PHYSIOLOGY OF MECHANICAL STRESS-INDUCED NEEDLE LOSS IN POSTHARVEST BALSAM FIR (*ABIES BALSAMEA* L.)

by

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Submitted in partial fulfilment of the requirements
for the degree of Doctor of Philosophy

at

Dalhousie University
Halifax, Nova Scotia
April 2018

DEDICATION

This work is dedicated to my

Dear wife, Anita Quaye

Children, Dede and Kwame

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Postharvest needle loss in balsam fir, *Abies balsamea* L., presents a major challenge for the Christmas tree industry. It was hypothesized that postharvest needle abscission is caused by mechanical stresses such as shaking, baling and storage temperatures triggered by certain physiological and biochemical processes and modulated by the storage environment. We discovered that baling cause, 1.03°C increase in needle temperature, 2-folds, 5-folds, and 2-folds increases in membrane injury index, ethylene and VTC evolutions, respectively. Control trees had a 16% increase in needle loss compared to higher shaking duration (60 sec.), while baled of 1 tree lost 13% more needles compared to what control. These trends corresponded with ethylene and VTC evolutions. Trees stored continuously at 20°C and 30°C lasted for 7 and 14 days at a higher humidity of 83% and 85%, respectively. Exposing baled trees to a low temperature of 3°C promoted NRD through maintaining high AWU and reducing ethylene and VTC (3-carene) evolution. Storage of trees that were shaken and baled at vapor pressure deficit of 0.26 kPa also increased NRD and AWU by 2-folds, and decreased ethylene by 2-folds and a near perfect relationship between total VTC and individual VTCs (3-carene and β-pinene) and NRD was observed. XPP was significantly ($p=0.05$) higher (-0.61MPa) in trees that were exposed to a combined treatment of 60sec shaking and bale of 5 trees compared to their respective controls. A 34.6% increase in XPP was recorded in the upper tier of trees compared to the lower tier. While a 12.12% decrease in impedance was recorded in the upper tier of control trees, trees shaken and baled showed a 31.49% decrease in impedance as a result of damaged tissues. These results indicate that balsam fir trees respond to mechanical stress caused by shaking and/or baling, by a decline in water uptake, increase in ethylene and VTCs specifically, 3-carene, β-pinene and β-terpene leading to postharvest needle abscission, which was modulated by storage of trees at low temperature (3°C) and low VPD (0.26 kPa).

Keywords: Christmas tree, shaking, baling, stress, temperature, MII, ethylene, VTC, needle abscission

LIST OF ABBREVIATIONS AND SYMBOLS USED

ACC	1-aminocyclopropane-l-carboxylic acid
ANOVA	Analysis of variance
AVG	Aminoethoxyvinylglycine
AWU	Average water use
AZ	Abscission zone
CO_2	Carbon dioxide
CRC	Christmas tree Research Centre
CTCNS	Christmas tree Council of Nova Scotia
DPP	Dimethylallyl pyrophosphate
FID	Flame ionization detector
GC	Gas chromatography
GGPP	Geranylgeranyl pyrophosphate
GAP	Glyceraldehyde 3-phosphate
GPP	Geranyl pyrophosphate
IAA	Indole-3-acetic acid (auxin)
IPP	Isopentenyl pyrophosphate
JA	Jasmonic acid
JA	Jasmonic acid
LSD	Least significant difference
MeJA	Methyl jasmonate
MEP	2-C-methyl-D-erythritol 4-phosphate
MVA	Mevalonic acid
NAR	Needle abscission resistance
NRD	Needle retention duration
NSERC	Natural Sciences and Engineering Research Council of Canada
NV	Needle volatiles
O_2	Oxygen
PA	Peak area
PGR's	Plant growth regulators
PIN	Production of proteinase inhibitor
PR	Pathogenesis-related
SAM	S-adenosyl-methionine
SAS	Statistical analysis system
SPME	Solid-phase microextraction
TPP	Thiamine pyrophosphate
USDA	United States Department of Agriculture
VTCs	Volatile terpene compounds
XPP	Xylem pressure potential

ACKNOWLEDGEMENT

After four and a half years of intense research, technology development and writing, I can sit back and thank God. Writing this note of acknowledgement marks the end of my journey through the academic ladder. It has been for me, a period of intense learning, not only in the area of scientific research but also on personal and professional levels. This journey has had a big impact on my life and would like to reflect on that and acknowledge those who have supported and helped me to make this achievement possible.

I would like to give thanks to the Almighty God for how far he has brought me, without HIM I am nothing. My sincere gratitude goes to my supervisor, Prof. Rajasekaran R. Lada for the continuous support, for his patience, motivation, immense knowledge and the best of funding support. His guidance helped me from beginning of the research to writing of this thesis. I could not have imagined having a better advisor and mentor for my Ph.D. study. I would like to thank the rest of my supervisory committee: Prof. Samuel K. Asiedu and Prof. Claude Caldwell, for their insightful comments and encouragement, but also for the hard questions which incented me to widen my research from various perspectives.

Thanks also go to fellow lab mates and staff of the Christmas Tree Research Centre (CRC), the stimulating discussions, for the sleepless nights we worked together before deadlines, and for all the fun we had during this period. In addition, this research wouldn't be possible without the support of several funding agencies, most notably the Natural Science and Engineering Research Council (NSERC), Atlantic Innovation Fund (AIF), and Christmas Tree Council of Nova Scotia (CTCNS) to Dr. Rajasekaran Lada.

Last but not the least, I would like to thank my family: my dear wife, parents, brother and entire family for supporting me spiritually throughout this journey and my life in general.

CHAPTER 1: INTRODUCTION

The following has been published as a review paper from parts of this Section.

Ernest A. Korankye, Rajasekaran R. Lada, Samuel K. Asiedu and Claude Caldwell. 2017. Plant Senescence: The Role of Volatile Terpene Compounds (VTCs). American Journal of Plant Science. 8.3120-3139. Doi: https://doi.org/10.4236/ajps.2017.812211.

In recent years, many studies have addressed the issue of postharvest needle abscission and proposed possible factors that trigger needle abscission in Christmas trees (MacDonald et al. 2008; 2010; Thiagarajan and Rajasekaran 2006; Thiagarajan at al. 2013). The common factors attributed to postharvest needle abscission are mostly environmental, however, attention has never been given to the techniques farmers have adopted over the years for easy harvesting, handling, and transporting of trees and the cumulative physiological effects on postharvest needle abscission. On most farms, the harvest of Christmas trees starts as early as the last week of October to take advantage of the early cold weather of the fall season for trees to acclimatize in the cold (Thiagarajan et al. 2013). In general, harvesting of trees is mechanically done for the most part by chainsaw, the mechanics of the process tends to inflict mechanical wounds on tree trunks, which is believed to pose a great mechanical stress to trees, resulting in stress responses such as ethylene and VTC evolutions, which are proposed to trigger postharvest physiological changes in trees through needle abscission (MacDonald et al. 2010 and Korankye, 2013).

After harvesting, trees are processed by shaking, baling, loading and transportation. Shaking is basically done to remove any detached or dead needles and possible insects on the trees to meet the international market regulation. Since this practice is rigorous, it tends to break needles and some branches, which may eventually result in mechanical or invasive stress because of wounding. Baling is done to wrap one or several trees for easy handling and transportation. It is done using plastic rope, which tends to cause needle loss and breakage of branches, possibly leading to stress. After that they are loaded in all forms of transportation equipment, these trees are mostly transported by open trucks or across the ocean in non-refrigerated containers, which are speculated to cause severe mechanical damage to the trees, resulting in needle losses by the time they get to the destination. Environmental conditions in these trucks and containers are not well documented however, it is established that plants do not do well under extreme temperatures and humidity (Jalkanen et al. 1995).

Since postharvest processing, handling and transportation of trees over a period of days or sometimes weeks in international shipments are integral components in the Christmas tree industry, they are commonly practised by the majority of farmers. Injuries on trees such as needle breakage and bruises of branches due to shaking and baling can induce water loss and subsequently reduce transpiration causing increased temperatures (Blum et al. 1982; Jackson et al. 1981). Blum et al. 1982, have reported an increase in temperature among genotypes of wheat when they are exposed to water stress. Imposed mechanical stress in pine trees have been reported to induce over 50% membrane injury and therefore, electrolyte leakage (Hinesley, 1991).

It is also reported that shaking increases the stomatal conductance and lowers the water potential of plants (Chapter 4), which can lead to postharvest needle abscission. It has been shown that mechanically induced stress in plants tend to reduce stomatal aperture (Akers et al. 1984). It is also speculated that shaking and baling cause cavitation of water columns in the xylem, disrupting the pathway for water transport, leading to water stress (Schulze and Hall, 1982; Meinzer and Grantz, 1990). Similar responses could be induced in balsam fir trees, eventually promoting postharvest needle abscission.

It is believed that the effect of the mechanical stimulus on a plant is mediated by the presence of ethylene (MacDonald et al. 2010). At the same time, ethylene is a known plant hormone that can trigger postharvest needle abscission in balsam fir trees with or without the presence of volatile terpene compounds (VTCs) (Korankye, 2013). A similar study by Korankye (2013), also elaborated on the significant roles of ethylene and VTCs in controlling the timing as well as the actual process of postharvest needle abscission in balsam fir. However, these studies were not conducted to examine the effects of mechanical stresses such as shaking and baling of trees. Mechanical stresses may be the causal factor for the induction of ethylene and VTC synthesis.

It is, therefore, prudent to identify and understand the key postharvest processes that trigger mechanical stresses in balsam fir trees and their detrimental role in reducing the postharvest lifespan of these trees. It is hypothesized that mechanical shaking, baling and transportation of balsam fir trees alone or in combination cause mechanically- induced stress to the trees, triggering changes in water status, membrane stability, ethylene and VTCs that may promote postharvest needle abscission.

1.1 WHY DO PLANTS ABSCISE?

As a beneficial process in most plants’ development, abscission serves as a mechanism for the removal of senescing or otherwise damaged or infected organs such as leaves, stems and roots to help promote growth (Bleecker and Patterson, 1997) and survival. One way plants respond to stress is the shedding of leaves. In situations of water stress, plants shed their leaves to maintain balanced gas exchange rates to balance transpiration and photosynthesis (Taylor and Whitelaw, 2001). Invasive stresses from wounds, feeding by insects, pathogen attack and diseases also result in accelerated abscission of infected plant parts to avoid the spread of infection and sequential cell death (Taylor and Whitelaw 2001). However, in some cases, intact and healthy organs may shed as a component of plant defence after harvest because of physical injuries and mechanical-induced stress (Kendall, 1918; Bleecker and Patterson, 1997). Several changes may take place in the leaves prior to abscission; development of anthocyanin; disappearance of chlorophyll; depletion of nitrogen, phosphorous, potassium, iron and magnesium; change in form or disappearance of carbohydrates or both; decrease in moisture; decrease in auxin (Hall and Lane 1952; Shoji et al. 1951) and increase in ethylene and VTC levels (Macdonald et al. 2010; Korankye, 2013). Abscission is a coordinated sequence of multiple changes in cell structure, metabolism and gene expression (Brown and Addicott, 1950) leading to wall digestion in well-defined cell layers known as the abscission zone (AZ)**.** These zones often evolve during the development of organ systems and are generally characterized as a band of small, square-shaped, densely cytoplasmic cells ranging from a few too many cell layers thick (Sexton and Roberts, 1982; Taylor and Whitelaw, 2001). The separation of organs from the parent plant is often preceded by the enlargement of cells in the AZ and then followed by a dissolution process. In combination with mechanical forces such as wind, abrasion or internally generated force by the cells, effective abscission is achieved. Abscission is then followed by the continuous enlargement of the cells on the proximal face of the fracture plane and, ultimately, the differentiation of these cells into suberized scar tissue (Addicott, 1982). The role of many enzymes and phytohormones has been implicated in abscission, nevertheless, the role of biotic (genotypic variation) and abiotic (environmental) factors in abscission are well established.

1.2 FACTORS LINKED TO POSTHARVEST NEEDLE ABSCISSION

1.2.1 Mechanical Stress

Mechanical stress has been described as a force per unit area, which can be manifested as pressure and or compression exerted inward upon a structure, surface shear through friction, and tensile reactive forces acting outward and often to equalize on externally imposed stress (Davies and Tripathi, 1993). Plants are persistently challenged with numerous mechanical stresses from the pressures exerted by winds, water, animal movement and feeding. Production agriculture and landscape practices such as pruning, trimming, pinching, shaking and tying (baling) have all been shown to impose mechanical stress (Mitchell, 1996). These are common stress factors known to cause a regular disturbance in many ecosystems (Puijalon et al. 2011).

Plant response to mechanical stress begins seconds to minutes, following the onset of stress (Jaffe, 1973; Mitchell, 1996). Deposition of callose (β-1,3-polyglucan) in the cell-wall space just outside the plasma membrane is recorded to occur within seconds after the onset of mechanical stress (Jaffe, 1984). This same area is thought to be the site of ethylene synthesis (Mattoo et al., 1982), therefore, the formation of callose may stimulate the ethylene-forming enzyme complex. It is also proposed that soluble, translocatable oligosaccharides arise in the wall space of rubbed plants, as breakdown products of wall polymers or as their precursors, eliciting the formation of ethylene in response to mechanical stress (Takahashi and Jaffe, 1984). Studies by Macdonald et al. 2011 elaborate on the key role of ethylene in promoting needle abscission.

1.2.2 Mechanical Stress-induced Injuries Influence Needle Abscission

During invasive stresses, such as penetration of the plant tissue either through wounding or pathogen attack, the plant responds by inducing a defence response through a substantial alteration in gene expression. This reinforces the cell wall by deposition of callose, lignin and hydroxyproline-rich in glycoproteins, synthesis of antimicrobial compounds such as phytoalexins and production of proteinase inhibitor (PIN) and pathogenesis-related (PR) proteins such as chitinases and endo-β1-1,3-glucanases (Taylor and Whitelaw, 2001). If the defence response is unsuccessful and pathogen invasion occurs, then the plants shed their infected organ, to prevent the spread of infection throughout the affected plant and its neighbours (Taylor and Whitelaw, 2001). Such a defence is proposed to have a relationship with organic volatile compounds. Studies by Carlow et al. (2006) showed that infected Fraser fir trees produce 50% more volatile compounds than non-infected trees. Studies by Korankye, 2013, discovered that VTCs such as β-Pinene, β–Terpinene, Fenchyl acetate, Camphene and 3-Carene were released in significant quantities prior to initiation of needle abscission, suggesting VTCs could play a role in triggering needle abscission. Thus, it is postulated that handling processes such as shaking, baling, transportation and other activities that are widely known to cause wounding of the trees may consequently result in an invasive stress triggering needle drop. Reports of biosynthesis and or release of ethylene and VTCs have been discussed in studies by Macdonald et al. (2011) and Korankye (2013), however, whether these compounds are synthesized as a result of a direct or indirect effect of the stress caused by factors such as plant water status is inconclusive.

1.2.3 Postharvest Water Consumption and Dehydration Plays Key Role in Needle Abscission

Balsam fir tree are recorded to consume approximately 0.15-0.20 $mL·g^{-1}·d^{-1}$ and a stomatal conductance of 20-25 $mmol·m^{-2}·s^{-1}$ immediately after harvest, but these factors are known to decrease after harvest (MacDonald and Lada, 2014; MacInnes, 2015). The effect of stress begins with the response of decreased stomatal conductance by 50% within the first 4 days of harvest and 80% within the first 1 week (MacInnes, 2015). Postharvest needle abscission in balsam fir in most cases has been reported to start when water consumption drops below 0.05 $mL·g^{-1}·d^{-1}$, it has therefore been suggested that improvement in water status or water uptake prior to this threshold may delay abscission (Rajasekaran et al. 2015; MacDonald and Rajasekaran, 2015). Although the exact cause of diminishing water consumption is not known, cavitation, embolism, stomatal dysfunction, bacteria contamination, or blockage of xylem vessels have all been speculated (Rajasekaran and MacDonald 2015b). Water use by a plant also indicates its health status. Proper hydration ensures an adequate supply of moisture to meet the demand of transpiration and basic physiological functions. In general, a tree weighing around 5 kg can consume approximately 1L of water per day (Rajasekaran et al. 2005a). However, despite a large initial uptake, constant consumption of water indicates normal water status maintenance activity. Abnormal water status can be observed in trees with very low water consumption, which indicates embolism that may lead to irreversible drying. In addition, excessive water consumption is also indicative of enhanced transpiration or poor stomatal control, which may perhaps be due to low synthesis, supply, or release of ABA (Rajasekaran et al. 2005b). While water flux is important to prevent dehydration, induced senescence and needle drop, hydration alone cannot prevent needle drop suggesting that a lack of some root-derived factor that prevents or delays senescence. For example, cytokinins are believed to be synthesized in meristematic tissues such as root apices then transported to needles *via* the xylem (Srivastava 2002). A decline in cytokinin supply can increase ethylene sensitivity, which may initiate the senescence (Meir et al. 2007; 2010) and continued increase in ethylene to trigger needle abscission (Macdonald et al. 2010). Recent studies have also established VTCs as a possible trigger factor in needle abscission. Korankye (2013) established that prior to needle abscission when tree water consumption is low, there was always a consistent surge in VTCs.

Moisture loss from trees harvested during warm periods is also known to rapidly lead to heavy needle losses (Hinesley and Chastagner, 2004). For that reason, baling of trees with moisture-proof materials during storage and transportation is suggested to decrease the moisture loss and hence improve the needle retention qualities (CTCNS, 2011). Transportation inside refrigerated containers has also been anecdotally found to decrease moisture loss due to decreased vapor pressure deficit. Although detailed information on postharvest handling, storage and transport conditions is not available for balsam fir, controlled atmospheric storage has been shown to effectively reduce the rates of oxidation, transpiration, respiration, and ethylene production to delay abscission in fruits and vegetables (Gorny, 1997).

1.2.4 Preharvest Exposure of Trees to Low Temperature (Cold Acclimation) Influence Needle Abscission

During the fall season through to the winter when photoperiods become shorter, the initial phase of hardening is initiated. The drop of temperature then controls the second and dormant stage of hardening (Greer et al. 2000). Studies by Macdonald and Rajasekaran

(2008) established that exposure of balsam fir trees to lower temperatures (0 to -15°C) during the late fall and early winter results in freeze hardening and predisposes trees towards better needle retention. In the process of tree cold hardening, several biochemical and physiological changes such as membrane composition and fluidity (Quinn 1985), accumulation of sugars and starches (Beck et al. 2004), cold-induced proteins (Close 1997) and boosting of free-radical scavenging potentials of cells (Tao et al. 1988) are observed. Studies of Norway spruce by Blodner et al. (2005) established that drought tolerance and cold hardiness occur concurrently. This phenomenon indicates that cold hardiness may potentially render drought tolerance and consequently influence the needle retention characteristics of trees. The mechanism for the co-existence of tolerance to cold temperatures and drought is unknown, however, evidence exists that cold hardening enhances needle retention qualities of many conifers (Thiagarajan et al. 2013). In fact, delay at the onset of cold temperatures can result in ineffective cold hardening and subsequently result in poor needle retention (Chastagner and Riley 2003). Nothing is known about the interactive effect of cold exposure and mechanical stress on postharvest needle abscission of trees. However, many studies have shown that trees with low needle retention when harvested in the fall and winter seasons at subzero temperatures do better in retaining their needles than those in the warmer seasons despite the same exposed handling processes (Macdonald and Rajasekaran, 2008; Thiagarajan et al. 2013). Although research findings exist on several physiological mechanisms of cold hardening on metabolism, there is limited information available on the direct influence of cold hardening on mechanical stresses and postharvest needle retention. Studies conducted by Thiagarajan et al. 2013 found out that artificial cold hardening at 0°C and 5°C in a year old, root-intact balsam fir seedling facilitated needle retention better than the root-detached seedling, suggesting that a yet unknown factor could facilitate cold acclimation leading to enhanced needle retention. Seedlings that were exposed to temperatures below 0°C, despite gradual acclimation, manifested needle loss. They concluded that exposing harvested trees to lower temperature (5°C) preserves the quality and extends the needle retention period of the trees. However, extremely high or low temperatures (subzero) during harvesting and transportation can adversely affect their postharvest qualities (Thiagarajan et al. 2013).

In another study, balsam fir trees that were exposed to natural cold acclimation, that is, grown in an orchard over the fall months, received benefits to needle retention even in subzero temperatures (MacDonald and Rajasekaran 2008). This same study also suggested that one can limit water loss in trees by storing them at 5°C and 60% humidity, which subsequently extend the needle retention duration significantly in the absence of ethylene. It was also shown that low temperatures and high humidity significantly delay the evolution of ethylene and increase xylem pressure potential (XPP) and therefore, delayed needle abscission significantly. These results suggest that declining water status because of high temperature and low humidity may be a signal required to trigger ethylene evolution and subsequently needle abscission. Although none of these studies subjected samples to mechanical stress, one could still speculate that regulation of environmental or storage temperature and humidity of the trees can mitigate the problem of dehydration during the period of processing and transportation. The role of ethylene, VTCs and interaction with the above factors in understanding the effects of mechanical stresses on postharvest needle abscission is not clear.

1.2.5 Postharvest Handling, Storage and Transportation Conditions Influence on Needle Abscission

It has been established that environmental conditions after harvest may have a significant bearing on the postharvest quality of Christmas trees. Mechanical stresses imposed by shaking, baling, storage and transportation of trees after harvest have all been speculated to have significant effects on postharvest qualities of trees. Christmas tree farmers are forced to shake freshly harvested trees to meet the international market and quarantine standards. The tree shaker is the common equipment used in shaking of trees. It is a mechanically built stand, equipped with a bowl in which the bottom of the tree trunk sits. Using an oscillating mechanism and powered by a motor the trees are shaken for mostly ten seconds. It is believed that shaking of the trees helps remove dust, pollen, debris and moulds of the trees to prevent allergies. The process is also known to remove any dead needles, broken branches and hidden pests. Preliminary studies at the Christmas tree Research Center (CRC) have shown that the shaking process just like any mechanical stress imposes a detrimental effect on the postharvest quality of the trees (Korankye, 2013). Baling is commonly done using ropes to tie up the trees to shrink the size for easy handling, loading and a cost-effective transportation. This process is achieved using an electrically powered bailer. It is also believed to cause breakages of tree branches and needles, although this is not proven by any research. Just like shaking, it is also speculated to have a damaging effect on the trees because of wounding and the mechanical stress it poses to the trees.

International transportation of trees is done in containers *via* the ocean except for trees that make their way to the United States. Transportation of trees in containers is believed to pose a bigger issue to the postharvest qualities of the trees due to uncontrolled environmental conditions in the containers, such as temperature and humidity that are known to regulate water content in trees and eventually needle abscission (Jalkanen et al. 1995; Hinesley and Chastagner 2004). It is believed that due to respiration of trees, humidity and temperature levels build up in the shipping containers. As discussed previously, high temperature and low humidity (or high VPD) speed up ethylene evolution and thereby, increase needle abscission in addition to causing dehydration (Hinesley and Chastagner 2004). It is well documented that temperature, light intensity and vapor pressure deficit are critical factors that could radically affect the postharvest qualities of Christmas tree. Studies by Jalkanen et al. (1995) found that higher temperature reduces the needle longevity of *Pinus sylvestris*. They concluded that elevated temperatures tend to increase the vapor pressure deficit in atmospheric air, heightening transpiration losses, and subsequently a surge in the xylem pressure potential in the trees, which have been speculated to cause postharvest abscission (Macdonald et al. 2010).

Although not proven, it has been suggested that baling of trees with moisture-proof covers during storage and transportation would decrease the moisture loss and hence indirectly improve the needle retention qualities, though increases in temperature could prevent this from being a viable option (CTCNS, 2016). Transportation inside refrigerated containers has been found to decrease moisture loss due to decreased vapor pressure deficit (CTCNS, 2016). Regulated atmospheric storage is another alternative, usually involving storage at cool temperatures (approximately 5°C) with modified oxygen and carbon dioxide ratios, that has been known to significantly reduces senescence in fruits, vegetables, and flowers (Gorny et al. 2002). While detailed information on the postharvest storage and transport conditions is not available for balsam fir, regulated atmospheric storage has been shown to effectively reduce the rates of oxidation, transpiration, respiration, and ethylene production to delay senescence in species of pear (Gorny 1997). However, the interactive effects of various mechanical stress and storage effects on postharvest needle abscission and the physiological responses in balsam fir are yet to be uncovered.

1.3 THE ROLE OF PHYTOHORMONES IN PLANT RESPONSE TO STRESS AND NEEDLE ABSCISSION

Virtually, every environmental parameter either abiotic or biotic to which plants are exposed affects plant growth and development. Whether the responses are mediated through direct or indirect effects on phytohormones are being debated. Nonetheless, hormones are known to respond to the environment and control the biochemical, physiological, and morphological aspects of development. Several aspects of hormone metabolism are operative in plants relative to plant response and development. Control of hormone synthesis, balance, concentration, sensitivity, degradation, conjugation, and transport are all factors that can affect plant growth response. This review will focus on the biochemical and physiological roles of ethylene and volatile terpene compounds that are implicated in stress and abscission.

1.3.1 Ethylene as a Key Signal for Postharvest Needle Abscission

Ethylene, a simple unsaturated hydrocarbon, regulates many diverse metabolic and developmental processes in plants. It is derived from the amino acid, methionine, which is converted to S-adenosyl-methionine (SAM) by SAM synthase. SAM serves as an intermediate in a variety of synthetic pathways, including polyamines. SAM can be converted into 1-aminocyclopropane-l-carboxylic acid (ACC) through ACC synthase, and it is the first committed step in the production of ethylene (Adams and Yang 1979). The final step converts ACC to ethylene with the enzyme ACC oxidase in the presence of oxygen.

Ethylene has a profound effect on plant growth and development, although it is usually associated with fruit ripening. Other senescence processes regulated by ethylene are fading of flowers and abscission of petals (Bleecker and Kende 2000). From the limited work done on the role of ethylene in needle abscission in conifers, it is clear that ethylene induces needle abscission in much the same manner it induces fruit, petal, and leaf abscission in other species (Macdonald et al. 2009, 2010, 2011). Ethylene evolution was significantly increased in jack and white pines due to drought (Rajasekaran and Blake, 1999; Islam et al., 2003), in silver fir due to biotic stresses (Fuhrer, 1985).

Several approaches confirmed the role of ethylene in abscission, such as, the use of ethylene inhibitor, aminoethoxyvinylglycine (AVG) and ethylene receptor blocker, 1-methylcyclopropene (1-MCP) to inhibit the conversion of S-adenosyl-L-methionine (SAM) to 1-aminocyclopropane- 1-carboxylic acid (ACC) to delay senescence and reduce abscission (Beyer, 1976; Rath et al. 2006; Macdonald et al. 2010). There is strong evidence that ethylene is not only associated with organ abscission in angiosperms but also induces abscission in balsam fir. Prolonged exposure to exogenous ethylene has consistently decreased needle retention by 60–70% (MacDonald et al. 2010, 2011a, b). Exogenous ethylene also accelerated abscission uniformly regardless of NAR genotype (MacDonald et al., 2012b) and regardless of storage temperature or humidity (MacDonald et al. 2012a). It is believed that ethylene facilitates abscission by promoting the production of cell wall hydrolytic enzymes, cellulase in the abscission zone (Wang, 2017; Tucker et al. 1988). This mode of ethylene action may also occur in balsam fir, as it has been shown that both endogenous and exogenous ethylene exposures induce an eight and twelve-fold increase in cellulase activity (MacDonald et al. 2011a). However, it is still unclear whether in balsam fir ethylene may also stimulate the production of other hydrolytic enzymes that may contribute to postharvest abscission.

Pre-and postharvest handling of balsam fir trees such a shaking, baling, storage and transportation are suspected to have practical implications on the role of ethylene in postharvest needle abscission. The major concern has been the imposed mechanical stress by these activities and their association with ethylene synthesis and abscission in many species (Mitchell and Myers, 1995) thus, it is possible that Christmas tree handling processes contribute to postharvest abscission in balsam fir. However, there is no information available in this area, thus, one of the objectives of this study is to bridge the knowledge gap between postharvest handling practices, the mechanical stresses, synthesis and role of ethylene in postharvest needle abscission of balsam fir trees.

1.3.2 Volatile Terpene Compounds and Their Role in Postharvest Needle Abscission

Terpenes are toxins and feeding deterrents to a large number of plant-feeding insects and mammals, thus they play important defensive roles in the plant kingdom (Gershenzon and Croteau, 1991). Many monoterpenes and their derivatives are important agents of insect toxicity, for example, pyrethroids, which are monoterpene esters produced in the leaves and flowers of *Chrysanthemum* species show very striking insecticidal activity and therefore are used in commercial insecticides (Buchanan et al. 2000). Although ethylene has been shown to be a key signal involved in postharvest balsam fir needle abscission (MacDonald et al., 2010), ultimately needle abscission still occurs in studies where ethylene biosynthesis has been inhibited immediately after harvesting (MacDonald et al., 2010, 2012a). It has been suggested that there might be an ethylene independent pathway(s) for postharvest needle abscission. Studies by Korankye (2013) discovered twelve distinct VTCs in balsam fir trees that are synthesized throughout the postharvest period. The same study reported that prior to the initiation of postharvest needle abscission, concentrations of certain VTCs such as β-Pinene, β–Terpinene, Fenchyl acetate, Camphene and 3-Carene were observed to peak in significant concentrations. The concentration of volatile terpenes released was also correlated with percentage needle loss in trees, despite no significant difference in ethylene evolution rates (Korankye, 2013). Although not yet known, one can speculate that volatile terpenes could be part of an ethylene-independent abscission pathway, thus a part of this study is directed towards testing the theory of VTCs dependent or ethylene independent and mechanical stress induced needle loss.

1.4 THE RESEARCH PROJECT

The project was funded by Natural Sciences and Engineering Research Council of Canada (NSERC), Atlantic Canada Opportunities Agency (ACOA) and Christmas Tree, Council of Nova Scotia. The main objectives of the project were;

1. To uncover the physiological responses of mechanical stress induced by shaking and baling in postharvest balsam fir.
2. To establish the physiological effect of mechanical shaking and baling on postharvest needle abscission of balsam fir trees.
3. To determine the influence of postharvest storage temperature on baling-induced changes in needle abscission.

4. To uncover various effects of vapor pressure deficit (VPD) on physiological changes such as water use, ethylene and VTC in trees exposed to shaking and baling.
5. To understand the changes in water relations and membrane stability on different tiers following imposed mechanical stress on balsam fir trees.

The result of this research project is expected to improve the current postharvest handling procedures that have been adopted by farmers over the years. Currently, balsam fir Christmas trees are exposed to high level of mechanical stress during harvest, shaking and bailing before it gets to the consumer. These common practices have been identified as catalysts to poor postharvest qualities of balsam fir Christmas trees such as needle freshness, and shelf life of trees.

