White earlywood rings in black spruce trees from the boreal plains region of western Manitoba

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Abstract

Tree-ring anomalies are increasingly used in dendrochronology to aid in reconstructions of past environments and to obtain a better understanding of current and future climate. White earlywood rings (WER) are an anomaly previously undescribed in the literature and are thought to result from decreased tracheid wall thickness. The objectives of this study were, i) to examine the distribution of WER in black spruce stems, ii) to characterize and compare tracheid dimensions in WER and control rings and iii) to investigate potential causes associated with WER formation. Sampling was conducted in Porcupine Provincial Forest and Duck Mountain Provincial Park in western Manitoba. Two sites were sampled for each region and ten black spruce trees were selected from each site for stem analysis. Results indicated that WER were most frequent in Porcupine Provincial Forest and were often preceded by a frost ring in the year prior to WER formation. Tracheid dimensions were measured in wood samples from the site with the highest frequency and intensity of WER. At the tree-ring scale, WER were found to have reduced cell wall thickness in the earlywood and they also differed in several latewood characteristics, including the diameter of the lumen and diameter of the tracheid as compared to control rings. It is speculated that the formation of WER may be related to reduced hormone and carbohydrate availability in the early growing season. The potential linkage between WER and severe frost damage to the crown prior to ring formation merits further investigation.

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1.0 Introduction

Dendrochronology is the science related to the interpretation of information stored in tree-rings by analyzing the temporal signature left by abiotic and biotic factors affecting tree-ring formation (Schweingruber 2007). Dendrochronological research has proven invaluable in a range of research fields, including climatology, ecology, glaciology, archaeology, and hydrology, to name only a few (Grissino-Mayer 1996). Analysis of tree-ring characteristics is an important area of research as it provides a period of reference for forest environments that facilitates a better understanding of current climate and aids in prediction of future climate (Fritts 1976). Historically, the majority of tree-ring research has been limited to the interpretation of ring-width variations (Schweingruber 2007). However, unfavourable growing conditions can also lead to anomalous tree-ring development and the specific year and cause of the anomaly can often be identified (Fritts 1976). Therefore, tree-ring anomalies can be used in addition to ring-width analyses to gain information on the growing conditions at the annual scale of resolution. In addition, analysis at the tracheid level within a tree-ring may allow for intra-annual resolution (Panyushkina et al. 2003; Vaganov 2005). Insight into the complexity of growth and development in trees can also be obtained by studying tree-ring anomalies and interpretation of the anomaly requires knowledge of tree-ring anatomy and development (Fritts 1976).

1.1 Annual growth of trees

The formation of annual growth layers in trees follows complex biochemical and physiological processes influenced by abiotic and biotic factors (Schweingruber 2007; Pallardy 2008). The influence of abiotic and biotic factors are typically most detrimental to growth and development once growth initiation has occurred, though trees can also be severely damaged by winter conditions (Parker 1963; Man et al. 2009). In the fall and spring trees undergo a process of hardening and dehardening, respectively. Dehardening occurs much faster than hardening and tree tissues can become damaged by severe weather events if dehardening occurs prematurely (Horntvedt and Venn 1980). Initiation of growth occurs in spring following dehardening of the stem and is temperature dependant (Antonova and Stasova 1997; Ko Heinrichs et al. 2007; Man et al. 2009). The dehardening process involves the conversion of sugars to starch, melting of extra-cellular ice and the subsequent return of turgor to the cells (Parker 1963; Krasowski et al. 1993). In addition, renewed sap flow following dehardening facilitates the mobility of carbohydrates and growth hormones required for the growth and maturation of developing tissues (Savidge 2000). There are two general phases in carbohydrate allocation in conifers. Early in the growing season, developing buds and vascular tissue are a carbon sink for photosynthates produced by older needles and remobilized reserve carbohydrates from previous years growth, respectively. Later in the growing season developed buds become a carbon source, which allows accumulation of carbohydrates that are used in subsequent growth (Hansen and Beck 1994; Pallardy 2008). The development of these tissues is influenced by growth hormones that can have a synergistic or antagonistic effect on growth and development (Harrison and Klein 1979).

