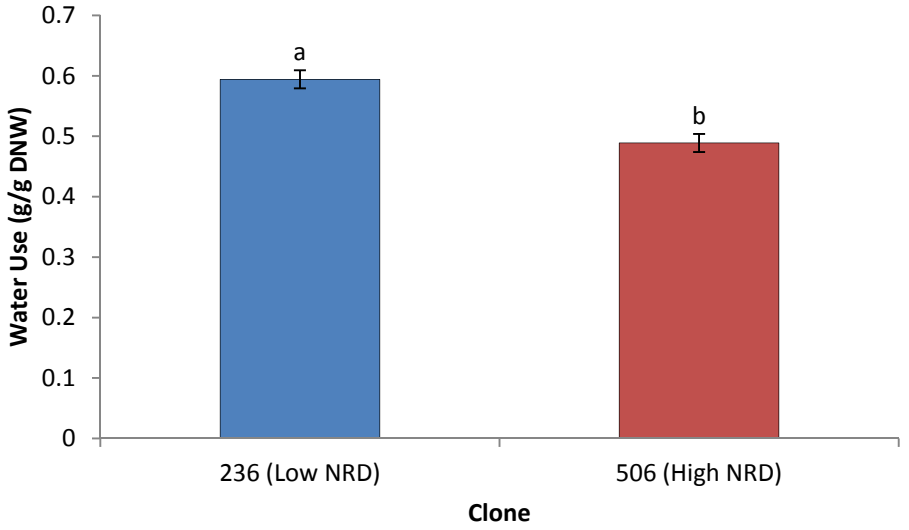



Figure 6.4 Comparison of average water use between a low-NRD and a high-NRD clone. Results are an average over the 5 months following harvest. Means with the same letter are not significantly different. n = 10

### 6.3.3 Needle Loss

Results revealed an interaction effect between the time factor and clone. Because of this interaction, it was not possible to directly compare the low-NRD and high-NRD clones for significant differences in needle loss.

Observation of the dynamics of needle loss over time (Figure 6.5) shows that up until approximately the 45$^{th}$ day following harvest, branches of the low-NRD clone were shown to lose less needles on a dry weight basis than branches of the high-NRD clone. Following this date, percent needle loss increased in low-NRD branches. By examining Figure 6.5, it can be estimated that needle loss initiation for the low-NRD clone took place by the 43$^{rd}$ day following harvest. It is more difficult to determine the point of needle loss initiation for the high-NRD clone, since the majority of the high-NRD branches did not lose needles by the conclusion of the experiment, instead drying out and browning after approximately 100 days postharvest. Some of the low-NRD branches also experienced this effect, which resulted in the mean cumulative needle loss terminating at approximately 60% rather than the expected 100%.

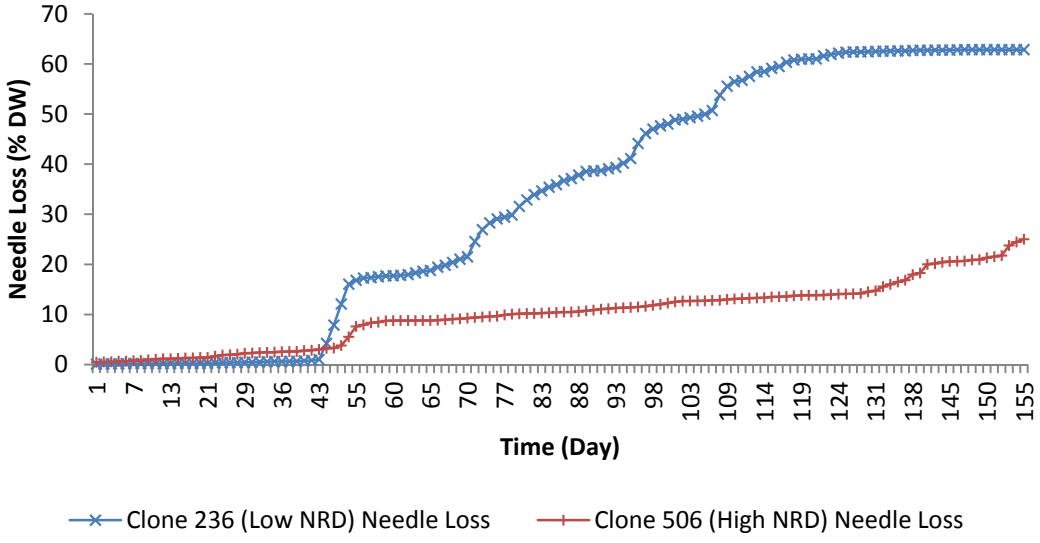
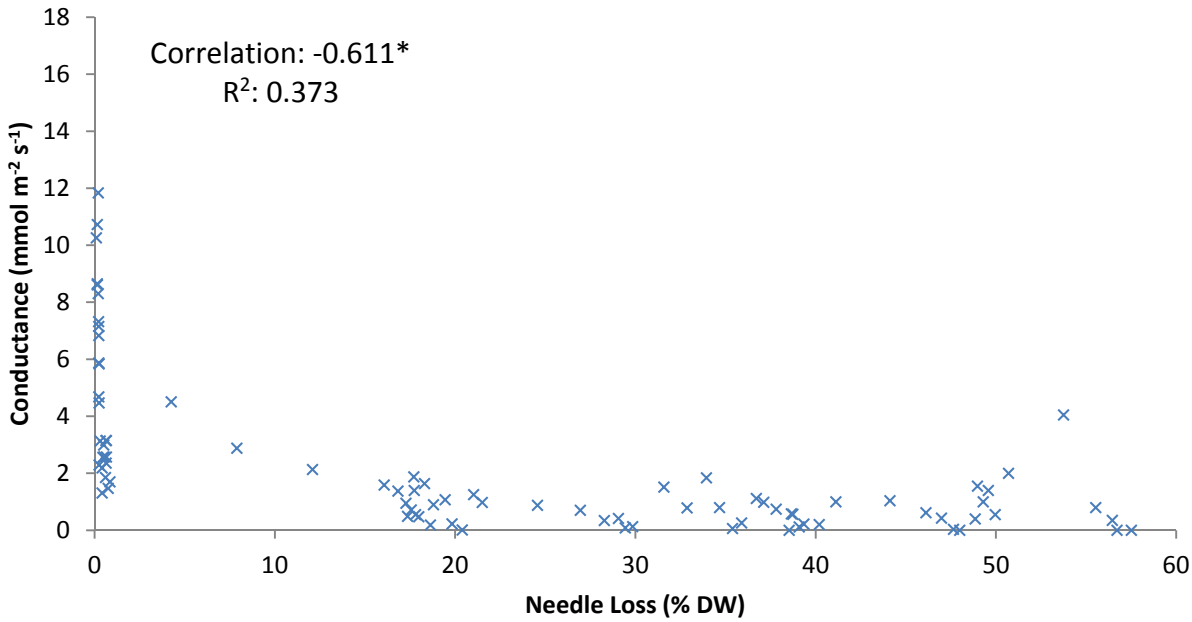



Figure 6.5 Dynamics of percent needle loss for a low-NRD and high-NRD clone over the 5 months following harvest. n = 10

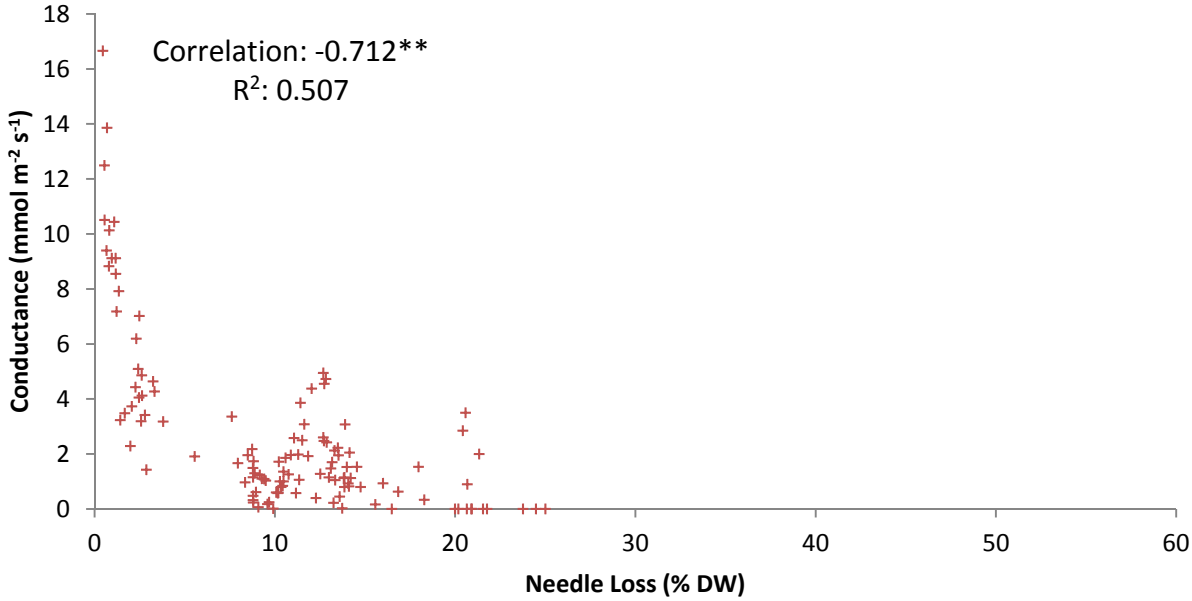
Stomatal conductance was plotted against percent needle loss for each of the clones (Figure 6.6 and 6.7) to observe the relationships between these two factors for each clone. In both clones, a negative nonlinear relationship can be seen, with the highest needle losses occurring at the point in time where stomatal conductance was the lowest. The nonlinear nature suggests that needle loss commences after stomatal conductance reaches a certain minimum, and increases with little to no change in stomatal conductance. The major differences between the two relationships are the point at which needle loss stops increasing, since most branches of the high-NRD clone did not reach complete needle abscission by the conclusion of the experiment, and the peak conductance, which is greater in the high-NRD clone.

Water use was plotted against percent needle loss in a similar fashion (Figure 6.8 and 6.9). As expected from the positive linear relationship between stomatal conductance and water use found in the stem cutting experiment (Chapter 5), results were similar to those of stomatal conductance versus needle loss, suggesting that after water use reaches a certain point, needle loss commences and increases with little to no change in water use.



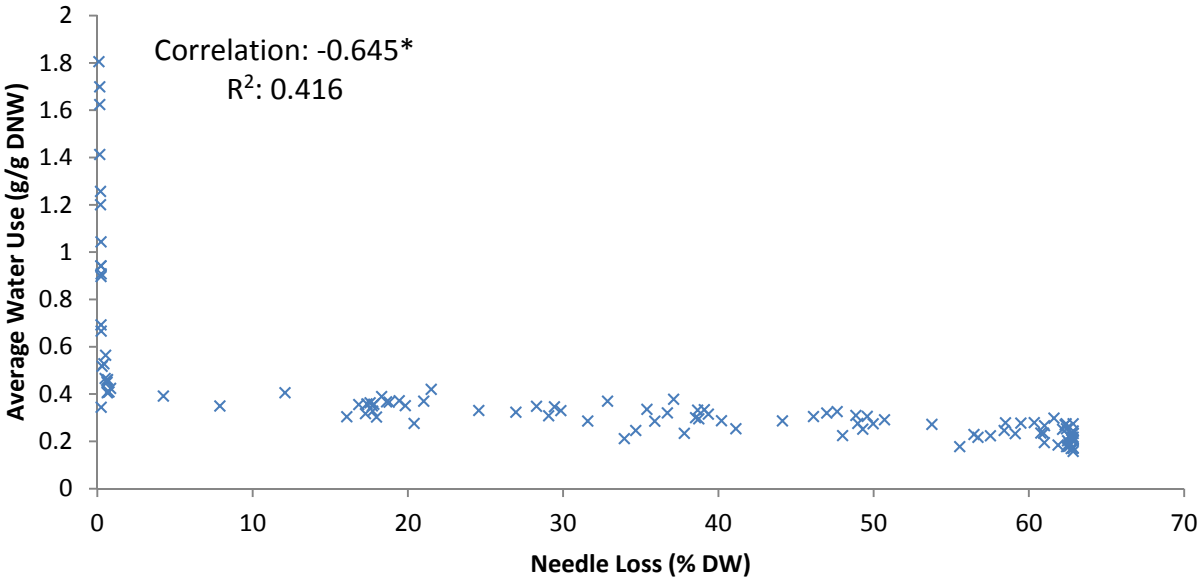

* indicates a weak relationship, ** indicates a strong relationship.

Figure 6.6 Dynamics of stomatal conductance against percent needle loss for low-NRD branches over the 5 months following harvest. Results are from the first 112 days following harvest. n = 10



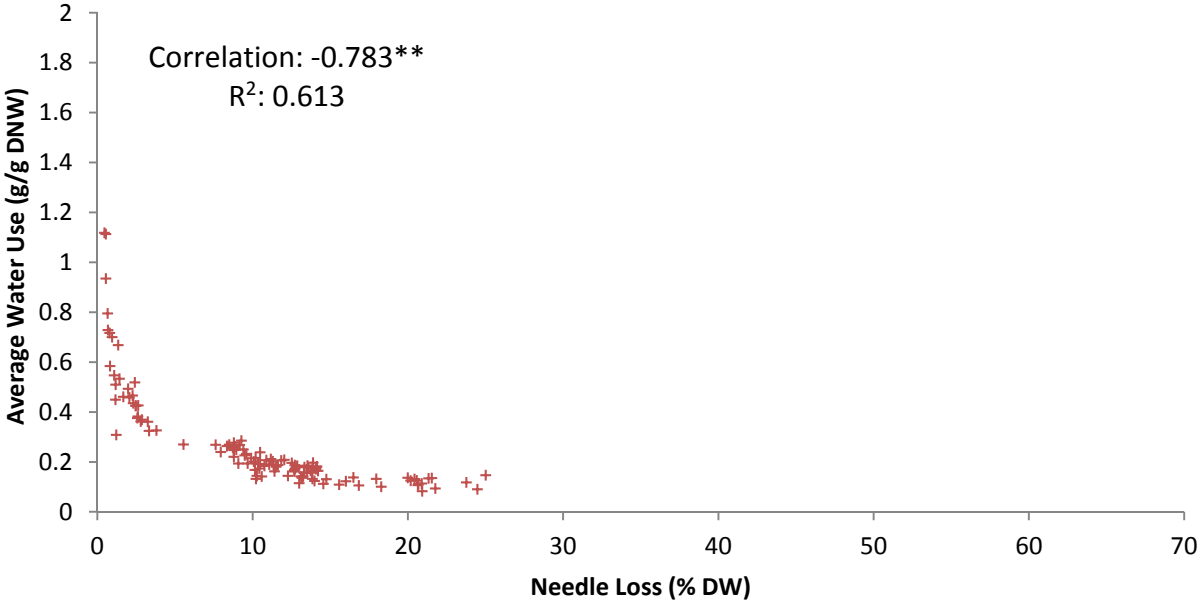

* indicates a weak relationship, ** indicates a strong relationship.