In search of efficient methods of handling balsam fir Christmas trees, the research moved towards the understanding role of various handling processes in commonly identified physiological responses such as water relations, ethylene and volatile organic compounds evolution. I noted that in the past, studies have not focused on these postharvest practices in our selected study material.

The project first identifies and describes the various stress indicators after exposure of balsam fir trees to low and high levels of shaking and baling by mimicking farmer's practices. It describes and analyzes the immediate and long-term physiological responses of balsam fir trees to these stresses, while at the same time exploring new storage technologies through modification of storage factors such as water relations, temperature and vapor pressure deficit.

1.5 THE STRUCTURE OF THE THESIS

The first part of the thesis is focused on the socio-economic importance of the Christmas tree industry, in particular in relation Balsam fir trees. It also elaborated on some of the challenges the industry faces against the artificial tree market, its impact on the Canadian economy and threw more light on common postharvest practices in the Christmas tree industry which are proposed to play key roles in this bottleneck. Chapter 2 describes methods and protocols developed to effectively achieve objectives set for this study. Technologies such as thermal infrared imaging, solid phase microextraction, headspace ethylene extraction and gas chromatography were among methods used in monitoring stress responses such as membrane injury (MII), tree core temperature, ethylene and VTC evolution, among others. Chapter 3 describes the identified stress indicators; MI, tree temperature, carbon dioxide emission, ethylene and VTC evolution after exposure of balsam fir trees to shaking and baling. In chapter 4, balsam fir trees are analyzed based on physiological responses to mechanical stress that ultimately lead to postharvest needle abscission. Chapter 5, 6 and 7 explore new storage and transportation technologies through temperature, vapor pressure deficit (VPD) and water relations, establishing the extent of the positive impact these technologies have on postharvest needle loss. The general discussion and conclusions, chapters 8 and 9 describe the role of imposed stress through shaking and baling and their negative effect on postharvest needle loss, touching on areas like water regulation in trees, tissue damage and stomatal changes. The role of phytohormone, ethylene and secondary metabolites like 3-carene, β-Pinene and β–Terpinene are discussed and concluded to be involved in signalling the trigger of postharvest needle abscission. We concluded that technologies that focus on improving balsam fir tree water use, storage and transportation temperature, and vapor pressure deficit can also improve postharvest tree qualities and ultimately needle loss. The last chapter focuses on future research by discussing key questions raised throughout these studies and proposed specific studies that can be developed to answer them.

CHAPTER 2: METHODS AND PROCEDURES

The following has been published as a review paper from parts of this Section.

Ernest A. Korankye, Rajasekaran R. Lada, Samuel K. Asiedu and Claude Caldwell. 2017. Plant Senescence: The Role of Volatile Terpene Compounds (VTCs). American Journal of Plant Science. 8.3120-3139. Doi: https://doi.org/10.4236/ajps.2017.812211.

2.1 THERMAL INFRARED IMAGING, STOMATAL CONDUCTANCE AND PLANT STRESS

Application of thermal imaging in plant physiology has been used to establish a good understanding of the basic features of plant and leaf energy balance and its regulation by plant and environmental factors. Measurement of plant and leaf temperatures using thermal infrared (IR) sensing is predominantly used in the study of plant water relations, stress and specifically stomatal conductance (Cohen et al. 2005; Hamlyn, 2004). This is because a major determining factor of leaf temperature is the rate of evaporation or transpiration from the leaf. The cooling effect of transpiration arises because a substantial amount of energy (the latent heat of vaporization, λ; $Jmol^{-1}$) that is required to convert each mole of liquid water to water vapor, and this energy is then taken away from the leaf in the evaporating water and, thus, cools it (Cohen et al. 2005). In rare cases, leaf temperature may be affected by other physiological processes, a common example is the heat generated as water when a leaf freezes; it can then be readily imaged (Wisniewski *et al*. 1997). As found in *Arum maculatum* (spadix), raised temperatures can be used as a measure of increased respiration rates (Seymour 1999). In most cases, however, the heat generated by respiration is too small to have a detectable effect on leaf temperature (Breidenbach et al. 1997).

Although this method has been used extensively for the study of plant water stress and other plant responses such as evaporation and conductance (Cohen et al. 2005; Hamlyn, 2004), it has never been used to identify mechanical stress in plants and specifically balsam fir trees. In this study, the method will be applied in monitoring tree temperature and stomatal conductance before and after mechanical stress induced treatments. The idea behind it is to use temperature and stomatal conductance as indicators of mechanical stress of the various trees.

2.2 VTC IDENTIFICATION AND QUANTIFICATION (SOLID-PHASE MICROEXTRACTION)

Solid-phase microextraction (SPME) is a relatively new extraction method, developed by Pawliszyn and co-workers in 1989 and made commercially available in 1993. SPME is a non-solvent extraction method that employs a fused silica fiber coated with a thin film of extraction phase, which can be either a liquid (polymer) or a solid (sorbent), to extract volatile analytes from a sample matrix by adsorption or absorption of the compounds from the headspace (Wells, 2003; Korankye, 2013). The fibre is housed within a syringe needle that protects the fibre and allows for easy penetration of sample and GC vial septa. Most published SPME work has been performed with manual devices (Vereen et al. 2000; Beck et al. 2008; Korankye, 2013), although automated systems are also available. There are two approaches to SPME sampling of volatile organics: direct and headspace. Indirect sampling, the fibre is placed directly into the sample matrix, and in headspace sampling, the fibre is placed in the headspace of the sample (Korankye, 2013). SPME has several advantages in the analysis of volatile organics; no additional instruments or hardware required, and fibres can be reused from several to thousand times, depending on extraction and desorption conditions (Korankye et al. 2017). For the purposes of this study, VTC evolution is monitored once a week using the solid phase microextraction (SPME). After the headspace extraction, SPME fibre is transferred to the GC, where the fibre is inserted into the heated injection port for 3min for easy desorption of VTCs into the column for analysis (Korankye, 2013). Chromatographic data is generated as a graph of peak area (pA) against retention time (minutes) (Vereen et al. 2000; Korankye, 2013). The standard is occasionally analyzed between sample analysis, especially after a treatment. It is a good practice to begin and end the GC analysis by testing with the internal standard prepared (β-pinene). The concentration of VTCs is in mM per fresh weight of branch used in grams (g).

2.3 ETHYLENE IDENTIFICATION AND QUANTIFICATION

Identification of ethylene begins with the ability to trap the ethylene gas in an airtight space. Most studies have invented such spaces to suit their experiment depending on factors like sample kind and size. A closed system where ethylene gas from the plant can accumulate over a period is widely used. This allows for the withdrawal of concentrated gas from the headspace, using a gastight syringe (Klintborg et al. 2001). A study by Korankye, 2013 used airtight jars to trap ethylene gas prior to quantification. In this current study, airtight plastic bags with total volume of 109.2 L has been adopted to accommodate the size of approximately 3ft trees. The use of flame-ionization detectors (FID) for ethylene analysis has been a standard practice in plant physiology since it has a considerable improvement over bioassays when accuracy and sensitivity are a priority. Another advantage of FID is the ability to confirm identified peak(s) with a GC-mass spectrometer (Ward et al. 1978). Photoionization detectors (PID) have been shown to enhance the sensitivity, therefore extend the limits of detection and analysis for several compounds (Bassi and Spencer, 1985). In this study, 1ml air sample was drawn from the airtight bag with a gastight syringe (Sigma-Aldrich Co. LLC, Canada) and injected into a FOCUS 3420 gas chromatograph (Thermo Scientific, Canada) GC equipped with an Agilent J&W GC 30m×0.32mm, GS-Gaspro column (Chromatographic Specialties Inc, Canada) packed with fused silica (Korankye, 2013). Helium was used as a carrier gas at 1.5 mL min^{-1}, with injector, column and detector temperatures at 200, 65 and 250°C, respectively. A total run time of 5 minutes was used (Korankye, 2013).

2.4 GAS CHROMATOGRAPHY

Gas chromatography (GC) is a quantitative analytical tool used to determine sample components that can be vaporized without decomposition (Carlow et al. 2006; Korankye, 2013). The sample is first injected into the injection port of the already temperature programmed GC. The sample is vaporized onto the head of the chromatographic column. The sample molecules are then transported through the column by the flow inert (nitrogen, helium, argon and carbon dioxide) gaseous mobile phase. When they come in contact with the microscopic layer of liquid or polymer on an inert solid support (solid phase) inside the column, molecules are separated into its individual component and recorded by a detector and chromatographic data generated as graphs (chromatogram) of detector response against retention time (Verren et al. 2000). Since each type of molecule has a different rate of progression, they elute at different times (retention time). There are several detectors that are used in GC but the common one is the flame ionization detector (FID). In some cases, the GC is connected to a mass spectrophotometer (MS) which acts as a detector popularly known as GC-MS. A study by Korankye (2013) adopted mass spectrometry in the determination of 13 volatile terpene compounds in balsam fir Christmas trees.

2.5 MECHANICAL STRESS MEASUREMENTS

Measurement of stress in many cases has been focused on measuring various response signals of plants, pre-and post-exposure to the intended stress. As part of this study, the emphasis has been placed on the measurement of response signals such as membrane injury, plant temperature, CO_2 emission, ethylene and VTC evolution post-exposure of trees to the mechanical stresses of shaking and baling.

2.5.1 Membrane Injury Index (%)

Membrane integrity quantified the percentage of cell membrane electrolyte that is leaked into solution and was performed on 0.4g of balsam fir needles. To achieve that, stripped needles were soaked in 30mL of distilled water and allowed to adjust to room temperature (25°C) overnight. Using an electrical CDM 2e Conductivity Meter (Bach-Simpson, London, ON) electrical conductivity of electrolytes leaked were measured (EC). The test tubes were then placed in a hot air oven for 4 h, maintained at 90°C to enforce complete lysis of cell tissues. The solutes were measured for their electrical conductivities (EC_a). Membrane injury index (MII) was calculated by using the formula:

$$MII = \left(\frac{EC_a - EC}{EC_a}\right) \times 100$$

2.5.2 Whole Tree Temperature (°C)

To estimate the amount of heat generated by the trees as a result of the mechanical stress, an infrared thermal imaging technology was adopted using Fluke Ti400 60Hz Thermal Imager (Fluke, Mississauga, Canada). Thermal images taken were processed with digital image processing tools (Fluke, Mississauga, Canada). The raw thermal images were obtained in FLUKE Systems' proprietary format, gave the spot temperatures for each tree and estimated average temperature for each tree in the frame. Images were then processed by converting them to grey-scale images in which each grey level represented 0.1°C. This conversion is performed by setting the temperature span of the images and saving them as bitmap images (BMP format) by means of Fluke SmartView®, version 3.7 software (FLUKE, Canada) and also converted the bitmap images to 8-bit uncompressed TIFF format by means of Adobe Photoshop CC 2015 software (Adobe Inc.).

2.5.3 CO_2 Emission ($\mu mol.mol^{-1}$)

Carbon dioxide emission was also measured after a 12-hour incubation of trees in an airtight plastic bag chamber. After incubation, an LI-6400XT portable photosynthesis system (LI-COR, Canada) was used in measuring CO_2 emission (Norman et al. 1997).

2.5.4 Ethylene Evolution ($\mu L \cdot g^{-1} \cdot h^{-1}$)

Ethylene evolution was determined by first incubating trees in airtight plastic bag chambers for 12 hours. 1µl air samples were collected from the chamber and analyzed using GC equipped with FID. Evolution rates from the trees were calculated by the following equation:

$$Ethylene\ evolution = \frac{Ethylene\ concentration\ (Initial - Final) \times Volume\ of\ incubation\ chamber\ (L)}{12h \times Mass\ (weight\ of\ tree)}$$

Where ethylene evolution is in $\mu L \cdot g^{-1} \cdot h^{-1}$, concentration is in $\mu L \cdot L^{-1}$, and mass is the fresh weight (g) of a tree. The volume of airtight incubation plastic bag (109.21 L) (Appendix II).

2.5.5 VTC Evolution ($mM \cdot g^{-1} \cdot h^{-1}$)

To analyze VTC, whole trees were sealed in airtight plastic bag chambers for 30mins. Within that period, solid phase micro-extraction (SPME) fibre was exposed to the headspace for VTC extraction. GC analysis was performed and the chromatographic data of peak area (PA) against retention time (minutes) for each of the samples were defined. Confirmation of terpene compounds was established by comparison of retention times and peak areas with that of a known standard. The respective relative abundances for each sample were normalized against a β-pinene standard (Sigma-Aldrich Co. LLC, Canada). The concentration of individual and total VTCs in mM per g of fresh weight of trees was estimated and the evolution was calculated per the incubation period of 30mins (Appendix I).

2.5.6 Percentage Needle Loss

To account for the needle loss on each tree, fresh dropped needles were weighed. Since balsam fir needles have the ability to stay connected to the branches even after abscission, a process known as ‘finger runs test’ has been adopted. This is achieved by rubbing fingers through the tree to cause abscised needles to drop. Fresh dropped needles are weighed and placed in an oven for 48hrs at 80ºC and final dry weight taken (MacDonald et al. 2010).

Needle loss was monitored throughout the experimental duration to estimate the percentage needle loss for each treatment. Days for the commencement of needle loss, 1, 5, 10, 20 and 40 percent needle loss to occur were also determined.

2.5.7 Needle Retention Duration (NRD)

The primary assessment of needle abscission will be NRD, which is defined as the number of days required for a complete needle abscission per tree (MacDonald et al. 2009; MacDonald et al. 2010).

2.5.8 Average Water Use

Average daily water use ($mL \cdot g^{-1} \cdot d^{-1}$) is also monitored as the sum of the change in mass of the apparatus (excluding mass loss due to abscission) per unit fresh weight of needles over the duration of the experiment or until a branch had lost all needles (MacDonald et al. 2010). AWU was calculated by the following equation:

$$AWU = \frac{(Initial\ Mass - Final\ Mass) - \ Needle\ Mass}{Time\ (Days\ to\ complete\ needle\ loss)}$$

AWU was expressed as the average of all daily measurements.

2.5.9 Xylem Pressure Potential (XPP)

XPP of branches was measured using a Plant Moisture System Pressure Bomb (PMS Instrument Co., Corvallis, USA). A clipping from the primary branch was mounted upside down inside a pressure chamber and the pressure was increased gradually at 0.01MPa per minute until water droplets appeared on the cut surface. The pressure required to release a water droplet was recorded. To minimize disturbance to a branch, XPP was recorded at the end of each experiment (unless otherwise noted). This method was adopted from MacDonald et al., (2010).

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CHAPTER 3: STRESS PHYSIOLOGY OF POSTHARVEST BALSAM FIR TREES AS INFLUENCED BY SHAKING AND BALING

The following has been published as an abstract and manuscript from this Section.

Ernest A. Korankye, Rajasekaran R. Lada, Samuel K. Asiedu and Claude Caldwell. 2016. Stress physiology of post-harvest balsam fir trees as influenced by shaking and baling. International Conference of American Society of Horticultural Sciences. August 2016. Atlanta, Georgia (Poster presentation).

Ernest A. Korankye, Rajasekaran R. Lada, Samuel K. Asiedu and Claude Caldwell. Stress physiology of postharvest balsam fir trees as influenced by shaking and baling. Trees. (Submitted for publication, January 2018). In Press.

3.1 ABSTRACT

As part of postharvest handling processes of balsam fir trees, farmers shake and bale trees to remove dead needles, branches, making them easier to transport. Since shaking and baling of trees tend to break needles, branches and cause bruises on tree trunks, it has been postulated to cause extreme mechanical stress to the trees. This is speculated to negatively impact postharvest qualities of the trees. To test this theory, fifty 6-year old trees were randomly harvested and exposed to shaking durations of 0 (control), 10, 15, 30 or 60 seconds and baling treatment at no bale (control), 1, 2, 3, or 4 trees per bale. Stress indicators; tree temperature, membrane injury index, CO_2 emission, ethylene and VTC evolutions were monitored. The prolonged shaking of trees caused a minimal stress on the trees; therefore, trees can adjust and use that to their survival advantage. Baling of trees, on the other hand, resulted in 1.03 °C, 2-folds, 5-folds, and 2-folds increases in tree temperature, membrane injury index, ethylene and VTC evolutions, respectively. These changes suggest that although shaking in most cases could be of benefit or of no negative impact to trees, baling caused mechanical stress on trees and thus, may play a critical role in postharvest needle loss of balsam fir trees.

Keywords: Christmas tree, shaking, baling, stress, temperature, MII, ethylene, VTC, abscission

3.2 INTRODUCTION

Postharvest shaking and baling are believed to produce mechanically-induced stress (MIS) in balsam fir Christmas trees when branches and needles bend, touch and collide with each other. Just like any other plant, when a balsam fir tree is exposed to these mechanical perturbations, it responds by modifying its growth, development and biochemical processes. This phenomenon has been referred to as thigmomorphogenesis (Jaffe 1973; Biro et al. 1980; Chehab et al. 2009). It is now well established that most plants can initiate long-term responses to mechanical stimuli by decreases in elongation and increases in shoot diameter (Jaffe 1973; Boyer, 1967). In other studies, where plants like *Capsella bursa-pastoris* and *Prunus avium* were exposed to mechanical stimuli, they showed long-term responses by developing extensive root systems (Niklas 1998; Coutland et al. 2008). On the other hand, short-term responses such as stomata, photosynthesis and chlorophyll alterations have been reported in plants post-mechanical stress (Biddington, 1986), which may contribute to the process of organ abscission. Handling of cocklebur leaves has been found to reduce growth and cause premature yellowing and senescence of leaves (Salisbury 1963). In addition, it has been reported that rubbing of *Bryonia. dioica* stems correlates with an increase in peroxidase activities like what happens during the process of senescence (Boyer et al. 1973; 1983). It has been shown that bending of papaya (*Carica papaya*) plants lead to reduced chlorophyll concentration in the hypocotyl (Porter et al. 2009), although there have been studies suggesting that the effects of MIS on chlorophyll content are opposite to what occurs during senescence (Biddington and Dearman 1985; Mitchell et al. 1975).

Although the direct effects of MIS on photosynthesis are unclear, it has been reported that shaking of plants reduces net assimilation rate, a measure of net CO_2 fixation (Akers and Mitchell 1984). It has also been postulated that there is a reduced respiration in plants post-exposure to MIS (Brito et al. 2014). It is also suggested that a reduction in respiration is a response to reduced CO_2 net assimilation, which is relevant to abscission and linked to ethylene biosynthesis as discussed in a study by Giovani et al. 2014. In the same study, the use of ethylene inhibitors (1-MCP and AVG) increased net assimilation rate and maintained high photosynthesis rates although plants were subjected to stress (Giovani et al. 2014; Brito et al. 2014). A study by Coutand, 2010 also confirmed similar reports of decreased photosynthetic activity in response to MIS-reduced chlorophyll concentration post-MIS.

When plants are injured, it is known to provoke long-distance signals that also trigger responses in unwounded tissues (Chaki et al. 2011). These responses have been linked to the biosynthesis of various compounds such as systemin, ipomoelin, oligouronides, volatile terpene compounds (VTCs) and phytohormones including abscisic acid, ethylene, and jasmonic acid (Jih et al. 2003; Stratmann, 2003). For the purposes of this study, ethylene and VTCs are discussed. Ethylene is suggested to play a role in controlling plant responses to mechanical-induced stress. High levels of ethylene have been recorded following rubbing stems of *Pinguicula vulgaris* and this has been related to increases in ethylene precursor ACC (Biro and Jaffe 1984). In studies where bean, apple and orange were exposed to MIS like bending and shaking resulted in several folds of a short-term increase in ethylene evolution in tissues (Robitaille 1975; Skene 1980; Biro and Jaffe 1984). Callose production is known to increase in area under stress, leading to activation of rate limiting

ACC-oxidase that catalyzes the conversion of ACC to ethylene (Jaffe, 1984). In the case of VTCs, little is known about their roles in MIS signalling, however, in several studies, their biosynthesis or evolution have been reported to increase after imposed mechanical stress such as insect bite. Under stresses such as insect attack and mechanical injuries, balsam firs are known to produce excess VTCs for their defence (Carlow et al. 2006). Previously, the emission of plant volatiles had been speculated to be dependent on both ethylene and jasmonic acid (Schmelz et al. 2003). However, studies by Korankye (2013) clearly showed that when ethylene is successfully inhibited by AVG, significant concentrations of VTCs still evolved prior to needle abscission. This suggests that VTC evolution and its role in needle abscission may be independent of ethylene and needle abscission can occur independently of ethylene action. In the same work, it was expected that if ethylene is inhibited then needle abscission should stop; however, needle abscission continued eventually with high VTC evolution. This also suggests that even in the absence of ethylene, needle abscission can occur and VTCs such as β-Pinene, β–Terpinene, Camphene and 3-Carene may be the potential candidate signal molecules triggering postharvest needle abscission.

In the case of mechanical perturbation of Christmas trees, as a result of shaking and baling, it is speculated that trees go through a short-term response that can cascade into multiple long-term effects on photosynthesis, water relation, ethylene and VTC synthesis. All these factors have been established to have a consequence on postharvest qualities of trees. Hence, the importance to establish and confirm the dynamic relationship of the imposed shaking and bailing, mechanical stress and effect on quality of trees. It was therefore hypothesized that shaking and baling of balsam fir trees impose MIS, and tree response is indicated through an increase in membrane injury, tree temperature, CO_2 emission, ethylene and VTC biosynthesis. The objective of this study is to uncover the physiological responses of mechanical stress induced by shaking and baling in postharvest balsam fir.

3.3 MATERIALS AND METHODS

Root-detached trees of 91cm average height and 6-year-old were used in this study. All the trees were harvested in August 2015. Prior to harvesting, visual inspection was conducted to avoid pest or disease infestation on the trees. Harvesting of trees was done using a handheld chainsaw as per the normal practice. The experimental setup followed a randomized design with five shaking duration levels of 0 (control), 10, 15, 30, 60 seconds, baling treatment of no baling (control), 1, 2, 3, 4 trees. Each of the experimental units had 5 trees, hence a total of 50 trees were randomly selected for the study.

3.3.1 Measured Response Variables

(I) Membrane Injury Index (%): Membrane integrity quantified the percentage of cell membrane electrolyte that is leaked into solution and was performed on 0.4g of balsam fir needles. Stripped needles were soaked in 30mL of distilled water and allowed to adjust to room temperature (25°C) overnight. Using an electrical CDM 2e Conductivity Meter (Bach-Simpson, London, ON) electrical conductivity of electrolytes leaked were measured (EC). The test tubes were then placed in a heat air oven for 4 h maintained at 90°C to enforce complete lysis of cell tissues. The solutes were measured for their electrical conductivities (EC_a). Membrane injury index (MII) was calculated by using the formula:

$$MII = \left(\frac{EC_a - EC}{EC_a}\right) \times 100$$

(II) Whole Tree Temperature (°C)**:** To estimate the amount of heat generated by the trees as a result of mechanical stress, an infrared thermal imaging technology was adopted using Fluke Ti400 60Hz Thermal Imager (Fluke, Mississauga, Canada). Thermal images taken were processed with digital image processing tools (Fluke, Mississauga, Canada). The raw thermal images were obtained in FLUKE Systems' proprietary format. Spot temperatures for each tree and estimated average temperature for each tree in the frame was estimated. If need be, images were processed by converting them to grey-scale images in which each grey level represented 0.1°C. This conversion was performed by setting the temperature span of the images and saving them as bitmap images (BMP format) by means of Fluke SmartView®, version 3.7 software (FLUKE, Canada) and also converted the bitmap images to 8-bit uncompressed TIFF format by means of Adobe Photoshop CC 2015 software (Adobe Inc.).

(III) CO_2 Emission (μmol. mol^{-1})**:** Carbon dioxide emission was also measured after a 12 h incubation of trees in an airtight plastic bag chamber. After incubation, an LI-6400XT portable photosynthesis system (LI-COR, Canada) was used in measuring CO_2 emission (Norman et al. 1997).

(IV) Ethylene Evolution ($\mu L \cdot g^{-1} \cdot h^{-1}$)**:** Ethylene evolution was determined by first incubating trees in airtight plastic bag chambers for 12 h. 1µL air samples were collected from the chamber using an airtight needle and analyzed using GC equipped with FID. Evolution rates from the trees were calculated by the following equation:

$$\text{Ethylene evolution} = \frac{\text{Ethylene concentration (Initial – Final)} \times \text{Vol. of incubation chamber (L)}}{12h \times \text{Mass (weight of tree)}}$$

Where ethylene evolution is in $\mu L \cdot g^{-1} \cdot h^{-1}$, concentration is in $\mu L \cdot L^{-1}$, and mass is the dry weight (g) of a tree. The volume of airtight incubation plastic bag (109.21 L).

(V) VTC Evolution ($mM \cdot g^{-1} \cdot h^{-1}$)**:** To analyze VTC, whole trees were sealed in airtight plastic bag chambers for 30mins. Within that period, solid phase micro-extraction (SPME) fibre was exposed to the headspace for VTC extraction. GC analysis was performed and the chromatographic data of peak area (PA) against retention time (minutes) for each of the samples were defined. Confirmation of terpene compounds was established by comparison of retention times and peak areas with that of a known standard. The respective relative abundances for each sample were normalized against a β-pinene standard (Sigma-Aldrich Co. LLC, Canada). The concentration of individual and total VTCs in mM per fresh weight of trees in g was estimated and the evolution was calculated per the incubation period of 30mins.

For all statistical analyses, assumptions such as constant variance, independence and normality were tested prior to ANOVA using Minitab 17 (Minitab 17, Minitab Inc., PA, USA). The main effects tested were shaking and baling. Membrane injury index, temperature, CO_2 emission, ethylene and VTC evolution met normality assumptions and hence ANOVA was performed on these parameters. A Tukey's LSD test was used to establish the treatment differences upon significant effects.

3.4 RESULTS

3.4.1 Effect of Postharvest Mechanical Stress (Shaking and Baling) On Whole Tree Temperature

Duration of tree shaking had a significant effect on whole tree temperature (Table 1a). Whole tree temperatures were detected in the range of 23.8°C - 26.5°C when trees were subjected to five different shaking durations. Regression analysis suggested a significant relationship between temperature and duration of tree shaking (r^2=0.42) (Fig. 1). Control (0 seconds of shaking) was observed to have a lower temperature of 24.27°C, but temperature increased to 24.56°C and 25.13°C (i.e. by 0.29°C and 0.86°C) when trees were shaken for 10 and 15 seconds, respectively (Fig. 2). A drop in temperature to 24.80°C and 24.81°C was observed when tree shaking duration was increased to 30 and 60 seconds, respectively, and these were significantly higher than control (Fig. 2).

The study on a number of trees per bale suggests a significant effect of baling on tree temperature as depicted in Table 1a. Although average temperature of control trees was 24.27°C, a continuous increase in tree temperature to 24.80°C, 25.03°C, 25.28°C and 25.30°C was observed when the number of trees per bale was increased to 1, 2, 3 and 4, respectively (Fig. 3). A regression analysis supported the theory of increasing tree temperature with increasing number of trees in a bale, with a highly significant, strong, positive relationship between tree temperature and a number of trees per bale (r^2=0.7773) (Fig. 4).

3.4.2 Can Mechanical Stress Through Shaking and Baling Reduce Membrane Integrity of Trees

MII of balsam fir trees was significantly ($p=0.041$) influenced by shaking duration of trees (Table 1a). Membrane injury indices were detected in the range of 14.50 to 20.70% and were 20.80% higher shaken trees compared to control (Fig. 5). At 10 sec. shaking, MII of 20.70% was the highest recorded. Although there was a decline in MII to 16.90, 17.60 and 14.90% when shaking duration was increased to 15, 30 and 60 seconds, they were relatively higher than the control (14.5%) (Fig. 5). Regression analysis showed a poor relationship between the shaking duration and MII (Fig. 6).

Baled trees exhibited higher membrane disruptions compared to shaken trees, with membrane injury indices ranging between 14.54 to 28.08%. Membrane injury index was detected to be significantly influenced by the baling of trees ($p=0.002$) (Table 1a), where the bale of three trees was significantly higher than control (Fig. 7). Regression analysis also suggested a significant ($p=0.037$), strong and positive relationship between membrane injury and baling of trees ($r^2=0.6257$) (Fig. 8).

3.4.3 Mechanical Stress Effects on CO_2 Emission in Balsam Fir Trees

Data analysis did not suggest any significant changes in CO_2 emission when trees were shaken or baled. However, we observed higher CO_2 emission (1.53 μmol.mol^{-1}) in trees shaken for 60 seconds, compared to 1.21, 1.28, 1.09, 1.050 μmol.mol^{-1} in trees shaken for 0, 10, 20, and 30 seconds, respectively (Fig. 9). We also observed higher CO_2 emission in the bale of 2 (1.04 μmol.mol^{-1}) trees and the control (1.21 μmol.mol^{-1}), compared to 0.72 and 0.63 μmol.mol^{-1} in a bale of 1 and 3 trees, respectively (Fig. 10). Nonetheless, there was no indication of a relationship between CO_2 emission and shaking or baling of balsam fir trees (Table 1a). It is also worth mentioning that observation of high standard errors was made (Figs. 9 & 10), this could be attributed to higher variations in trees and instrumentation error.

3.4.4 Mechanical Stress Effects on Ethylene Evolution in Balsam Fir Trees

Data analysis suggested a significant (p=0.039) influence of tree shaking duration on ethylene evolution (Table 1a). Although we did not observe any significant trends, we recorded the highest levels of ethylene evolution (1.06 $\mu L \cdot g^{-1} \cdot h^{-1}$) at 10 sec. shaking, while 15 sec shaking emitted the lowest ethylene of 0.60 $\mu L \cdot g^{-1} \cdot h^{-1}$ (Fig. 11). Control trees emitted 14% more ethylene than shaken trees. On the other hand, we recorded a significant (p=0.048) effect of baling on ethylene evolution (Table 1a). A trending increase in ethylene evolution with increasing trees in a bale was observed. The lowest ethylene evolution was recorded in control trees at 0.99 $\mu L \cdot g^{-1} \cdot h^{-1}$ and a bale of four trees recorded the highest at 5.64 $\mu L \cdot g^{-1} \cdot h^{-1}$ (Fig. 11). Comparatively, it was observed that baled trees emitted higher levels of ethylene than shaken trees.