The main hormones that promote tracheid growth and development are auxin (Harrison and Klein 1979; Little and Savidge 1987), gibberellins (Harrison and Klein 1979; Wang et al. 1997) and cytokinins (Harrison and Klein 1979; Gaspar et al 2003). Auxin and gibberellins are produced in active meristems in the crown (Wang et al. 1997), while cytokinins are produced in developing tissues through out the tree, including roots, foliage and fruit (Sheldrake 1973). The annual growth of vascular tissue contains the record of the growing conditions for a particular year and variability in tree-ring width and anatomy can be interpreted by dendrochronologists.

1.2 Conifer tree-ring formation

Annual secondary growth of conifer trees is accomplished by division of cells in the apical and lateral meristems that increases the height and girth of the tree, respectively (Raven et al. 2005). The lateral meristems consist of a thin layer of cells, termed the vascular cambium, that forms a continuous layer through the roots, stem and shoots (Vaganov 2005). Division of cells within the vascular cambium adds xylem to the inside and phloem to the outside (Panshin and De Zeeuw 1970; Lachaud et al. 1999; Pallardy 2008). It is widely accepted that cell division and development begins in the crown in spring with basipetal progression from the crown to the roots with the reverse, acropetal progression observed for cessation of tracheid development (Thibeault-Martel et al. 2008). However, while this general progression has been observed, Thibeault-Martel et al. (2008) have reported that onset of tracheid development was highly variable, occasionally beginning in the stem. Initiation of cambial development begins in May and

is temperature dependant, with cessation of growth occurring in August and September (Ko Heinrichs et al. 2007; Rossi et al. 2008). In conifers, the annual growth ring is composed of xylem and consists of closed ended cells called tracheids that are connected by pits and provide a pathway for the flow of water and nutrients upwards in the tree (Panshin and De Zeeuw 1970; Gray and Parham 1982). Resin and parenchyma cells also occur, but to a much lesser degree (Vaganov 2005). Tracheid development is often divided into three phases: division, expansion and maturation. During the expansion and maturation phase, the secondary cell wall is developed and is often divided into an S1, S2 and S3 component (Pallardy 2008). The first tracheids produced are termed earlywood tracheids and are characterized by large lumen area and thin cell walls (Wimmer 2002). The thinner cell walls of the earlywood have been attributed to rapid maturation of the tracheids which coincides with the active growth phase of shoots and needles (Pallardy 2008). As the growing season progresses temperature and day length decrease and latewood tracheids are produced (Plomion et al. 2001). They typically have small lumen diameters and thick cell walls (Wimmer 2002). The production of thick tracheid walls has been attributed to slower tracheid maturation, which allows increased cell wall deposition and coincides with growth cessation of shoots and needles (Pallardy 2008). The development of earlywood and latewood can be disrupted during the growing season, leading to the formation of tree-ring anomalies that can potentially be assessed using dendrochronological techniques.

1.3 Tree-ring anomalies

The presence of tree-ring anomalies in the stem of woody plants allows for a more detailed interpretation of past environments than could be achieved with tree-ring widths alone. Increasingly, dendrochonological research has examined tree-ring anomalies including false rings, light rings and frost rings in relation to abiotic and biotic influences (Gurskaya and Shiyatov 2006; Schweingruber 2007). False rings, also known as density fluctuations, are a tree-ring anomaly indicative of moisture stress occurring during the growing season (Schweingruber 2007). Summer drought can lead to the formation of tracheids that have a smaller lumen and thicker cell walls, followed by larger tracheids when moisture conditions improve, giving the annual growth ring a double-ringed appearance (Hoffer and Tardif 2009; Figure 1A). Wimmer et al. (2000) used the presence of false rings in Austrian pine (*Pinus nigra* Arn.) to reconstruct precipitation at the beginning of the growing season. They found that false ring formation was related to a wet May, dry April and wet June sequence. Hoffer and Tardif (2009) observed similar results for jack pine (*Pinus banksiana* Lamb.) and black spruce (*Picea mariana* (Mill) B.S.P.) growing in eastern Manitoba. As the environmental conditions of false rings have been found to be related to moisture conditions at the beginning of the growing season (Wimmer et al. 2000; Hoffer and Tardif 2009), the appearance of false rings in the tree-ring sequence can be used to infer these conditions at the time of formation.