Figure 6.7 Dynamics of stomatal conductance against percent needle loss for high-NRD branches over the 5 months following harvest. n = 10




* indicates a weak relationship, ** indicates a strong relationship.

Figure 6.8 Dynamics of average water use against percent needle loss for low-NRD branches over the 5 months following harvest. n = 10




* indicates a weak relationship, ** indicates a strong relationship.

Figure 6.9 Dynamics of average water use against percent needle loss for high-NRD branches over the 5 months following harvest. n = 10

## 6.4 Discussion

Results of correlation and regression analysis between stomatal conductance and average water use (Chapter 5) show that as stomatal conductance decreases, an associated decrease in average water use can be seen. Thus, it is clear that stomatal conductance and average water use are tightly linked, and it is expected that a clone that experiences higher stomatal conductance will lose more water through transpiration. However, the results of this experiment show that the low-NRD clone consumed more water on a daily basis than the high-NRD clone, despite giving lower readings for stomatal conductance over the first 76 days. This suggests that a high-NRD clone may be able to react more quickly than a low-NRD clone to the change in water status caused by harvest and removal of the root system, thus more effectively conserving water than a clone with lower NRD, and providing a possible explanation for a high-NRD clone to better retain its needles.

There are a number of possible explanations for the higher stomatal conductance, but lower water use in the high-NRD clone. Stomatal size and density were not measured in this experiment, but as stomatal conductance is a function of both, differences in these attributes between clones should result in differences in stomatal conductance. Another possibility is a clone-specific response of stomatal conductance to the water stress situation caused by the removal of the root system. This response may be unrelated to total daily water loss, causing a clone of high NRD to experience higher stomatal conductance than a low-NRD clone while conserving more water. A study by Thiagarajan et al. (2013) investigated a possible link between ABA concentration, photoperiod, temperature and needle retention duration in a low and high-NRD clone, and found that ABA concentrations were slightly higher in the low-NRD clone investigated, but that each genotype had a specific response to the changes in ABA levels brought about by photoperiod and temperature. Since ABA can have an inhibitory effect on stomatal opening (Dubbe et al., 1978; Raschke, 1975), this suggests that ABA levels postharvest may be linked to the closure to stomata, but that the response may be genotype-dependent. Daily water loss may also include losses not accounted for by

transpiration. Clones may vary in terms of cuticle thickness, xylem diameter and number of tracheids, all of which could affect water use.

The variation in conductance observed between low-NRD and high-NRD clones may also be due to differences in size and density of needles between clones. Though measures were taken to eliminate gaps between needles during readings, this became more difficult as needles were lost. This may have possibly led to lower conductance readings in the low-NRD branches, due to the exposure of less leaf surface to the porometer's sensor. In addition, conductance readings were consistently taken from only one particular region of each branch. It is possible results could be improved if conductance readings are instead taken from multiple regions of the branch, and compiled to give an "average" conductance reading for that branch.

Due to the interaction between the time factor and clone, it is difficult to draw conclusions on whether differences in NRD between clones can be explained by differences in stomatal conductance and water use. Both clones appear to have a nonlinear negative relationship with stomatal conductance, showing that regardless of clone, the highest loss of needles will occur when stomatal conductance has reached its minimum. Initiation of needle loss in the low-NRD clone took place at least 20 days after the initial decline in stomatal conductance, so it cannot be concluded from these results that stomatal conductance directly influences the date of needle loss initiation. It can, however, be suggested that stomatal closure is promoted by an increase in ABA following the removal of the root system, and that this response to ABA may be genotype-specific.

## 6.5 Conclusions

The high-NRD clone examined in this study was found to experience a significantly lower average water use than the low-NRD clone, showing that the high-NRD clone may be more efficient at conserving water in a situation of postharvest water stress than the low-NRD clone, thus delaying dehydration and needle abscission. However, the high-NRD clone was also found to experience significantly higher stomatal conductance than

the low-NRD clone, which was not expected based on the linear relationship between stomatal conductance and needle abscission found in the stem cutting experiment (Chapter 5).

The results of this study point to a possible clone-specific response of the stomata to the postharvest water stress caused by the removal of the root system. They also suggest that the water lost by postharvest branches may not be completely accounted for by transpiration, possibly resulting in a higher average water use in low-NRD branches than high-NRD branches, despite their lower stomatal conductance.

The upcoming experiment further examines the link between hydraulic status and needle loss by placing branches in particular environmental conditions and monitoring their patterns of stomatal conductance, water use and needle abscission. Two such conditions that influence stomatal behaviour are light intensity and humidity (Section 2.3.5). It is expected that manipulating these conditions to increase stomatal conductance will cause an increase in water use, and possibly a delay in needle abscission.

## 6.6 References

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# CHAPTER 7 EFFECTS OF LIGHT INTENSITY AND HUMIDITY ON STOMATAL CONDUCTANCE, WATER USE AND NEEDLE ABSCISSION


## ABSTRACT

In this two-part experiment, branches of two-year growth from a low needle retention duration (NRD) balsam fir clone were exposed to various conditions of light intensity and humidity to determine the effects of these conditions on stomatal conductance, water uptake and short-term needle abscission of postharvest balsam fir. The first part investigated the effects of three levels of light intensity, while the second part compared two levels of light intensity and two levels of humidity. Measurements of stomatal conductance, average water use, and needle loss were taken daily for seven days. Results suggest that conditions of moderate light intensity will keep stomata open for a longer period of time and reduce short-term needle loss, while conditions of low and very high light intensity may promote short-term needle loss. In addition, keeping branches under conditions of high humidity allows stomata to remain open for a longer period of time, but does not prevent the eventual decline in stomatal conductance. Even while being kept under conditions of constant high light intensity and high humidity, stomatal conductance was still shown to decline within the first seven days of harvest.


## 7.1 INTRODUCTION

An investigation involving the measurement of xylem blockage, water use and percent needle loss postharvest (Chapter 4) showed that water use in balsam fir declines within the first 10 days following harvest. Though the presence of xylem blockage appeared to be random, having no correlation with time, water use or needle loss, the postharvest decline in water use indicated the presence of a barrier to water uptake that manifests shortly after harvest, possibly from the removal of the root system.

Investigations into patterns of stomatal conductance postharvest (Chapters 5 and 6) suggested a possible link between stomatal conductance, water use and needle abscission. An attempt at removing the barrier to water uptake by giving stems a fresh cut on set

days (Chapter 5) resulted in an increase in water consumption, but with no significant change in stomatal conductance. However, comparison of a high-NRD and low-NRD clone over the first 75 days of a five-month experiment (Chapter 6) showed a significantly higher ($P < 0.001$) stomatal conductance in the high-NRD clone than the low-NRD clone. This supports the possible link between hydraulic status and the ability for balsam fir to retain its needles.

This study further examines this link by measuring the effects of light intensity and humidity on stomatal conductance, water use and needle loss in postharvest balsam fir. Light intensity can be linked to stomatal behaviour, since stomata open during conditions of high light intensity to assimilate $CO_2$, and close during conditions of low light intensity to prevent the loss of water through transpiration (Kramer and Boyer, 1995). A study by Veitch et al. (2012) has also shown evidence of a link between light intensity and needle retention, with NRD increasing significantly in branches exposed for 48 hours to red, white or blue LEDs when compared to those under white fluorescent lightning or darkness. Humidity can also be linked to stomatal behaviour, since the humidity of the atmosphere determines its water potential (with lower atmospheric humidity resulting in a more negative water potential), and thus its influence on water loss through transpiration (Sperry et al., 1994, Ewers and Cruiziat, 1990). In addition, humidity can independently influence stomatal opening and closure. Under conditions of high humidity, and therefore low vapour pressure deficit (VPD), stomatal guard cells remain turgid, keeping stomata open. Under conditions of high VPD, stomata close as their guard cells become less turgid (Ketellapper, 1963; MacDonald et al., 2012).

If a link exists between stomatal conductance, water use and needle loss, manipulating conditions of light intensity and humidity would allow us to examine this relationship better. It was hypothesized that keeping branches under constant conditions of high light intensity and high humidity will keep stomata open for a longer period of time after harvest, allowing the branches to maintain higher stomatal conductance and water use, and possibly delaying needle abscission.

## 7.2 Materials and Methods

### 7.2.1 General Methodology

#### 7.2.1.1 Preparation of the Sampling Units

This experiment consisted of four separate runs, each lasting seven days. Eight replicates were used for all levels of each treatment. For each run, sixteen branches were collected and prepared using methods described in Section 3.1. Eight branches were placed into each of two growth chambers for the duration of the experiment (Figure 7.1).

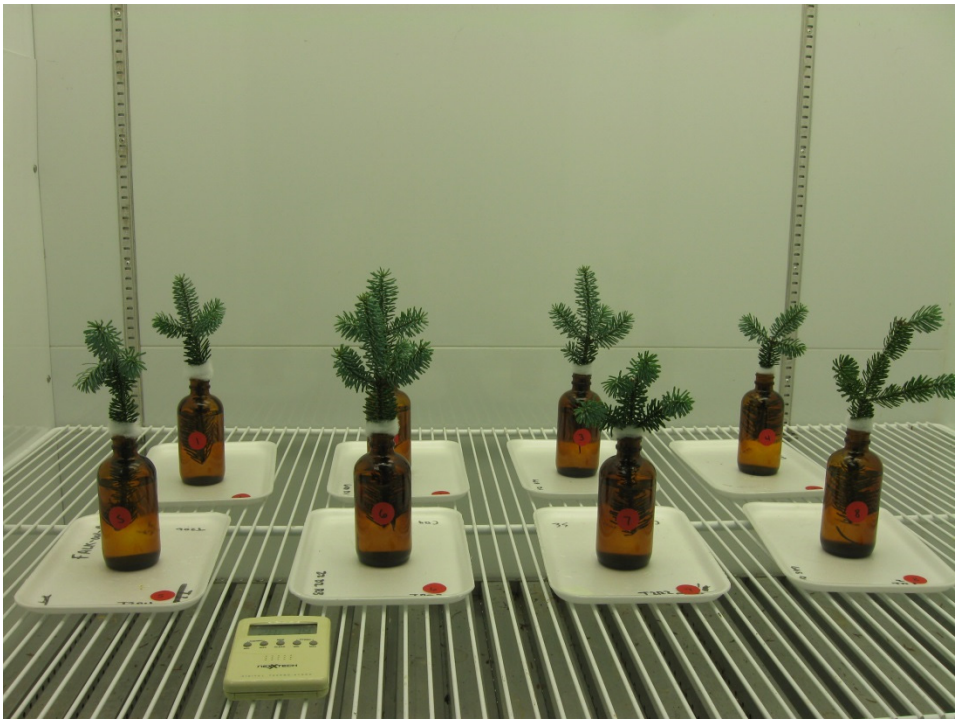

Figure 7.1 Experimental setup of the treated branches in the growth chambers.

#### 7.2.1.2 Preparation of the Growth Chambers

To ensure branches were kept under constant environmental conditions for the duration of the experiment, all branches were contained in growth chambers (Conviron Model S10H, Controlled Environment Ltd., ND, USA). Two growth chambers were able to be operated per week. Temperature, humidity and light intensity of the chambers were set 24 hours in advance, and conditions remained constant for the following seven days. All treatments used fluorescent lighting only (420-450 nm).

#### 7.2.1.3 Measurement of Needle Loss and Water Use

Measurements of needle loss were taken daily using methods described in Section 3.2, with percent needle loss calculated using formulas from Section 3.2.1. Using the methods outlined in Section 3.3, daily water consumption was measured and calculated using formulas from Section 3.3.1.

### 7.2.2. Experiment 7.1: Effects of Light Intensity on Stomatal Conductance, Water Use and Needle Abscission

#### 7.2.2.1 Variables Studied

The parameters investigated in this experiment were stomatal conductance, water loss, and percent needle loss over a seven-day time period. The treatment was level of light intensity (50, 100 and 150 μmol $m^{-2}$ $s^{-1}$).

#### 7.2.2.2 Growth Chamber Conditions

Between the two weeks, three levels of light intensity (T1, T2 and T3) were used inside the chambers, along with a fourth level (C) to mimic the conditions of a control room. The conditions used within each chamber are detailed in Table 7.1.

Table 7.1 Growth chamber conditions for the two weeks over which this experiment was run. Eight replicates were used for all levels of the treatment.

| **Week I** | | | | |
|---|---|---|---|---|
| **Chamber** | **Treatment** | **Light Intensity** ($\mu mol\ m^{-2}\ s^{-1}$) | **Temperature** (°C) | **Humidity** (%) |
| I | T2 | 100 | 27 | 40±2 |
| II | T3 | 150 | 27 | 40±2 |
| **Week II** | | | | |
| **Chamber** | **Treatment** | **Light Intensity** ($\mu mol\ m^{-2}\ s^{-1}$) | **Temperature** (°C) | **Humidity** (%) |
| I | T1 | 50 | 27 | 40±2 |
| II | C | 14 | 27 | 40±2 |

#### 7.2.2.3 Measurement of Stomatal Conductance

Measurements of stomatal conductance were taken daily using methods described in Section 3.5.

#### 7.2.2.4 Statistical Methods

A factorial design with two factors, time and light intensity, was used, with eight replicates for each level of the treatment. Relationships between stomatal conductance, water use, percent needle loss, and the factor of interest, light intensity, were investigated using repeated measures analysis. Data were analyzed in SAS (version 9.3, SAS Institute Inc.) using the MIXED procedure. The Least Squares means of each treatment were compared to determine which treatments resulted in the highest stomatal conductance and water use.

### 7.2.3 Experiment 7.2: Effects of Light Intensity and Humidity on Stomatal Conductance, Water Use and Needle Abscission

#### 7.2.3.1 Variables Studied

The parameters investigated in this experiment were stomatal conductance, water loss, and percent needle loss over a seven-day time period. The factors of interest were level of light intensity (50 and 150 $\mu mol\ m^{-2}\ s^{-1}$) and humidity (40% and 75%).

### 7.2.3.2 Growth Chamber Conditions

Two levels of light intensity (T1 – 50 μmol $m^{-2}$ $s^{-1}$ and T2 – 150 μmol $m^{-2}$ $s^{-1}$) were used inside the chambers each week. During the second week, humidifiers were placed inside the growth chambers (Figure 7.2) in order to achieve the desired humidity conditions. The conditions used within each chamber are detailed in Table 7.2.