3.4.5 Effect of Mechanical Stress on VTC Evolution

VTC profile in this study was similar to what has been reported in our earlier study (Korankye, 2013). In this study, a total of 12 VTCs were consistently identified in balsam fir trees irrespective of the treatments or treatment levels. On the other hand, dynamic changes in individual VTCs of trees exposed to mechanical stress were established. Out of the twelve identified VTCs, ten (peaks 1 to 10) were monoterpenes whereas the remaining two (peak 11 and 12) were terpenoids (Figure 12). The concentration of individual VTC released by trees was dependent on treatment. Among characterized monoterpenes, 3-

Carene was recorded to be significantly (p=0.014) higher in baled trees (0.39 mM·g^{-1}·h^{-1}), compared to the control (0.20 mM·g^{-1}·h^{-1}), although it was not significantly different from shaken trees (0.35 mM·g^{-1}·h^{-1}). Other monoterpenes such as β-pinene were significantly (p=0.026) higher (0.34 mM·g^{-1}·h^{-1}) in baled trees when compared to control (0.08 mM·g^{-1}·h^{-1}) and shaken trees (0.09 mM·g^{-1}·h^{-1}) (Table 1b). Despite the similarity in trends of VTCs in all treated trees, VTCs such as D-limonene β-terpinene, α-pinene, 3-thujene, camphene, β–phellandrene, fenchyl acetate and bornyl acetate were not significantly different compared to the control.

Our results also showed a gradual decrease in total VTC with an increase in duration of tree shaking. Although I did not record any significant differences in total VTC, control trees emitted the highest VTC concentration of 0.87mM·g^{-1}·h^{-1} while 30 sec shaken trees emitted the lowest concentration of 0.67 mM·g^{-1}·h^{-1} (Figure 13a). On the contrary, baled trees showed a consistent and significant (p=0.045) increase in total VTC with an increase in the number of trees per bale (Table 1a). With the control trees emitting the lowest concentration of VTC (0.87 mM·g^{-1}·h^{-1}), the treatments where four trees were baled together emitted higher levels of VTC of 1.63 mM·g^{-1}·h^{-1} than control (Figure. 13b). VTC concentrations in baled trees were also found to be significantly higher than that of shaken trees.

Table 1a: Statistical P values for the main effects of shaking duration and number of trees per bale tree temperature, membrane injury, carbon dioxide emission, ethylene and volatile terpene evolutions of balsam fir based on ANOVA

Source of variation	Tree Temp.	MII	CO_2	Ethylene	VTC
Shaking duration	**<0.001**	**0.041**	0.587	**0.039**	0.104
Number of trees per bale	**<0.001**	**0.002**	0.444	**0.048**	**0.045**

Highlighted p-values were shown to be significant

Table 1b: Comparison of VTC profiles of balsam fir trees exposed to mechanical stress

	Stress Treatment		
VTC	Control	Shaking	Baling
3 – Carene	0.20^{b}	0.35^{a}	0.39^{a}
β – pinene	0.08^{b}	0.09^{b}	0.34^{a}

Means that do not share the same letters in a row are significantly different (*P*≤0.05).

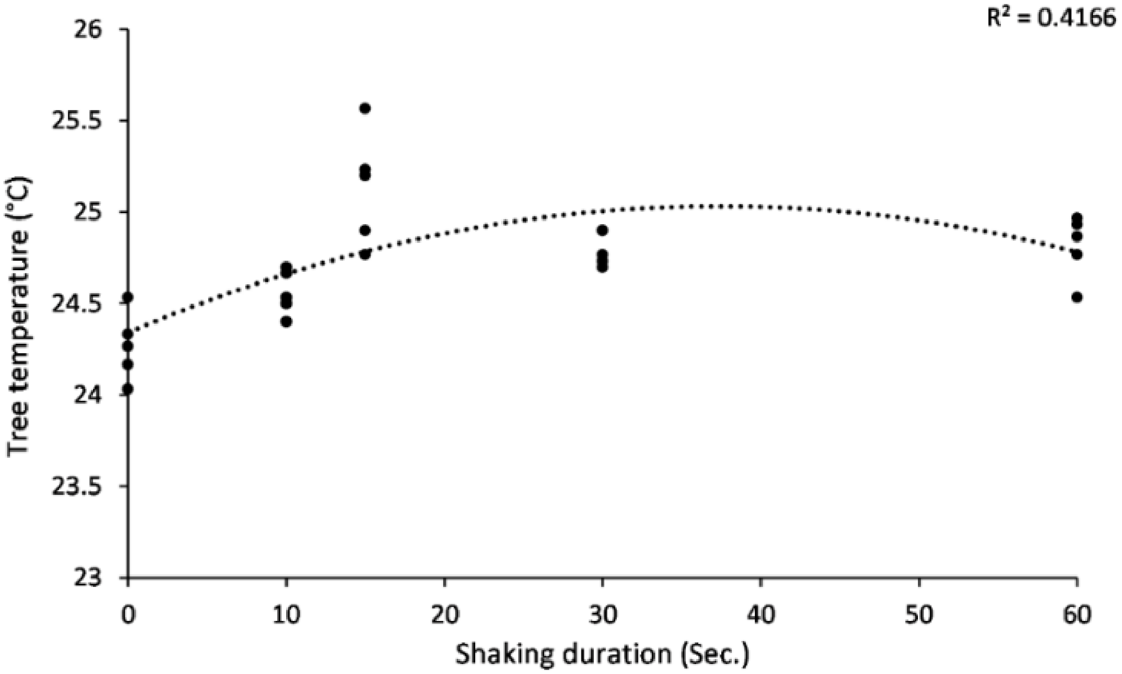


Figure 1: Relationship between the tree temperature (°C) and the shaking duration (seconds) of balsam fir (Abies balsamea L.) trees.

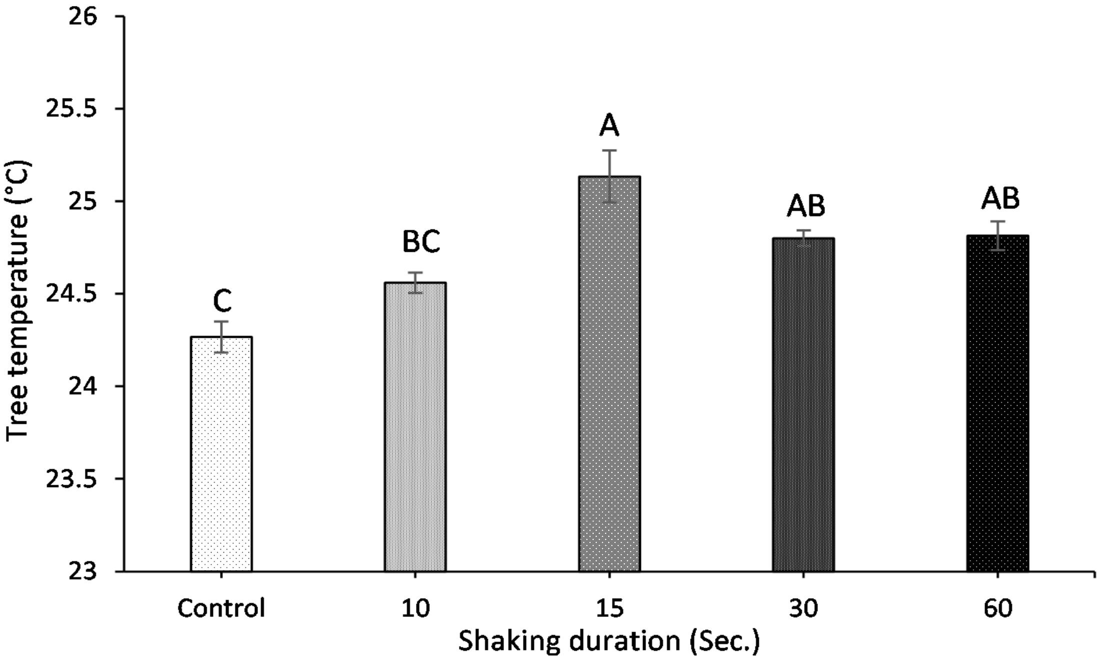


Figure 2: Temperature of shaken balsam fir trees. Any two means with the same letters are not significantly different (p≤0.05).

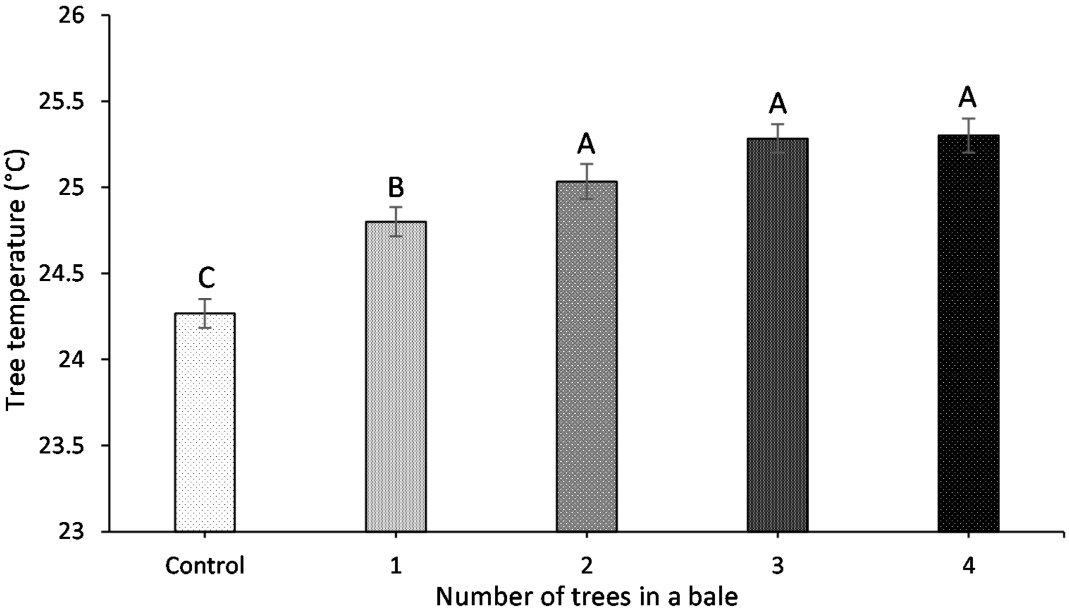


Figure 3: Temperature of baled balsam fir trees. Any two means with the same letters are not significantly different (p≤0.05).

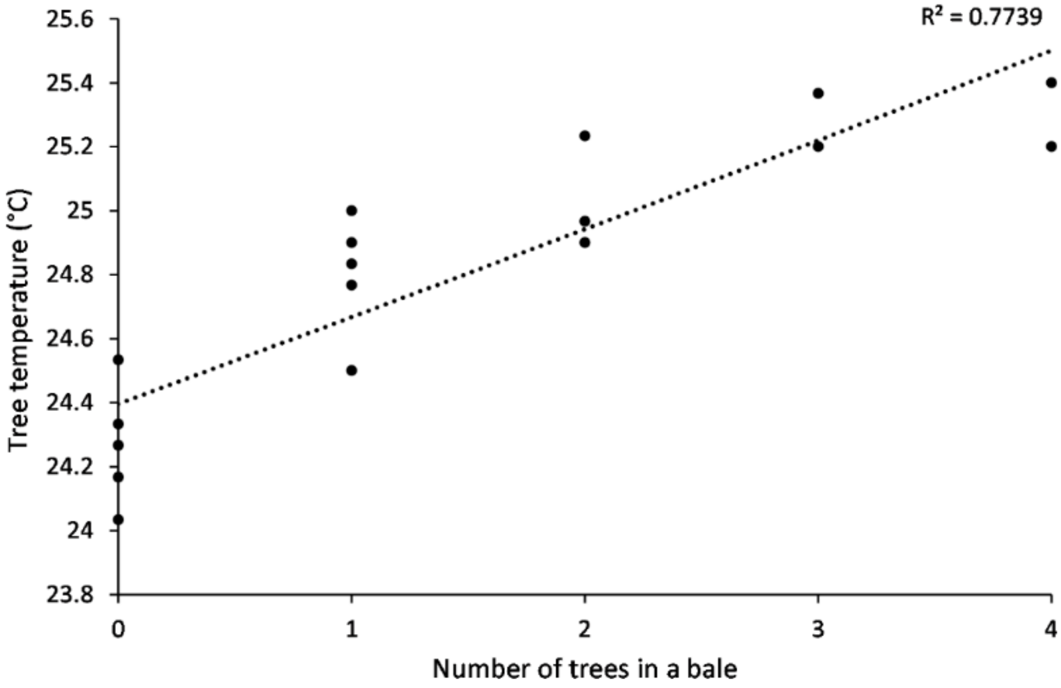


Figure 4: Relationship between the tree temperature (°C) and the number of trees per baled of balsam fir (Abies balsamea L.) trees.

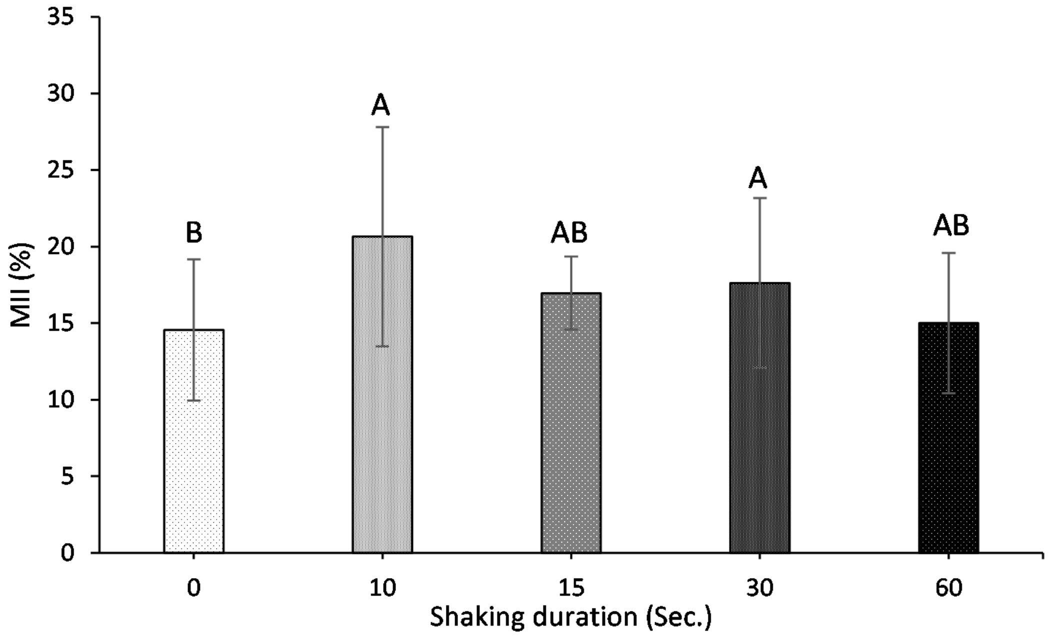


Figure 5: Membrane injury index of shaken balsam fir trees. Any two means with the same letters are not significantly different (p≤0.05).

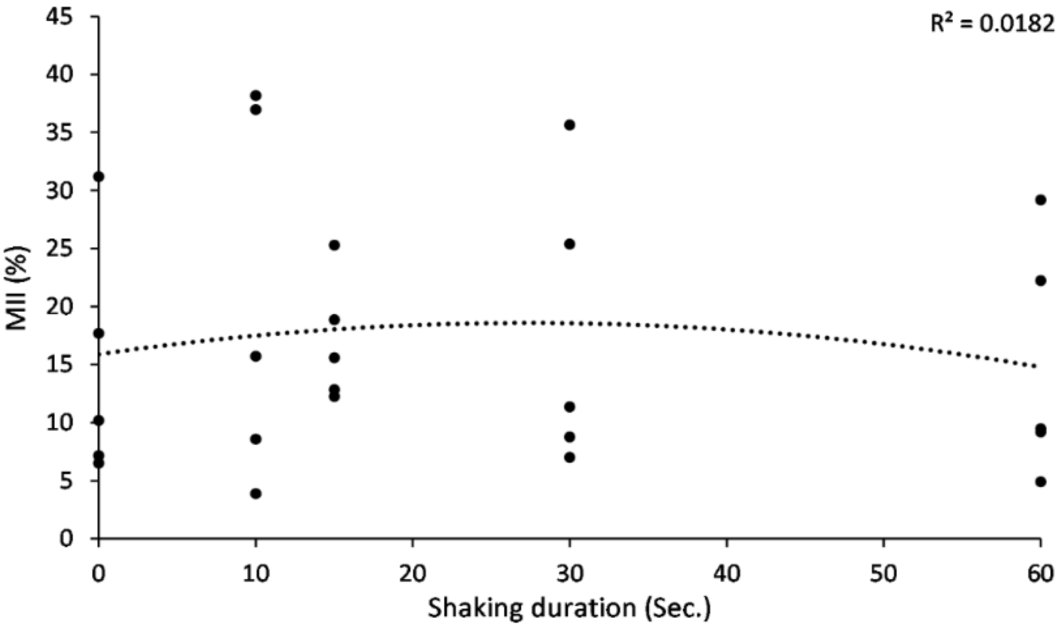


Figure 6: Relationship between the tree temperature (°C) and the number of trees per baled of balsam fir (Abies balsamea L.) trees.

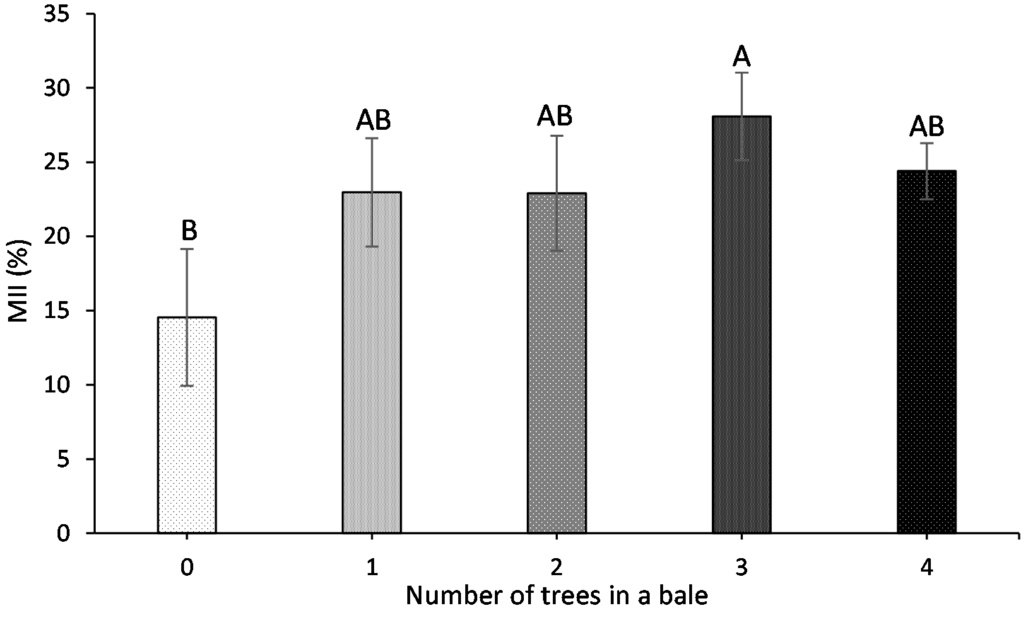


Figure 7: Membrane injury index of baled balsam fir trees. Any two means with the same letters are not significantly different (p≤0.05).

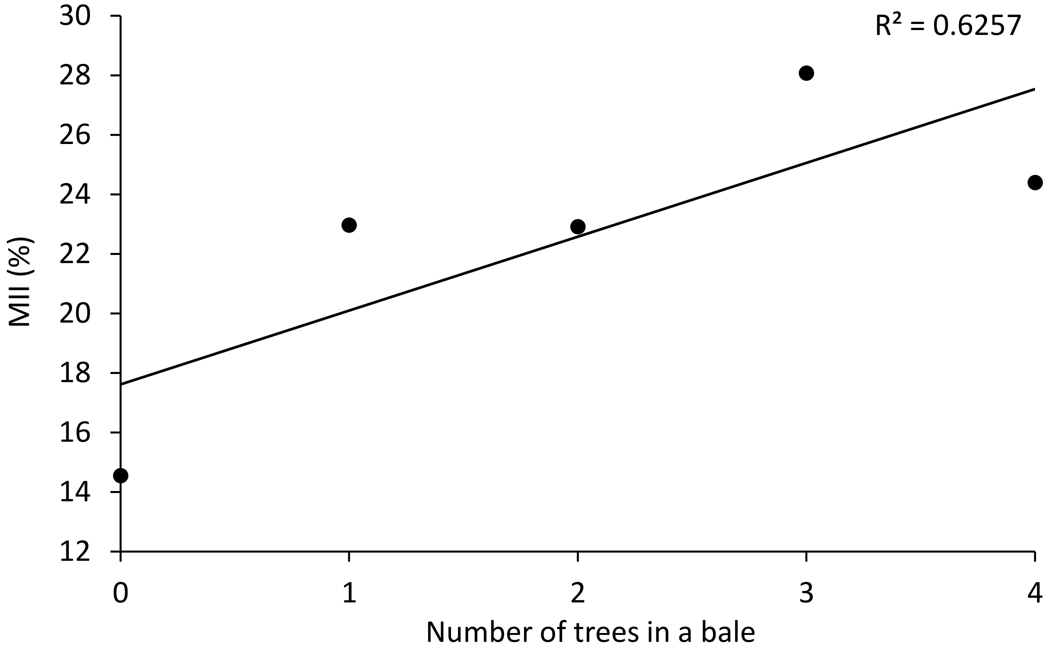


Figure 8: Relationship between the membrane injury index (%) and the number of trees per baled of balsam fir *(Abies balsamea* L.) trees, (p=0.037).

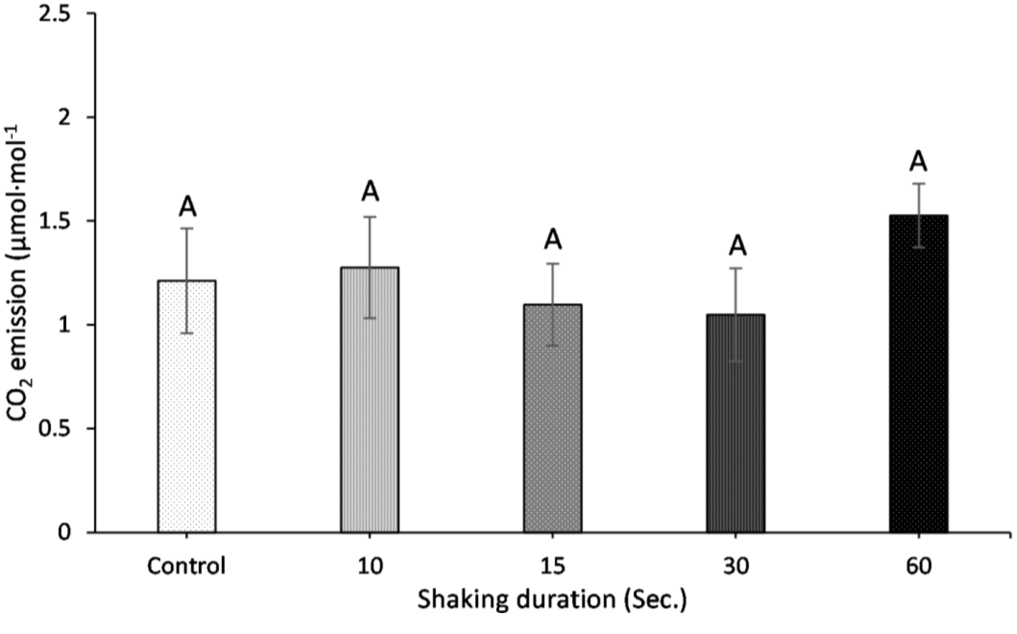


Figure 9: CO_2 emission in shaken balsam fir trees after a period of 0, 10, 15, 30 and 60 Sec. Any two means with the same letters are not significantly different ($p \leq 0.05$).

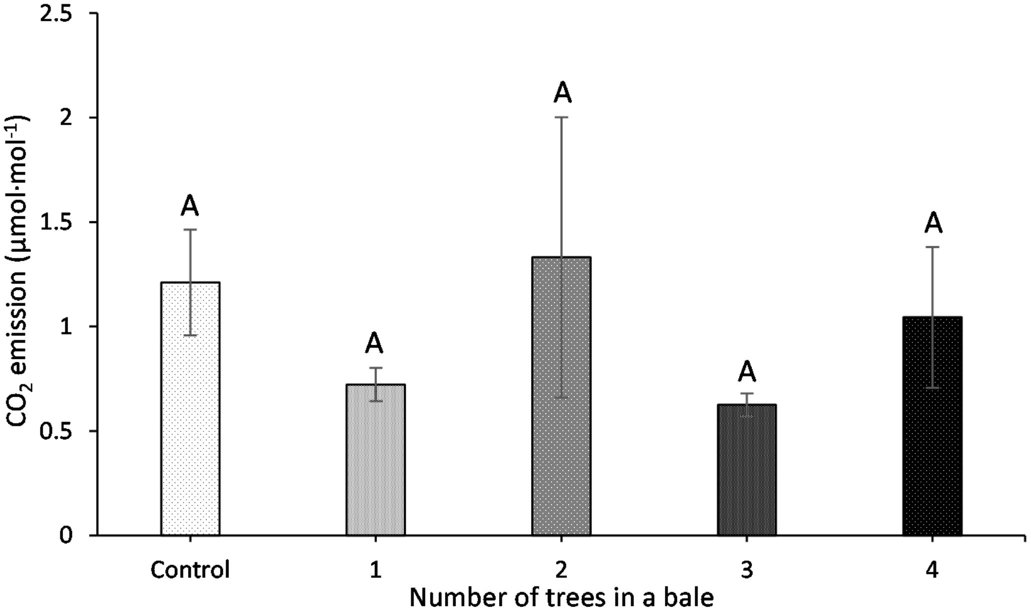


Figure 10: CO_2 emission in baled balsam fir trees of 1, 2, 3 and 4 trees. Any two means with the same letters are not significantly different ($p \leq 0.05$).

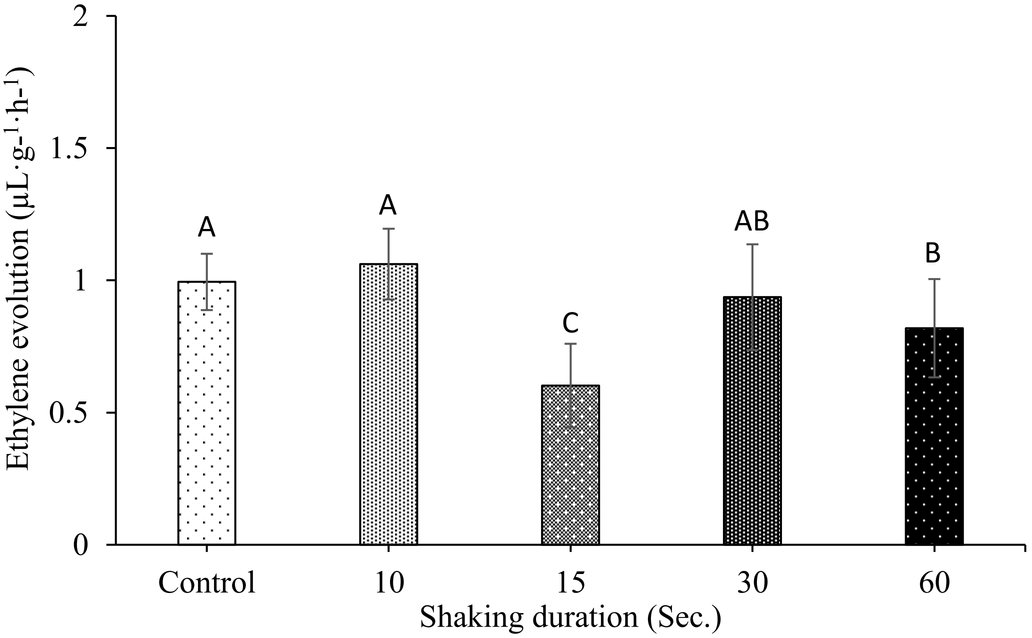


Figure 11: Ethylene evolution of shaken balsam fir trees. Any two means with the same letters are not significantly different ($p \leq 0.05$).

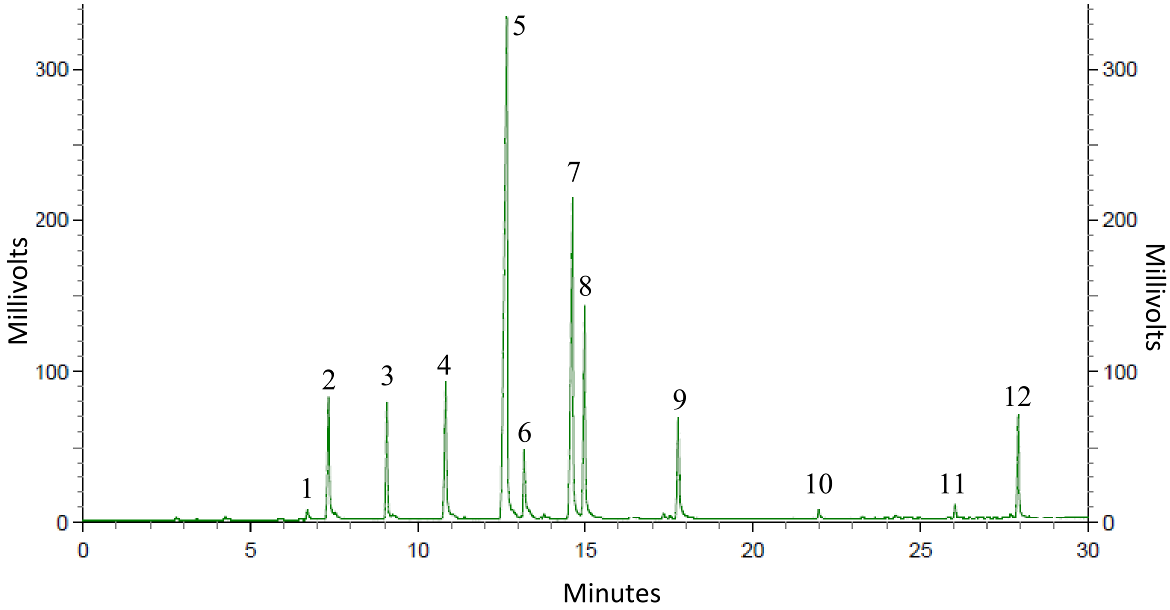
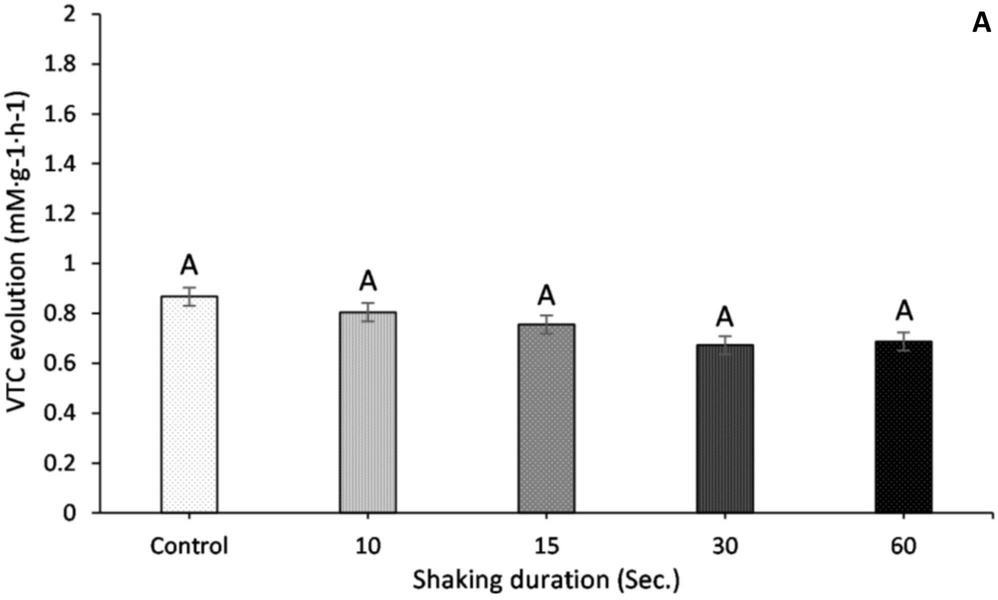


Figure 12: Volatile terpene compound profiles for balsam fir trees subjected to using SPME with headspace sampling after 30 mins equilibration. Compounds determined were: (1) α-Pinene; (2) 3-Thujene; (3) Camphene; (4) β-Pinene; (5) 3-Carene; (6) β–Terpine; (7) D-Limonene; (8) β –Phellandrene; (9) ɣ -Terpinene; (10) Terpinolene; (11) Fenchyl acetate; (12) Bornyl acetate.