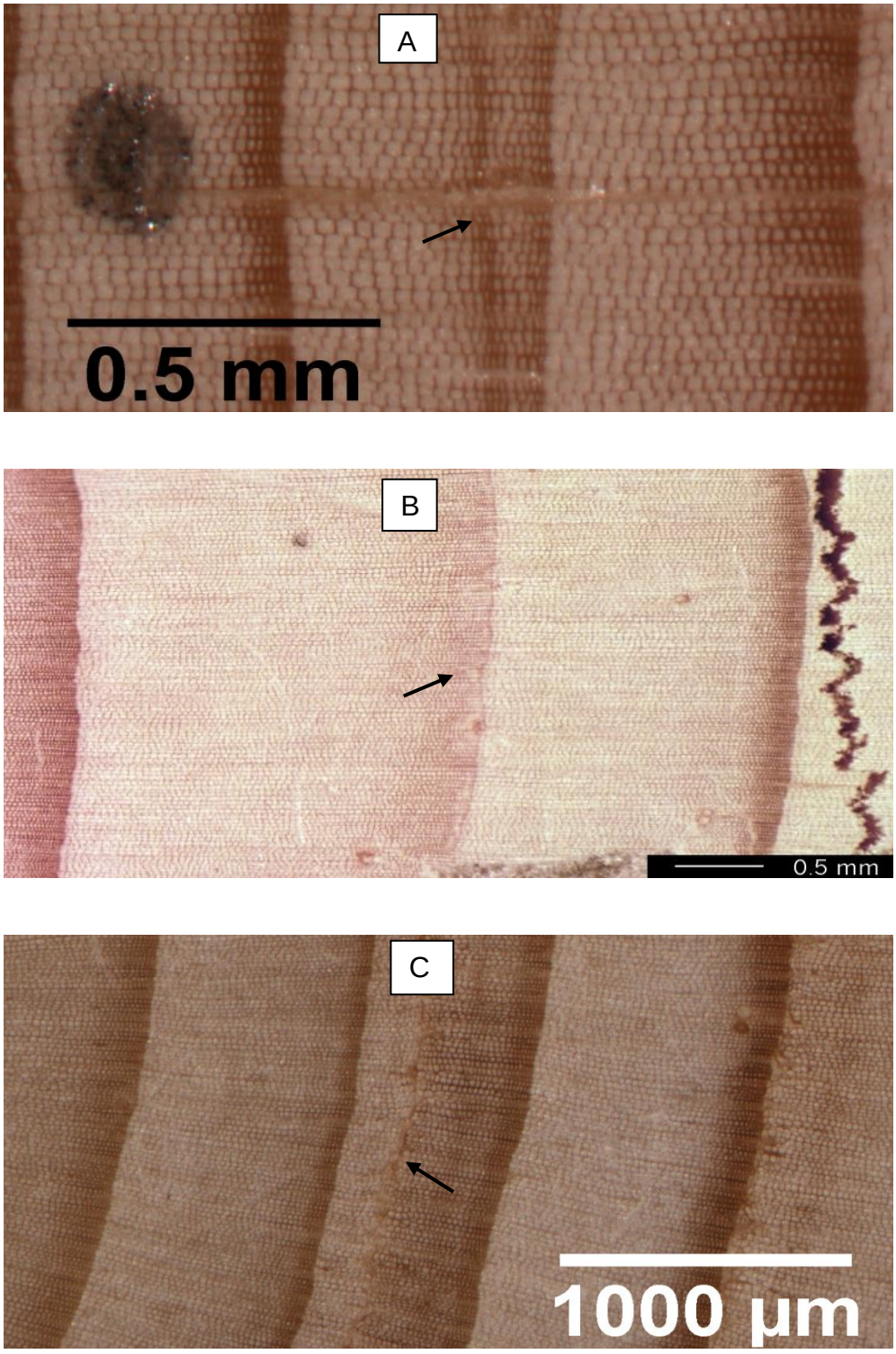

Figure 1: Tree- ring anomalies in black spruce. Top image presents a false ring (A), middle image presents a pale latewood (B) and bottom image presents a frost ring (C). Each anomaly is indicated by an arrow.