Table 7.2 Growth chamber conditions for the two weeks over which this experiment was run. Eight replicates were used for all levels of the treatment.

| **Week I** | | | | |
|---|---|---|---|---|
| **Chamber** | **Treatment** | **Light Intensity** (μmol $m^{-2}$ $s^{-1}$) | **Temperature** (°C) | **Humidity** (%) |
| I | T1L | 50 | 27 | 40±2 |
| II | T2L | 150 | 27 | 40±2 |
| **Week II** | | | | |
| **Chamber** | **Treatment** | **Light Intensity** (μmol $m^{-2}$ $s^{-1}$) | **Temperature** (°C) | **Humidity** (%) |
| I | T1H | 50 | 27 | 75±2 |
| II | T2H | 150 | 27 | 75±2 |

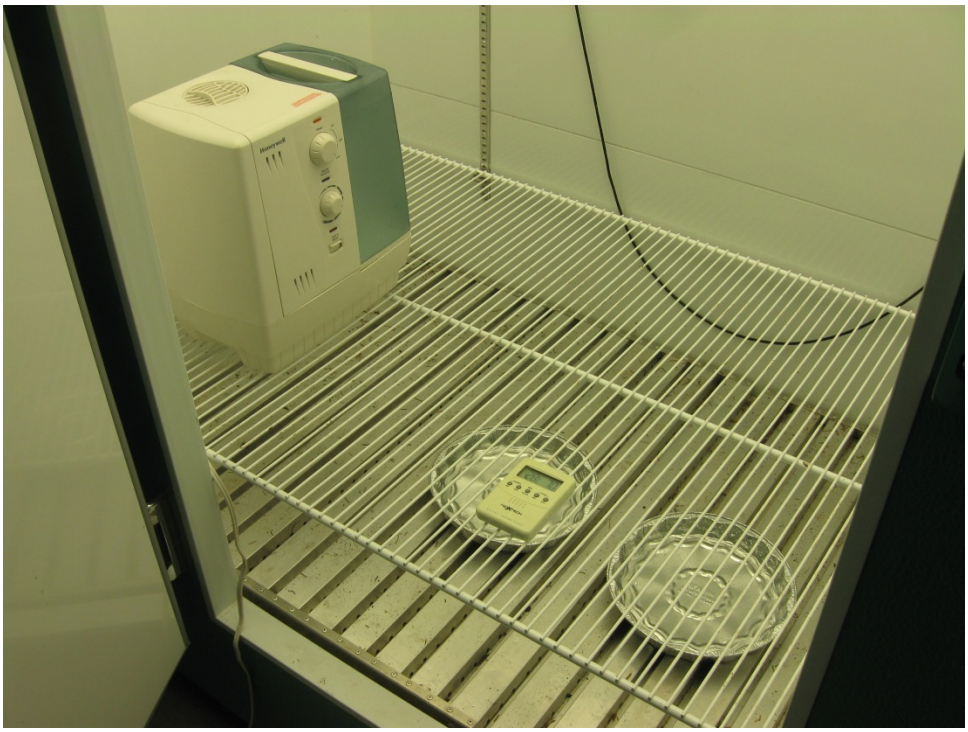

Figure 7.2 Humidifers were placed inside the growth chambers during the second week in order to achieve desired humidity conditions.

#### 7.2.3.3 Measurement of Stomatal Conductance

Measurements of stomatal conductance were taken daily using methods described in Section 3.5. Any moisture on the surface of the needles was wiped off before taking readings in the second week.

#### 7.2.3.4 Statistical Methods

A factorial design with three factors – time, light intensity and humidity - was used, with eight replicates for each level of the treatment. Relationships between stomatal conductance, water use, percent needle loss, and the factors of interest, light intensity and humidity, were investigated using repeated measures analysis. Data were analyzed in SAS (version 9.3, SAS Institute Inc.) using the MIXED procedure. The Least Squares means of each treatment were compared to determine which treatments resulted in the highest stomatal conductance and water use.

## 7.3 RESULTS

### 7.3.1. Experiment 7.1: Effects of Light Intensity on Stomatal Conductance, Water Use and Needle Abscission

#### 7.3.1.1 Stomatal Conductance

Repeated measures analysis revealed a two-way interaction between time and treatment. This suggests that stomatal conductance is influenced by time on any level of light intensity – despite being kept under constant conditions of light intensity for the duration of the experiment, stomatal conductance decayed over time. Least Squares Means comparisons are presented in Table 7.3, and graphed data are shown in Figure 7.3.

Table 7.3 Comparison of stomatal conductance for all branches over each of the 7 days following harvest and the 4 levels of the treatment (14, 50, 100 and 150 μmol $m^{-2}$ $s^{-1}$).

| Light Intensity (μmol $m^{-2}$ $s^{-1}$) | Time (Day) | Conductance (mmol $m^{-2}$ $s^{-1}$) |
|---|---|---|
| 14 | 0 | $8.3^{ab}$ |
| 14 | 1 | $5.9^{b}$ |
| 14 | 2 | $4.5^{bc}$ |
| 14 | 3 | $3.9^{bc}$ |
| 14 | 4 | $1.9^{c}$ |
| 14 | 5 | $3.0^{c}$ |
| 14 | 6 | $0.8^{d}$ |
| 14 | 7 | $3.2^{c}$ |
| 50 | 0 | $3.9^{bc}$ |
| 50 | 1 | $10.2^{ab}$ |
| 50 | 2 | $5.4^{bc}$ |
| 50 | 3 | $4.7^{bc}$ |
| 50 | 4 | $1.9^{c}$ |
| 50 | 5 | $4.4^{bc}$ |
| 50 | 6 | $0.0^{e}$ |
| 50 | 7 | $2.8^{c}$ |
| 100 | 0 | $2.8^{c}$ |
| 100 | 1 | $11.0^{a}$ |
| 100 | 2 | $10.1^{ab}$ |
| 100 | 3 | $9.1^{ab}$ |
| 100 | 4 | $7.3^{ab}$ |
| 100 | 5 | $5.9^{b}$ |
| 100 | 6 | $5.3^{bc}$ |
| 100 | 7 | $4.6^{bc}$ |
| 150 | 0 | $6.2^{b}$ |
| 150 | 1 | $11.1^{a}$ |
| 150 | 2 | $8.2^{ab}$ |
| 150 | 3 | $7.4^{ab}$ |
| 150 | 4 | $6.6^{b}$ |
| 150 | 5 | $5.5^{bc}$ |
| 150 | 6 | $3.1^{c}$ |
| 150 | 7 | $3.2^{c}$ |

Means with the same letter are not significantly different. $\alpha = 0.05$, 8 replicates

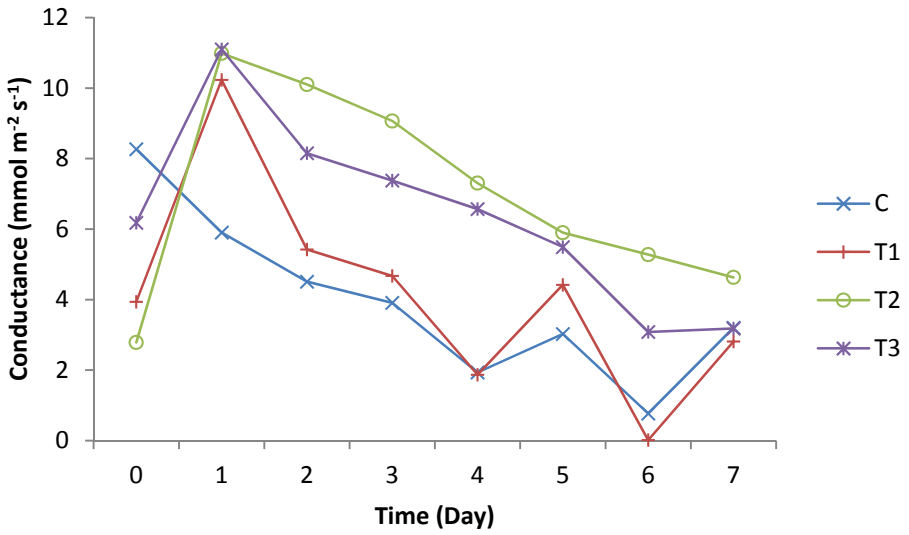


Figure 7.3 Effects of four levels of light intensity (including a control) on stomatal conductance for the first seven days following harvest. C (control) = 14 µmol $m^{-2}$ $s^{-1}$, T1 = 50 µmol $m^{-2}$ $s^{-1}$, T2 = 100 µmol $m^{-2}$ $s^{-1}$, T3 = 150 µmol $m^{-2}$ $s^{-1}$. 8 replicates were used.

Due to the interaction effect between time and treatment, it is difficult to determine which treatment results in the highest stomatal conductance from the comparison of means. However, trends can be seen in Figure 7.3. There was a contrast between the two higher levels of the treatment (100 and 150 µmol $m^{-2}$ $s^{-1}$) and the lowest treatment level (50 µmol $m^{-2}$ $s^{-1}$) and control (14 µmol $m^{-2}$ $s^{-1}$), with the former treatments producing higher stomatal conductance. Control branches under a light intensity of 14 µmol $m^{-2}$ $s^{-1}$ had the lowest stomatal conductance until Day 4, after which both these branches and those under a light intensity of 50 µmol $m^{-2}$ $s^{-1}$ had the lowest conductance. Examination of the means by letter grouping shows that the control and 50 µmol $m^{-2}$ $s^{-1}$ tend to have lower-ranked means than 100 and 150 µmol $m^{-2}$ $s^{-1}$, showing that the higher light intensity treatments result in higher stomatal conductance, though the interaction effect provides some uncertainty.

### 7.3.1.2 Water Use

Repeated measures analysis revealed a two-way interaction between time and treatment. This shows that water use is influenced by time on any level of light intensity. Least Squares Means comparisons are presented in Table 7.4, and graphed data are shown in Figure 7.4.

Table 7.4 Comparison of average water use for all branches over each of the 7 days following harvest and the 4 levels of the treatment (14, 50, 100 and 150 μmol $m^{-2}$ $s^{-1}$).

| Light Intensity (μmol $m^{-2}$ $s^{-1}$) | Time (Day) | Average Water Use (g/g DNW) |
|---|---|---|
| 14 | 1 | $0.223^{bc}$ |
| 14 | 2 | $0.164^{cd}$ |
| 14 | 3 | $0.124^{d}$ |
| 14 | 4 | $0.101^{de}$ |
| 14 | 5 | $0.116^{de}$ |
| 14 | 6 | $0.074^{e}$ |
| 14 | 7 | $0.094^{de}$ |
| 50 | 1 | $0.311^{b}$ |
| 50 | 2 | $0.217^{bc}$ |
| 50 | 3 | $0.157^{cd}$ |
| 50 | 4 | $0.124^{d}$ |
| 50 | 5 | $0.139^{cd}$ |
| 50 | 6 | $0.088^{e}$ |
| 50 | 7 | $0.110^{de}$ |
| 100 | 1 | $0.507^{a}$ |
| 100 | 2 | $0.293^{b}$ |
| 100 | 3 | $0.234^{bc}$ |
| 100 | 4 | $0.183^{c}$ |
| 100 | 5 | $0.186^{c}$ |
| 100 | 6 | $0.163^{cd}$ |
| 100 | 7 | $0.111^{de}$ |
| 150 | 1 | $0.613^{a}$ |
| 150 | 2 | $0.253^{bc}$ |
| 150 | 3 | $0.177^{c}$ |
| 150 | 4 | $0.125^{d}$ |
| 150 | 5 | $0.123^{d}$ |
| 150 | 6 | $0.113^{de}$ |
| 150 | 7 | $0.076^{e}$ |

Means with the same letter are not significantly different. α = 0.05, 8 replicates

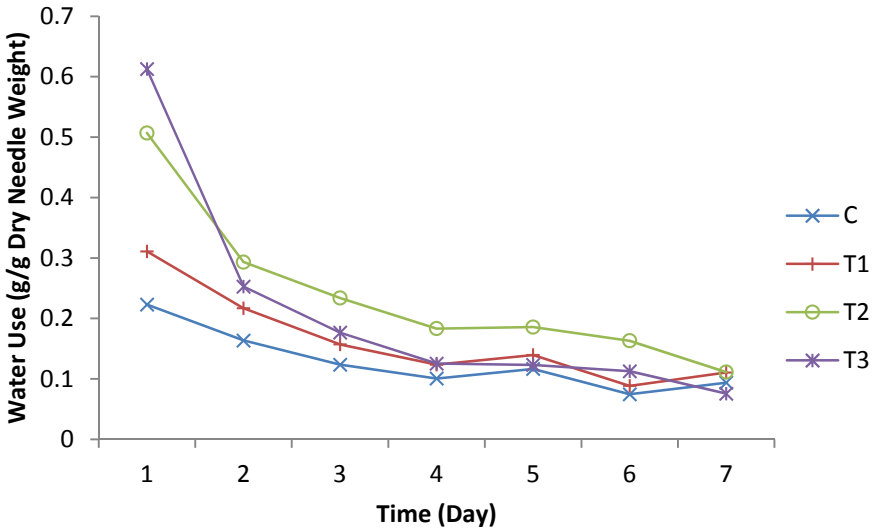


Figure 7.4 Effects of four levels of light intensity (including a control) on average water use for the first seven days following harvest. C (control) = 14 µmol $m^{-2}$ $s^{-1}$, T1 = 50 µmol $m^{-2}$ $s^{-1}$, T2 = 100 µmol $m^{-2}$ $s^{-1}$, T3 = 150 µmol $m^{-2}$ $s^{-1}$. 8 replicates were used.

As with stomatal conductance, the interaction effect between time and treatment makes determination of the optimal treatment level difficult. Figure 7.4 suggests that 100 µmol $m^{-2}$ $s^{-1}$ may be the optimal treatment, as it results in slightly higher water use over the seven-day period when compared with the other levels of the treatment. Figure 7.4 also shows that a light intensity of 100 µmol $m^{-2}$ $s^{-1}$ was able to maintain higher water uptake for a longer period of time, and that water use was lowest in control branches, though by the seventh day all treatments are statistically similar (as shown by the similar letter groupings for Day 7 in Table 7.4). Both Figure 7.4 and the comparison of means in Table 7.4 show that water use decreases significantly by the seventh day following harvest, regardless of treatment level.

### 7.3.1.3 Percent Needle Loss

Only treatment level was found to have a significant effect on needle loss, there was no significant interaction between time and treatment levels, which means that the light and humidity were not interactively affecting needle loss. Least Squares Means comparisons are found in Table 7.5, with graphed data shown in Figure 7.5.