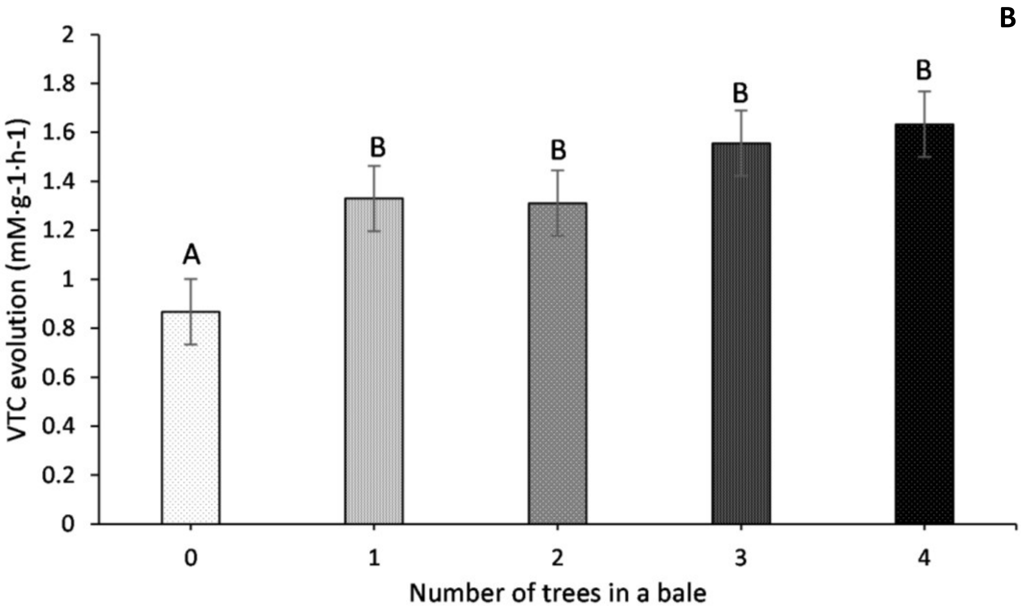


Figure 13: (A) Total VTC evolution of shaken balsam fir trees. Any two means with the same letters are not significantly different ($p \leq 0.05$). (B) VTC evolution of baled balsam fir trees. Any two means with the same letters are not significantly different ($p \leq 0.05$).

3.5 DISCUSSIONS

Plant temperature has long been recognized as an indicator of plant stress mostly linking it to increased respiration and decrease in transpiration (Leinonen et al. 2004). It is believed that when plants are under stress, such as water stress, transpiration is reduced or halted thereby, increasing plant or leaf temperature (Jackson et al. 1988; Chaves et al. 2002). This means that any physiological activity that leads to stomatal closure or cavitation can initiate an increase in plant temperature. A decline in stomatal closure within 2h after balsam fir branches were cut was reported (MacInnes, 2015). Similar conditions could be seen in this study. After harvesting, trees did not have access to water and therefore, they could be presumed to have been exposed to water stress prior to shaking or baling treatments. Injuries of needle breakage and bruises of branches due to shaking and baling could have also induced water loss and reduced transpiration causing increased temperatures by 0.86°C and 1.03°C, respectively, compared to the control. This phenomenon has been confirmed in various studies. In Blum et al. 1982, leaf temperature ranges of 1-2°C within genotypes and 4-8°C among genotypes of wheat were recorded when plants were under water stress. Similar findings were reported by Kumar and Tripathi 2008 where a decrease in transpiration rate by 7.6 µg $\mathrm{cm}^{-2} \cdot \mathrm{sec}^{-1}$ with an increase in temperature of 3.8°C was recorded when wheat plants were stressed.

In both imposed mechanical stresses due to shaking and baling in this study, a varying increase of 20.7% and 28.1% in membrane injury indices was observed. In potato (*Solanum tuberosum* L.) and tomato (*Solanum lycopersicum*) plants, cell lysis has been shown to increase by 70%, 4 h after wounding, resulting in 90% decrease in protoplast yield (Walker et al. 1984). In black spruce, exposure of trees to mechanical stress has been reported to increase electrolyte leakage by 51% (Zwiazek and Blake, 1990). In both cases of imposed mechanical stresses of shaking and baling in our study, we observed a varying increase of 20.7% and 28.1% in membrane injury indices, respectively. The major cause of the loss of membrane integrity has been attributed to weakened and damaged cell membranes after most mechanical stresses (Walker et al. 1984; Leon et al. 2001).

Our study did not give much clarity on the topic of CO_2 emission due to inconsistencies in results and high margins of error. However, studies over the years have suggested increased transpiration rate with increase in imposed mechanical stress. In a study by Kahl, 1951, he reported a 35% increase in transpiration above normal as a result of increased CO_2 assimilation in a shaking treatment of lettuce plants. On the other hand, a study by Akers et al. 1984 reported 18% reduction in CO_2 assimilation and eventual decrease in relative growth rate of *Pisurn sativurn* L. cv. Alaska. It has been speculated by few studies involving tomato and pea that the basis for diminishing CO_2 in mechanically stressed plants is a result of reduced stomatal aperture post-MIS (Mitchell et al. 1977: Akers et al. 1984). There is a reason to believe that mechanical stress in plants induces the production of gaseous plant hormones, ethylene. Although a study by Telewski, 1990 suggested otherwise, studies such as Jaffe and Biro (1979), Biro and Jaffe (1984) have shown that mechanical stress imposed through rubbing or flexing of plants cause substantial tissue damage thereby inducing the production of high ethylene levels. Goeschl et al. (1966) also demonstrated that when peas are pushed through glass beads, tissue damage occurs, inducing production of higher ethylene levels. In this study, similar trends were observed. Although shaking did not clearly show such a phenomenon, we saw more trees in a bale causing higher ethylene evolution. This could be speculated to be a result of varying intensities of these two postharvest practices. Although shaking is known to disturb tree tissues, there is no evidence of the intensity of tissue disruption. However, there is evidence to show that strings used in baling cause bruises and breakage of tree branches and needles, therefore, a more intense mechanical stress is anticipated to occur in baling treatments of trees. This is also evident in our results where baled trees evolved higher ethylene levels than shaken trees.

Although much is not known about the exact role of VTCs in plant defence, their synthesis and or emission in either herbivore-attacked or mechanically injured plants cannot be questioned. Studies such as Carlow et al. 2006 reported the emission of 3-carene and β-pinene in both Fraser fir (*Abies fraseri*) and Balsam fir (*Abies balsamea*) when trees were exposed to insect attack by the balsam woolly adelgid (*Adelges piceae*). After artificial wounding of Fraser fir (*Abies fraseri*) trees, Arthur and Hain, 1987 also detected α and β-pinene, 3-carene and limonene at the injury site. Similarly, in this study exposure of trees to baling resulted in 95% increase in 3-carene compared to control. Again, β-pinene increased by 3-folds when trees were baled compared to control. Several studies have shown that plant responses to forms of mechanical stress is similar, irrespective of what is causing the damage or wounding (Dudareva et al. 2004; Arimura et al. 2004). In a plant's response to mechanical injury, the transcription of (-)-germacrene D synthase gene *PtdTPS1*, that codes for various VTCs, sesquiterpenes have been found to be induced, similar to what happens in plants response to methyl jasmonate (MeJA) treatment (Arimra et al. 2004). Biosynthesis of VTCs occurs in the epidermal cells of the plant's tissues, from which they escape into the atmosphere (Chen et al. 2004) or into the glandular trichomes as found in peppermint, *Artemisia annua*. (Lu et al. 2002) after being synthesized. This explains the consistent induction of VTCs synthesis in response to both mechanical stresses of shaking and baling of balsam fir trees reported in this study.

3.6 CONCLUSIONS

The prolonged shaking of trees at or above 15 seconds lowered tree temperature, membrane injury, ethylene and in the case of VTC, a continuous decline with increasing shaking period was observed. Baling increased all stress indicators by 1.03°C in temperature, membrane injury by 2-fold, ethylene by 5-fold and VTC by 2-fold. This trend suggests that baling induces mechanical stress in postharvest trees. One of the weaknesses of this study was the lack of monitoring dynamic changes of these factors and how they affect needle abscission over a long period. Our next study focused on establishing the physiological effect of these handling practices on postharvest needle abscission of balsam fir trees, by looking at selective indicators such as NRD, AWU, ethylene and VTC and their role in stressed-imposed trees.

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CHAPTER 4: MECHANICAL SHAKING AND BALING OF BALSAM FIR TREES INFLUENCE POSTHARVEST NEEDLE SENESCENCE AND ABSCISSION

The following have been published as abstract and manuscript from this Section.

Ernest A. Korankye, Rajasekaran R. Lada, Samuel K. Asiedu and Claude Caldwell. 2015. Physiology of Postharvest Needle Abscission as influenced by Mechanical Stress. International Conference of American Society of Horticultural Sciences. August 2015. New Orleans, Louisiana (Poster presentation).

Ernest A. Korankye, Rajasekaran R. Lada, Samuel K. Asiedu and Claude Caldwell. 2018. Physiology of Postharvest Needle Abscission as influenced by Mechanical Stress. American Journal of Plant Sciences, 9, 339-352.

4.1 ABSTRACT

This study investigated the influence of both shaking duration and number of trees per bale on postharvest needle characteristics such as percentage needle loss, needle retention duration and explored the physiological roles of endogenous ethylene and volatile terpene compounds. To accomplish this task, two experiments were conducted. In the first experiment, 25 six-year-old trees were detached and exposed to a range of shaking durations (0 to 60 sec.). The second experiment was comprised of 30, six-year-old detached trees exposed to baling treatments from 0 to 5 trees. Response variables measured were percent needle loss, needle retention duration, average daily water use, ethylene evolution and VTC evolution. There was a general decrease in percent needle loss with increasing shaking duration. A 16% increase in needle loss of control trees was observed when compared to trees shaken for 60 seconds. On the contrary, baling increased postharvest needle loss in trees. A 13% increase in needle loss was recorded when 1 tree was baled, compared to the control. These trends corresponded with ethylene and individual VTCs (3-Carene, β-Pinene and β-Terpinene) evolutions, where longer the shaking duration or larger number of trees in a bale, higher the ethylene and VTC evolutions. One can, therefore, draw the inference that mechanical perturbation as a result of shaking and baling induce biosynthesis and regulation of ethylene and VTC in balsam fir trees in an effort to regulate postharvest needle abscission.

Keywords: Shaking, baling, ethylene, volatile terpene compounds, needle loss, thigmomorphogenesis

4.2 INTRODUCTION

Common practices among Christmas tree growers and exporters are shaking and baling of the trees. Shaking of trees is done to meet international-market quarantine standards since the practice is known to reduce or remove dust, pollen, debris and moulds off the trees, and remove any dead needles, broken branches and hidden insects (CTCNS, 2016). Baling of trees help shrink the sizes of trees, for easy handling and create more room for storage and transportation leading to an eventual reduction in storage and transportation costs. However, postharvest shaking and baling of trees have been suggested to cause mechanical stress, contributing to a significant negative effect on postharvest qualities of trees (Parkhurst et al. 1972). Mechanical stresses caused by shaking and baling of trees can cause physiological perturbations, which may cause postharvest needle abscission. Such a mechanically-induced postharvest stress has been reported in several species (Biddington, 1986; Chehab et al. 2009; Jaffe and Forbes, 1993). Mechanical perturbations as a result of wind, rain, handling and predations are among the many environmental stimuli to which plants respond (Biro et al. 1980; Chehab et al. 2009).

Plants have the ability to sense a wide range of forces from very intense and physical damage to more moderate ones and respond to these mechanical stimuli immediately or over time, depending on the level of stress perceived (Chehab et al. 2008; Chen et al. 2005; Karban and Baldwin 1997) by a phenomenon called “Thigmomorphogenesis” (Biro et al. 1980; Chehab et al. 2009). Plants mostly do so by synthesizing an array of phytohormones and other VTCs in addition to expressing defence-related genes (Chehab et al. 2009; Green and Ryan, 1972) mostly resulting in growth retardation, leaf senescence and possibly organ abscission (Jaffe and Biro, 1979). It has been reported that shaking of cocklebur plants cause an increase in the rate of leaf senescence (Salisbury, 1963). Similar studies have shown that plants respond to mechanical stimuli by an alteration in chlorophyll content and stomata closure leading to senescence and abscission (Biddington, 1986). It has also been established that after mechanical damage by harvesting of balsam fir trees there is an initial increase in ethylene evolution, a major contributing factor to postharvest needle abscission in balsam fir trees (Macdonald et al. 2010).

A similar phenomenon of mechanical perturbation is speculated to occur in root-detached balsam fir trees, from the harvesting, shaking to baling of the trees. The trees go through mechanical perturbation and then initiate immediate survival responses such as stomatal closure with the consequence of lower photosynthetic rate, initiating the synthesis of phytohormones such as ethylene (Macdonald et al. 2010) in the long term, and volatile terpene compounds (VTCs) (Korankye, 2013) all leading to the process of senescence and abscission in an attempt to communicate its distress to their community surrounding them and survive (Dicke et al. 2003; Jaffe and Forbes, 1993). It is hypothesized that shaking and baling of root-detached balsam fir trees is the initial trigger of postharvest needle abscission. The objective of this study was to establish the physiological effect of mechanical shaking and baling on postharvest needle abscission of balsam fir trees.

4.3 MATERIALS AND METHODS

Full root-detached trees of 91cm average height and 6-year-old were used in this study. All the trees were harvested during the regular harvesting season of Christmas trees in

December 2015. Prior to harvesting, visual inspection was conducted to avoid pest or disease infestation on the trees. Harvesting of trees was done using a handheld chainsaw as per the normal practice. Trees were then loaded into trucks and transported to the mechanical treatment site. In this study, there were two separate experiments. The first experiment which focused on the effect of shaking durations on postharvest needle retention followed a completely randomized design. Tree shaking durations ranged from 0 (control), 10, 15, 30 and 60 seconds. Each of the 5 experimental units had five trees, hence a total of 25 trees were selected for the experiment. After treatment, trees were weighed and set up in 4L glass jars containing 3L of water. The glass jars were refilled whenever water levels dropped significantly, and the amount of water added recorded and factored into the estimation of water uptake by trees.

The second experiment was focused on the effect of baling on postharvest needle retention and followed a completely randomized experimental design with the number of trees in a bale as the only factor. Six levels of tree baling were selected, and these were 0 (no baling-control), 1, 2, 3, 4 and 5 trees per bale and replicated 5 times, bringing the total to 30 trees selected. The trees were weighed and set up in 4L glass jars containing 3L of water. The glass jars were refilled whenever water levels dropped significantly, and the amount of water added a note.

Response variables were (i) percent needle loss – PNL (%), (ii) needle retention duration - NRD (day), (iii) average daily water use (AWU) per gram of fresh weight ($mL\cdot g^{-1}\cdot d^{-1}$), (iv) ethylene evolution ($\mu L\cdot g^{-1}\cdot h^{-1}$) and (v) VTC evolution ($mM\cdot g^{-1}\cdot h^{-1}$). These variables were measured following modified procedures from Macdonald et al. 2010 and Korankye, 2013. For all analyses, statistical assumptions such as constant variance, independence and normality were tested prior to ANOVA using Minitab 17 (Minitab 17, Minitab Inc., PA, USA). The main effects tested were shaking and baling. PNL. NRD, AWU, ethylene and VTC evolution met normality assumptions and hence ANOVA was performed on these parameters. A Tukey's LSD test was used to establish the treatment differences upon significant effects.

4.4 RESULTS

4.4.1 Percent Needle Loss (PNL)

ANOVA test performed showed that duration of tree shaking and number of trees in a bale had a significant effect on PNL (Table 2). Control trees recorded a 16% more needle loss compared to trees shaken for 60 seconds. Trees shaken for 10, 15 or 30 seconds lost 66.2, 62.0 and 57.2 % of needles compared to the control, respectively (Figure 14a). On the contrary, baling of trees significantly increased PNL (Table 2). A significant increase in PNL by 13% was observed when one tree was baled compared to the control (Figure 15a). PNL was 2.5-fold higher in shaken trees compared to baled trees (Figure 19b). Cumulative PNL trends in shaken trees showed that a significant needle loss commenced within the first week of postharvest, while in baled trees significant needle loss commenced 3 weeks postharvest (Figure 16). Within that period, a significant increase in needle loss was observed. There was up to 35% in needle loss of shaken trees, on the contrary, baled trees that lost 10% needles. It was also observed that shaken trees lasted 7 weeks after harvest and losing 80% of total needles, while baled trees lasted 9 weeks, losing a total of 57% needles (Figure 16).

4.4.2 Needle Retention Duration (NRD)

NRD was estimated as the number of days to 10% needle loss since most trees lose their market value by 10% needle loss. The data showed that duration of tree shaking, and hydration condition had a statistically significant effect on NRD ($p<0.001$) (Table 2). A pattern of increased days to 10% needle loss by shaking up to 15 seconds was seen. After 15 seconds of shaking trees, there was a decline in needle loss as days progressed. The best-performed trees were those shaken for 15 seconds, which took an almost two-fold increase in days to lose 10% of its needles, compared to the unshaken, control (Figure 17). Baling of trees had a significant effect on NRD ($p=0.002$) (Table 2). A linear trend of increased days to 10% needle loss with increasing number of trees per bale until 3 trees in a bale was seen. After that, a decline in days to 10% needle loss in 4 and 5 trees per bale was observed. Among the 5 levels of treatments, approximately two-fold increase in days to 10% needle loss in the bale of 3 trees compared to the control was observed (Figure 18).

4.4.3 Average Water Use (AWU)

Water consumption increased by 11% (0.11 mL g^{-1} day^{-1}) in baled trees compared to control (0.09 mL g^{-1} day^{-1}). On the other hand, there was no significant difference in water consumption when shaken trees were compared to control. However, there was an increase in water consumption by 2.5% when shaken trees (0.10 mL g^{-1} day^{-1}) were compared to baled trees (0.11 mL g^{-1} day^{-1}) (Figure 19).

4.4.4 Ethylene Evolution

Data showed that shaking trees had a significant effect on ethylene evolution (Table 2). We observed that on average, the control trees emitted 32.65% more (0.65 $\mu L \cdot g^{-1} \cdot h^{-1}$) of ethylene compared to the shaken trees (0.49 $\mu L \cdot g^{-1} \cdot h^{-1}$). There was no significant difference between the different levels of shaking duration. However, an increase in ethylene evolution with an increase in shaking duration (Figure 14B) was observed. On the other hand, data on baling showed that baling of trees had a significant negative effect on ethylene evolution (Table 2). It was observed that baling of only one tree resulted in a two-fold increase in ethylene concentration (0.48 $\mu L \cdot g^{-1} \cdot h^{-1}$) compared to the control (0.21 $\mu L \cdot g^{-1} \cdot h^{-1}$). There was also a decrease in ethylene evolution with an increase in number of trees per bale with five trees per bale recording the lowest ethylene evolution (0.20 $\mu L \cdot g^{-1} \cdot h^{-1}$) (Figure 15B).

4.4.5 Volatile Terpene Compound Evolution

Baling of trees had a significant effect on total VTC evolution (Table 2). It was evident that baled trees emitted 10% more total VTCs (7.25 $mM \cdot g^{-1} h^{-1}$) compared to the control (6.61 $mM \cdot g^{-1} h^{-1}$). The maximum total VTC concentration was observed in baling of 2 trees (11.24 $mM \cdot g^{-1} h^{-1}$) on the contrary, the lowest was observed in baling of 4 trees (5.04 $mM \cdot g^{-1} h^{-1}$) (Figure 20). Analysis of individual VTCs in this study showed that the amounts of β-Pinene, 3-Carene and β-Terpinene released were significant ($p=0.001$) higher in baled trees compared to control. The highest evolution was with 3-Carene (10.40 $mM \cdot g^{-1} \cdot h^{-1}$), followed by β-Pinene (5.12 $mM \cdot g^{-1} \cdot h^{-1}$) and β-Terpinene (3.19 $mM \cdot g^{-1} \cdot h^{-1}$), while control trees showed 0.04, 0.08, and 0.12 $mM \cdot g^{-1} \cdot h^{-1}$ respectively. We consistently observed a release of these three VTCs a week prior to increasing needle loss. However, when trees were shaken, no significant differences in individual VTCs were identified (Table 3).

4.4.6 Relationship Between Ethylene, VTC and Postharvest Needle Characteristics

There was a significant positive relationship between ethylene evolution and NRD. A significant correlation was observed between NRD and ethylene evolution in baled balsam fir trees with R^2 value of 0.052 and p-value of 0.048 (Table 4). Ethylene evolution peaked at 3.36 $\mu L \cdot g^{-1} \cdot h^{-1}$ four weeks postharvest, nonetheless, a week after that decreasing evolution was recorded in trees until complete needle loss was attained (Figure 21). On the other hand, a significant correlation was detected between NRD and VTC evolution with R^2 of 0.159 and p-value of 0.019 (Table 4). Like ethylene, peak VTC evolution was observed in the fourth week at a rate of 13.45 $mM \cdot g^{-1} h^{-1}$ (Figure 21).

Table 2: Statistical p-values for the main effects of shaking duration, hydration condition and number trees per bale on postharvest characteristics of balsam fir based on ANOVA

Source of variation	PNL	NRD	AWU	Ethylene Evo.	VTC Evo.
Shaking duration	<**0.00**1	<**0.001**	0.587	**0.041**	-
Number of trees per bale	<**0.001**	**0.002**	0.444	**0.048**	**0.034**

Table 3: Comparison of VTC profiles of balsam fir trees exposed to mechanical stress

	Stress Treatment		
VTC	Control	Shaking	Baling
3 – Carene	0.04^a	1.15^a	10.40^a
β – pinene	0.08^a	0.99^a	5.12^b
β – Terpinene	0.12^a	1.03^a	3.19^c

Means that do not share the same letters in a row are significantly different.

Table 4: The R^2 and statistical p-values for the relationship between ethylene and total volatile terpene compound evolution and NRD of baled balsam fir trees

	NRD of trees	
	R^2	P
Ethylene	0.052	**0.048**
VTC	0.159	**0.019**

**denotes a significant effect where p≤0.05

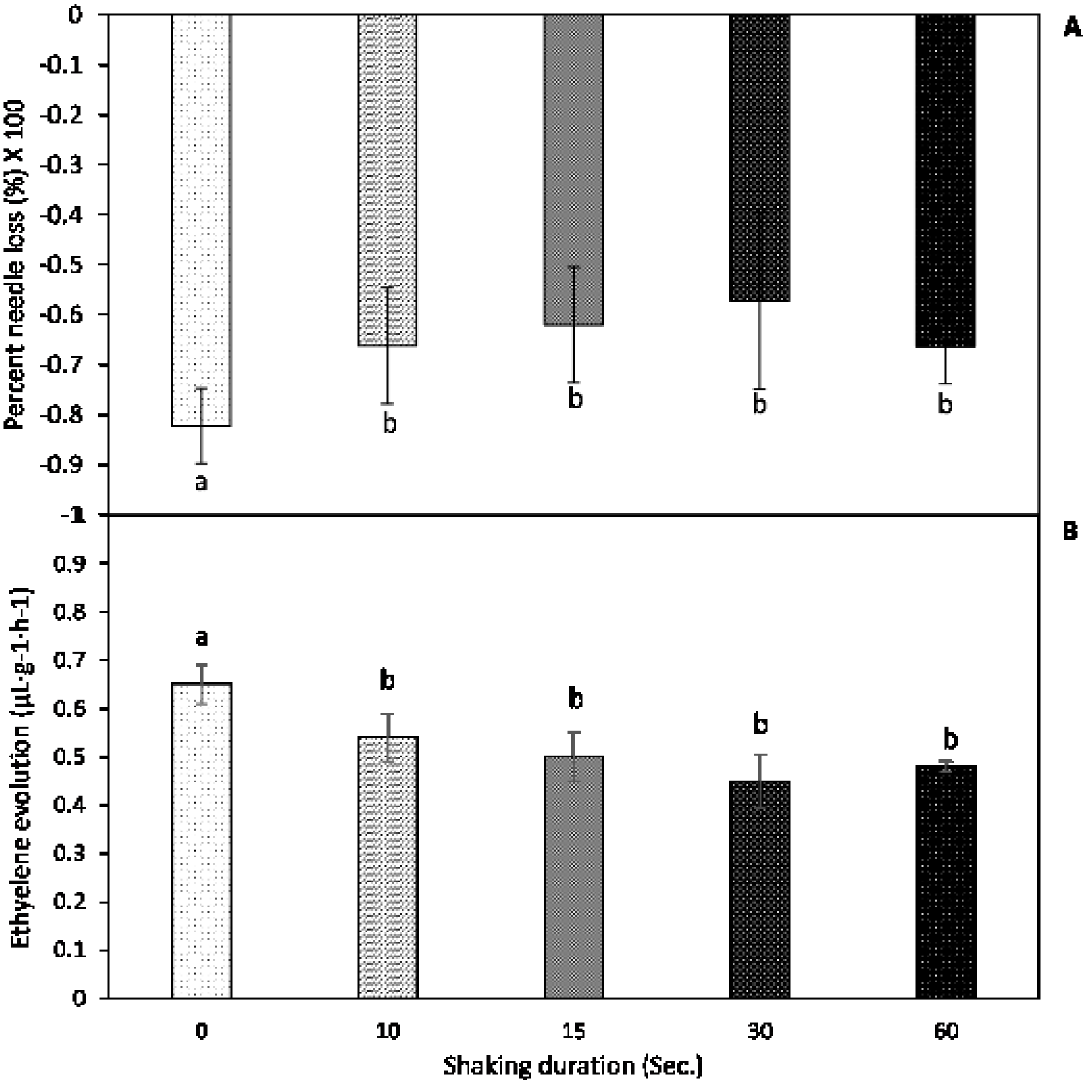

Figure 14: (A) Percent needle loss (%) and (B) Ethylene evolution (µL·g^{-1}·h^{-1}) of shaken balsam fir trees for a duration with standard error bars (n=5). Any two means followed by same letters are not significantly different ($p \leq 0.05$).

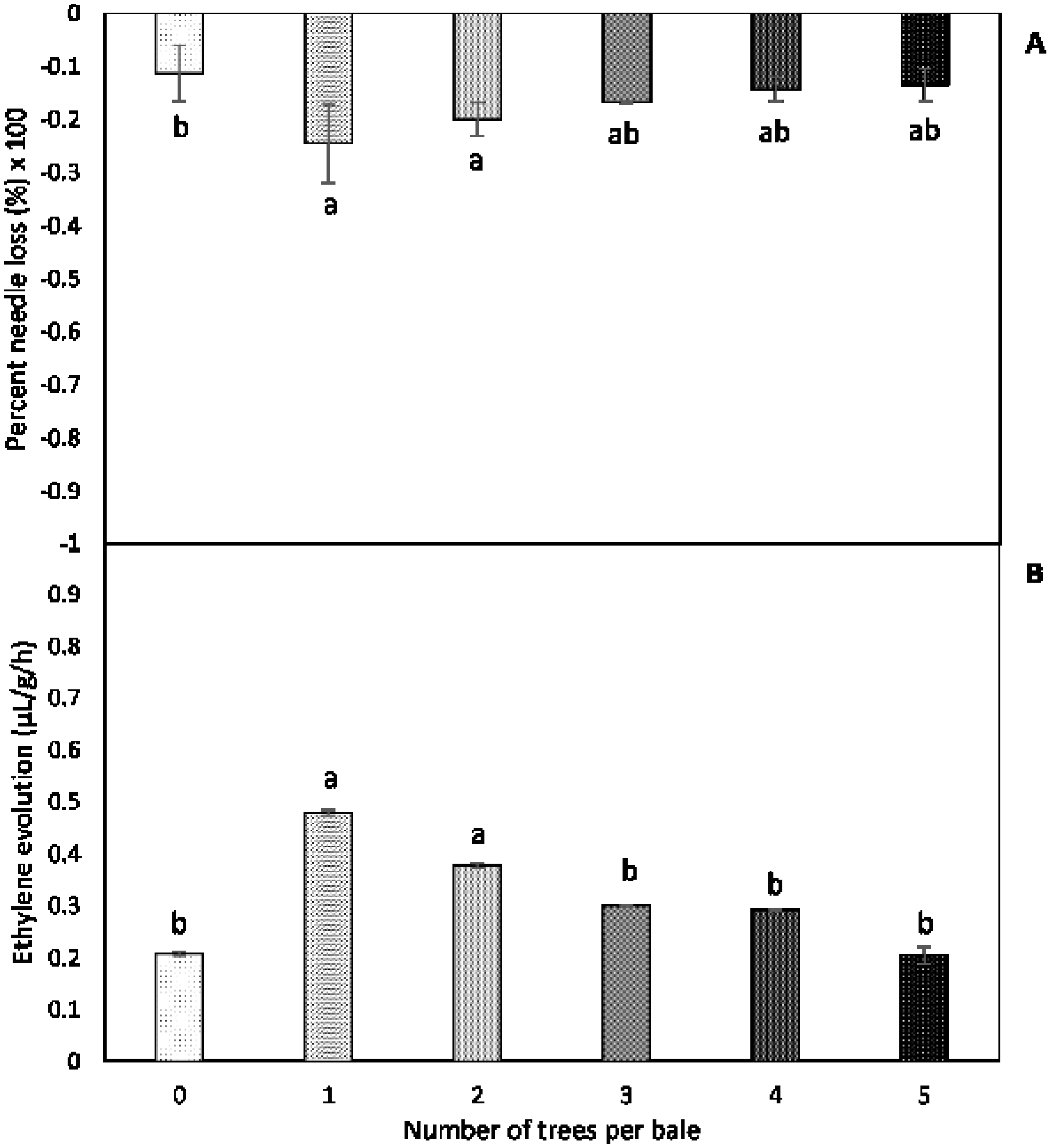

Figure 15: (A) Percent needle loss (%) and (B) Ethylene evolution (μL·g^{-1}·h^{-1}) of baled balsam fir trees with their standard error bars (n=5). Any two means followed by same letters are not significantly different (p≤0.05).