Light rings, also known as pale latewood, are a tree-ring anomaly indicative of climatic or biotic disturbance during the growing season. Light rings consist of latewood with reduced width and density, causing a light appearance to the latewood (Wang et al. 2000; Girardin et al. 2009; Figure 1B). Dendrochronologists have observed that light ring formation can be associated with a temperature, precipitation or defoliation signal. Girardin et al. (2009) used light rings in jack pine to reconstruct climate in western Manitoba for the past 300 years. They found that light rings were formed in jack pine in relation to a late start to the growing season and a cool end to the growing season, with below average temperatures generally observed for the entire growing season. Liang and Eckstein (2006) studied light rings in Chinese pine (*Pinus tabulaeformis* Carr.) in a semi-arid region of northern China and observed an association with summer drought conditions. They found that ring-width and latewood width contained a strong precipitation signal in this region and so could gain insight into precipitation patterns. Liang et al. (1997) studied light ring formation in eastern larch (*Larix laricina* (Du Roi) K. Koch) and used anatomical differences to distinguish between climatically and biotically induced light rings. They found that climatically induced light rings had a greater reduction in growth compared to the biotically induced light rings, particularly for cell wall thickness and number of tracheids. Therefore, upon careful analysis of light rings in the tree-ring chronology, insight into climatic and biotic processes can be obtained.

Frost rings (FR) are a tree-ring anomaly indicative of frost events occurring during the growing season. Frost rings appear in the annual ring as a layer of crushed xylem and

bent rays (Schweingruber 2007; Figure 1C). The crushed xylem is thought to be the result of extra-cellular ice formation and contraction of external tissues (Parker 1963; Schweingruber 2007). In addition to physical damage, FR formation halts developmental processes, including lignification of the tracheids (Glerum and Farrar 1966). Gurskaya and Shiyatov (2006) used FR occurrence in a tree-ring chronology to examine the distribution of FR spatially as well as temporally within individual stems of conifers. They found that FR formation occurred over a large area, but was highly dependent on the height of the tree, the circumference of the stem and the thickness of the bark. The majority of FR in their samples were associated with trees less than 7m in height and less than 5cm in diameter with a bark thickness less than 3mm. The association of FR with small diameter, thin barked trees has been reported in other studies as well (Rhoads 1923; Fayle 1981; Payette et al. 2010).

1.4 White earlywood ring and potential causes

Through extensive sampling conducted in Manitoba over the last decade by the dendroecology laboratory at the University of Winnipeg, a previously "undescribed" tree-ring anomaly, termed white earlywood rings (WER), has been observed in black spruce, jack pine, eastern larch and northern white-cedar (*Thuja occidentalis* L.) throughout Manitoba (F. Conciatori pers. comm. 2009). At the macroscopic level, these rings present a whitish colour and appear to have a lighter appearance in the earlywood (Figure 2).