Table 7.5 Comparison of percent needle loss between the four levels of the treatment (14, 50, 100 and 150 µmol $m^{-2}$ $s^{-1}$). All branches and days following harvest are included in the means.

| Light Intensity (µmol $m^{-2}$ $s^{-1}$) | Needle Loss (%) |
|---|---|
| 14 | 2.065[a] |
| 50 | 0.513[b] |
| 100 | 0.274[b] |
| 150 | 1.116[ab] |

Means with the same letter are not significantly different. α = 0.05, n = 7, 8 replicates

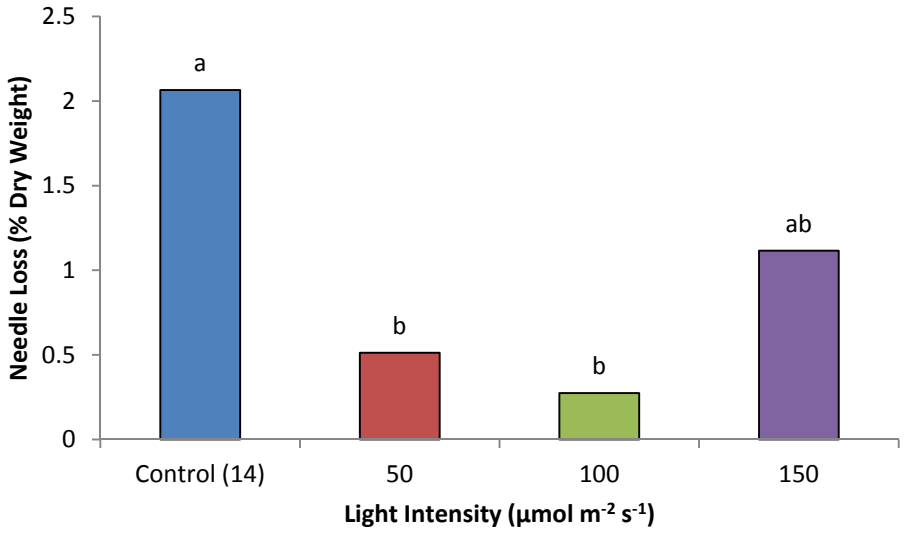


Figure 7.5 Effects of four levels of light intensity (including a control) on percent needle loss. n = 7, 8 replicates.

Even after only a seven-day period, there was a significant difference in percent needle loss between the levels of the treatment. The controls and the branches under the highest level of the light intensity treatment (150 µmol $m^{-2}$ $s^{-1}$) were shown to experience the greatest needle loss, with the branches under low to moderate light intensity (50 and 100 µmol $m^2$ $s^{-1}$) experiencing significantly less needle loss.

### 7.3.2 Experiment 7.2: Effects of Light Intensity and Humidity on Stomatal Conductance, Water Use and Needle Abscission

#### 7.3.2.1 Stomatal Conductance

Repeated measures analysis revealed a two-way interaction between time and treatment, suggesting that stomatal conductance is influenced by time on any level of light intensity and humidity. Least Squares Means comparisons are presented in Table 7.6, and graphed data are shown in Figure 7.6.

Table 7.6 Comparison of stomatal conductance for all branches over each of the 7 days following harvest and each of the four treatment combinations. Levels of light intensity were 50 (50 μmol $m^{-2}$ $s^{-1}$) and 150 (150 μmol $m^{-2}$ $s^{-1}$). Levels of humidity were L (40% Humidity) and H (75% Humidity).

| Light Intensity (μmol $m^{-2}$ $s^{-1}$) | Time (Day) | Conductance (mmol $m^{-2}$ $s^{-1}$) |
|---|---|---|
| 50L | 0 | $5.4^{ef}$ |
| 50L | 1 | $14.3^{cd}$ |
| 50L | 2 | $12.0^{de}$ |
| 50L | 3 | $8.0^{e}$ |
| 50L | 4 | $6.3^{e}$ |
| 50L | 5 | $7.1^{e}$ |
| 50L | 6 | $5.6^{ef}$ |
| 50L | 7 | $5.2^{ef}$ |
| 50H | 0 | $8.3^{de}$ |
| 50H | 1 | $52.2^{a}$ |
| 50H | 2 | $38.3^{ab}$ |
| 50H | 3 | $30.4^{b}$ |
| 50H | 4 | $23.6^{bc}$ |
| 50H | 5 | $17.5^{cd}$ |
| 50H | 6 | $14.1^{cd}$ |
| 50H | 7 | $13.9^{cd}$ |
| 150L | 0 | $2.9^{f}$ |
| 150L | 1 | $24.1^{bc}$ |
| 150L | 2 | $13.1^{cd}$ |
| 150L | 3 | $10.3^{de}$ |
| 150L | 4 | $3.9^{f}$ |
| 150L | 5 | $6.1^{e}$ |
| 150L | 6 | $6.2^{e}$ |
| 150L | 7 | $9.5^{de}$ |
| 150H | 0 | $7.7^{e}$ |
| 150H | 1 | $31.5^{b}$ |
| 150H | 2 | $25.0^{bc}$ |
| 150H | 3 | $19.5^{c}$ |
| 150H | 4 | $17.8^{cd}$ |
| 150H | 5 | $16.8^{cd}$ |
| 150H | 6 | $12.7^{d}$ |
| 150H | 7 | $11.8^{de}$ |

Means with the same letter are not significantly different. $\alpha = 0.05$, 8 replicates

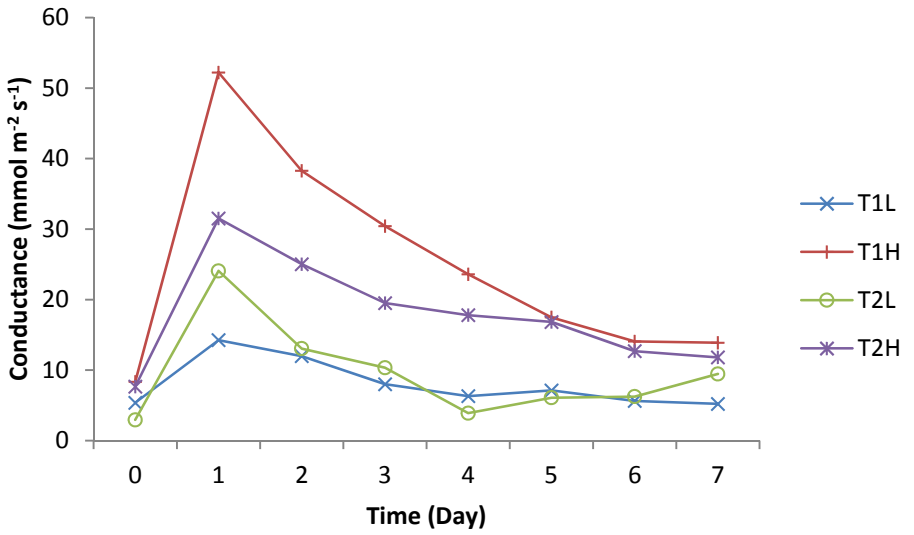


Figure 7.6 Effects of four combinations of light intensity and humidity on stomatal conductance for the first seven days following harvest. Levels of light intensity were T1 (50 µmol $m^{-2}$ $s^{-1}$) and T2 (150 µmol $m^{-2}$ $s^{-1}$). Levels of humidity were L (40% Humidity) and H (75% Humidity). 8 replicates were used.

Though the interaction between time and treatment prevented the direct comparison of main effects, Figure 7.6 shows that the two high-humidity treatments resulted in overall higher stomatal conductance when compared to the low-humidity treatments. Both Figure 7.6 and the comparison of means in Table 7.6 show that the highest stomatal conductance appears to result from a combination of lower light intensity and higher humidity.

### 7.3.2.2 Water Use

Repeated measures analysis revealed a two-way interaction between time and treatment. Least Squares Means comparisons are presented in Table 7.7, and graphed data are shown in Figure 7.7.

Table 7.7 Comparison of average water use for all branches over each of the 7 days following harvest and each of the four treatment combinations. Levels of light intensity were 50 (50 μmol $m^{-2}$ $s^{-1}$) and 150 (150 μmol $m^{-2}$ $s^{-1}$). Levels of humidity were L (40% Humidity) and H (75% Humidity).

| Light Intensity (μmol $m^{-2}$ $s^{-1}$) | Time (Day) | Average Water Use (g/g DNW) |
|---|---|---|
| 50L | 1 | 1.498[a] |
| 50L | 2 | 1.307[a] |
| 50L | 3 | 0.840[bc] |
| 50L | 4 | 0.822[bc] |
| 50L | 5 | 0.497[cd] |
| 50L | 6 | 0.527[cd] |
| 50L | 7 | 0.498[cd] |
| 50H | 1 | 1.395[a] |
| 50H | 2 | 1.328[a] |
| 50H | 3 | 0.796[bc] |
| 50H | 4 | 0.808[bc] |
| 50H | 5 | 0.610[c] |
| 50H | 6 | 0.556[cd] |
| 50H | 7 | 0.419[d] |
| 150L | 1 | 1.508[a] |
| 150L | 2 | 1.192[ab] |
| 150L | 3 | 0.654[bc] |
| 150L | 4 | 0.676[bc] |
| 150L | 5 | 0.496[cd] |
| 150L | 6 | 0.429[cd] |
| 150L | 7 | 0.417[d] |
| 150H | 1 | 1.131[ab] |
| 150H | 2 | 0.905[b] |
| 150H | 3 | 0.648[bc] |
| 150H | 4 | 0.693[bc] |
| 150H | 5 | 0.542[cd] |
| 150H | 6 | 0.494[cd] |
| 150H | 7 | 0.367[d] |

Means with the same letter are not significantly different. α = 0.05, 8 replicates

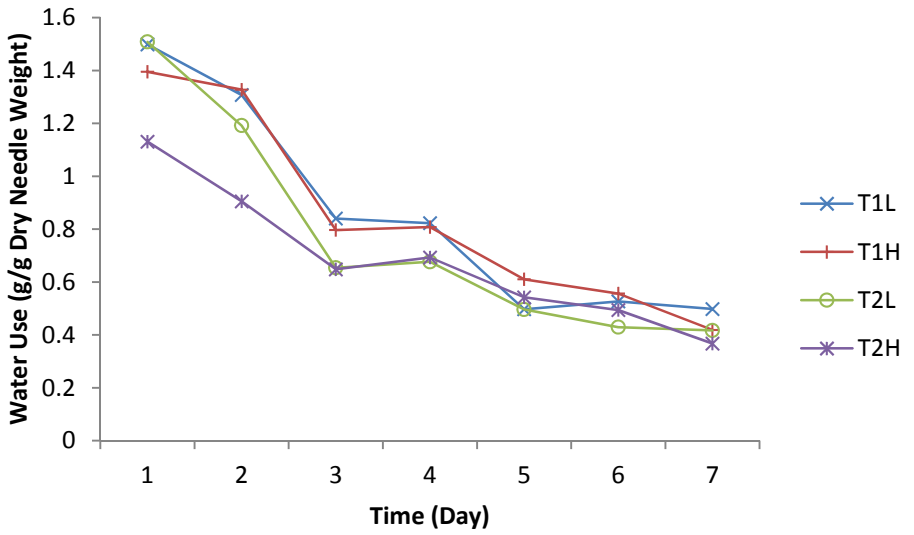


Figure 7.7 Effects of four combinations of light intensity and humidity on average water use for the first seven days following harvest. Levels of light intensity were T1 (50 µmol $m^{-2}$ $s^{-1}$) and T2 (150 µmol $m^{-2}$ $s^{-1}$). Levels of humidity were L (40% Humidity) and H (75% Humidity). 8 replicates were used.

Due to the interaction effect between time and treatment, there is some uncertainty in determining which combination of light intensity and humidity results in the highest water use. Figure 7 shows that a combination of high light intensity and high humidity (T2H) resulted in lower water use up until the second day. Following the second day, there was no significant difference between treatments. Both Figure 7 and the comparison in means in Table 7 show that there is a significant decline in water use by the seventh day in all levels of the treatment.

### 7.3.2.3 Percent Needle Loss

The main effects of treatment and time were found to have a significant effect on needle loss, but their interaction was not significant. Least Squares Means comparisons are found in Table 7.8 and 7.9, with graphed data shown in Figure 7.8 and 7.9.

Table 7.8 Comparison of percent needle loss between the four combinations of the light intensity and humidity treatments. Levels of light intensity were 50 (50 µmol $m^{-2}$ $s^{-1}$) and 150 (150 µmol $m^{-2}$ $s^{-1}$). Levels of humidity were L (40% Humidity) and H (75% Humidity). All branches and days following harvest are included in the means.

| Light Intensity (µmol $m^{-2}$ $s^{-1}$) | Needle Loss (%) |
|---|---|
| 50L | 0.349[b] |
| 50H | 0.367[b] |
| 150L | 0.228[c] |
| 150H | 0.480[a] |

Means with the same letter are not significantly different. α = 0.05, n = 7, 8 replicates

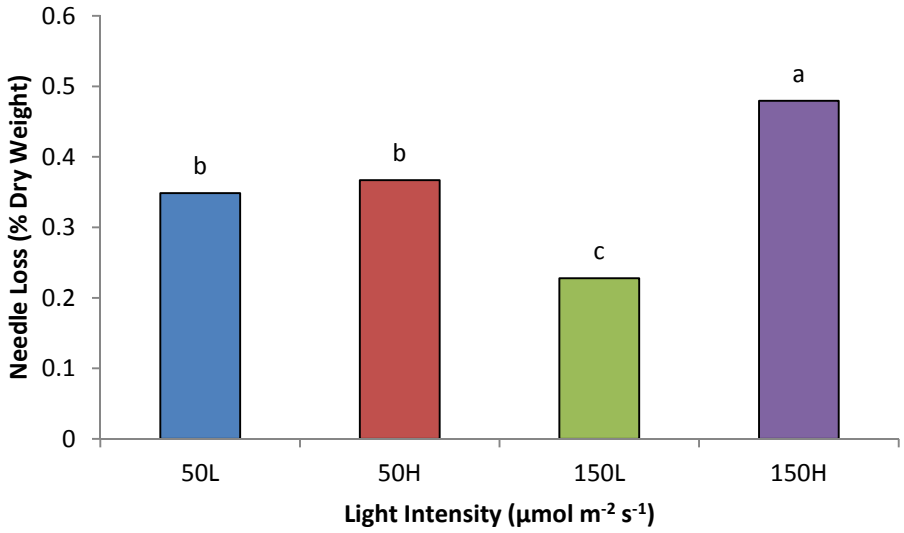


Figure 7.8 Effects of four combinations of light intensity and humidity on percent needle loss. Levels of light intensity were T1 (50 µmol $m^{-2}$ $s^{-1}$) and T2 (150 µmol $m^{-2}$ $s^{-1}$). Levels of humidity were L (40% Humidity) and H (75% Humidity). n = 7, 8 replicates.