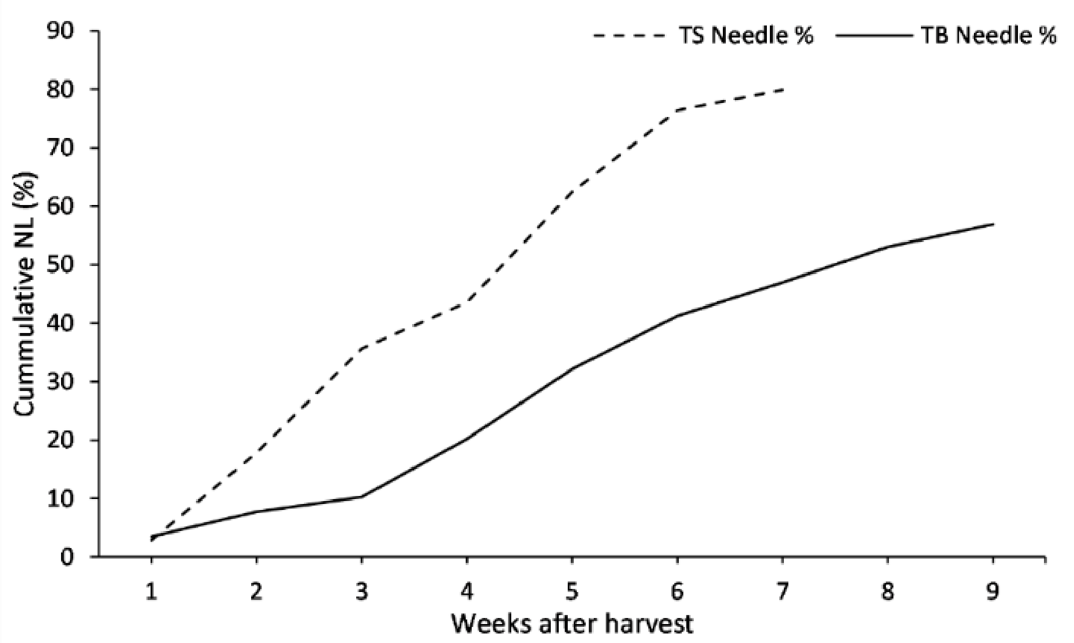

Figure 16: Cumulative PNL of the shaken tree (TS) and baled tree (TB). The continuous line indicates trend observed with PNL of the baled treatment. The broken lines indicate the PNL of shaking treatment.

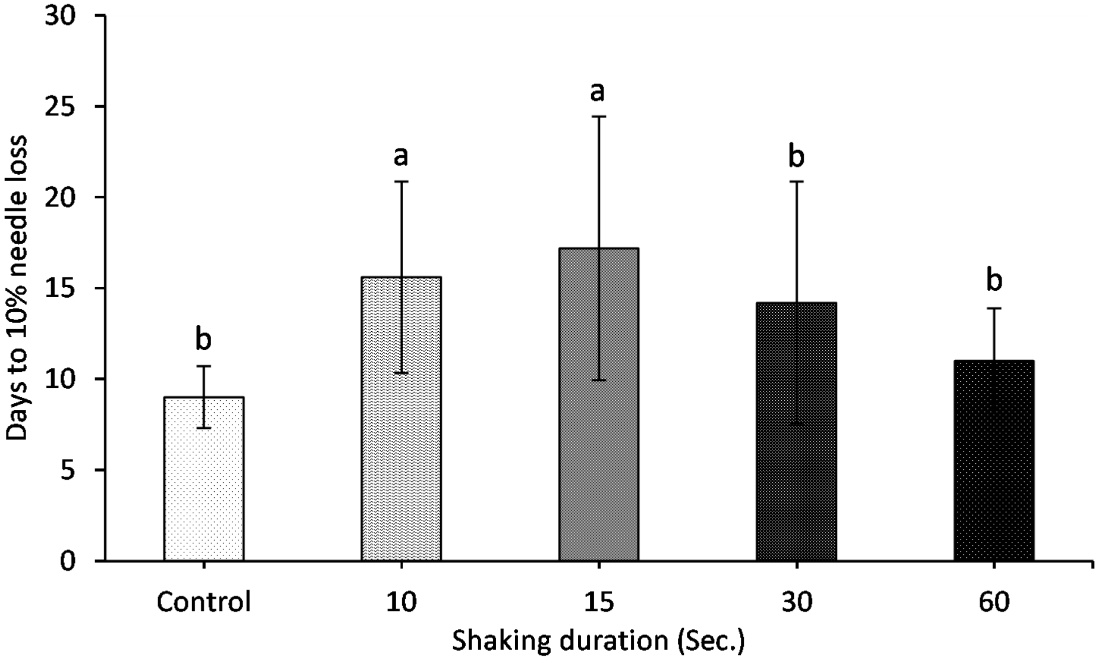

Figure 17: Days to 10% needle loss of shaken balsam fir trees for a duration with standard error bars (n=5). Any two-means followed by same letters are not significantly different (p≤0.05).

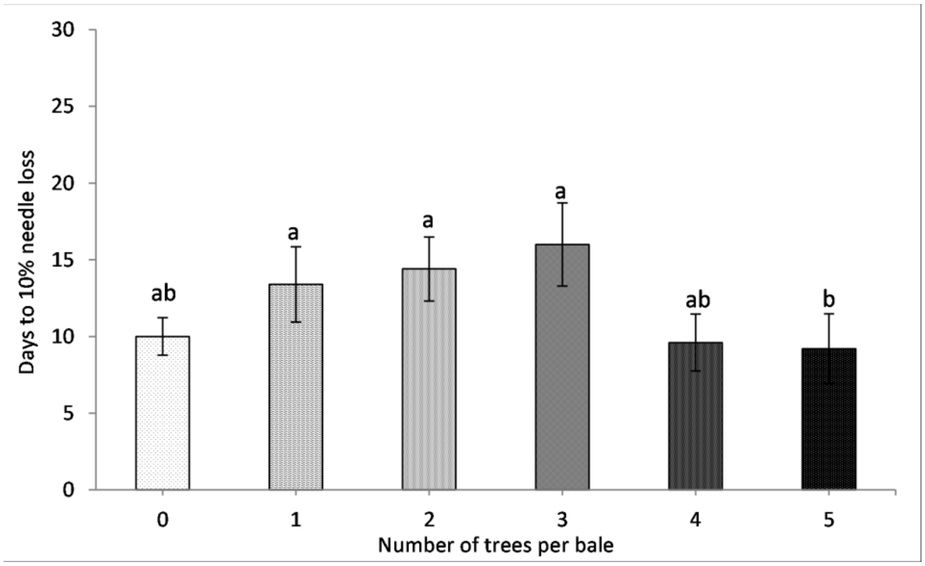

Figure 18: Days to 10% needle loss of baled balsam fir trees with standard error bars (n=5). Any two-means followed by same letters are not significantly different (p≤0.05).

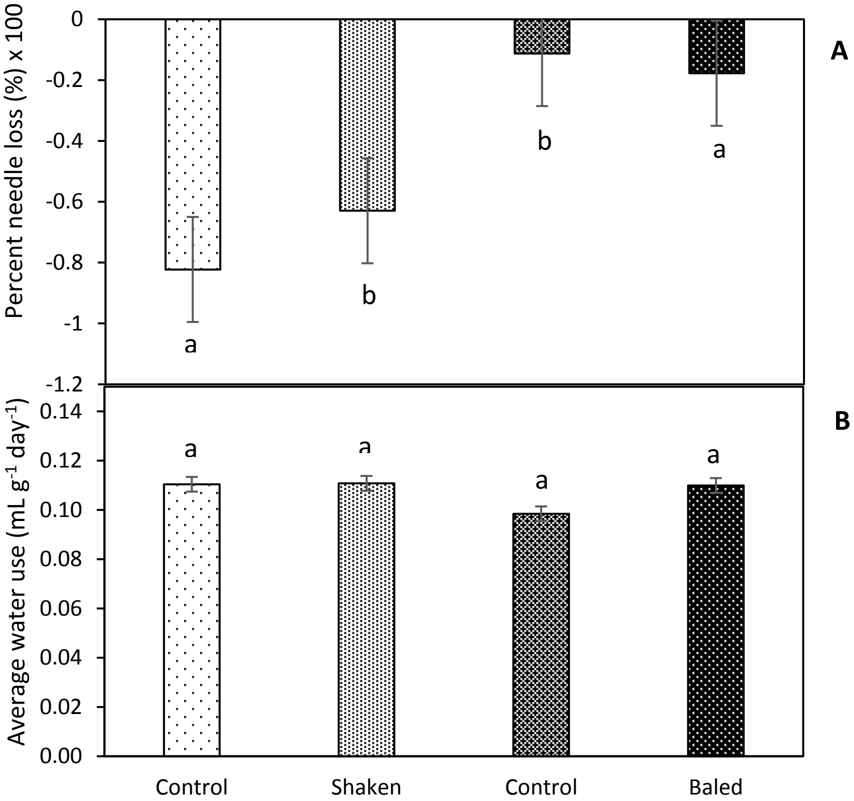

Figure 19: (A) Average water use of shaken and baled balsam fir trees. (B) Percentage needle loss of shaken and baled balsam fir trees with standard error bars (n=5). Any two means followed by same letters are not significantly different (p≤0.05).

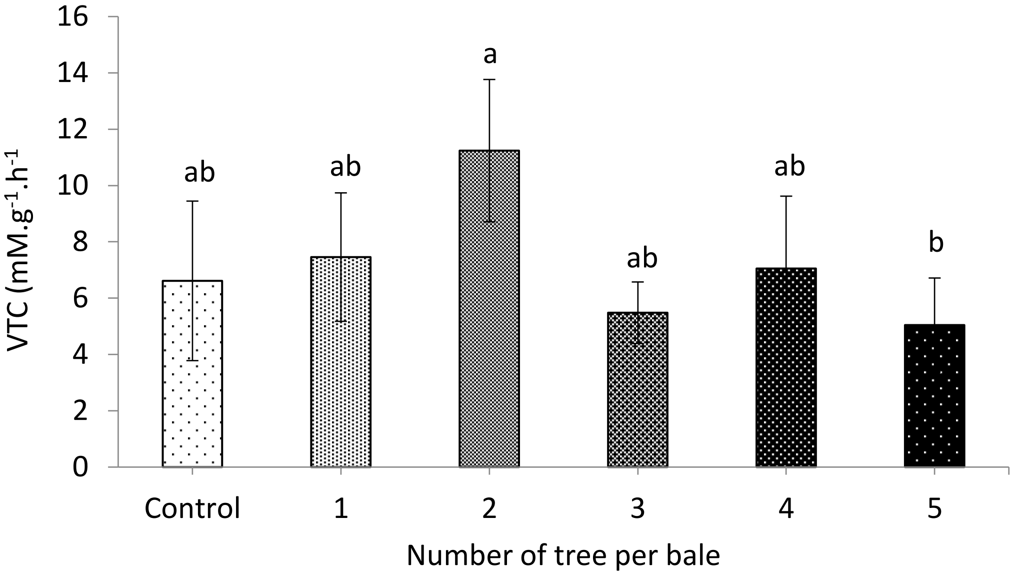

Figure 20: Volatile terpene compounds evolution of baled balsam fir trees with standard error bars (n=5). Any two means followed by same letters are not significantly different ($p \leq 0.05$).

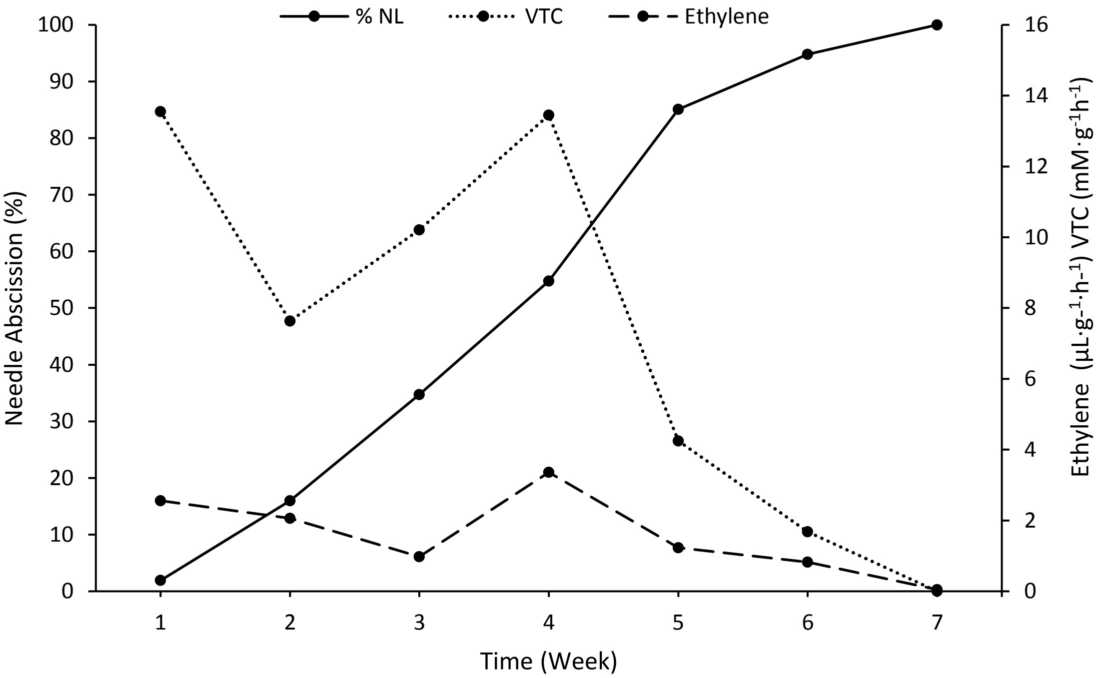

Figure 21: Dynamics of postharvest ethylene and VTC evolution in relation to needle abscission of balsam fir (Abies balsamea L.) trees

4.5 DISCUSSION

The ability of a tree to withstand any postharvest stress depends largely on the ability to rehydrate (Chastagner 1986; Rajasekaran and Blake, 1999). Therefore, it has been suggested that exposure of trees to any mechanical activity such as shaking, and or baling that can disrupt the water pathway and upstream transport of water will negatively influence the postharvest quality of the tree (Grace *et al*. 1982; Rajasekaran and MacDonald, 2015). One would expect that increasing the shaking duration of trees and number of trees in a bale will result in a significant decrease in water use compared to their respective controls. However, our results on water use by trees did not depict water stress (Figure 19). A possible explanation will be lower levels of shaking and baling imposed on trees, which may not be extreme enough to cause a significant mechanical damage, and consequent loss of turgor of the epidermal cells of needles as reported in Grace et al. (1982). However, we saw a significant 16% and 13% decrease in PNL of both shaken and baled trees respectively, in comparison to the control. A decrease in PNL with an increase in shaking duration (30 and 60 sec.) and a number of trees (4 and 5 trees) in a bale was also observed. This can be explained by the physiological perturbation where plants respond to mechanical-induced stress (MIS) by going through several physiological changes such as regulation of transpiration (Chehab et al. 2009). Plants respond to mechanically-induced stress is through stomatal regulation. We can, therefore, speculate that in response to baling, these trees kept stomata open to allow water uptake, as evident by a 11% increase in water use by baled trees compared to the control. This is beneficial to the trees' survival, which is evident in reducing postharvest needle loss and increasing NRD. Similar results have been reported by Grace et al. (1982) and Grace, (1974) where MIS in *F. arundinacea* through exposure to wind had more widely opened stomata than the control. Other studies in the past have also supported this phenomenon, where plants like *Prunella vulgaris* have been reported to recover from stress by rubbing of the stem (Jaffe and Forbes, 1993). Suge, (1980) also concluded that stroked leaves of *Phaseolus vulgaris* lost less water and reduced leaf abscission compared to untreated plants.

Historically, ethylene evolution has been reported to significantly increase with drought in jack and white pines (Rajasekaran and Blake, 1999; Islam et al. 2003), with biotic stresses in silver fir (Fuhrer, 1985) and ozone stress in Norway spruce (Van den Driessche and Langebartels, 1994). Though it is involved in a host of physiological processes, ethylene evolution due to stress is often associated with senescence and abscission as a stress response (Brown, 1997; Rajasekaran and Blake, 1999; Macdonald et al. 2010). Although there was no indication of water stress in this study, we recorded a significant increase in ethylene evolution (0.05 – 3.36 $\mu L \cdot g^{-1} \cdot h^{-1}$) from the beginning to the end in baled trees compared to the control. One would then ask if there could be an alternative signal(s) other than water stress that can trigger ethylene evolution. Studies such as Kende 1993; Lincoln et al 1993 and O'Donnelle et al, 1996 have confirmed that ethylene independently or together with JA serve as signal molecules to regulate the *pin* gene expression in response to wounding of plants. Ethylene release has also been found to increase in many different plant species following mechanical perturbation (Biro and Jaffe, 1984; Telewski, 1995). This could explain the detection of higher levels of ethylene in our study as a result of plant wounding through shaking and baling. Lower ethylene evolution rate was observed in the first few weeks but reached a peak several weeks after harvest. This pattern of ethylene evolution is also similar to studies by Alvarez-Moctezuma et al. 2007 and MacDonald et al., 2010, supporting the finding of the detrimental role of ethylene in postharvest needle abscission in mechanically stressed trees.

Prior to peak needle loss, peak VTC evolution was observed, irrespective of the treatment or treatment level, similar observations have been made in a study by Korankye (2013). We also observed as high as 10% increase in total VTC evolution in baled trees compared to control as well as significant increase in 3-Carene, β-Pinene and β-Terpene. VTCs have been described as protective secondary molecules, synthesized to protect the photosynthetic apparatus against oxidative damage (Loreto and Velikova, 2001; Lorento et al. 2004). Mechanical stress, wounding and environmental stress such as drought have been reported to trigger the active production of reactive oxygen intermediates (ROIs) by NADPH oxidases (Hammond-Kosack and Jones 1996; Orozco-Cardenas and Ryan 1999). This is known to pose a threat to cells and act as a signal for the activation of stress response and defence pathways (Knight and Knight 2001). Since oxidative stress is known to be linked to cell degradation and abscission (Valko et al. 2005), VTCs have been speculated to be synthesized and or evolved by plants during cell degradation to slow down or stop abscission. This could explain the increase in total VTC, and more specifically, increases in β-Pinene, 3-Carene and β-Terpinene that are previously speculated to induce postharvest needle loss in balsam fir trees. The trend for these VTCs to increase postharvest in balsam fir is similar to those reported by Korankye (2013).

4.6 CONCLUSION

In summary, shaking and baling of balsam fir trees affect postharvest needle retention characteristics. PNL was significantly lower when trees were generally shaken however, there is possibly some physiological benefit of lower PNL and higher NRD when trees are shaken for 30sec. PNL was significantly low in non-baled trees compared to the baled trees. It is therefore undoubtedly better not to bale the trees at all however, if baling is imperative, it is therefore recommended to bale at least 5 trees since our study showed that trees from the bale of 5 retained the highest percentage of needles for a longer period. Ethylene and VTC evolution were consistent with the trends in PNL. We observed an increase in the evolution of ethylene and VTCs with an increase in either baling period or number of trees per bale. We also observed a peak in both ethylene and VTC evolution in week four, a week prior to peak needle loss. These findings on ethylene and VTC and their impact on postharvest abscission significantly enhance our understanding of the intrinsic relationship between postharvest handling processes such as shaking, baling and endogenous hormonal and secondary metabolite changes in balsam fir trees. Despite these discoveries, there is still a knowledge gap in our understanding of the causes of postharvest needle abscission and technologies that can either stop or slow down the process of needle abscission in balsam fir trees and this still eludes scientists. In the next few chapters, we will explore environmental factors such as; temperature, vapor pressure deficit, water relations and membrane stability to understand how they regulate postharvest needle abscission and the ability to manipulate these factors to extend the shelf life of stress-imposed balsam fir trees.

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CHAPTER 5: THE ROLE OF STORAGE TEMPERATURE AND HANDLING ON POSTHARVEST STRESS IMPOSED ON BALSAM FIR TREES

The following has been published as an abstract and manuscript from this Section.

Ernest A. Korankye, Rajasekaran R. Lada, Samuel K. Asiedu and Claude Caldwell. 2017. Low-temperature Storage Overcomes the Negative Effects of Baling on Postharvest Needle Abscission in Balsam Fir Trees. International Conference of American Society of Horticultural Sciences. September 2017. Waikoloa, Hawaii (Poster presentation).

Ernest A. Korankye, Rajasekaran R. Lada, Samuel K. Asiedu and Claude Caldwell. Low-temperature Storage Overcomes the Negative Effects of Baling on Postharvest Needle Abscission in Balsam Fir Trees. Trees (Submitted for Publication, January 2018). In Press.

5.1 ABSTRACT

Needle abscission after harvest is a serious postharvest problem in balsam fir. Baling of trees has been shown to negatively affect postharvest needle abscission, however, exposure of trees to low temperature has been shown to delay needle abscission. In this study, we tested the hypothesis that low temperature can over-ride the negative effect of tree baling. Bales of six-year-old trees in groups of 3 and non-baled trees was exposed to varying temperatures of 5, 10, 20, or 30°C for 30 days while the absolute control was kept outside the laboratory at an ambient temperature of 3°C. After that trees were transferred to 4L jars containing 3L water at room temperature (22°C) until a minimum of 60% needle loss was achieved. Trees were observed for percent needle loss PNL (%), needle retention duration (NRD), average water use (AWU), ethylene and volatile terpene compound (VTC) evolution. The control (non-baled trees) irrespective of the storage temperature, lost needles within 14 days of storage primarily through discolouration. Trees stored at 20°C and 30°C lasted for a duration of 7 and 14 days at a significantly higher humidity of 83% and 85%, respectively. Our results showed that postharvest storage of trees under varying temperatures (absolute control - 3°C, 5°C and 10°C) strongly modulate NRD through its effect on AWU, ethylene and VTC evolutions. At lower temperature of 3°C, needle drop reduced possibly by higher AWU. This suggests that the ability of trees to take up water as depicted in high AWU can be beneficial to balsam fir trees by slowing down needle abscission, irrespective of the presence of ethylene and VTC.

Keywords: Balsam fir, temperature, needle abscission, ethylene, VTC

5.2 INTRODUCTION

Abscission is a reasonably well-studied phenomenon that is known to occur in all plant systems (Addicott, 1982), yet there is still a lot unknown. It is known to be accelerated by dehydration of plants (Escudero et al. 2008). Christmas trees after harvesting are normally baled and transported in open trucks and containers, which is speculated to accelerate and or contribute to the process of postharvest needle abscission predominantly as a result of dehydration. Our prior studies have suggested imposed mechanical stress by baling balsam fir trees. This is known to interact with water statuses such as water content and hydraulic properties causing dehydration, ethylene and VTC biosynthesis that accelerate postharvest needle abscission. Changes in ambient temperature are known to affect transpiration and therefore, water losses (Hardenburg et al. 1986; Cochard et al 2000). In a study by Cochard et al. 2000, plant transpiration responded linearly to temperature changes, where a decrease in temperature from 40°C to 7°C saw 80% decrease in transpiration. On the other hand, the temperature has been shown to greatly affect root and shoot hydraulic conductance as elaborated by Carvajal et al. 1996. Changes in hydraulic conductance with temperature have been attributed to changes in membrane fluidity (Ameglio et al. 1990) and water viscosity (Lopez and Nobel, 1991). Studies have shown that at temperatures between 15°C and 30°C, changes in hydraulic conductance ensues and is explained by mostly the modification in membrane fluidity (Cochard et al. 2000) and or membrane degradation in shoot or needle. A study by Sperry and Pockman, 1993 has drawn a correlation between changes in hydraulic conductance and transpiration as a result of ambient temperature changes. For example, when hydraulic conductance of *Betula occidentallis* was reduced as a result of low-temperature exposure, it caused stomatal closure. These studies have illustrated the significance of temperature on plant water relations and therefore, postharvest abscission. Similar inference can be drawn in reported postharvest NAS during and after transportation of baled trees, either in trucks or enclosed containers.

Accordingly, it was hypothesized that exposure of balsam fir trees to a low ambient temperature in shipping containers augment postharvest needle retention, irrespective of the mechanical stress imposed on trees. To test the hypothesis, this investigation was conducted with a general objective to determine the influence of postharvest storage temperature on baling-induced changes in needle abscission. The following individual objectives were set: (i) determine the influence of postharvest storage temperature on baling-induced changes in needle abscission; (ii) identify the optimal temperature that delays needle abscission under baling-induced conditions, and (iii) to establish the relationship between postharvest temperatures and needle abscission in balsam fir.

5.3 MATERIALS AND METHODS

Six-year-old trees from a single geographical location in New Germany, NS were used for this study to avoid any ecotype effects. Prior to harvesting, visual inspection was conducted to avoid pest or disease infestation on the trees. An average of 91cm tall trees with similar girth sizes was harvested using a chainsaw in September 2014. The experiment followed a 2 x 5 factorial design with 2 levels of baling, bale of 3 trees and control (no baling) and 5 levels of temperature storage at 5, 10, 20 and 30°C, control (room temp. at 22°C) and absolute control (trees outside at an average temperature of 3ºC). Nine replicates were run for each treatment unit.

Baling of tress was achieved using a Christmas tree baler (Howey Model, 210RC) that tied up trees in a bunch with a plastic twine. Under temperature treatments, trees were stored in a temperature-controlled growth chamber for 4 weeks with absolute control trees set outside. This duration was chosen to mimic the average duration trees stayed in storage containers during road transport within Canada and US and ocean shipment to other parts of the world. Although relative humidity (RH) in the chambers was not regulated, they were monitored throughout the entire period with 19% and 88% as a minimum and maximum RH respectively within various growth chambers. The controls (n=9) were kept in a separately controlled chamber for the same duration, where the ambient temperature and RH were held at 22°C and 40%, respectively. Following treatments, all trees were given a fresh cut at the trunk using aseptic secateurs. The excised trees were wrapped in a 2.5-inch thick foam and then placed in 4L glass jars containing 3L of sterile distilled water. The foam was used to limit the loss of water by evaporation from the glass jars. The fresh weight (kg) of each tree and the mass of the whole assembly were recorded for all the experimental units. Nine replicates from each of the treatments were then transferred to a controlled room with ambient temperature and RH of 22°C and 40%, respectively until trees loss all needles.

Percent needle loss – PNL (%), needle retention duration - NRD (day), average weekly water use (AWU) per gram of fresh weight ($mL \cdot g^{-1} \cdot w^{-1}$), ethylene evolution ($\mu L \cdot g^{-1} \cdot h^{-1}$) and VTC evolution ($mM \cdot g^{-1} \cdot h^{-1}$) were measured as per protocol described in Chapter 2.5. Before statistical analysis, all data were checked for normality. Whenever necessary, transformations were performed to achieve normality. All the response variables were analyzed using Minitab 17 (Minitab 17, Minitab Inc., PA, USA). Upon significant p-values, Tukey's LSD test was adopted to separate means.

5.4 RESULTS

5.4.1 Effect of Storage Temperature on Postharvest Needle Retention

By visual description, the non-baled trees, irrespective of storage temperature were completely dead within 14 days of storage, primarily through discolouration. NRD during storage was significantly influenced by storage temperature ($p<0.001$; Table 5). In temperature-controlled storage, all trees stored at 20°C and 30°C lasted for a duration of 14 and 7 days (Figure 22b) at a significantly higher humidity of 83% and 85%, respectively. Needle loss was manifested through discolouration as shown in Figure 22. Post-storage NRD was negatively related to storage temperature. NRD at 23 days was recorded for the absolute control (trees stored outside at an average temperature of 3°C) and was significantly higher than trees subjected to 5°C and 10°C storage temperatures. An increase in temperature to 5°C and 10°C saw a decrease in NRD to approximately 8 days in both treatments with average relative humidity of 60% (Figure 23).

5.4.2 Effect of Storage Temperature on Water Uptake by Trees

Average water use (AWU) was strongly regulated by storage temperature of trees ($p<0.001$: Table 5). I observed a significant drop in the amount of water consumed by trees from 110 $\mathrm{mL{\cdot}g^{-1}{\cdot}w^{-1}}$ to 30 $\mathrm{mL{\cdot}g^{-1}{\cdot}w^{-1}}$, when storage temperature was increased from control (3°C) to 5°C. A further drop in water use to 20 $\mathrm{mL{\cdot}g^{-1}{\cdot}w^{-1}}$ in trees stored at 10°C, although that was not significantly different from trees stored at 5°C (Figure 23).

5.4.3 Effect of Storage Temperature on VTC Evolution

Data on VTC and ethylene evolution monitored during the four-week storage showed a significant effect of storage temperature on total VTC. As illustrated in Figure 24, a significantly lower VTC evolution (2.03 $mM\cdot g^{-1}\cdot h^{-1}$) was observed in control trees, compared to 5°C (7.62 $mM\cdot g^{-1}\cdot h^{-1}$) and 10°C (4.95 $mM\cdot g^{-1}\cdot h^{-1}$), although there were no significant differences in VTC among trees stored at 10, 20 and 30°C (Figure 24). Despite the significant effect of storage temperature on total VTC, there were no significant differences recorded among individual VTCs during the first four weeks of storage. After four weeks of storage and set up of the experiment under room temperature, total VTC was again shown to be significantly affected by storage temperature (Table 5). VTC evolution in control trees was 51 % higher (5.44 $mM\cdot g^{-1}\cdot h^{-1}$) than trees stored at 5°C (3.60 $mM\cdot g^{-1}\cdot h^{-1}$) and 2-fold higher than trees stored at 10°C (2.50 $mM\cdot g^{-1}\cdot h^{-1}$) (Figure 25). However, there was no significant difference in total VTCs among trees stored at 5°C and 10°C (Figure 25). Upon further analysis, it was observed that individual VTC, 3-carene was significantly ($p=0.025$) lower by 16-fold in control trees (0.52 $mM\cdot g^{-1}\cdot h^{-1}$) and 3-fold lower in trees stored at 5°C (2.3 $mM\cdot g^{-1}\cdot h^{-1}$), compared to trees stored at 10°C (8.90 $mM\cdot g^{-1}\cdot h^{-1}$) (Table 6). A spike in the concentration of 3-carene a week prior to peak needle abscission was observed in all treatments and was found to be significantly higher (0.66 $mM\cdot g^{-1}\cdot h^{-1}$, $p<0.001$) in trees stored at 10°C when compared to control trees. Although β-pinene and β-terpinene levels were also recorded to increase prior to peak needle loss, no significant differences were observed.