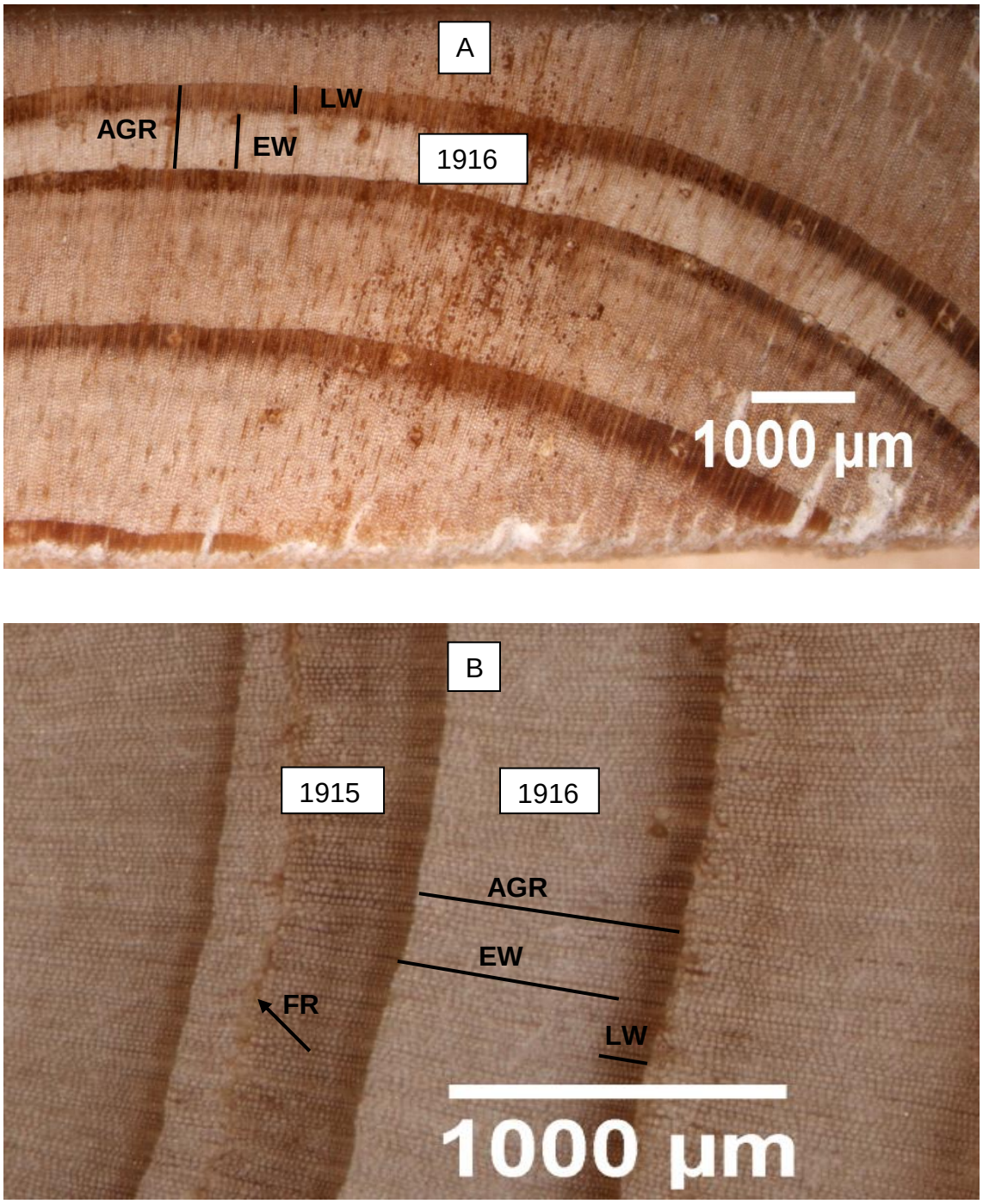

Figure 2: White earlywood ring of 1916 in jack pine sample (A) and white earlywood ring of 1916 associated with the frost ring (FR) of 1915 in a black spruce sample (B). Tree-ring components indicated are annual growth ring (AGR), earlywood (EW) and latewood (LW).

One hypothesis to explain the formation of WER is that they are caused by a decrease in carbohydrate availability associated with defoliation. The presence of white rings in trembling aspen (*Populus tremuloides* Michx.) has been described by Sutton and Tardif (2005). They reported that defoliation by forest tent caterpillar (*Malacosoma disstria* Hbn) led to a reduction in fibre cell wall development with a corresponding increase in fibre lumen area related to reduced carbohydrate availability and reduction in growth hormones. This reduction in the cell wall thickness is also supported by research regarding light ring formation in eastern larch in response to insect and/or climate disturbances. Liang et al. (1997) suggested that defoliation and/or cool seasonal temperatures resulted in less photosynthate available for latewood cell wall development and thus to reduced cell wall thickness. Interestingly, similar reductions in cell wall development have been observed in earlywood by Polge and Garros (1971) following experimental defoliation of maritime pine (*Pinus pinaster* Aiton) prior to growth initiation. They found that wood density decreased in the earlywood due to thinner cell walls, particularly for completely defoliated trees and related this to reduced carbohydrate availability. It is therefore possible that WER formation could also result from decreased carbohydrate availability following defoliation events. Defoliation events can result from several forest disturbances, including frost events, insect outbreaks, fire and extreme weather events (i.e. wind and hail) (Schweingruber 2007).