Table 7.9 Comparison of percent needle loss over the first 7 days following harvest. All branches and treatment combinations are included in the means.

| Time (Day) | Needle Loss (%) |
|---|---|
| 1 | 0.155[d] |
| 2 | 0.222[cd] |
| 3 | 0.301[c] |
| 4 | 0.356[bc] |
| 5 | 0.470[b] |
| 6 | 0.527[ab] |
| 7 | 0.622[a] |

Means with the same letter are not significantly different. α = 0.05, n = 4, 8 replicates

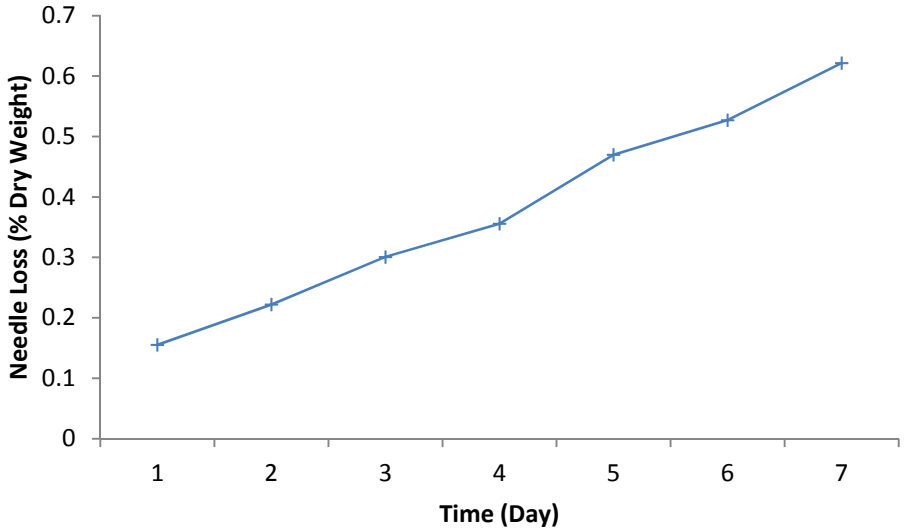


Figure 7.9 Dynamics of cumulative percent needle loss over the first 7 days following harvest. All treatment combinations are included. n = 4, 8 replicates.

Though no branches experienced a great deal of needle loss during this part of the experiment, there appeared to be a significant difference between levels of the treatment. The greatest difference was between humidity levels of the high light intensity treatment (T2L and T2H), with the lower humidity level resulting in the lowest overall needle loss and the higher humidity level causing the highest overall needle loss. These results contradict literature, with the study by MacDonald et al. (2012) finding that conditions of high humidity promoted needle retention. However, since measurements were only taken

over the first seven days postharvest, it is possible this needle loss is only a short-term effect. Humidity levels of the low light intensity treatment (T1L and T1H) had statistically similar results for percent needle loss. Figure 9 shows that needle loss appeared to increase at a rather linear rate over the seven-day period.

## 7.4 Discussion

The results of Experiment 1 in this chapter suggest that keeping branches under a light intensity of 100 or 150 μmol $m^{-2}$ $s^{-1}$ will keep stomata open for longer, allowing a balsam fir branch to maintain higher water uptake. Though there was a significant interaction between the time factor and treatment, preventing the direct comparison of main effects, it suggested that both stomatal conductance and water use decay over time even while kept for several days under a constant light intensity. Examination of the dynamics of stomatal conductance and water use over time showed some evidence that branches under conditions of higher light intensity are able to maintain higher stomatal conductance and water use, particularly those under conditions of 100 μmol $m^{-2}$ $s^{-1}$ and above.

A higher atmospheric humidity results in a lower VPD and a less negative water potential to drive the water potential gradient, which should theoretically reduce water loss from the stomata. However, stomatal guard cells remain turgid under conditions of high humidity, causing stomata to remain open and increasing stomatal conductance (Ketellapper, 1963). Even though a less negative atmospheric water potential reduces water loss through a reduction in the differential water potential gradient, the stomata respond independent of water use, as shown by the dynamics of stomatal conductance seen in Experiment 7.2 (Figure 7.6). Though branches maintained at 75% humidity experienced higher stomatal conductance at the beginning of the experiment, the treatment did not prevent the decline in conductance. This suggests that within the first two days postharvest, the turgidity of the stomatal guard cells declines even under conditions of high humidity, indicating the needles may be starting to experience water deficit.

Due to each run of the experiment lasting only seven days, there was not enough information to determine whether maintaining higher stomatal conductance and water use will delay needle abscission in the long run. Experiment 1 suggests that both low and very high light intensity may promote short-term needle abscission, as both the control branches and the branches under the highest light intensity treatment were shown to lose significantly more needles than those under moderate light intensity conditions. However, it should also be mentioned that all lost needles in Experiment 1 were from current-year growth. This may indicate that having too low or too high light intensity may be detrimental to new growth in balsam fir, possibly due to drying of needles. Branches in Experiment 2 experienced little to no needle loss compared to those in Experiment 1. This may be due to the branches for Experiment 2 being harvested approximately one month after those collected for Experiment 1, allowing the current-year needles an additional month of growth before harvest. However, due to the fact that only some of the replicates in Experiment 1 experienced needle loss, while others lost few to no needles, the cause of the difference in needle loss cannot be determined from the results of this experiment.

Based on these results, a moderately high light intensity appears to have a beneficial effect on maintaining stomatal conductance and water use, and reducing short-term needle abscission, while keeping branches under high humidity promotes stomatal conductance. Experiment 1 showed that keeping branches under a light intensity of 100 $\mu mol\ m^{-2}\ s^{-1}$ resulted in the highest stomatal conductance and water use, and the lowest cumulative needle loss, when averaged over the seven-day period. As discovered in Experiment 2, keeping branches at 75% humidity increased stomatal conductance, but did not prevent its decline over the first 7 days postharvest. In conclusion, keeping balsam fir branches under constant conditions of high light intensity and high humidity will keep stomata open for a longer period of time, but both stomatal conductance and water use decay over time even under these constant conditions.

## 7.5 Conclusion

A significant interaction was found between time and treatment on both stomatal conductance and water use. This indicates that stomatal conductance and water use are influenced by time on any level of the light intensity and humidity treatments used in this experiment. Experiment 1 shows some evidence that keeping branches under conditions of high light intensity will keep stomata open for longer, maintaining high stomatal conductance and water use for a longer period of time, though the short-term nature of this experiment makes it difficult to determine whether this will delay needle abscission. Water use and stomatal conductance were still shown to decline within the first seven days following harvest, despite higher stomatal conductance as influenced by high humidity and high light intensity, suggesting that there is still a barrier to water uptake possibly the lack of root pressure.

The following experiment attempts to use simulated positive water pressure to remove this barrier to water uptake. Previous experiments in this project have shown that water use and stomatal conductance will still decline within the first 10 days following harvest, even when a fresh cut of the stem is made on a daily basis, or when branches are exposed to conditions of constant high light intensity. The final experiment in this project attempts to improve stomatal conductance and water uptake by providing a constant positive pressure through the use of a pump system, meant to simulate the root pressure provided by attached roots prior to harvest.

## 7.6 References

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# CHAPTER 8 EFFECTS OF SIMULATING ROOT PRESSURE ON STOMATAL CONDUCTANCE, XYLEM BLOCKAGE AND NEEDLE ABSCISSION


## ABSTRACT

In plants with intact root system, in addition to stomatal regulation, and water potential gradients, water uptake (water use) is regulated by root pressure. However, in a postharvest Christmas trees, roots are detached and thus, root pressure component is eliminated totally. This might have a significant implication in water movement, especially if stomata remain open or partially closed, leading to gradual dehydration thus triggering needle abscission. This experiment examined the hypothesis that simulating root pressure and maintaining water pressure will sustain stomatal conductance and thereby, delay needle abscission postharvest. Three levels of a water pressure treatment (10 psi, 30 psi and 45 psi) were compared for effectiveness in maintaining stomatal conductance and improving needle retention in a low-NRD clone, Clone 236, over a 6-month period. Eighteen branches were mounted in a simulated root pressure system (SRPS) consisting of three manifolds, each set to deliver and maintain a specified water pressure to the cut end of the contained branches. Two controls were used: one being an "absolute" control, and the other with branches having a short section of pipe fitted around the base of the stem to simulate the possible restricting effect of the SRPS set up without pressure element. Water use was measured in control branches only, and branches fitted with the section of pipe were found to have significantly lower water use than the absolute controls, showing that the SRPS can restrict water uptake. However, a water pressure treatment may be able to compensate for this restriction, since needle loss even in the 45 psi pressure treatment was similar to that in the absolute controls. The SRPS treatment at 10 psi was the most effective among the three treatments, to delay and reduce needle loss by 77%. Cross-sections of the cut end of each branch were taken at the conclusion of the experiment and rated for degree of xylem blockage showed no significant differences among the treatments, suggesting that xylem blockage, at least at the cut end, may not be linked to postharvest needle abscission. Compensating root pressure promoted needle retention independent of stomatal regulation. Root pressure

compensation had limited influence on stomatal conductance suggesting that the possibility that stomatal regulation may be regulated independent of root pressure signals.

## 8.1 Introduction

A possible link between stomatal conductance, water use and needle abscission has been suggested from the results of the previous experiments in this study (Chapters 5, 6 and 7). A stem cutting experiment (Chapter 5) showed that giving a fresh cut to the end of the stem had a positive effect on water use, and that cutting branches every second day decreased the rate of needle abscission after the point of initiation. The results of an experiment involving light intensity (Chapter 7, Part A) showed that branches exposed to conditions of moderately high light intensity (100 μmol $m^{-2}$ $s^{-1}$) experienced higher average stomatal conductance and lower average needle loss than untreated branches.

However, both stomatal conductance and average water use were always shown to decline over the first 7 days following harvest, even with a fresh cut being given to the end of the stem on a daily basis. In addition, XPP and RWC both have been shown to be negatively linked with abscission (MacDonald and Lada, 2012, MacDonald et al., 2014). Even while keeping branches under conditions of high light intensity, which resulted in a higher average stomatal conductance and water use, the decline in stomatal conductance and water use persisted postharvest. Furthermore, there were no significant trends in the degree of xylem blockage over the first 60 days following harvest (Chapter 4), indicating that blockage at the cut end may not be a contributing factor to the postharvest decline in water use. This suggests that there is yet another factor involved. Postharvest trees lack roots. In a root-detached system, the root pressure element is completely removed. In trees, in general, a root pressure of 0.3 to 21.5 psi equivalent is generated and in combination with negative water potential gradient and transpirational pull, water flow continues (Fisher et al, 1997; Kramer and Boyer, 1995; Sperry et al., 1994)). However, in a root-detached system, root pressure is completely eliminated. With stomata closing in a postharvest situation, the transpirational pull of water greatly decreases, reducing hydraulic flow throughout the plant. Depending on the stomatal conductance, the trees/branches may lose water, leading to dehydration, promoting needle loss. In order to

optimize both stomatal conductance and water use, and possibly delay the decline in the tree's water status, the onset of dehydration, and needle abscission, this positive water pressure, normally supplied by the root system, must be restored in some form.

This experiment investigates the effects of exposing branches to various levels of constant water pressure at the cut end, simulating root pressure by building an artificial root pressure system. It is hypothesized that exposure to a constant positive pressure at the cut end will counter the decline in water consumption caused by the postharvest closure of the stomata, allow better water use for a longer period of time, thus, delay needle abscission.

## 8.2 Materials and Methods

### 8.2.1 Variables Studied

The parameters investigated in this experiment were stomatal conductance and percent needle loss over six months. Water use was measured in two control treatments only. Three levels of water pressure treatments, 10 psi, 30 psi and 45 psi, were applied to the cut end of the stem. These pressure levels were chosen based on a range of root pressures observed in trees in the field (Fisher et al., 1997), with one pressure level chosen to greatly exceed the upper level of this range. Two controls were used, one with a collar attachment intended to simulate any constriction caused by the root pressure system on treated branches (C1) and one with no constriction (C2 – Absolute control). Six replicates were used for each level of the treatment. Xylem blockage was measured in all branches upon termination of the experiment.

### 8.2.2 Preparation of the Sampling Units

Thirty branches of the low-NRD clone 236 were collected and prepared using methods described in Section 3.1. Six branches were designated for each of the controls, while the remaining eighteen (six per treatment) were installed into the root pressure system (Section 8.2.3).

Six of the control branches were fitted with rubber caps and inserted into a short section of 3/4” clear braided vinyl tubing, which was tightened with a hose clamp (Figure 8.1). The branches were then placed in amber bottles containing 100mL of distilled water, and secured using Parafilm (Figure 8.2). These controls were used to measure the possible restricting effect of the pipes in the root pressure system on water uptake. The six remaining control branches, designated as “total controls”, were simply placed in amber bottles containing 100mL of distilled water (Figure 8.2).

Needles were removed from approximately 1.5 inches of stem at the cut end of branches intended for treatment, in order to ensure a tight seal was formed upon insertion into the root pressure system. The same procedure was used for control branches fitted with rubber caps and tubing. When re-cutting branches, the end was cut in a “v” shape to maximize exposure of the vascular tissue to water.

Figure 8.1 Six control branches were fitted with a short section of 3/4" clear braided vinyl tubing to simulate the possible restricting effect of the root pressure system.

Figure 8.2 Setup of the control branches. Branches in the back row were fitted with a section of tubing meant to simulate the possible restricting effect of the root pressure system on water uptake. Branches in the front row were not subjected to this restriction.

### 8.2.3 Building the Simulated Root Pressure System (SRPS)

The SRPS consisted of a series of three manifolds containing water at a pre-determined, fixed pressure, applied to the cut ends of 18 inserted branches (Figure 8.3 and 8.4). The manifolds were constructed of sections of 1/2” blue Pex tubing and 3/4" clear braided vinyl tubing, connected by T-fittings and 90-degree elbows. Each manifold was able to accommodate six branches, which served as replicates. Branches were first fit with rubber caps, then inserted into the open end of each pipe and tightened with a hose clamp to create a seal.