5.4.4 Effect of Storage Temperature on Ethylene Evolution

A significantly lower ethylene evolution (0.77 $\mu L \cdot g^{-1} \cdot h^{-1}$) was observed in trees stored at 5°C during the 4-week storage. Although it was not significantly different from the control, 10°C and 30°C, trees stored at 20°C had a significantly higher ethylene evolution (1.1 $\mu L \cdot g^{-1} \cdot h^{-1}$) compared to the trees stored at 5°C (Figure 24). After four weeks of storage, ethylene evolution followed a similar trend to that of the VTC evolution, which was significantly influenced by storage temperature (Table 5). Data analysis showed an approximately 2-fold decrease in total ethylene evolution from 2.57 $\mu L \cdot g^{-1} \cdot h^{-1}$ in control compared to 1.5 $\mu L \cdot g^{-1} \cdot h^{-1}$ when storage temperature was set to 5°C. A drop of approximately 3-folds (1.11 $\mu L \cdot g^{-1} \cdot h^{-1}$) was also recorded when storage temperature was at 10°C (Figure 25).

5.4.5 Relationship Between the Effects of AWU, VTC and Needle loss

Figure 26 illustrates the trend observed with cumulative water consumption of the trees stored at control, 5°C and 10°C. There is a consistent ability of control trees to consume significantly more water compared to trees stored at 5°C and 10°C. It also illustrates a sharp increase in water consumption among the control trees throughout the study in comparison to 5°C and 10°C storage that had a consistent level of water consumption. There was a strong relationship between AWU, VTC and postharvest NRD ($R^2 \geq 0.624$: Figure 27a, and b). NRD had a positive relationship with AWU with R^2 of 0.62 with high NRD of 27 days and at AWU of 0.062 $mL \cdot g^{-1} \cdot w^{-1}$ for control trees (Figure 27a). A similar positive relationship was illustrated with VTC with R^2 of 0.67 with high needle loss of 27.52g and at VTC of 60.47 $\mu L \cdot g^{-1} \cdot h^{-1}$ for control trees (Figure 27b).

Table 5: Statistical p-values for the effect of storage temperature on various factors that influence needle quality

Source of Variation	PNL	NRD	AWU	Ethylene	VTC
Storage temperature	1.00	**<0.001**	**<0.001**	**0.003**	**0.006**

Values indicated in bold face indicate significant difference at p≤0.05

Table 6: Comparison of VTC profiles of balsam fir trees exposed to temp-control storage

	Storage Temperature		
VTC	Control (3°C)	5°C	10°C
3 – Carene	0.52^{c}	2.30^{b}	8.90^{a}

Mean VTC ($mM \cdot g^{-1} \cdot h^{-1}$) that do not share the same letters are significantly different.

Control 5°C 10°C 20°C 30°C Control 5°C 10°C 20°C 30°C Control 5°C 10°C

Figure 22: Illustration of postharvest needle abscission characteristics as influenced by storage temperature (A) Before storage treatment (B) After 30 days in storage (C) 21 days after storage treatment.

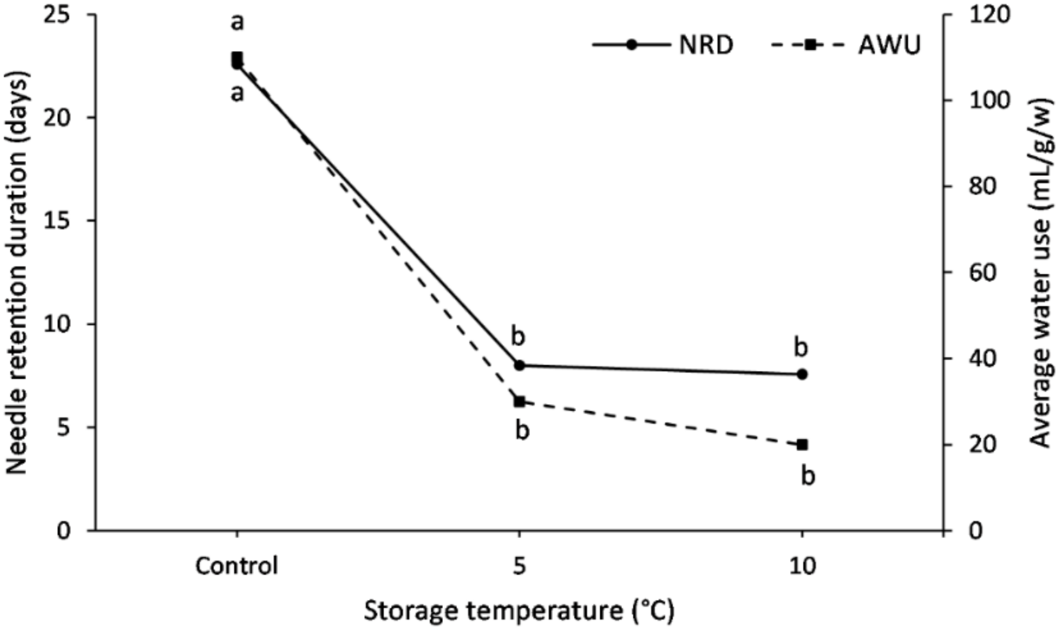

Figure 23: The effect of storage temperature control, 5°C and 10°C on needle retention duration (days) and average water use (mL/g/w) of balsam fir trees. Each point represents an average of seven replicates. Same letters represent no significant difference at p≤0.05.

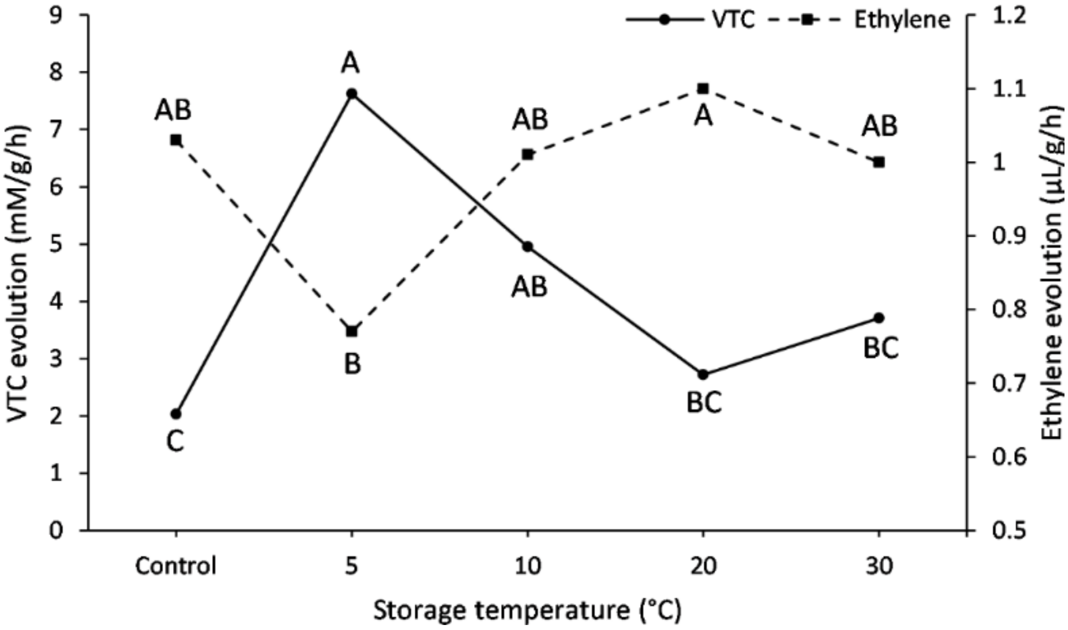

Figure 24: The effect of storage temperature 5, 10, 20, 30°C and control on volatile terpene compounds (mM·g-1·h-1) and ethylene (µL·g-1·h-1) evolution during 4-weeks controlled temperature storage. Each point represents an average of seven replicates.

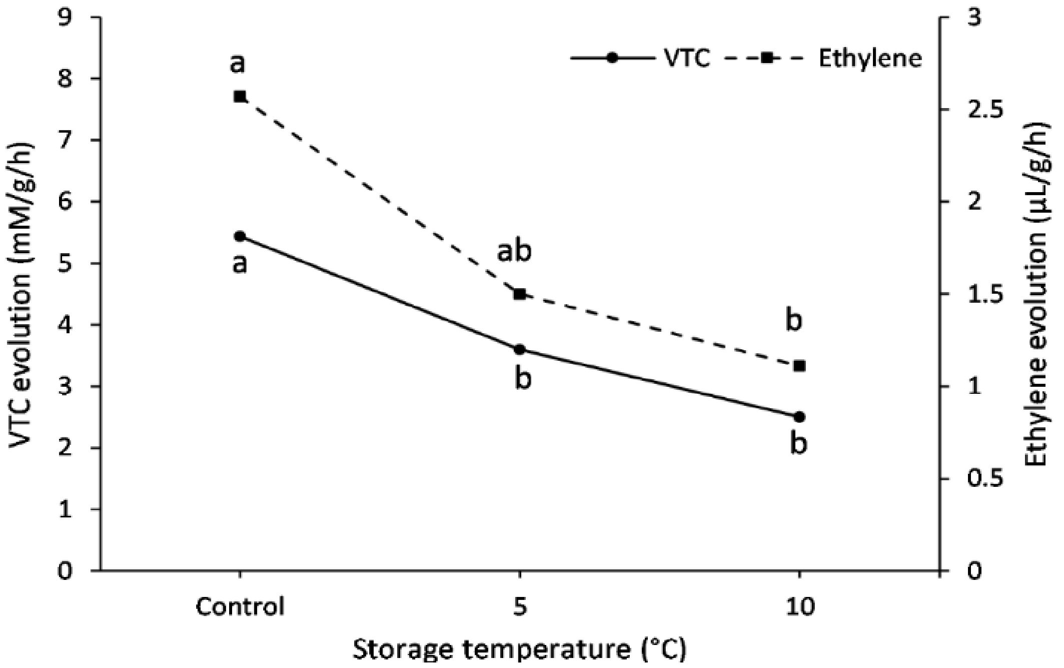

Figure 25: The effect of storage temperature control, 5°C and 10°C on volatile terpene compounds ($mM \cdot g^{-1} \cdot h^{-1}$) and ethylene ($\mu L \cdot g^{-1} \cdot h^{-1}$) evolution after four weeks of storage. Each point represents an average of seven replicates. Same letters represent no significant difference at $p \leq 0.05$.

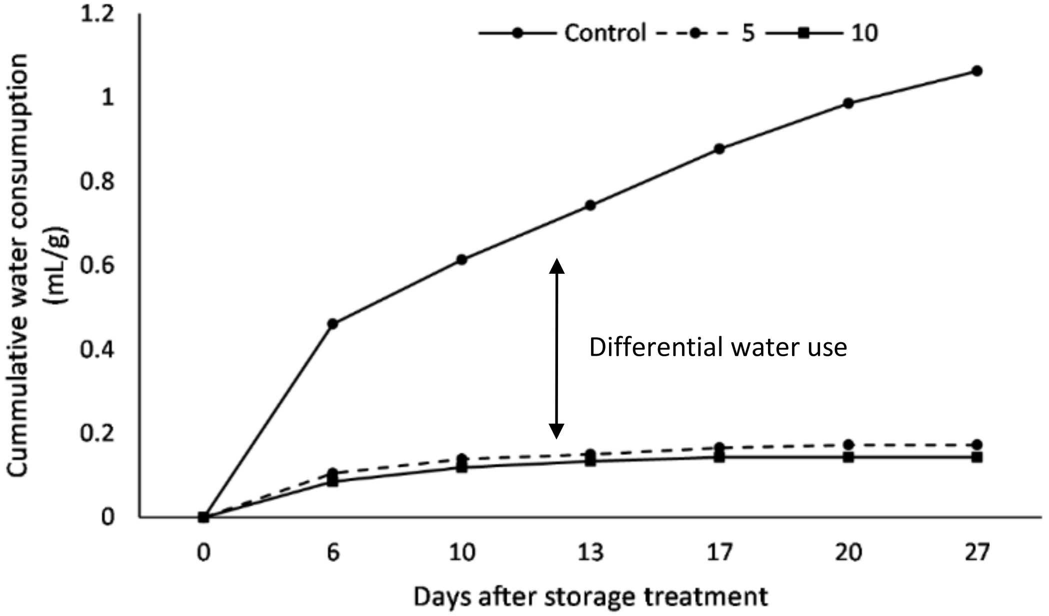

Figure 26: Cumulative water consumption ($mL \cdot g^{-1}$) of balsam fir as influenced by storage temperature (5°C, 10°C and control). Each point represents an average of 3 replicates.

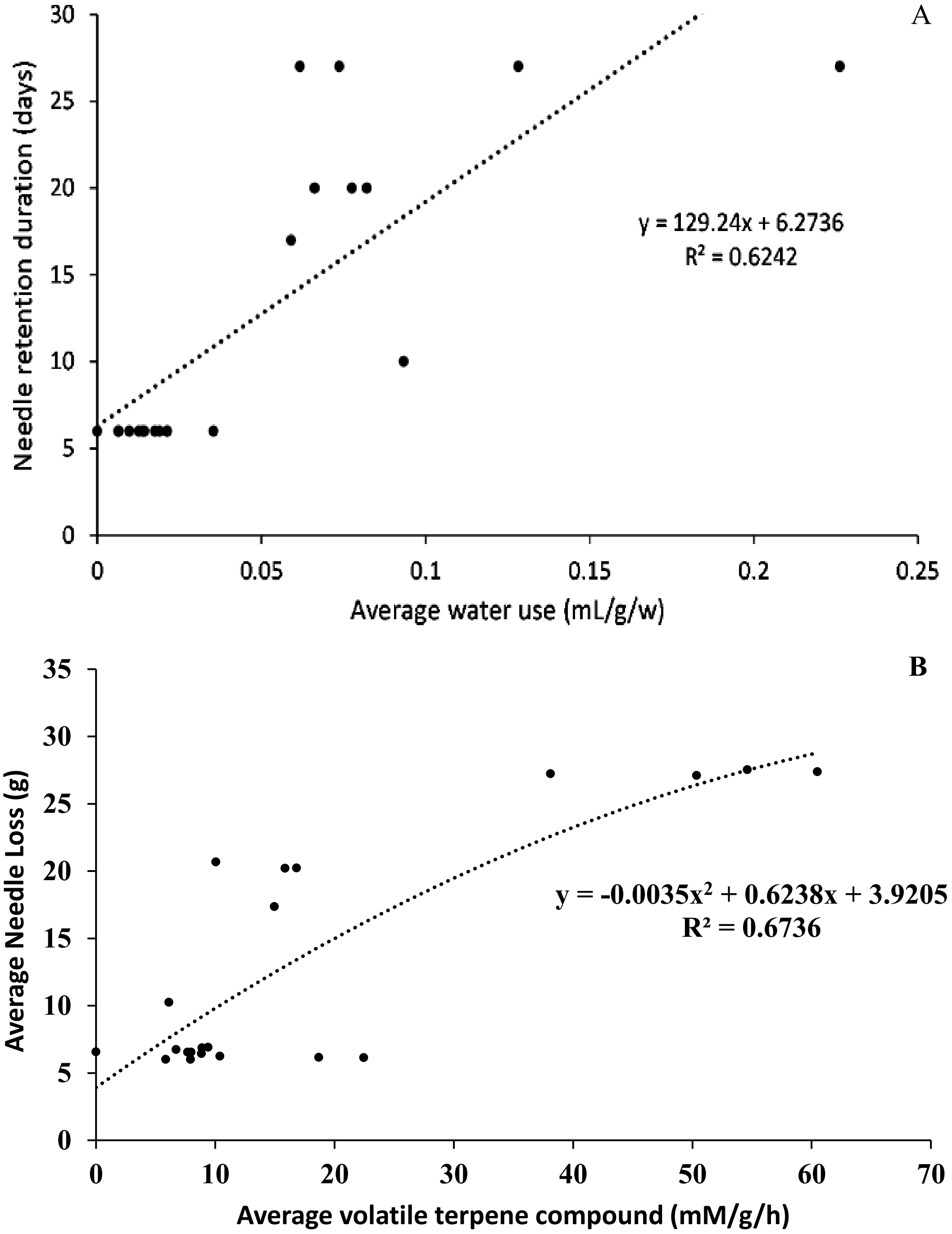

Figure 27: (A) Relationship between the average water use ($mL \cdot g^{-1} \cdot w^{-1}$) (B) average volatile terpene compound (mM/g/h) and needle retention duration (days) in balsam fir.

5.5 DISCUSSION

The drop in average water use with increases in storage temperature was expected from this study and it is supported by previous findings by Macdonald and Lada, 2012 and Thiagarajan et al. 2015. In both studies, trees exposed to a lower temperature (5°C) saw an increase in AWU and therefore, the higher average water content. Plant transpiration responds linearly to temperature changes, where a decrease in temperature from 40°C to 7°C is recorded to decrease transpiration by 80% (Cochard et al. 2000). This can explain the trends observed in this study of a significantly higher AWU (110 $mL \cdot g^{-1} \cdot w^{-1}$) in control trees stored outside at an average temperature of 3°C, although AWU at 5°C and 10°C were not significantly different despite the variance in temperature. Exposure of trees to lower temperatures has been recorded to augment abscisic acid (ABA) biosynthesis (Chen and Gusta, 1983) and in Balsam fir, ABA is reported to increase by 2.5-folds when exposed to a lower temperature (Thiagarajan et al., 2013). ABA as a plant response phytohormone is well known to downregulate water losses, keeping trees at higher water content therefore, extending the needle retention duration (Thiagarajan et al. 2013). This corresponds to NRD data elaborated in this study. The early death of trees stored at 20°C (NRD = 14days) and 30°C (NRD = 7days) regardless of it having been baled or not can also be explained by high levels of VPD and eventual dehydration of trees. The results from this study general indicate the benefit in NRD because of lower storage temperature.

Under controlled temperature storage, the study showed a significantly lower level of ethylene (0.77 $\mu L \cdot g^{-1} \cdot h^{-1}$) at a lower temperature of 5°C, compared to the higher temperatures of 10°C, 20°C, 30°C at 1.01 $\mu L \cdot g^{-1} \cdot h^{-1}$, 2.72 $\mu L \cdot g^{-1} \cdot h^{-1}$ and 3.71 $\mu L \cdot g^{-1} \cdot h^{-1}$ respectively. Since lower temperatures tend to extend postharvest NRD (Thiagarajan et al.

2013; Macdonald et al. 2014b), one would then expect lower levels of needle abscission-hormone, ethylene in baled trees stored at lower temperatures. However, it was observed that when trees were brought to room temperature post-treatment, a higher ethylene concentration was observed. A similar phenomenon was reported by Knee et al. (1983) and Lelievre et al. (1997), where postharvest production and release of ethylene are higher and quicker in trees such as apple and pear when they are kept at 20°C, after 3°C cold storage. This is explained by a strong enhancement of ACC oxidase activities and mRNAs hybridizing to ACC synthase leading to postharvest ethylene synthesis when plants are transitioned from cold to warmer conditions (Larrigaudiere, 1993).

At room temperature, a continuous significant decline in total VTC evolution was observed with an increase in temperature. Although an increase in total VTC with an increase in storage temperature was expected, a 2-fold decrease in total VTC from 5.44 $mM \cdot g^{-1} \cdot h^{-1}$ in control trees to 2.5 $mM \cdot g^{-1} \cdot h^{-1}$ in 10°C storage trees was observed. VTCs are largely known to be stored in pools in the glandular trichomes (Penuelas et al. 1995) and diffuse through epidermis cells into extracellular airspace and therefore the substomatal cavities. This suggests that changes in stomatal conductance could regulate the emission of VTCs. Schuh et al. 1997 reported a positive relationship between VTC emission and stomatal conductance. In this study, dehydration as a result of an increase in storage temperature was discovered thus, it is possible that the decline in total VTC may perhaps be due to lower stomatal conductance, in trees stored at a relatively higher temperature (10°C).

On the other hand, studies such as Schuh et al. (1997) and Huang et al. (2005) have reported temperature dependence on the release of individual VTCs. This was evident in this study, where individual VTC, 3-Carene was found to be significantly higher in trees stored at a relatively higher temperature (10^{o}C) and increase among other VTCs such as β-pinene and β-terpinene prior to postharvest needle loss. This finding is similar to findings in a study by Korankye, 2013, where the detrimental role of some individual VTCs such as 3-Carene, β-pinene and β-terpinene in postharvest needle loss was speculated.

5.6 CONCLUSION

The beneficial effects of low temperature on postharvest NRD was observed due to changes in AWU, ethylene and VTC evolutions. Storage of baled trees outside at an average temperature of 3°C improved water use and needle retention ability by lowering evolution of individual volatile terpene compounds such as 3-carene, which is known to cause postharvest needle abscission. This suggests that trees can take up more water when stored outside in open air before shipping, at a relatively lower temperature of 3°C. This can be beneficial to balsam fir trees by slowing down needle abscission, irrespective of the level of ethylene and VTC although these plant metabolites are recorded to induce postharvest needle abscission in balsam fir trees. This study also suggests that baled trees do better under temperature-controlled storage by extending NRD compared to non-baled trees. However, the physiological response of trees to controlled temperature and humidity storage is still unknown. Our next study explores various VPD levels and monitors changes in trees such as VTC and ethylene and how that affects postharvest needle abscission.

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CHAPTER 6: MECHANICAL – INDUCED STRESS; RESPONSE OF BALSAM FIR TREES TO CHANGES IN VAPOR PRESSURE DEFICIT (VPD)

6.1 ABSTRACT

Negative effects of shaking and baling of Balsam fir trees have been well elaborated in our previous studies. Therefore, research focused on the physiological effects of key storage environmental factors on postharvest qualities of stressed-imposed trees is imminent. This study explored the hypothesis that lower VPD enhances the postharvest abilities of trees to retain needles, despite mechanical stresses. The selected experimental design was a 2 x 4 factorial design with 6 replicates. A total of 48 trees were studied by subjecting them to mechanical stress; control (no shaking or baling) and 15sec shaking and baling of 3 trees. All trees were stored in VPD regulated chambers at 0.26, 0.44, 0.61 kPa and a control (trees stored in the non-VPD regulated chamber). Generated results showed lower VPDs of 0.26 and 0.44 to be effective in delaying postharvest needle abscission of Balsam fir trees. With more than 2-fold increase in NRD, a 2-fold increase in AWU and 2 and 3-folds decrease in ethylene and VTC evolutions, respectively. The storage of trees at 0.26 kPa storage was more effective in improving postharvest physiological characteristics of the trees. It can be concluded that maintaining good water relations through VPD regulation slowed down needle abscission processes by lowering ethylene and VTC, irrespective of the imposed stress.

Keywords: Mechanical stress, vapor pressure deficit, postharvest needle abscission.

6.2 INTRODUCTION

Postharvest needle abscission is one of the common challenges facing Balsam fir and Christmas tree industry in general (Chastagner, 1986; MacDonald et al., 2010). Years of research has proved that postharvest needle abscission is a complex process, influenced by several abiotic, biotic and management factors. Our previous studies have shown that root detachment of trees by harvesting exposes trees to mechanical, pathogenic and drought stress factors, considered to be the initial stimuli for postharvest abscission (MacDonald et al., 2015, MacInnes, 2015).

After harvest, the availability of moisture to trees cannot be overemphasized, therefore the role of dehydration has been proven to be critical in postharvest needle loss. Studies like Chastagner and Riley, 2003; MacDonald and Lada, 2014; MacInnes, 2015 and Chapter 5 of this thesis have strongly proposed a link between water consumption and postharvest needle loss. Alternatively, factors such as preharvest nutrition, ethylene and volatile terpene compounds are known to play critical roles in postharvest needle abscission (Georgeson et al., 2013; MacDonald et al., 2010; Korankye, 2013). Beyond these established factors, the complete mechanisms and pathways that eventually culminate in abscission remain unknown.

One of the areas that have not been extensively explored is the role of vapor pressure deficit (VPD) on the water status of postharvest trees and its cumulative effect on needle abscission. VPD has been described as a known moisture gradient between leaves and external air temperature (Grange and Hand, 1987; Schulze, 1986). Changes in humidity and temperature have an important influence on stomatal conductance and therefore stomatal response. The sensitivity of stomatal response to humidity and temperature has been well recorded and linked to plant water relations (Losch and Tenhunen, 1981; Addington et al. 2004). The decrease in VPD moves water into guard and subsidiary cells increasing turgor potential of stomata, causing stomata to open. Studies like Johnson and Ferrell, 1983 reported a dynamic stomatal response to changing VPD. As a factor of transpiration, changes in VPD is strongly linked to plant water status and as a direct physical response (Day, 2000; Addington et al. 2004). Stomatal response to VPD varies among species depending on the ambient conditions. Douglas fir is known to respond to low VPD by closing stomata at 20°C. Contrary, at 35°C strawberry plants respond to humidity by closing its stomata (Johnson and Ferrell, 1983).

The direct effect of changing water status of plants on the dynamics of stress hormones; ABA, ethylene and secondary compounds such as volatile terpene compounds and their role in postharvest abscission have been discussed in various studies. Korankye (2013) reported consistent increasing levels of both ethylene and VTC with changing Balsam fir water status. Studies like MacDonald et al. 2010 and Rajasekaran and Blake 1999 also reported the pattern of ethylene evolution coinciding with tree water status and postharvest needle loss and drawing the conclusion that ethylene in conifers is triggered by water stress. Despite these findings, the role of vapor pressure deficit in postharvest needle abscission during storage and transportation of balsam fir trees is unknown. In this study, we hypothesize that high VPD during storage and transportation causes higher levels of water loss and therefore early postharvest needle loss. The main objective of this study was to uncover various effects of vapor pressure deficit (VPD) on physiological changes such as water use, ethylene and VTC in trees exposed to shaking and baling. Based on that these individual objectives were developed to uncover;

a. VPD threshold where stored Balsam fir trees can sustain water status to slow down postharvest needle abscission
b. levels of VTC and or ethylene evolution of Balsam fir trees in response to varying VPD storage
c. role of VPD on physiological qualities of postharvest Balsam fir trees

6.3 MATERIALS AND METHODS

Six-year-old trees from Delong Farms in New Germany were used for this study to avoid any ecotype effects. Prior to harvesting, visual inspection was conducted to avoid pest or disease infestation on the trees. An average of 91cm tall trees with similar girth sizes was harvested using a chainsaw in December 2016. The experiment followed a 2 x 4 factorial design with 6 replicates, where a single tree served as a replicate. The first factor was a mechanical-imposed stress on trees; control (no shaking or baling) and 15sec shaking and baling of 3 trees while the second factor was vapor pressure deficit (VPD); control (trees stored at no regulated VPD), 0.26, 0.44, 0.61 kPa.

Shaking and baling of trees were achieved using Christmas tree oscillating shaker and baler (Howey Model, 210RC), respectively. VPD treatment was conducted in a temperature and relative humidity (RH) controlled chambers. The set VPDs were achieved at a temperature of 5ºC and varying RH of 70%, 50% and 30%, respectively. Controls (*n*=6) were kept in separate chambers for the same duration, where temperature and RH were not regulated. Treatment was sustained in the growth chamber for 4 weeks. This duration was chosen to mimic the average duration trees stay in storage containers during road transport within Canada and US and ocean shipment to other parts of the world.

Following the treatments, all trees were given a fresh cut at the trunk using aseptic secateurs. The excised trees were wrapped in 5cm thick foam and then placed in 4L glass jars, containing 3L of sterile distilled water. The Foam was used to limit the loss of water by evaporation from the glass jars. The fresh weight (kg) of each tree and mass of the whole assembly were recorded for all experimental units. All surviving replicates from each of the treatments were transferred to a controlled room with ambient temperature and RH of 22°C and 40%, respectively until trees lost all needles.

Needle retention duration - NRD (day), average weekly water use (AWU) per gram of fresh weight ($mL \cdot g^{-1} \cdot w^{-1}$), ethylene evolution ($\mu L \cdot g^{-1} \cdot h^{-1}$) and VTC evolution ($mM \cdot g^{-1} \cdot h^{-1}$) were measured as per the protocol described in Chapter 2.5. Before statistical analysis, all data were checked for normality. Whenever necessary, transformations were performed to achieve normality. All the response variables were analyzed using Minitab 17 (Minitab 17, Minitab Inc., PA, USA). Upon significant p-values, Tukey's LSD test was adopted to separate the means.

6.4 RESULTS

6.4.1 Effect of VPD on Postharvest Needle Retention

There was a significant ($p<0.001$) interaction between mechanical-imposed stress and VPD and NRD. All trees that were not shaken or baled lost needles within 7 days of storage, irrespective of the storage VPD levels. These trees lasted for 3, 3, 7 and 7 days under non-regulated VPD (Control), 0.61, 0.44, and 0.26 kPa VPD, respectively. On the contrary, trees that were shaken and baled remained fresh and green over the 4-weeks storage, except those stored in non-regulated VPD chamber, lost needles after 21 days of storage. Needle loss was manifested through discolouration as shown in Figure 28. Our study focused on trees that maintained their green colouration after the 4-weeks storage.

There was a significant interaction between mechanical-imposed stress and VPD for NRD ($p=0.012$), ethylene ($p<0.001$) and VTC ($p<0.001$) evolution. Exposure of mechanical-imposed stress trees to 0.61 kPa VPD decreased needle retention by 9.6% compared to control. However, trees stored at 0.26 and 0.44 kPa VPD showed an increase in needle retention by approximately 57% (45 days) and 24% (36 days), respectively compared to the control (29 days) (Table 7).