Preliminary observations of WER indicated that frost rings were often observed in the year prior to WER formation (Figure 2B). This suggests that frost events may have an influence in WER formation. The potential influence that frost events may have is

through damage to the foliage either during the dormant season or once growth initiation has begun. Foliage damage may reduce photosynthate production, interfere with hormone production and require redistribution of carbohydrate reserves to replace damaged foliage (Sutton and Tardif 2005). Man et al. (2009) observed widespread foliage damage to the crowns of black spruce, white spruce (*Picea glauca* (Moench) Voss), jack pine, balsam fir (*Abies balsamea* (L.) Mill), northern white cedar and eastern larch in Ontario prior to bud break of 2007. They found that up to 70 percent of needles and up to 30 percent of buds were killed on affected trees. They attributed the needle death to winter dessication, stand structure, and cold temperatures. Horntvedt and Venn (1980) observed similar results following frost damage to Norway spruce (*Picea abies* (L.) Karst.). They found that affected trees had up to 60 percent foliage damage and reported that damage rarely led to mortality of the tree. They speculated that the frost event occurred as a result of abnormal fall and winter conditions that made the trees susceptible to frost damage. Lamontagne et al. (1998) studied the effect of frost on photosynthesis by artificially frosting branches at varying intensities and observed that photosynthesis was reduced by up to 95% two days after frost application and had only recovered to 50% capacity 23 days following the frost application.

In addition to frost events, foliage damage can result from other biotic and abiotic factors. Defoliation from biotic factors is largely through insect activity. As in frost events, needle damage by insects reduces the production of photosynthates and hormones, and carbohydrate reserves are redistributed to replace lost needles, resulting in less carbohydrate reserves available for tracheid production and maturation. Characteristics of

annual ring growth resulting from insect outbreaks include reduced latewood development, reduced radial growth (Tailleux and Cloutier 1992; Filion and Cournoyer 1995; Krause and Morin 1995; Liang et al. 1997), and resin duct formation (Schweingruber 2007).

Finally, defoliation can also result from forest fires and weather damage to foliage and has also been proposed as a cause of WER formation (F. Conciatori pers. comm. 2009). In both cases characteristic features include formation of callus tissue, reduction in ring width over several years, and a fire scar may sometimes be formed following a fire (Schweingruber 2007). Severe crown damage by fire often leads to tree mortality and weather events, such as hail, are often only directly observable for several years following the event.

1.5 Objectives and hypotheses

The objectives of this study were threefold. First, the distribution of WER in black spruce stems were examined following stem analysis. It was hypothesised that WER would occur throughout the black spruce stems. Second, WER were characterized and compared to control rings using measurements of ring and tracheid dimensions. Measurements of tracheids were compared for earlywood, latewood and the entire ring. It was hypothesised that the walls of earlywood tracheids would be thinner than those of the controls. Finally, potential environmental factors that may cause WER formation were examined.

2.0 Methodology

2.1 Study area

The study areas are located in Porcupine Provincial Forest and Duck Mountain Provincial Park in western Manitoba, Canada (Figure 3). Both regions are part of the Manitoba Escarpment and are located about 600 and 500 km northwest of Winnipeg, respectively. The geology of the region is composed of Cretaceous shales overlaid with glacial till, lacustrine deposits and fluvial-glacial deposits. Both regions are situated in the Mid-boreal upland ecoregion (Environment Canada 2009a). Dominant vegetation includes trembling aspen, white spruce, balsam poplar (*Populus balsamifera* L.), black spruce, paper birch (*Betula papyrifera* Marsh), jack pine, balsam fir and eastern larch (Sutton and Tardif 2005). The closest meteorological station is in Swan River (52° 2'N, 101° 13'W). For the period 1971-2000, the study area had a mean annual temperature of 1.6 °C and total annual precipitation of 530 mm (Environment Canada 2009b).