Water was distributed to the manifolds by a 16-gallon stainless steel shallow well jet pump (Burcam Model #506538SS, Burke Water Systems Manufacturing Inc., Quebec, Canada) (Figure 8.5), at a pressure of 45 psi. A storage tub was used to supply distilled water to the pump during the priming process. Pressure regulators were installed at the head of each manifold to deliver a specific water pressure to each set of branches. The

levels of water pressure in each manifold were 10 psi in the low-pressure treatment (T1), 30 psi in the medium-pressure treatment (T2) and 45 psi in the high-pressure treatment (T3).

Pressure gauges were used to monitor the water pressure in each manifold on a daily basis. In the case that the water pressure dropped in any of the manifolds, it was required that the pump be recharged. After shutting off the control valves to the three manifolds to ensure the pressure inside each did not change, the pump was drained to a pressure of 30 psi and refilled using water in the storage tub.

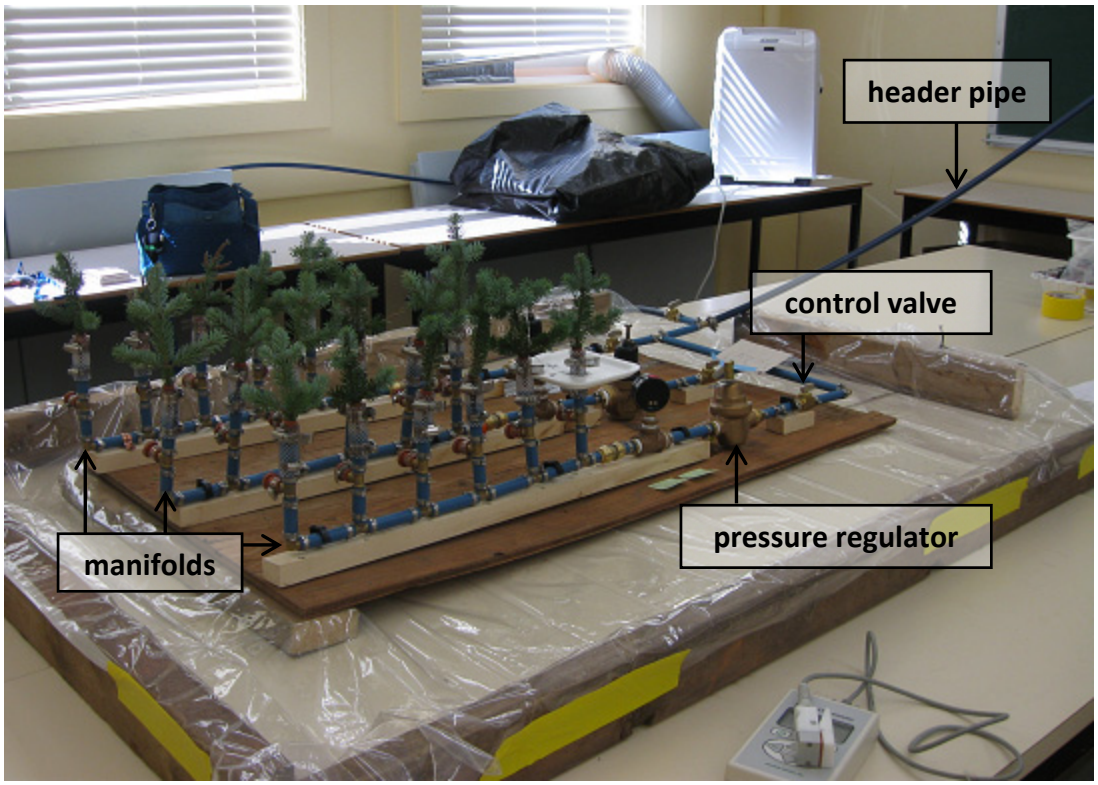


Figure 8.3 Setup of branches treated with water pressure at the cut end. Branches were inserted into three manifolds, each containing a specific water pressure. Water was distributed at 45 psi to the manifolds by a 16-gallon shallow well jet pump, and pressure was regulated in each manifold individually.

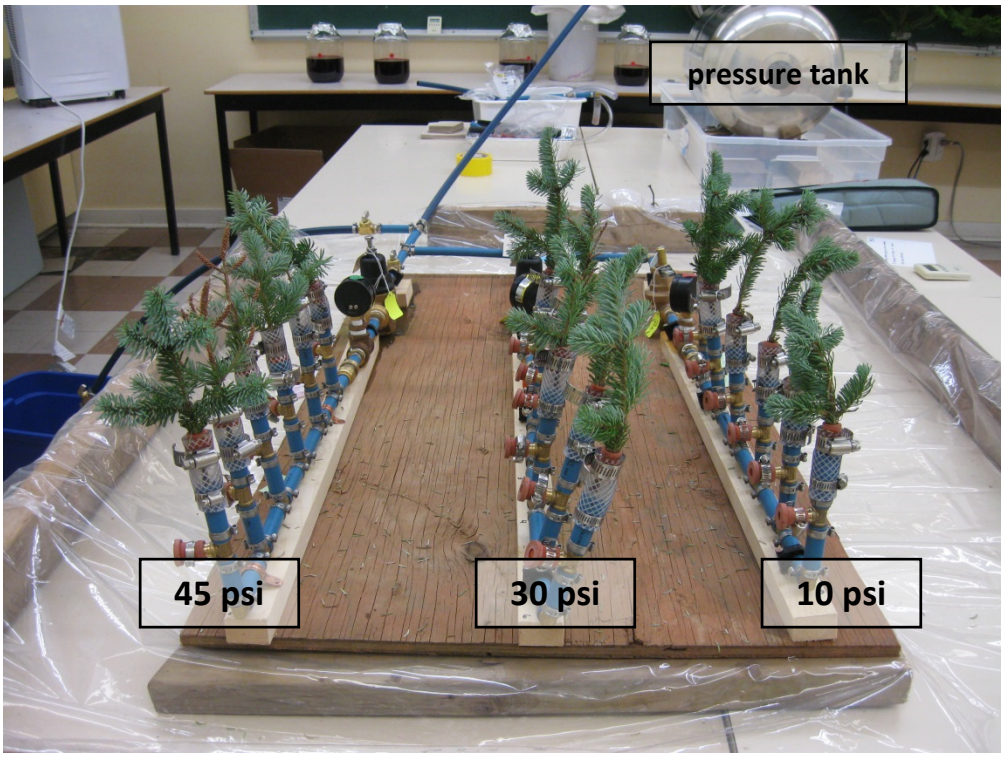


Figure 8.4 Setup of branches treated with water pressure at the cut end. The water pressure in each of three manifolds was regulated by pressure regulators.


Figure 8.5 Setup of the root pressure system. Water was distributed to the manifolds (left) by a 16-gallon shallow well jet pump (right).

### 8.2.4 Measurement of Needle Loss and Water Use

Measurements of needle loss were taken daily using methods described in Section 3.2, with percent needle loss calculated using formule from Section 3.2.1. For branches under each treatment, a tray was fitted under each branch to collect fallen needles during the finger-run test, as shown in Figure 8.6.


Figure 8.6. A section was cut from a foam tray to fit around the pipe containing the pressure-treated branch. The tray was fitted under the branch, and the section was replaced to secure the tray. The tray was transferred from one branch to the next during the finger-run test and needle collection.

Daily water consumption was measured in branches under both controls only, since treated branches could not be removed from the SRPS. Measurements were taken using methods outlined in Section 3.3, and calculated using formulas from Section 3.3.1.

### 8.2.5 Measurement of Stomatal Conductance

Measurements of stomatal conductance were taken daily, at approximately 10 A.M., using methods described in Section 3.5.

### 8.2.6 Microscopy

At the conclusion of the experiment, sections were taken from the stems of all branches under all treatments for examination of xylem blockage using microscopy. Cross-sections of each stem were prepared and examined using methods described in Section 3.4.

### 8.2.7 Statistical Methods

A factorial design with two factors, time and pressure treatment, was used, with six replicates for each level of the treatment. Stomatal conductance, water use and percent needle loss were analyzed using repeated measures analysis in SAS (version 9.3, SAS Institute Inc.). Xylem blockage was analyzed using a one-way analysis of variance (ANOVA) in Minitab (Version 17, Minitab Inc.). Where appropriate, means were compared using an LSmeans test to determine the effectiveness of each level of the treatment.

## 8.3 Results

### 8.3.1 Stomatal Conductance

The interaction between treatment and time was statistically significant. By examining the dynamics of stomatal conductance in Figure 8.7, it can be seen that within the first 40 days, conductance in branches under the Simulated Root Pressure System (SRPS) remained higher compared to the control branches. However, the SRPS did not prevent the decline in conductance, with all treatments reaching minimum conductance by Day 40.

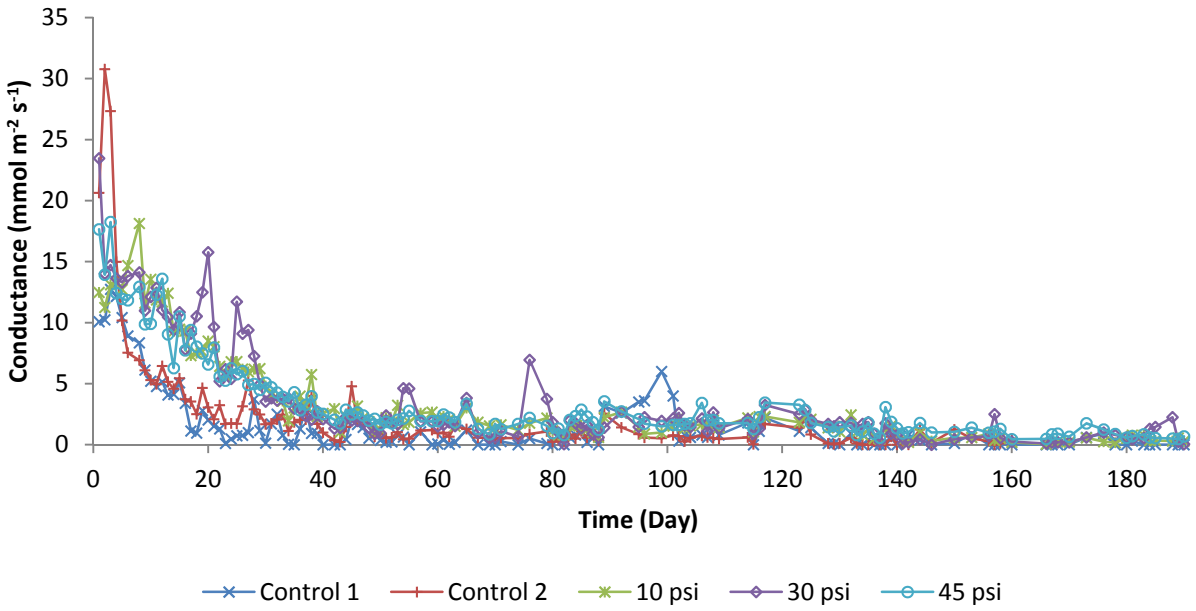


Figure 8.7 Dynamics of stomatal conductance for two controls and three levels of a water pressure treatment over the 6 months following harvest. Control 1 branches were fitted with a section of tubing meant to simulate the possible restricting effect of the root pressure system on water uptake, while Control 2 branches had no such restriction. n = 6

Without being able to directly compare main effects, it cannot be concluded whether or not stomatal conductance is significantly higher in pressure-treated branches than in control branches over all 190 days of the experiment. In order to further examine the link between water pressure treatment and stomatal conductance, three time points - Day 10, Day 15 and Day 20 - were selected, and conductance measurements for those three days were analyzed using ANOVA and multiple means comparison. On the three days investigated, stomatal conductance was significantly higher in all three levels of the pressure treatment (10, 30 and 45 psi), when compared to both absolute control branches and control branches fitted with the collar (Figure 8.8), suggesting that simulated root pressure did in fact increase stomatal conductance.

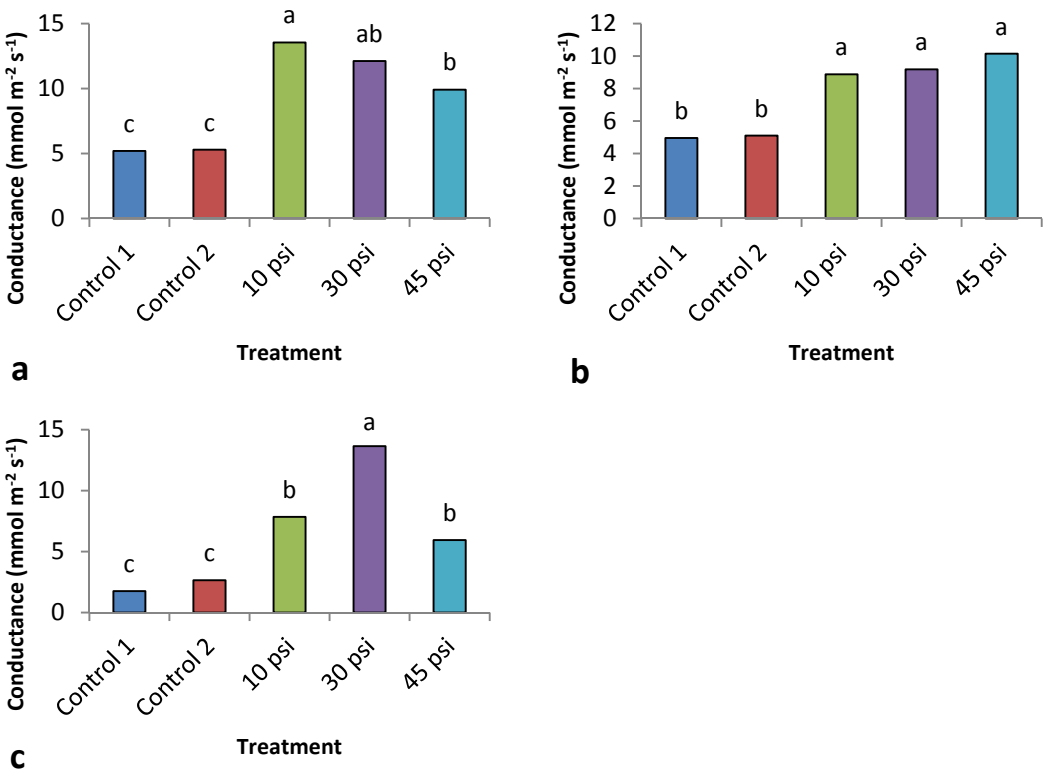


Figure 8.8 Comparison of mean stomatal conductance readings on the a) 10th, b) 15th and c) 20th day postharvest. Control 1 branches were fitted with a section of tubing meant to simulate the possible restricting effect of the root pressure system on water uptake, while Control 2 branches had no such restriction. Means with the same letter are not significantly different. n = 6

### 8.3.2 Water Use

Results of analysis on Day 1 to 190 showed an interaction effect between the time factor and treatment. This interaction effect became apparent starting from Day 80. Analysis of data taken up until Day 80 showed no interaction effect between factors, with both the time factor and treatment having a significant influence on average water use.