6.4.2 Effect of VPD on VTC, Ethylene Evolution and Water Relations

Consistent with NRD, significant ($p\leq0.05$) changes in both VTC and ethylene evolution in trees at varying VPD were observed (Table 7). All individual VTCs were analyzed and data showed similar trends in some individual VTCs. Data generated from baled trees stored at VPD (control) showed significantly higher β-pinene evolution ($1.80\ \mathrm{mM\cdot g^{-1}\cdot h^{-1}}$), compared to 0.26 kPa storage ($0.51\ \mathrm{mM\cdot g^{-1}\cdot h^{-1}}$). This was evident in the first week, prior to the commencement of significant needle loss. A similar trend of 3-carene ($1.58\ \mathrm{mM\cdot g^{-1}\cdot h^{-1}}$) in control trees at the peak of needle abscission was observed, whereas at 0.26 kPa storage there was no detection of 3-carene (Table 8). All other individual VTCs showed no significant differences among treatments. Significantly lower evolution of total VTCs by 3 and 2-fold was observed when trees were stored at 0.26 kPa and 0.44 kPa VPD, compared to control. At the same storage VPD, ethylene evolution decreased by 28% and 17% respectively, compared to control (Table 7). There was a near-perfect relationship between VTCs such as 3-carene and β-pinene, ethylene and NRD ($R^2=98.9\%$ and 96.02%) where peak VTC and ethylene evolutions were observed on an average of 5 and 7 days respectively before peak needle abscission (Figures. 29 and 30). AWU was significantly affected by VPD ($p<0.001$). When trees were stored at 0.26 kPa, AWU increased by 52% and 23% when trees were stored at 0.26 and 0.44 kPa respectively, compared to control but AWU decreased by 30% at 0.61 kPa compared to the control (Table 7).

Table 7: Effect of VPD on root-detached balsam fir trees. Treatment means were calculated from 6 replications and separated using least squares differences with $\alpha = 0.05$. NRD = needle retention duration; VTC = volatile terpene compounds; AWU = average daily water use.

VPD (kPa)	NRD (Day)	VTC ($mM\cdot g^{-1}\cdot h^{-1}$)	Ethylene ($\mu L\cdot g^{-1}\cdot h^{-1}$)	AWU ($mL\cdot g^{-1}\cdot w^{-1}$)
Control	28.7^{c}	1.47^{a}	0.82^{a}	2.13^{c}
0.26	45.0^{a}	0.52^{c}	0.44^{c}	3.23^{a}
0.44	35.7^{b}	1.06^{b}	0.68^{b}	2.63^{b}
0.61	26.0^{c}	1.85^{a}	1.31^{a}	1.49^{c}

*Means that do not share same letters are significantly different

Table 8: Comparison of VTC profiles of balsam fir trees exposed to VPD-control storage

	VPD Storage (kPa)	
VTC	Control	0.26
β – pinene	1.80^{a}	0.51^{b}
3 - carene	1.58	-

Mean VTC ($mM\cdot g^{-1}\cdot h^{-1}$) that do not share the same letters are significantly different.

Figure 28: Illustration of postharvest needle abscission characteristics as influenced by VPD (A) Absolute control (B) Control (C) 0.26 kPa, (D) 0.44 kPa, (E) 0.61 kPa.

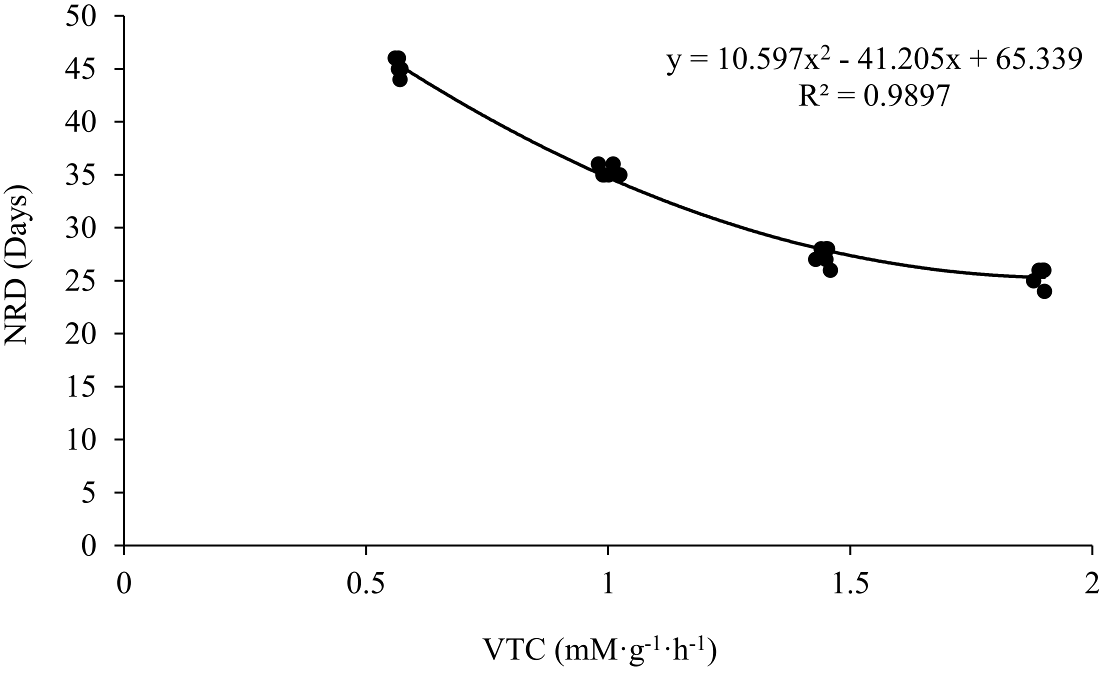

Figure 29: Significant ($p < 0.001$) relationship between VTC ($mM \cdot g^{-1} \cdot h^{-1}$) and NRD (days) in balsam fir. The relationship is best described by NRD = VTC + 5.

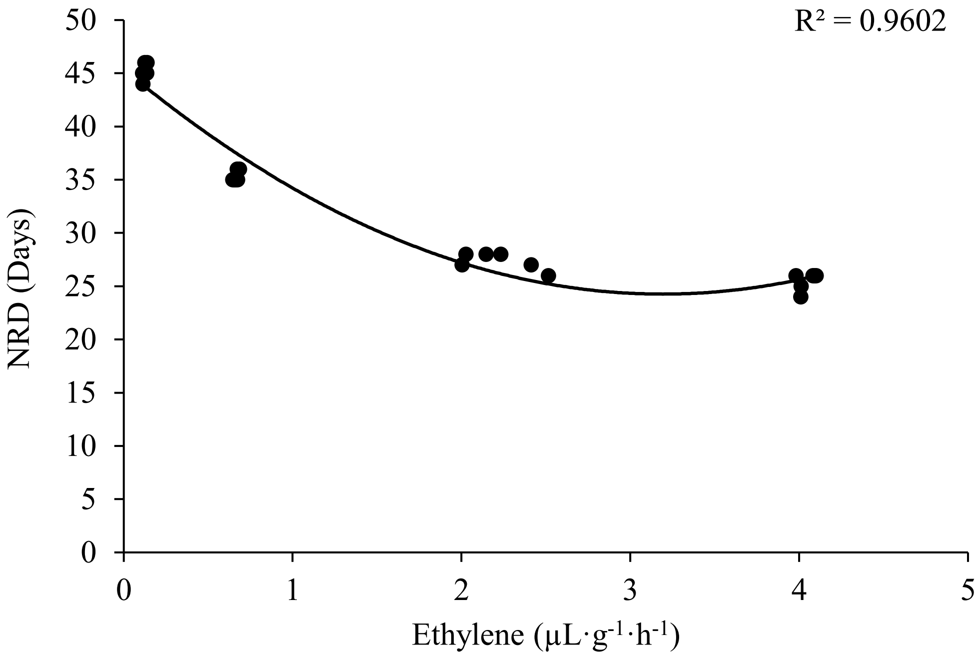

Figure 30: Significant ($p < 0.001$) relationship between Ethylene (µL·g^{-1}·h^{-1}) and NRD (days) in balsam fir. The relationship is best described by NRD = ethylene + 7.

6.5 DISCUSSION

In this experiment, trees with low AWU lost needles earlier than trees with high AWU. These findings were consistent with our earlier reports in chapter 5 however, it is contrary to reports by Rajasekaran and Thiagarajan (2005) and Macdonald et al. 2010. Differences in samples used and treatments could explain the differences in outcomes. Both studies by Rajasekaran and Thiagarajan (2005) and Macdonald et al. 2010 used tree branches as their sample materials. In this study however, full trees were used and exposed to various mechanical stress. Again, differences in AWU may be explained by the differences in transpiration of root-detached balsam fir trees. Studies by MacInnes (2015) reported a 50% decrease in stomatal conductance within the first 4 days and 80% decrease within the first week after harvesting of trees. Water use by trees has been linked to postharvest needle abscission in balsam fir trees. Initiation of significant postharvest needle abscission in balsam fir trees has been reported to occur when water consumption is below 0.05 mL·g^{-1}·d^{-1} (Rajasekaran et. al., 2015; MacDonald and Rajasekaran, 2015). Less research on balsam fir trees has suggested factors like cavitation, embolism, stomatal dysfunction, bacteria contamination, or blockage of xylem vessels (Rajasekaran and Macdonald, 2015; MacInnes, 2015) to be the main cause(s) of decreasing water consumption after the harvest of these trees. However, the effect of storage VPD on these factors and how it affects postharvest needle abscission of balsam fir trees exposed to mechanical stress has never been studied.

In this study, trees stored at VPD of 0.26 kPa (5ºC and 70% humidity) had a significant 57% increase in NRD compared to the control. A 9% decrease in NRD was observed when storage VPD was increased to 0.61 kPa (5 ºC and 30% humidity). This is similar to a 120% increase in NRD when balsam fir branches were exposed to 5°C and 60% humidity (Macdonald et al. 2010). In another study, storage at low vapor pressure deficit increased balsam fir needle retention duration by five folds (MacDonald et al., 2012). Our previous studies in Chapter 5 and other studies such as Mitcham-Butler et al. (1988) concluded that lower temperature storage of balsam and Fraser fir trees promote higher NRD, a combined exposure of whole trees to lowered temperature and high humidity promote higher NRD in this study. The significant delay in the evolution of ethylene and VTCs in trees exposed to lower VPD is in line with other studies such as Macdonald et al. (2010) and Korankye (2013). Individual VTCs such as β-Pinene and 3-Carene that have been identified to promote postharvest needle abscission (Korankye, 2013), was also significantly higher in trees that were exposed to higher VPD. These results suggest that declining uptake of water by balsam fir trees could be a signal that triggers ethylene and or VTCs evolution. Regulation of water loss by the trees through the augmentation of ABA at lower temperatures and in combination with higher humidity (Chen and Gusta, 1983) can explain high AWU and NRD, lower ethylene and VTC evolution in trees exposed to lower VPD.

6.6 CONCLUSION

Lower VPDs of 0.26 kPa and 0.44 kPa were effective in delaying postharvest needle abscission on balsam fir trees. Among the treatments, VPD of 0.26 kPa was the most effective in improving postharvest physiological characteristics of mechanically stressed trees with more than 2-fold increase in NRD, more than 2-fold increase in AWU and a 2-fold decrease in ethylene evolution, as well as a 3-fold decrease in VTC evolution. It can be concluded that maintaining good water relations through VPD regulation can slow down postharvest needle abscission processes by lowering ethylene and VTC in shaken and baled root-detached balsam fir trees. It was obvious that throughout the various chapters in this thesis, water status in trees has played a pivotal role in the response of trees to shaking and or baling. An unexpected discovery was the pattern of needle abscission, in most trees from top to the bottom. The mechanism of this pattern was unknown, therefore our next study focused on examining membrane damages and water status at different tiers of the tree after imposed stress. This was to uncover the link between imposed mechanical stress and membrane injury, water status and pattern of postharvest needle abscission in balsam fir trees.

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CHAPTER 7: WATER RELATIONS, ELECTRICAL IMPEDANCE AS INFLUENCED BY MECHANICAL STRESSES IN POSTHARVEST BALSAM FIR (*ABIES BALSAMEA, L*)

7.1 ABSTRACT

Common benefits of shaking and baling of balsam fir and most conifer Christmas tree are debris-free and pest-free trees as well as compact trees for easy and cost-effective transportation. Despite the benefits of these handling processes, trees are exposed to stresses because of damages to membranes and tissues. The experiment examined the hypothesis that postharvest needle abscission induced by mechanical stresses may be due to changes in tree water relations. To test this hypothesis, a 3 x 2 factorial experiment with 60 sec shaking, baling of 5 trees and control was set up with eighteen, six-year-old trees. Responses measured at three branch-tiers from top to bottom of the trees were XPP and electrical impedance. XPP was significantly (*P*=0.05) higher (-0.70 MPa) in trees that were exposed to the combined treatment of 60 sec shaking and a bale of 5 trees compared to control (-0.52 MPa) by 61.9%. A 34.6% increase (more negative) in XPP was recorded in the upper branch tiers of trees compare to the lower tiers. While a 12.12% decrease in impedance was recorded in the upper tier of control trees and the trees shaken and baled showed a 31.49% decrease in impedance. This suggests that handling processes such as shaking and baling trigger stress responses as a result of membrane and tissue damages, leading to possible postharvest dehydration and needle abscission of trees.

Keywords: Stress, dehydration, impedance, abscission

7.2 INTRODUCTION

Various methods of handling Balsam fir and other trees used as Christmas trees are known to impose mechanical stress. Common handling practices such as pruning, shaking and baling, impose stress mainly through injuries and perturbation (Chapter 3). Plant response to such practices has been documented in many studies (Biddington, 1986; Salisbury 1963; Porter et al. 2009). The mechanically-induced stomatal closure has previously been reported by Biddington, (1986) in other species, through changes in plant water status and therefore yellowing and abscission of cocklebur leaves (Salisbury 1963). Increase in peroxidase activities and reduction in levels of chlorophyll can lead to stomata closure and water loss. This is similar to what happens during senescence and has been observed in rubbing of bryony stems and bending of papaya tree (Boyer et al. 1973; 1983; Porter et al. 2009). Similar responses have been reported in balsam fir trees. Initial reports suggested that postharvest balsam fir branches that consume more water tend to lose needles quicker (Rajasekaran and Thiagarjan 2005; MacDonald et al. 2010). However, over the years further research such as Korankye (2013), Chapters 5 and 6 of this study has concluded that trees that consume less water within a short period after harvesting, are trees that lose more needles. However, this phenomenon has been consistently observed in studies involving whole trees (Chapter 5 and 6), rather than studies where branches were used (Rajasekaran and Thiagarajan, 2005; MacDonald et al. (2010). A constant observation has been the initiation of needle abscission from the apex of the tree to the bottom. It is speculated to be as a result of possible cavitation, embolism, stomatal dysfunction, or blockage of xylem vessels as a result of damages to tree tissues when trees are shaken and or baled after harvest. These factors can limit the hydraulic conductivity of root detached trees, leading to lower water uptake. Studies like MacInnes (2015), have focused on technologies to improve the hydraulic conductivity of trees by mounting balsam fir branches on simulated root pressure system, in an effort to maintain water flow by generating positive pressure. Low levels of positive hydraulic pressure generated by this system were sufficient in delaying postharvest needle abscission.

A common but effective method used in the past for the study of the hydraulic conductivity of plants has been xylem pressure potential (XPP). Studies like Rajasekaran and Thiagarajan, 2005; MacDonald et al., 2010 have successfully studied water relation in balsam fir trees. One of the proposed water relation mechanisms has been the inability of trees to maintain a positive pressure through the xylem, as a result of damages to tissues by the handling processes of shaking, baling and transportation (Chapter 4). In the past, the use of electrical impedance in monitoring tissue and membrane damage has been extensively studied. This technology has been successfully used in monitoring the integrity of extra- and intra-cellular, plasma membrane, cytoplasm, tonoplast, and vacuoles by measuring both the resistance and capacitance (Zhang and Willison, 1991; Gora and Yanoviak, 2014). Electrical impedance has been used to assess severa1 physiological changes in plants. Common among them have been cold acclimation in different plant organs (Glerum, 1973; Zhang et al., 1993), virus infections (Greenham et al., 1978), and apply bruises (Cox et al., 1993). A study by Glerum, 1969 reported an invariable decrease in impedance with an increase in tissue damages. Similar bruises and damages of conifer trees that occur during the process of baling, shaking as well as transportation can be assessed by this method. The application of electrical impedance has the capacity to detect severe tissue damage in trees and thus, damages within the cells as physical injuries or physiological disorders. The advantage of this technique has been the ability to assess damages non-destructively. With the need of further clarity on water relations in conifer species through the assessment of damaged tissues, it is imperative for this chapter of our study to uncover the dynamic changes in water status of balsam fir trees after exposure to mechanical stresses post shaking and baling. Thus, it was hypothesized that shaking and or baling cause tissue damages to balsam fir trees, limiting the ability of trees to take up all needed water for long-term survival.

Objectives of this study were to;

(i) determine the effects of shaking and baling on water relations at different tiers of balsam fir branches along the tree and

(ii) determine the impact of handling processes on trees at the membrane and tissue level through measurement of electrical impedance.

7.3 MATERIALS AND METHODS

Root-detached balsam fir trees of 91cm average height and 6-year-old were used in this study and these were harvested in September 2017. Prior to harvesting, visual inspection of each tree was conducted to avoid selection of pest or disease-infested trees. Harvesting of trees was done using a handheld chainsaw as per normal practices by farmers. Shaking was done using Christmas tree oscillating shaker while baling was achieved using a baler (Howey Model, 210RC). The trees were transported to the laboratory after shaking and baling treatments. The experiment followed a 3 x 2 factorial design with shaking (0 and 60 sec) as one factor, the second factor was baling (0 and 5 trees) and the third factor was the interaction of the two treatment factors (shaking + baling). Three replicates were run for each treatment unit.

Response variables monitored in this study were XPP and electrical impedance. Measurement of XPP followed a method adopted from MacDonald et al., 2010. XPP of branches was measured using a Plant Moisture System Pressure Bomb (PMS Instrument Co., Corvallis, USA). Measurement of electrical impedance was done using methods adopted from Jackson and Harker, 2000 and Glerum, 1969. Two electrodes made from rust-resistant steel needles 3.5mm long, 1mm in diameter were impaled in the tree trunk 35 mm apart and to an approximate depth of 3.0mm. The resistance and reactance components of electrical impedance were measured at frequencies between 100 Hz and 1 MHz using a DER EE precision LCR meter (model DE-5000; DER EE Electrical Instrument Co., Ltd, Taiwan). Electrodes were removed after reading on LCR stabilized and recorded. Measurements were repeated for 3 tiers of each tree. Tier 1, 2, and 3 of heights ranging between 29 and 31cm represented the upper, mid and lower tiers of trees as shown in the diagram below (Fig. 31).

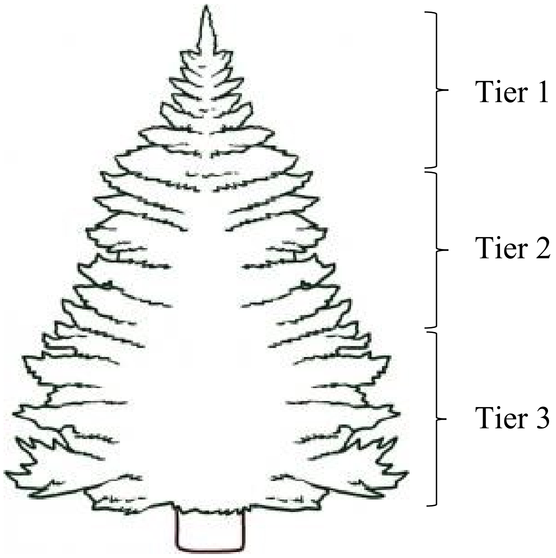

Figure 31: Illustration of three tiers at which XPP and impedance measurements are taken.

35 mm inter-electrode distance selected for this study was based on average sizes of bruises or damages on the tree trunks as a result of the handling process. Resistance measurements at 1KHz for the 3 different tiers are represented by the abbreviations $R_{1KHZ}1$, $R_{1KHZ}2$, $R_{KHZ}3$ respectively. Changes in resistance were represented by ΔR_{1KHz}. The resistance measurement made at 1 MHz was represented by $R_{1MHz}1$, $R_{1MHz}2$, $R_{1MHz}3$ and the changes in resistance was represented as ΔR_{1MHz}.

Before statistical analysis, all data were checked for normality. Whenever necessary, the transformation was performed to achieve normality. All the response variables were analyzed using Minitab 17 (Minitab 17, Minitab Inc., PA, USA). Upon significant p-values, Tukey's LSD test was adopted to separate the means.

7.4 RESULTS

7.4.1 XPP Indicates Stress-Induced Water Relations in Balsam Fir Trees

Tree handling showed a significant effect on XPP ($p \leq 0.05$). XPP was generally higher (less negative) in control trees (-0.39 MPa). Trees shaken exhibited a lower level of XPP (-0.49 MPa), similar to trees that were just baled (-0.54 MPa) (Fig. 32). Trees that were shaken and baled at the same time, exhibited 18.34% lower XPP than trees that were just shaken or baled. Consistent through all handling treatments, we observed lower XPP at the top (Tier 1) of trees compared to the base (Tier 3) of trees, although the level of XPP varied with different handling treatments (Table 9). A 34.6% significant decrease in XPP (more negative) was observed in Tier 1 of trees when shaken and baled, compared to control. A similar trend was recorded in Tier 3 where 88.5% decrease in XPP was observed when trees were shaken and baled as compared to control (Table 9).

7.4.2 Electrical Impedance, a Measure of Mechanical Stress-Induced Membrane Injury in Balsam Fir Trees

Impedance was significantly ($p<0.001$) influenced by the different types of stress imposed on the trees by handling. A significantly higher impedance in control (60317Ω) compared to baled (43984Ω) and combined shaking and baling trees (39333Ω) was observed (Fig. 33). Although there was no significant difference in impedance among shaken and control trees, the interaction of shaking and baling was significantly lower than shaking or control. A 12.12% decrease in impedance in control trees was recorded, while shaken and baled trees showed a decrease in impedance by 31.49%. This trend was consistent with impedance measurements recorded at a higher frequency (1MHZ). Despite the differences in tree weight, we observed no significant relationship between tree weight and electrical impedance. However, the data showed significant differences ($p\leq0.05$) in electrical impedance at different tiers of trees. We observed lower mean impedance of 40,038Ω at the upper tier of trees, while the lower tier showed higher mean impedance of 54,416Ω (Table 10). Although there was no relationship between tree weight and impedance, there was a strong positive significant ($p\leq0.05$) relationship between impedance and XPP (R^2 = 0.7097%) (Fig. 34).

Table 9: Influence of imposed mechanical shaking and baling on XPP at different tiers of trees

	XPP (MPa)		
Stress	Tier 1	Tier 2	Tier 3
Control	- 0.52^{b}	- 0.40^{c}	- 0.26^{b}
Shaking	- 0.59^{b}	- 0.53^{b}	- 0.37^{b}
Baling	- 0.61^{ab}	- 0.58^{b}	- 0.44^{a}
Shaking x baling	- 0.70^{a}	- 0.65^{a}	- 0.49^{a}

Any two means followed by same letters are not significantly different ($p\leq0.05$).

Table 10: Influence of imposed mechanical stress (shaking and baling) on measurements of electrical impedance. Values represent the means for 3 balsam fir trees.

Stress	Tree wt. (kg)	Impedance Measurements (ohms)					
		Tier 1		Tier 2		Tier 3	
		R_{1KHZ}	R_{1MHZ}	R_{1KHZ}	R_{1MHZ}	R_{1KHZ}	R_{1MHZ}
Control	1.71	55,350^{a}	7,225^{a}	62,620^{a}	7,820^{a}	62,982^{a}	7,980^{a}
Shaking	1.61	42,100^{b}	6,320^{b}	56,200^{b}	7,130^{b}	58,980^{a}	7,220^{b}
Baling	1.59	31,800^{c}	6,050^{b}	49,550^{c}	6,820^{b}	50,602^{b}	6,950^{b}
Shaking x Baling	1.72	30,900^{c}	5,200^{c}	42,001^{d}	5,900^{c}	45,100^{c}	5,990^{c}

Any two means with same letters are not significantly different ($p \leq 0.05$) within the same column.

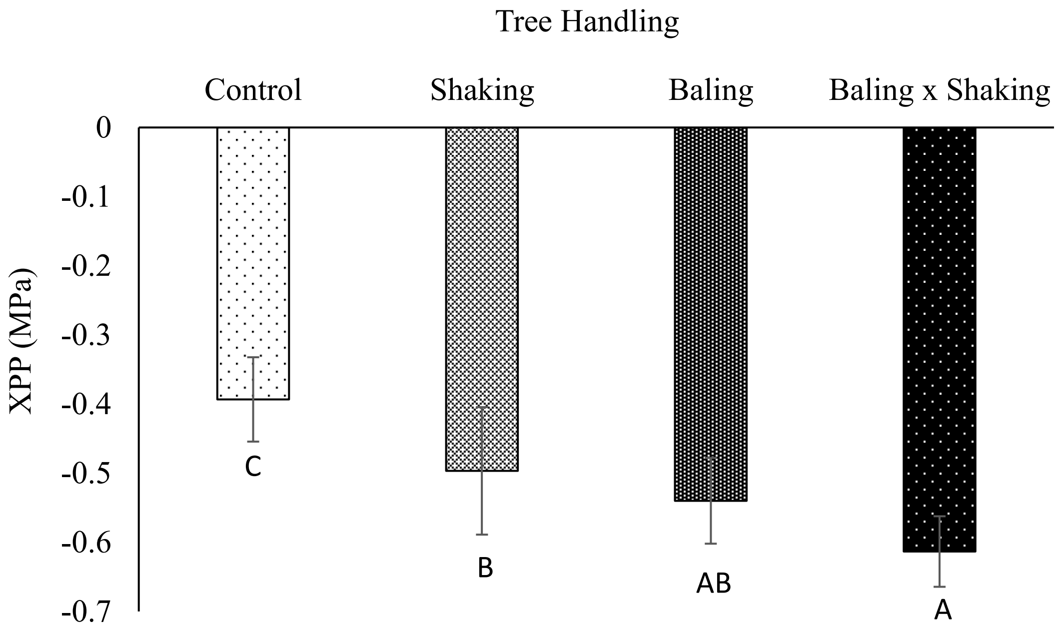

Figure 32: Average XPP (MPa) of balsam fir trees exposed to shaking and baling treatments with standard error bars (n=3). Any two means followed by same letters are not significantly different ($p \leq 0.05$).

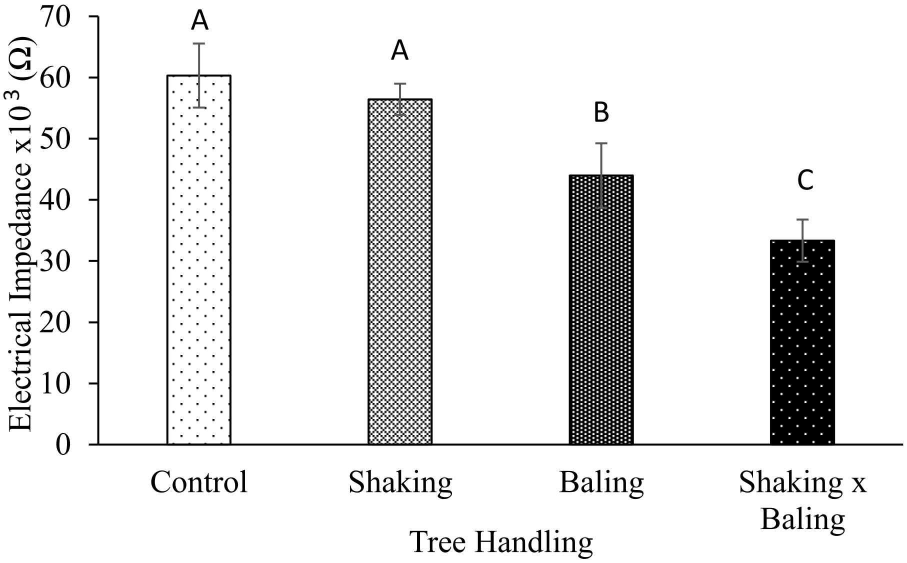

Figure 33: Average impedance (Ω) of balsam fir trees exposed to shaking and baling treatments with standard error bars (n=3). Any two means followed by same letters are not significantly different (p<0.001).

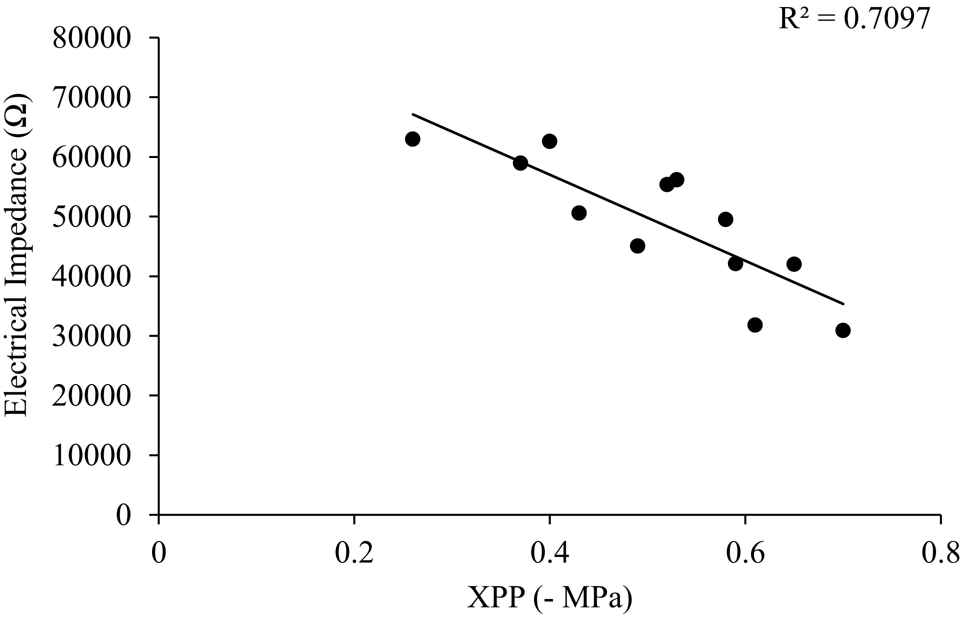

Figure 34: Significant ($p \leq 0.05$) relationship between XPP and impedance. The relationship is best described by impedance = -72194XPP + 85895

7.5 DISCUSSION

In the past, a study by Macdonald et al., 2010 has reported a decrease in water consumption of balsam fir trees in the range of 0.2 $mL \cdot g^{-1} \cdot d^{-1}$ to 0.05 $mL \cdot g^{-1} \cdot d^{-1}$ in approximately 2 weeks after harvest as a result of dehydration. Our previous study (Chapters 4 and 5) reported a trend of initial needle loss or tree death through needle discolouration from the top of the tree to the base. In another study, dehydration has been strongly linked to XPP changes (Hinesley and Snelling, 1991) and therefore, needle abscission in balsam fir (MacDonald and Rajasekaran, 2015; Rajasekaran et al., 2015; MacInnes, 2015). The result of this study has clearly demonstrated immediate (3 days after harvesting) decline in XPP with the increase in tree height as well as the varying handling processes of trees. The presence of roots creates an effective pressure system for water to travel up the trees, however, with the detachment of trees from root, in combination with extensive shaking and baling a lot of complications are speculated to occur influencing this hydraulic pressure, especially when stomatal conductance is at its lowest (MacInnes, 2015). When trees are shaken or baled they are more likely to be vulnerable to the formation of water free pockets in the xylem conduits forming cavitation therefore, limiting tree water uptake. Formation of gums and mucilage in the xylem conduits of cut conifer trees has also been reported by MacInnes. (2015); Sperry et al., (1994) and Van Ieperen et al., (2002). The possible obstruction of xylem by gums and mucilage can reduce hydraulic flow forcing trees to shut stomata to conserve water. Stomata closure has been extensively studied to limit water update by trees (Arve et al., 2011; Tombesi et al., 2015). This phenomenon can explain the varying XPP in different tires of trees after imposed shaking and bailing.