2.2 Sampling and data collection

The observation of WER from species sampled throughout Manitoba by the dendroecology lab were compiled into a database. From the compiled data, four sites (Figure 3) were chosen for further study based on the observed frequency of WER. Two sites were sampled in Porcupine Provincial Forest and two in Duck Mountain Provincial Park (Figure 3). The Porcupine Provincial Forest sites were labelled L2 and L3 and had

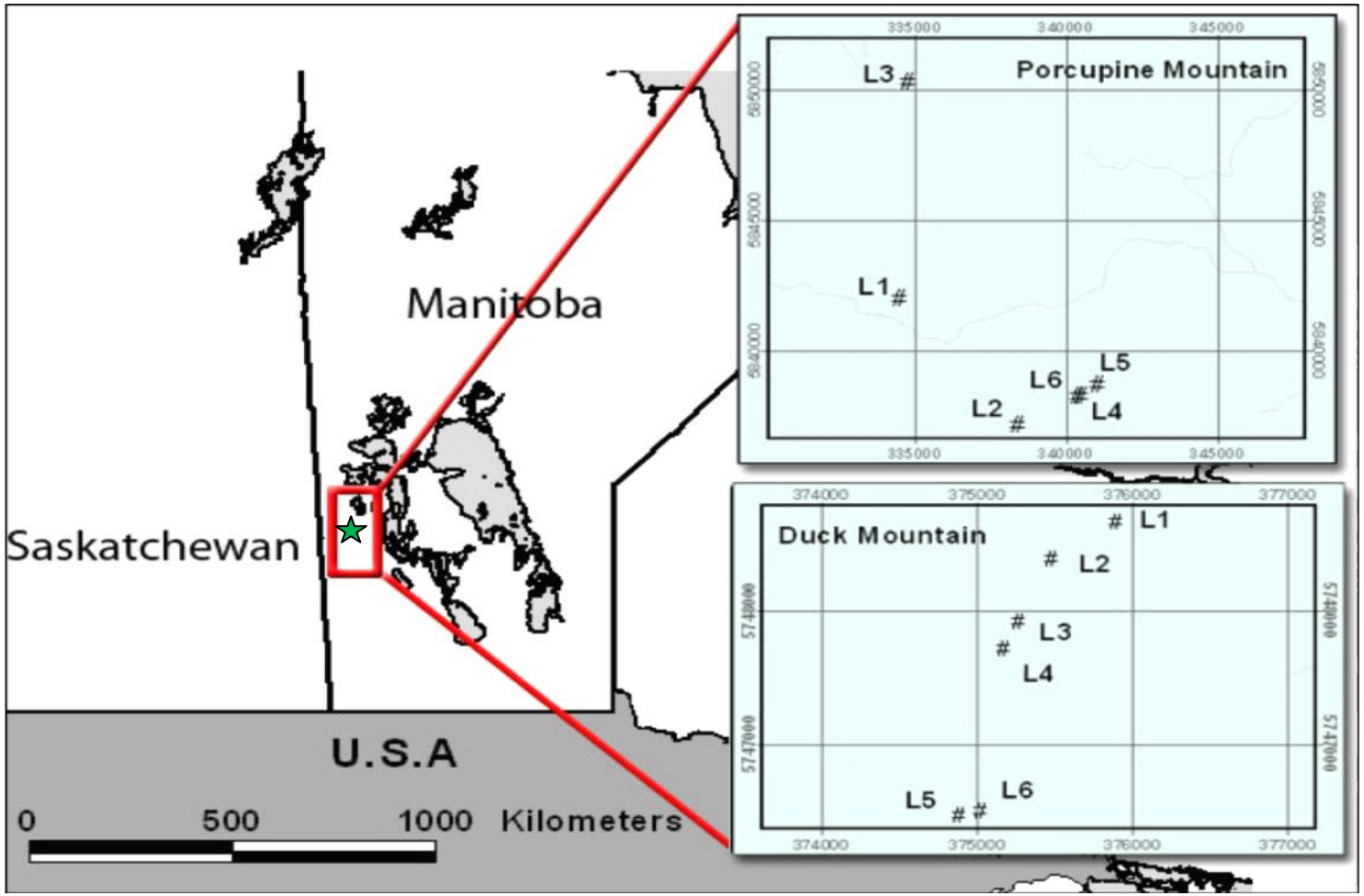

Figure 3: Map of the study area. The Porcupine Provincial Forest study area is shown in the upper map and the sampled sites are shown as L2 and L3. The Duck Mountain Provincial Park study area is shown in the lower map and the sampled sites are shown as L2 and L3. The green star indicates the location of the Swan River climate station.

an elevation of 752m and 655m, respectively as determined by GPS. The Duck Mountain Provincial Park sites were also labelled L2 and L3 and had an elevation of 734m and 729m, respectively (Figure 3). Sampling was conducted from June 15th to June 22nd 2009. Ten black spruce were chosen for stem analysis from each of the sites in Porcupine Provincial Forest and in Duck Mountain Provincial Park. For each tree, cross sections of the stem were taken at 0m, 0.5m, 1.3m, 2m and every 0.5m thereafter up to the top of the tree (Epp and Tardif 2004; Sutton and Tardif 2005). The 40 sampled trees had a mean origin date of 1894 (±3.18 StD) as determined at diameter breast height and had a mean height of 14.6m (±2.3 StD).