Branches fitted with a section of tubing meant to simulate the possible restriction caused by the root pressure system (C1) experienced a significantly lower average water use than branches without any such restriction (C2), according to results from the first 80 days (Figure 8.10). Figure 8.9 shows that average water use appeared to reach its minimum by

Day 60 in C1 branches, and Day 80 in C2 branches. The branches under absolute control had a significantly high water use (Figure 8.10) compared to C1 suggesting that the cufflings in the system may restrict water flow to the branches.

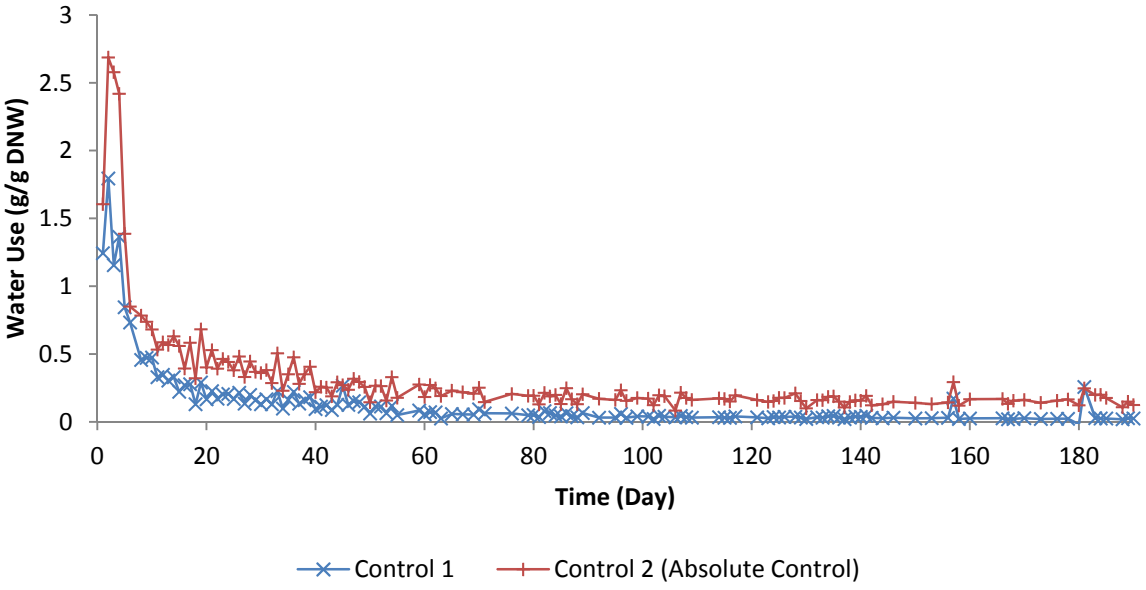


Figure 8.9 Dynamics of average water use for two controls over the 6 months following harvest. Control 1 branches were fitted with a section of tubing meant to simulate the possible restricting effect of the root pressure system on water uptake, while Control 2 (absolute control) branches had no such restriction. n = 6.

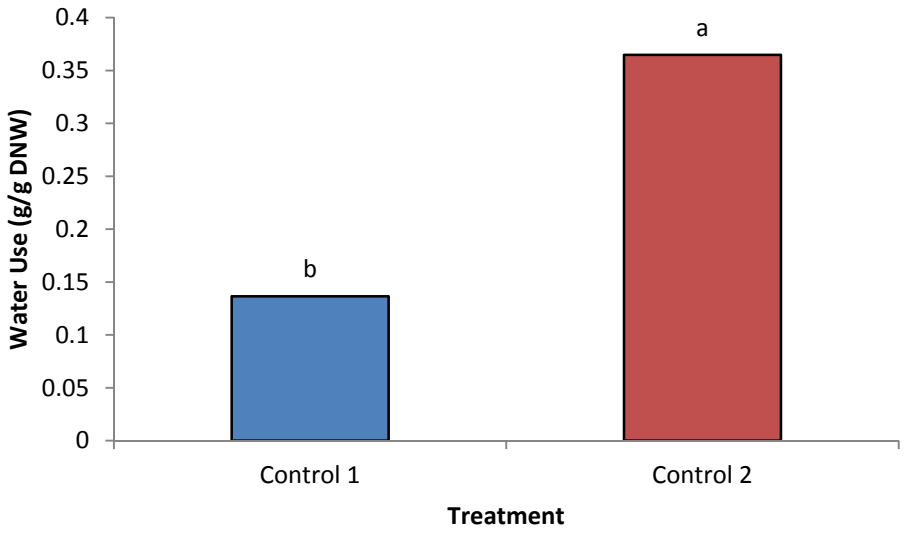


Figure 8.10 Comparison of average water use between two controls. Control 1 branches were fitted with a section of tubing meant to simulate the possible restricting effect of the root pressure system on water uptake. Results are an average over the first 80 days following harvest. Means with the same letter are not significantly different. n = 6

### 8.3.3 Needle Loss

Observation of the dynamics of needle loss showed that the initiation of needle loss began the earliest in the control branches fitted with a section of tubing and the branches treated with a water pressure of 45 psi, followed by the branches treated with a water pressure of 30 psi, and in the branches under absolute control. Branches under the lowest water pressure treatment (10 psi) delayed needle abscission initiation for 20 days (Figure 8.11), with a slow rate of needle loss following until the termination of the experiment, and the lowest needle loss percentage over the 6 months of experimentation (Figure 8.11; Figure 8.12). Furthermore, branches under the 10 psi pressure treatment had reached 20% needle loss by Day 116, while control branches fitted with the collars had exceeded 20% needle loss by Day 8 (Figure 8.11). The experiment had to be terminated at the end of 6 months to complete the thesis.

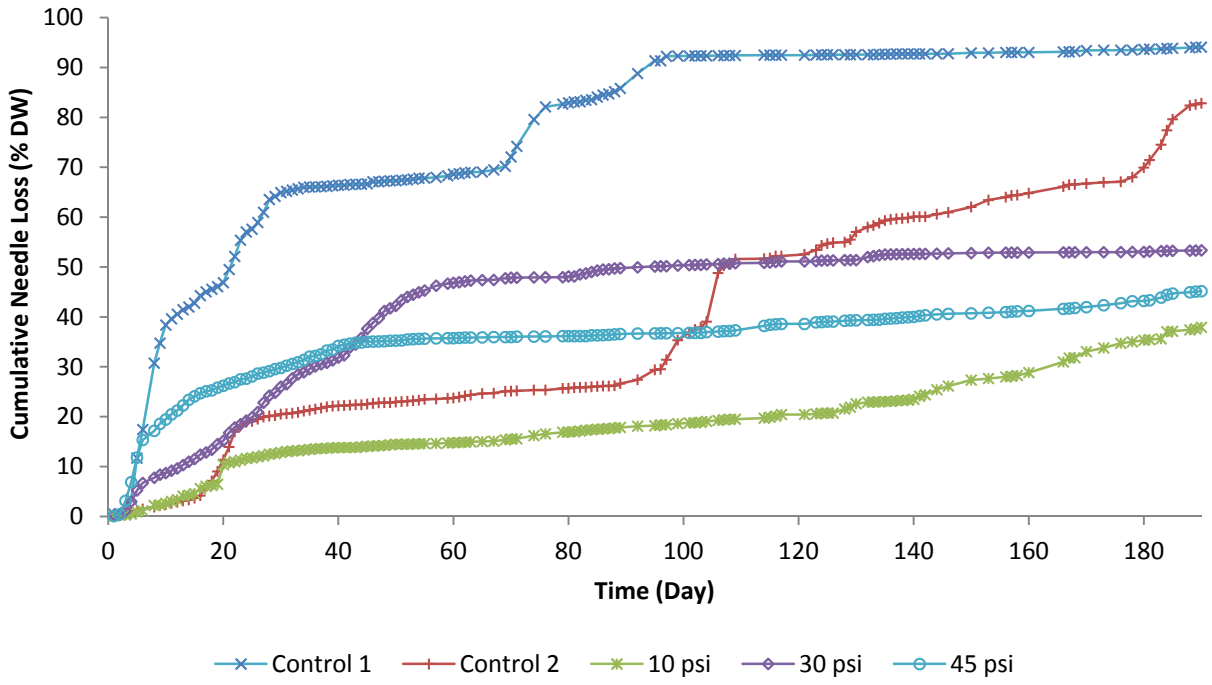


Figure 8.11 Dynamics of percent needle loss for two controls and three levels of a water pressure treatment over the 6 months following harvest. Control 1 branches were fitted with a section of tubing meant to simulate the possible restricting effect of the root pressure system on water uptake, while Control 2 branches had no such restriction. n = 6

Comparison of the mean percent needle loss between levels of the treatment (Figure 8.12) found the lowest needle loss found in the branches treated with a water pressure of 10 psi. Overall, branches treated with a water pressure of 10 psi experienced a 77% reduction in needle loss (Figure 8.12) when compared to control branches fitted with a collar. These results suggest that simulating root pressure at a 10 psi level delays needle drop and promotes needle retention.

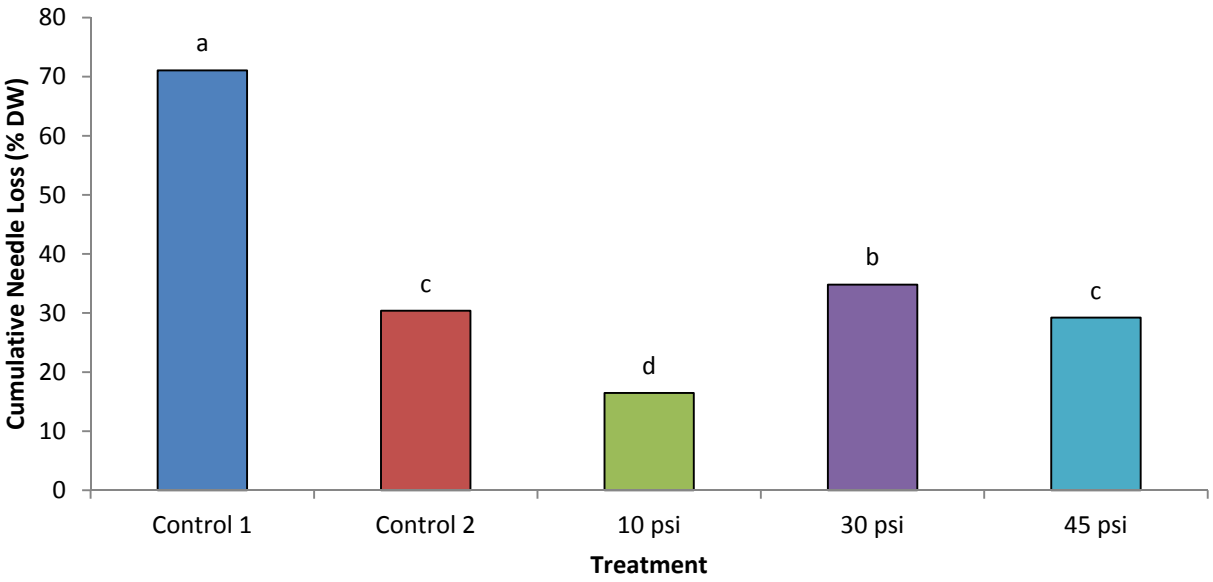


Figure 8.12 Comparison of percent needle loss between two controls and three levels of a water pressure treatment. Results are an average over the 6 months following harvest. Control 1 branches were fitted with a section of tubing meant to simulate the possible restricting effect of the root pressure system on water uptake. Results are an average over the 6 months following harvest. Means with the same letter are not significantly different. n = 6

Figure 8.13 shows that by the end of the 190-day experimental period, both controls had experienced nearly total needle loss, while many of the treated branches retained the majority of their needles. In the 10 psi treatment, none of the branches experienced complete needle loss over the experimental time frame, indicating that while all pressure treatments were effective in promoting needle retention, the 10 psi treatment resulted in the least overall needle loss.

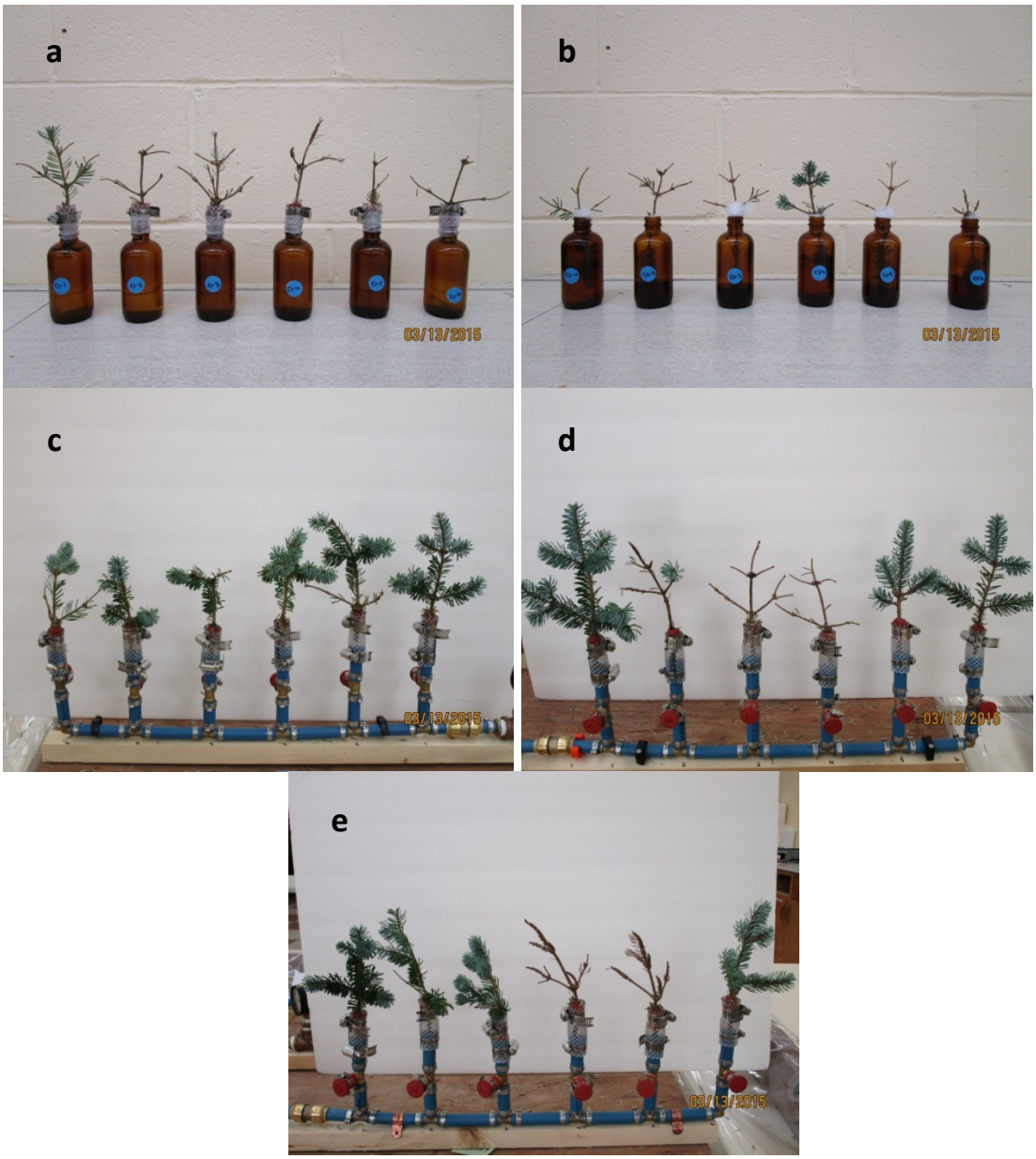


Figure 8.13 All branches at the conclusion of the experiment (Day 190). a) Control branches fitted with a section of tubing meant to simulate the possible restricting effect of the root pressure system on water uptake. b) Absolute control branches. c) Branches treated with a water pressure of 10 psi. d) Branches treated with a water pressure of 30 psi. e) Branches treated with a water pressure of 45 psi.