However, it cannot be limited to only the handling processes since control trees also exhibited similar XPP trend at lower levels.

The theory of electrical impedance comprises resistance and reactance. Apoplastic resistance is measured at lower frequencies and in this study at 1KHZ, while at higher frequencies such as 1MHZ used in this study, the resistance of entire tissue, including symplast, is measured (Cole, 1972; Harker and Maindonald, 1994; Stout, 1988). This technology has been tested in the measurement of tissue stability of stressed plants such as potato, apple and conifers (Zhang et al., 1993; Jackson and Harker, 2000; Glerum, 1973). To study the disturbance of entire plant tissue, electrical impedance has been one of the most viable tools. In our previous study (Chapter 3), we have speculated that shaking and or baling in addition to root detachment of balsam fir trees disturb tree tissues. In this study, trunks of trees tested showed significant changes in resistance following imposed mechanical stress. We observed a direct link between the postharvest handling of balsam fir trees and electrical impedance by the changes in impedance measurements. Impedance analysis of same frequency showed that tissue injury in trees increased as membrane capacitance and resistance decreased. Trees that were shaken, and at the same time baled showed significantly lower levels of impedance at a lower frequency of 1KHZ, indicating a more structural damage leading to membrane disintegration compared to control. Lower impedance measurements observed in the upper tier of trees suggest a more intense disturbance in the upper tier than lower tier. Similar trends are also uncovered at a higher frequency of 1MHZ suggesting that handling of trees by shaking, baling or worse case can disturb the entire plant tissue. Severe damage to the upper tier of trees and therefore, the low impedance can also be explained by younger tissue development at the upper tier of the tress than the more matured lower tier. Plants have the ability to recover from tissue damage as reported in a study by Xu et al., 2010. However, the disturbance or damage to vascular tissues can be detrimental to tree survival. Vascular tissues such as xylem and phloem are responsible for transporting water, hormones and nutrients throughout the plant. Disturbance of such tissue limit water, as shown by dehydration of trees in this study. The more tissue damage (lower impedance), the higher water loss or dryer the trees (lower XPP). Upstream transport of hormones or nutrient can also be limited as a result of a loss in the hydraulic pull, leading to nutrient or hormonal deficiency, and subsequently leading to cell/tree death as evidenced in this study by needle abscission or discolouration starting from the top of trees downwards.

7.6 CONCLUSION

Our data support the conclusion that balsam fir trees are truly exposed to mechanical and eventual water stress as a result of postharvest handling processes such as shaking and baling. These processes are speculated to trigger stresses through membrane disintegration and tissue damages, leading to dehydration and eventual death of trees. However, tissue damage is more prevalent at the top part of the trees leading to the initiation of dehydration and death from the upper tier of trees. Despite these discoveries, further research to investigate the extent of membrane and tissue damage, as well as the limits to which these trees will recover from such damages, will be imperative for the Christmas tree industry and science in general.

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CHAPTER 8: GENERAL DISCUSSION

Throughout this study, a lot of discoveries have been made that added new knowledge to our understanding of postharvest needle abscission in root detached balsam fir trees exposed to various mechanical stresses. It is undoubtedly evident that balsam fir Christmas trees experience a mechanical stress due to shaking and/ or baling as evident from the changes in tree temperature, loss of membrane integrity, the evolution of ethylene and VTCs. The cause and effect relationships of the mechanical stress and the effects of environmental alterations in mitigating mechanical stress effects have been illustrated in the schematic diagram below (Fig 35).

This study established a link between tree temperature and imposed stress as a result of shaking and baling. A significant ($p<0.001$) increase in temperature by 0.86°C and 1.03°C were recorded in shaken and baled trees compared to control respectively. Changes in temperature have long been recognized as a plant stress indicator mostly connecting it to transpiration (Jackson et al. 1988; Chaves et al. 2002; Leinonen et al. 2004). When plants are under stress, transpiration is reduced or halted in some cases, resulting in an increase in a plant or leaf temperature (Jackson et al. 1988; Chaves et al. 2002). Although transpiration measurements were not taken in this study, it is known that any abiotic stress that leads to stomatal closure and/or cavitation, can reduce transpiration, increase respiration and thus, increase in plant temperature (Leinonen et al. 2004).

Another important physiological factor that indicates mechanical stress in trees is membrane integrity, which is known to regulate stomatal conductance and therefore transpiration. A significant increase in membrane injury index (MII) by 20.7% ($p=0.041$)

and 28.1% (*p*=0.002) was observed in trees exposed to mechanical stress caused by shaking and baling tress, respectively. Studies in plants like potato (Walker et al. 1984), coffee (Compos et al. 2003) and pines (Zwiazek and Blake, 1990) have reported an increase in electrolyte leakage and therefore an increase in MII when plants were exposed to a mechanical stress. The major cause of loss of membrane integrity has been attributed to weakened and damaged cell membranes post-mechanical stresses (Walker et al. 1984; Leon et al. 2001) as a result of breakages of leaves and stems. Similar evidence was observed in our study, where bruises and breakages of tree branches and needles after shaking and baling may have caused a much more intense mechanical stress. Again, the initiation of needle abscission from the upper tier of trees was observed (Chapter 4). Electrical impedance measurements had revealed that a combination of shaking and baling lead to tissue disintegration, which was evident through lower impedance measurements at a decreasing percentage of 31.49% when trees were shaken and baled compared to the control. Younger tissues at the upper tier of trees were found to be more vulnerable to membrane and tissue damages therefore, recording extremely low impedance supporting the hypothesis that shaking and baling indeed cause mechanical stress.

This study was also established that mechanical stress through shaking and baling of balsam fir trees causes low water uptake therefore, it is important to understand the effect of such changes on trees water relations and some plant metabolites that are known to play a significant role in postharvest needle abscission. It is believed that the genesis of the problem of postharvest needle loss is the detachment of trees from the root, and if that is combined with extensive shaking and baling of trees, a series of physiological changes occur, including a reduction in water uptake leading desiccation, increased ethylene and VTC evolution leading to postharvest needle loss. Studies such as Chastagner 1986; Rajasekaran and Blake, 1999; Rajasekaran and MacDonald 2015 have suggested that the ability of trees to withstand postharvest stress relies largely on their ability to rehydrate. One can, therefore, postulate that mechanical stresses such as shaking and or baling can disrupt the process of rehydration directly or indirectly and will negatively influence the postharvest quality of trees. Based on this, one would expect that increased shaking duration and number of trees in a bale will result in a significant decrease in water use, resulting in significant needle loss. Part of our study has indeed shown that shaking and baling of trees reduce water status (Chapter 5) and therefore, more negative XPP of the tree (Chapter 7). We have also found a decrease in XPP (more negative) at the upper tier of the baled trees. Xylem of shaken and or baled trees may be vulnerable to the formation of water free pockets in the xylem conduits forming cavitation therefore, limiting tree water uptake. The possible obstruction of xylem by gums and mucilage as a result of tissue damages can also reduce hydraulic flow forcing trees to shut down stomata in an effort to conserve water. Previous research at CRC has shown that there is significant xylem blockage due to microbial biofilm formation therefore, cutting the bottom end of the branches once in two days improved water uptake. As well, providing a compensatory root pressure through a simulated root pressure system (SRPS) delayed the onset of needle abscission for nearly six months (MacInnes, 2015). Thus, mechanical stress-induced dehydration can promote needle abscission.

Plant responses to changes in water status that are linked to the postharvest quality of trees have been associated with the production and/or release of ethylene gas and volatile terpene compounds (VTCs). Studies like Jaffe and Biro (1979), Biro and Jaffe (1984) have shown that imposed mechanical stress through rubbing or flexing of plants causes substantial tissue damage thereby changing stomatal structure and elevation of ethylene levels. Goeschl et al., 1966 also demonstrated that when peas are pushed through glass beads, tissue damage occurs, and higher levels of ethylene are produced. Similarly, the mechanical stresses, shaking and baling induced ethylene evolution. Although shaking of trees did not clearly depict such a phenomenon, baling of more trees caused higher ethylene evolution suggesting that baling is more detrimental to trees than shaking. Previous studies by MacDonald et al 2010, observed a continuous spike in ethylene levels days prior to needle abscission in branches that are not subjected to mechanical stresses, throughout this study ethylene evolution was also observed due to shaking and baling, which may be a potential hormonal signal for postharvest needle abscission in balsam fir trees.

Emissions of VTCs such as 3-Carene, limonene and α, β-Pinene in Balsan fir (*Abies balsamea*) were discovered previously (Korankye, 2013). In this study, however, it was discovered that mechanical stresses also increased total VTC emission (chapters 3, 4, 5, 6). The consistent evolution of VTCs, more specifically, 3-carene, β-pinene and β-terpinene in response to mechanical stresses provide evidence that mechanical stress triggers biosynthesis of certain specific VTCs. These VTCs are then stored in the epidermal cells of the plant's tissues (Chen et al. 2004) or glandular trichomes (Lu et al. 2002) and released days prior to or during abscission when the tissues or trichomes are exposed. The mode of significant release of VTCs, only when needles abscise suggests a specific and an important role for VTCs in postharvest needle abscission.

If the mechanical stress-induced dehydration is the cause of the postharvest needle drop, then reducing dehydration through environmental manipulation would mitigate the mechanical stress effects and promote needle retention. The experiments in Chapters 6 and 7 explored various options to slow down dehydration and thus, delay postharvest needle abscission. The results showed that a decrease in storage temperature will increase the average water use by trees, which was supported by previous findings by MacDonald and Lada, 2012 and Thiagarajan et al. 2015. It is also known that exposure of trees to lower temperatures augment abscisic acid (ABA) biosynthesis (Chen and Gusta, 1983). In balsam fir, ABA increased by 2.5 folds under a lower temperature of 5°C (Thiagarajan et al. 2015). ABA as a plant response phytohormone is well known to downregulate water losses, keeping trees at higher water contents therefore, extending the needle retention duration (Thiagarajan et al., 2013). These results are similar to NRD data elaborated in Chapter 5, with the early death of trees stored at 20°C (NRD = 14days) and 30°C (NRD = 7days) regardless of it having been baled or not. Trees stored at 3°C, 5°C and 10°C remained green and maintained their needles throughout the 30-day storage. When all trees were brought to room temperature, trees that had been stored at the lower temperature of 3°C (control) lasted longer with an NRD of 23 days, while the trees stored at 5°C and 10°C only lasted for 8 days. This raises the question of why control trees lasted although they had 2 and 3-fold higher levels of ethylene than 5°C and 10°C. Could there be other factors apart from temperature, ethylene and VTC that can play a role in the postharvest needle? In this study, control trees were stored outside the lab and exposed to natural light in addition to lower temperature, while the trees of other treatments were stored in darkness. In addition to temperature, light has been reported to deactivate and or reduce the production of ethylene in plants (Goeschl et al. 1967), while in the dark ethylene production is known to be increased (Bassi and Spencer, 1982). One can, therefore, speculate that significant ethylene biosynthesis in control trees was only initiated after the 30-day storage when trees were transferred to the lab under room temperature. Therefore, the effect of ethylene on needle abscission in control trees was not until after 30-day storage, resulting in higher NRD. Ethylene production and effect on needle abscission of trees stored at 5°C and 10°C in the dark commenced prior to transfer of trees to the lab. A continuous significant decline in total VTC evolution with an increase in temperature by 2 folds from 5.44 $\mathrm{mM \cdot g^{-1} \cdot h^{-1}}$ in control trees to 2.5 $\mathrm{mM.g^{-1} \cdot h^{-1}}$ in 10°C storage trees was also observed. However, upon further analysis, a significant difference was recorded among individual VTCs. 3-Carene was found to be significantly ($\rho=0.025$) lower in control trees (0.52 $\mathrm{mM \cdot g^{-1} \cdot h^{-1}}$) and 5°C (2.3 $\mathrm{mM \cdot g^{-1} \cdot h^{-1}}$) compared to trees stored at 10°C (8.90 $\mathrm{mM \cdot g^{-1} \cdot h^{-1}}$). We also observed a spike in the concentration of 3-carene a week prior to peak needle abscission in all treatments, but it was found to be significantly higher (0.66 $\mathrm{mM \cdot g^{-1} \cdot h^{-1}}$, $\rho \leq 0.001$) in trees stored at 10°C compared to the control trees. Korankye (2013), observed that VTCs, β-Pinene and β-Terpinene evolve at higher amounts prior to needle loss, but in this study, these two VTCs did not change significantly among treatments. These suggest that 3-Carene could be the potential signal for the postharvest needle loss in mechanically stressed trees and reduce the evolution of 3-Carene by subjecting them to a low temperature of 3 degrees can reduce postharvest needle loss despite mechanical stress induced by shaking and baling.

Altering VPD could provide an advantage to manipulate storage environment to reduce water loss. While the impact of VPD on postharvest needle loss was already studied (Losch and Tenhunen, 1981; Addington et al. 2004 in balsam fir, the impact of mechanical stress under various VPDs were not understood and it was not known whether altering VPD would mitigate the postharvest needle loss due to mechanical stresses. It was found that trees stored at 0.26 kPa VPD (i.e. 5ºC and 70% humidity) had a significant increase (57%) in NRD compared to the control. A 9% decrease NRD was observed when VPD was increased to 0.61 kPa (5 ºC and 30% humidity). This is similar to a 120% increase in NRD when mechanically non-stressed balsam fir branches were exposed to 5°C and 60% humidity (Macdonald et al. 2010). The low VPD mitigated mechanical stress through higher AWU, lower ethylene and VTC evolution proving that the mechanical stress effects can to a certain extent be alleviated by subjecting the mechanically stressed trees to low VPD, enhancing needle retention duration.

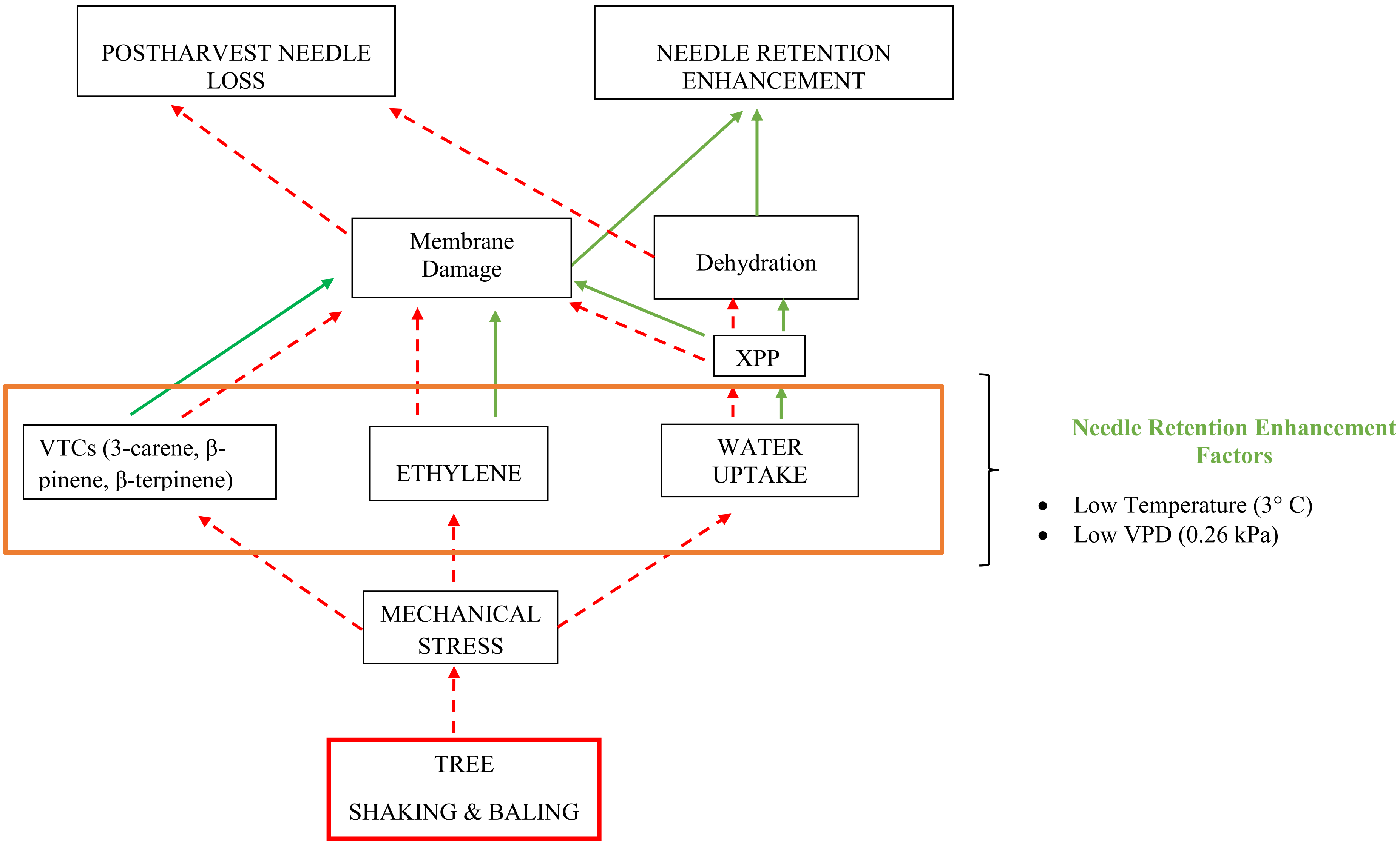

Figure 35: Illustration of proposed stress-induced postharvest needle abscission processes in Balsam fir tree. Red box represents postharvest activities and orange box represents key responses. Broken red lines represent response patterns that lead to needle abscission, solid green lines represent response patterns that lead to needle retention enhancement.

CHAPTER 9: CONCLUSIONS

The overall goal of this research was to understand the events leading to postharvest abscission in balsam fir trees exposed to mechanical shaking and baling with a specific interest in the role of stress indicators, ethylene and VTC and their interaction with water use, storage and transportation temperature, and VPD. The following briefly describes the key conclusions with respect to objectives presented in Chapter 1. For purpose of convenience, original objectives are re-stated before each conclusion.

1. To uncover the physiological responses of mechanical stress induced by shaking and baling in postharvest balsam fir.
    - The prolonged shaking of trees at or above 15 seconds, lower tree temperature, membrane injury, ethylene and in the case of VTC showed a continuous decline as the duration of shaking increased. Balsam fir trees adjust and overcome shaking stress for its benefit. On the other hand, baling increased all stress indicators by 1.03°C in temperature, membrane injury by 2-fold, ethylene by 5-fold and VTC by 2-fold. Baling of balsam fir trees poses a mechanical stress to the trees demonstrating that this process is the most detrimental stressor to the trees postharvest.
2. To establish the physiological effect of mechanical shaking and baling on postharvest needle abscission of balsam fir trees.
    - Postharvest needle loss (PNL) is significantly lower in shaken or non-baled trees compared to non-shaken and baled trees. The significant physiological benefit with low PNL and high needle retention duration (NRD) are more evident in trees shaken for 30 sec. and bales of 5 trees. This is supported by

evidence shown in this study, where trends of abscission-inducing compounds like ethylene and VTC (3-carene, β-pinene, β-terpene) are consistent with reported PNL and NRD. It is therefore undoubtedly better not to bale balsam fir trees at all however, if baling is imperative one can recommend for it to be done at a level of 5 trees in a bale.

3. To determine the influence of postharvest storage temperature on baling-induced changes in needle abscission
    - Beneficial effects of low temperature on postharvest needle retention interacted with AWU use, ethylene, total and individual VTC (3-carene) levels in trees. Storage of trees outside at 3°C induced high AWU, NRD, despite the presence of higher ethylene and lower 3-carene evolution. This suggests the ability of lower temperature storage to augment water uptake, beneficial to balsam fir trees by slowing down needle abscission.

4. To uncover various effects of vapor pressure deficit (VPD) on physiological changes such as water use, ethylene and VTC in trees exposed to shaking and baling.
    - VPD of 0.26 kPa was the most effective in improving postharvest physiological characteristics of balsam fir trees with more than 2-fold increase in NRD, more than 2-fold increase in AWU and a 2-fold decrease in ethylene evolution as well as a 3-fold decrease in total VTC and β-pinene evolution. It can be concluded that maintaining good water relations through lower VPD regulation slows down needle abscission processes by lowering ethylene and VTC to delay needle abscission of baled root-detached balsam fir trees.

5. To understand the changes in water relations and membrane stability at within different branch when tree is subjected to mechanical stress.
    - All trees tested responded to shaking and baling, increasing water potential by 34.6% in the upper tier (Tier 1) and 88.5% at the lower tier (Tier 3). This is consistent with electrical impedance, where we observed a lower impedance at the upper tier of trees, while lower tiers showed higher impedance and a 12.12% increase in impedance in control trees, while shaken and baled trees showed a decrease in impedance by 31.49%. Our data supports the concept that tissue damage is more prevalent at the upper tier of the trees leading to initiation of dehydration and death in comparison to the lower tier of Balsam fir trees.

FUTURE RESEARCH

Our research has generated an extensive amount of new knowledge fundamental to postharvest needle abscission in balsam fir, but there is still significant future research. Future research can focus strongly on areas such as physiological and practical factors influencing balsam fir needle abscission.

Physiological research should be targeted at key factors leading to needle abscission as discussed in these studies. It was proposed that mechanical stress through shaking and baling induces ethylene and or VTCs, but no work has been done on ethylene and VTC-induced gene expression. It will be interesting to establish the timeline of gene expression for these compounds after imposed stress. Research on higher levels of imposed stress above what was used in this research is also advised.

Practical research must focus on one important area, which is technologies that can mitigate the detrimental effects of shaking and or baling on postharvest needle abscission. Our studies have discovered four key methods that have the potential to delay postharvest needle abscission in balsam fir; reducing injuries to trees during shaking and baling processes, improvement of tree water relations, low-temperature storage of harvest trees before and during shipment and storage of trees at low VPD. New technologies can strongly focus on the development of new baling technologies, that includes baling materials that will pose less damage to trees. Factors like water, temperature and VPD have been tested in our studies, however further research focuses on different levels of these factors in combination with different levels of shaking and baling of trees will expand our knowledge in the area of postharvest needle abscission.

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APPENDIX I: Standardization of Gas Chromatograph Method for VTC Analysis

Standardization of GC was performed in each experiment by injecting 10µl of internal standard (β-pinene) onto a filter paper and placed in the incubation bag and left sealed with SPME fibre exposed to the headspace for 30mins. Since the volume of each incubation bag used was known, the concentration of injected terpene standard was calculated and plotted against terpene concentration determined by GC analysis. For each experiment, standards were made and tested each day prior to VTC analysis to account for the degradation of SPME fibre and the daily variations in our GC setup. All terpene standards were purchased from Sigma-Aldrich Co. LLC, Canada.

Figure 36 shows chromatogram with a 95.9% peak area at 6min. 6sec. and a baseline free of any noise. This shows the efficiency of the method developed for the terpene analysis.

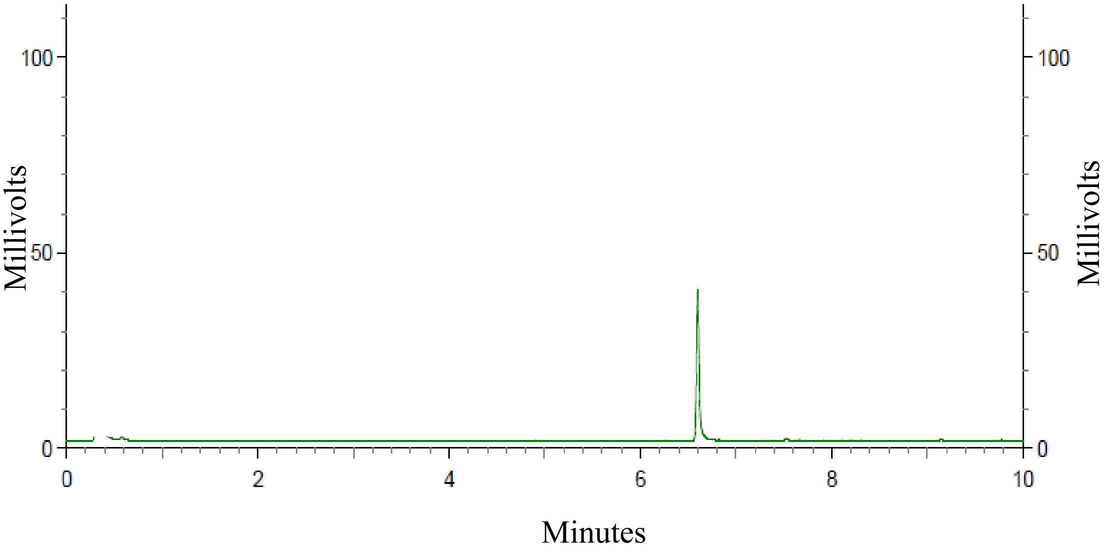

Figure 36: Chromatogram for 10mM β-pinene by SPME at 30 min equilibration time

A linear relationship was found between 0mM and 10mM β-pinene (Figure 36). Detected concentrations slightly diverged from the injected concentrations. The common discrepancy was detected at an injected concentration of 10mM which was measured at 9mM. The slope of the linear regression was determined to be 1.215 with $R^2 = 0.991$, a perfect linear relationship would have a slope of exactly 1. One can, therefore, conclude that GC setup used performed excellent, therefore can be said to accurately measure VTCs released from balsam fir.

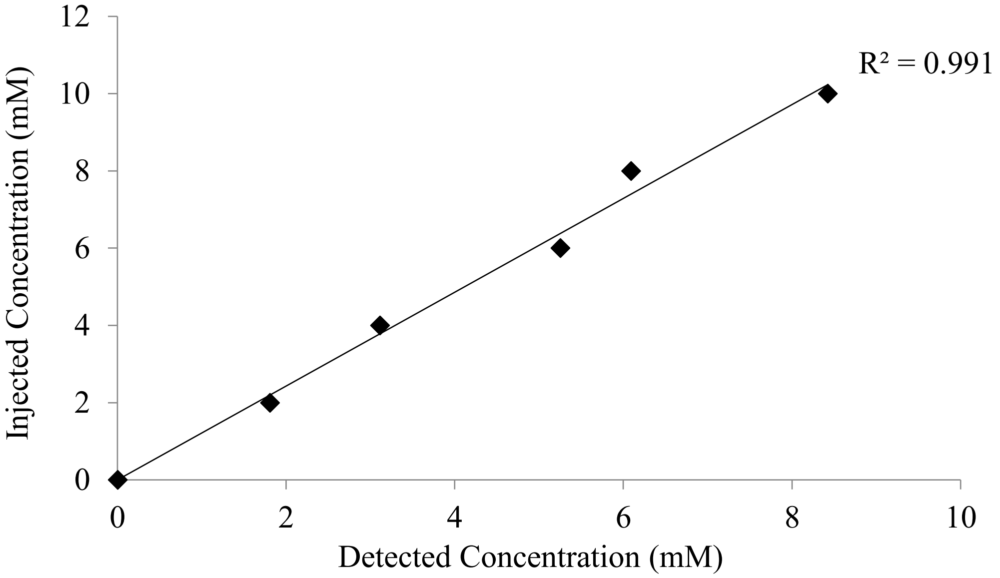

Figure 37: Standard curve for Gas chromatograph of VTC standard.

APPENDIX II: Standardization of Gas Chromatograph method for ethylene analysis

Ethylene was standardized by allowing incubation bags to sit for 12 hours after injecting known volumes of ethylene gas into the incubation bag. Since the volume of each incubation chamber was known (109.21 L), the concentration of injected ethylene was calculated and plotted against the concentration determined by the GC (Figure 37).

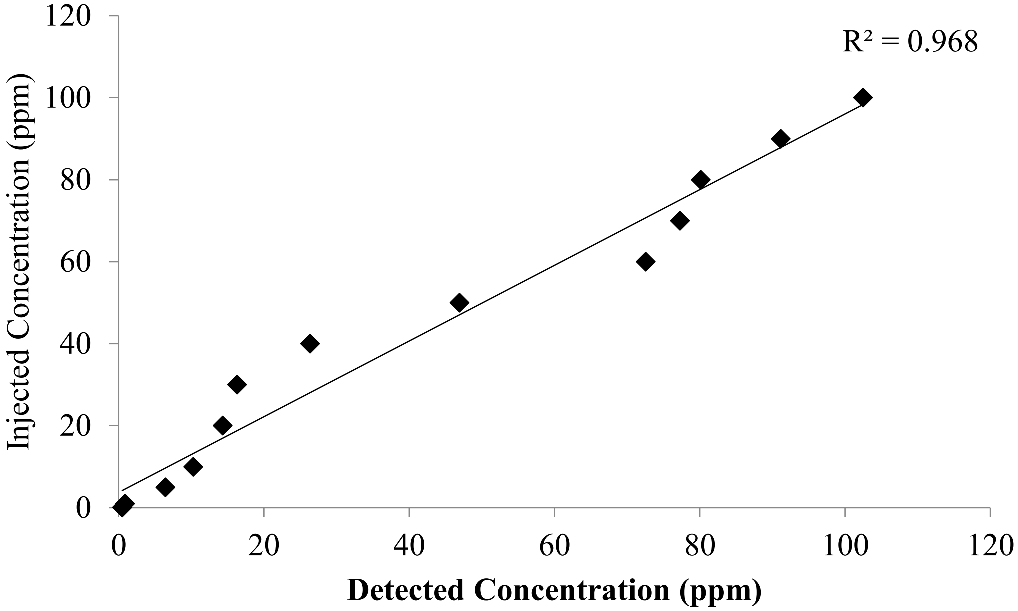

Figure 38: Standard curve for Gas chromatograph for ethylene standard.

A linear relationship was found between 0 ppm and 100 ppm ethylene. However, the detected concentrations begin to diverge from the injected concentrations after 10 ppm. For example, a concentration of 100 ppm was measured at 102 ppm by the GC. The largest discrepancy was found as the concentration of injected ethylene was increased. GC performed excellently between 0 ppm and 100 ppm. The slope of the linear regression was determined to be 0.02 with R^2 = 0.968. Based on data we concluded that the GC is near accurate in detecting ethylene released from balsam fir provided detected concentrations remain below 100 ppm.