### 8.3.4 Xylem Blockage

Although there was a low xylem blockage with the controls, there was no statistically significant difference in xylem blockage rating among various treatments (Figure 8.14), suggesting that xylem blockage, at least at the cut end, has no link with the level of water pressure applied at the cut end, and possibly no link with needle abscission. These results are similar to those found in the xylem blockage experiment (Chapter 4), in which there was no relationship between xylem blockage, patterns of needle loss, and patterns of water use over time.

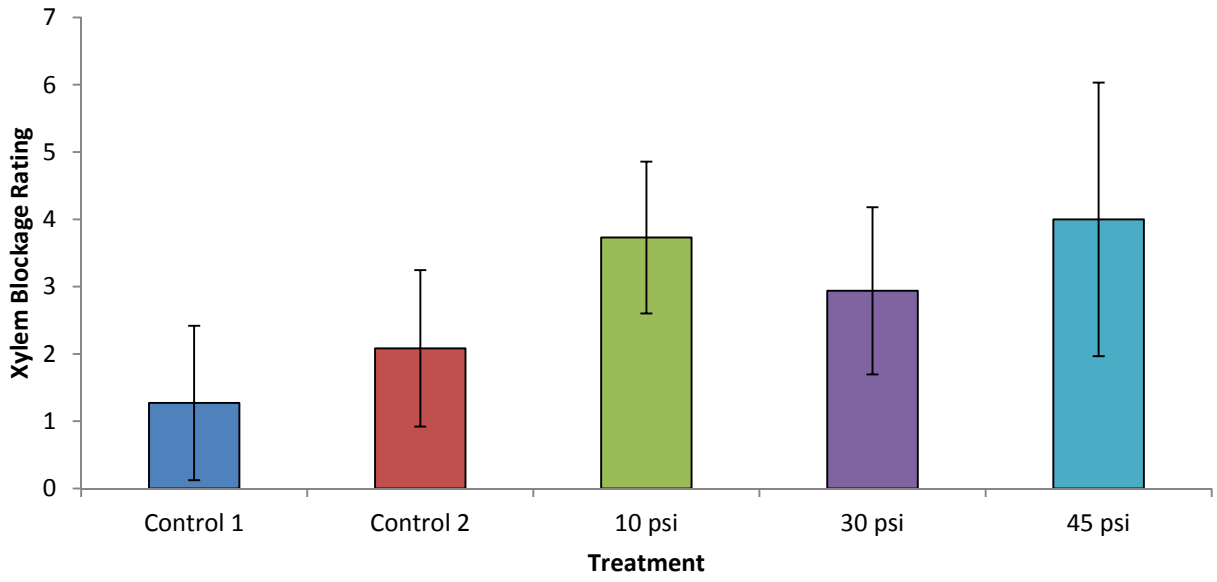


Figure 8.14 Comparison of xylem blockage rating, taken from the cut end on the final day of the experiment, between two controls and three levels of a water pressure treatment. Control 1 branches were fitted with a section of tubing meant to simulate the possible restricting effect of the root pressure system on water uptake. Error bars show the 95% confidence interval for the mean. n = 24

## 8.4 Discussion

Results suggest that applying a positive water pressure of 10 psi to the cut end of the stem has a significantly positive effect in delaying intiation and slowing needle loss postharvest. Control branches fitted with a section of pipe meant to simulate the possible restricting effect of the root pressure system were among those that lost needles the earliest, and had the overall greatest needle loss by the final day of the experiment. Branches treated with a water pressure of 10 psi at the cut end delayed needle loss initiation for 20 days, were found to lose needles at a slow rate throughout the experiment, and had the overall least needle loss by Day 190.

The dynamics of stomatal conductance over the 190 days following harvest (Figure 8.7) seem to suggest that the rate of decline of stomatal conductance in pressure-treated branches is lower than in untreated branches, but analysis revealed that stomatal conductance is dependent on an interaction between pressure treatment and the time factor. However, excessive noise may have led to a false significant interaction. There was a great deal of variation in conductance between branches of the same treatment. As each branch reached the point of abscission, that replicate was removed from its associated treatment level, which may have made any variation in conductance between remaining replicates more pronounced. This suggests that that branch behaviour may be independently regulated, in that the behaviour of one branch did not have any effect on the other branches undergoing the same water pressure treatment, especially since positive water pressure was constantly applied to the cut end of each branch in the manifolds, preventing the xylem sap of one branch from entering the vascular system of another in the same manifold. The fact that the SRPS did not prevent the eventual decline in stomatal conductance in any pressure-treated branches suggests that stomatal behaviour may be differentially regulated, and independent of root pressure.

Some of the branches treated with a water pressure of 45 psi lost their needles very early on, one undergoing nearly complete needle abscission after only 14 days. A similar effect was seen in branches treated with a water pressure of 30 psi, only at a later date. None of the branches treated with a water pressure of 10 psi experienced such a sudden loss of

needles during the first 190 days following harvest. This suggests that water pressures of 30 psi and higher may promote needle abscission, possibly by causing damage to cell membranes, particularly at the abscission zone. Fisher et al.'s survey of root pressure in plants (1997) found root pressures ranging from 0.3 to 21.5 psi in the 109 species of plants examined. The fact that only the lowest pressure treatment used in this study falls within this range, and that the branches under this treatment experienced the overall least needle loss when compared to the other levels of the treatment, suggests that applying water pressure within this range to the cut end may best simulate the original pre-harvest root pressure and delay abscission, while applying pressures in excess of this may be detrimental.

Water use was measured only in control branches, since the pressure-treated branches were anchored in the root pressure system and could not be weighed. Water use in branches fitted with the same tubing used in the root pressure system was significantly lower than that in branches without the tubing. When the branches were removed from the root pressure system on the final day of the experiment, a constriction was visible where the branch had been anchored into the pressure system (Figure 8.15); a similar constriction was seen in control branches fitted with the section of pipe. Possibly by reducing branch, xylem, and more likely, phloem diameter, this constriction may have led to the lower water use seen in control branches fitted with the pipe. These control branches also experienced the highest needle loss, while needle loss in absolute controls was considerably lower, similar to that of branches treated with a water pressure of 45 psi (Figure 8.12). Though water use was not measured in pressure-treated branches, needle loss results give some indication that the pressure treatment was successful in counteracting the constriction, and possibly the reduced water use leading to dehydration, caused by the mounting of the branches in the root pressure system.

There was no significant difference in degree of xylem blockage between levels of the treatment, suggesting that xylem blockage, at least at the cut end, has no link with the level of water pressure applied at the cut end, and possibly no link with needle abscission. These results are similar to those found in the xylem blockage experiment (Chapter 4), in

which there was no relationship between xylem blockage, patterns of needle loss, and patterns of water use over time.


Figure 8.15 A pressure-treated branch after being removed from the root pressure system. A constriction is visible near the cut end (a), where the branch was anchored in the pressure system.

## 8.5 Conclusions

The application of a positive water pressure of 10 psi to the cut end of the stem was successful in maintaining needles on most treated branches for at least 6 months. Some branches treated with water pressures of 30 psi and 45 psi lost needles early in the experiment, which indicates that higher water pressures may be detrimental, possibly even promoting abscission in some cases. Due to the nature of the pressure system used, a constriction at the base of each branch may have reduced water use in pressure-treated branches. The water pressure applied to the cut end appeared to counteract this based on needle loss results, but in order to confirm this, a means a measuring water use in

pressure-treated branches must be devised so that treated and untreated branches can be compared on the basis of water use.

Results on stomatal conductance showed evidence of an interaction effect between the time factor and the treatment level, indicating that stomatal conductance decays over time regardless of the level of water pressure applied to the cut end. A graph showing the dynamics of stomatal conductance over time suggests that applying water pressure to the cut end may slow the decline of stomatal conductance for some length of time.

In conclusion, a system that simulates the positive water pressure provided by the root system in a postharvest situation appears to be an effective means of prolonging needle retention postharvest, with a water pressure treatment of 10 psi resulting in the least needle loss overall.

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# CHAPTER 9 CONCLUSION

## 9.1 General Discussion and Conclusions

Xylem blockage at the cut end of the stem did not appear to have any sort of link with needle abscission. In the xylem blockage experiment (Chapter 4), there was no relationship between xylem blockage, water use and needle loss, or any visible patterns of xylem blockage over time. However, this does not rule out xylem blockage as a possible promoter of needle abscission, since only blockages at the cut end were examined, and it is possible that blockages may occur at other points in the hydraulic pathway. There may also be other types of blockages, such as embolisms, not visible through cross-sections or even with the light microscope used in these studies.

The stem cutting experiment (Chapter 5) found that giving a fresh cut to the end of the stem had some effect on water use, but did not appear to affect stomatal conductance. Branches cut every second day experienced a slower rate of needle loss following initiation, though patterns of needle loss in branches cut every day were not significantly different than those in uncut branches. This study also found a positive linear relationship between water use and stomatal conductance. An increase in water use can be associated with an increase in stomatal conductance, and both of these appear to decline within the first 10 days following harvest. However, the clone comparison experiment (Chapter 6) suggested the possibility of a clone-specific response in conductance. The high-NRD clone investigated experienced less water use over the 5 months following harvest, but greater stomatal conductance, which is unexpected based on the linear relationship between stomatal conductance and water use. It is possible each clone exhibits specific stomatal behaviour postharvest, particularly in the presence of abscission-signaling hormones such as ABA and ethylene. Other factors not investigated in this project, such as stomatal size, density and cuticle thickness, may also have a role to play in each clone's stomatal response to the postharvest situation.

The experiments involving light intensity, humidity and simulating root pressure (Chapters 7 and 8) attempted to improve and maintain stomatal conductance and water

use, thus delaying dehydration, by keeping branches under conditions known to keep stomata open. Increasing light intensity to 100 μmol $m^{-2}$ $s^{-1}$ had a beneficial effect on conductance and water use, even reducing short-term needle loss. Keeping branches under conditions of constant high humidity resulted in significantly higher stomatal conductance, but a decline in conductance was still seen within the first 7 days postharvest. Branches under a high light intensity of 150 μmol $m^{-2}$ $s^{-1}$ appeared to dry out and lose first-year needle growth. These results show that increasing light intensity, which causes stomata to open, can increase water use and decrease needle loss, but may be detrimental at higher light intensities.

Applying a positive water pressure to the cut end, meant to simulate the actual root pressure in a pre-harvest situation, may improve stomatal conductance and water use, possibly delaying the initial decline in both of these seen in the stem cutting and clone comparison experiments (Chapters 5 and 6). It is unclear whether or not stomatal conductance improves, as there was a great deal of variation between branches undergoing the same pressure treatment, but a graph of mean stomatal conductance readings taken over the first 6 months following harvest seems to suggest conductance in pressure-treated branches declines at a slower rate than untreated branches, and comparison of measurements taken on the $10^{th}$, $15^{th}$ and $20^{th}$ day postharvest show a significantly higher conductance in pressure-treated branches compared to control branches. As water use was not measured in pressure-treated branches, there is not enough information based on these results to determine whether water use actually improves when water pressure is applied at the cut end. However, the benefits of applying a positive water pressure to the cut end of the stem can be seen in the dynamics of needle loss, with most pressure-treated branches retaining needles even 6 months after harvest. Applying a water pressure of 10 psi had the most beneficial effect on needle retention, with pressures of 30 psi and 45 psi possibly promoting needle loss in some branches.

In conclusion, there is some evidence of a link between stomatal conductance, water use and needle loss. While it is not known whether or not the initial decline in both

conductance and water use directly promotes needle loss or works alongside other abscission-signaling factors to promote abscission, it has been seen that keeping branches under conditions known to improve stomatal conductance and water use visibly improves needle retention, showing that maintaining conductance and water use over a longer period of time can reduce needle loss.

## 9.2 Future Study

Experiments conducted throughout the course of these studies have found results that support a link between balsam fir's postharvest hydraulic status and needle abscission. Most notably, it has been found that applying a positive water pressure of 10 psi to the cut end, in order to simulate the pre-harvest root pressure, allows branches to retain needles for at least 6 months, and possibly slows the initial decline in stomatal conductance.

Results of the simulated root pressure system (SRPS) experiment suggest that needle retention promotion may not be regulated by stomatal opening and closure, since the SRPS appeared to slow, but not prevent the initial decline in stomatal conductance. However, since the SRPS did slow the decline in stomatal conductance, it is clear that applying a positive water pressure to the cut end of the stem has some positive effect on keeping stomata open. This brings about several questions of how root pressure influences stomatal behaviour and needle retention. When applying a water pressure of 10 psi to the cut end of the stem, how do the stomata perceive the pressure? Is root pressure perceived as a signal through cell membranes? Does the application of a positive water pressure to the cut end maintain the turgidity of the stomatal guard cells, allowing them to remain open? In a pre-harvest situation, is root pressure a biophysical trigger for stomatal opening? Currently, we do not know the mechanics of how root pressure induces stomatal opening in postharvest balsam fir, or whether root pressure is able to compensate for water loss postharvest, thus preventing needles from dehydrating and abscising. Further research is needed to better understand this field of study.

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