2.3 Dendrochronological methods and data analysis

In the laboratory all cross sections were air dried for several weeks before the samples were sanded using a progression of eight sandpaper grits beginning at 80 and ending at 600. The samples were crossdated by F. Conciatori following the list method (Yamaguchi 1991). All dating and crossdating was verified using the COFECHA computer program (Holmes 1983). Black spruce samples had four radii drawn on the cross sections that were labelled A, B, C and D and both WER and FR were systematically identified along each radii macroscopically using a binocular dissecting microscope. The use of four radii allowed assessing variability along the circumference of each tree. For each year and at each height the frequency of WER and FR was compiled for each of the four sites (maximum of 40 radii per site) and for each region (maximum of 80 radii per region). From this compilation, one site in the Porcupine Provincial Forest

(L3; Figure 3) was selected for anatomical study due to its high frequency of WER. From this site, three years exhibiting WER were selected for image analysis of tracheid dimensions.

2.3.1 Sample selection for microtome sectioning

The three years that were selected for anatomical analysis were 1916, 1943 and 1970. The samples pertaining to these years were selected at a height of 1.3 meters to compare different WER years at a constant height. In addition, samples for 1970 were selected at a height of 6 and 11 meters to compare the WER year at different heights. Since WER were not observed macroscopically in all samples for each year and height, it was decided to systematically couple each sample showing WER with another not showing WER to examine if difference existed at the microscopic scale. This was done for each year-height combination, resulting in 5 samples with WER (termed WER) and 5 samples with no-WER (termed NWER). For the year 1970, one replicate of WER/NWER were obtained, resulting in 8 WER and 8 NWER rings including samples from 9 of the 10 trees initially selected at that site. For each WER and NWER, control rings were also selected two years prior to the ring of interest and were termed CWER and CNWER, respectively. The control ring for one 1916 sample was located in 1919 because 1914 was not able to be obtained. In total, 16 slides were prepared for analysis of 32 rings (Appendix A). Prior to anatomical slide preparation, small wood samples were removed from the cross sections along the appropriate radius using a bandsaw and included the ring of interest as well as the control ring. All samples were labelled and placed in plastic

bags. Overall, there were 8 slides to be made at 1.3 meters, 4 slides to be made at 6 meters, and 4 slides to be made at 11 meters (Appendix A).

2.3.2 Microtome sectioning

For anatomical slide preparation, thin sections were obtained following methods outlined in other studies (Ruzin 1999; Chaffey 2002; Jones et al. 2004; Sutton and Tardif 2005). Prior to thin-sectioning, samples were boiled for ten minutes to soften the wood. Sections were obtained from the samples using a Euromex sledge and Leitz rotary microtome with the blade angle set at 15° and thickness set at 20µm. Three thin sections were taken from each sample that included the WER year and at least two tree-rings before and after the year of interest and included the tree-ring selected as a control. The staining procedure consisted of a 5 minute treatment of 1 % Safranin to highlight the lignin content of the cell walls, followed by rinsing the sections with distilled water to remove excess dye. The sections were then placed in successive ethanol baths to dehydrate the wood. Two 2 minute treatments of the wood were applied for both 50% and 95% ethanol. Samples were then treated with 100% ethanol reagent for two 30 second treatments. Following ethanol treatment, the sections were placed in D-limonene for two minutes to clear them of any remaining impurities. The three sections from each sample were mounted on a slide using Permount® and a cover slip placed over them. The slides were labelled and placed on a warming plate with weights placed on top to prevent the formation of bubbles during the drying process. Slides were left for at least a week to allow Permount® to dry.

2.3.3 Image analysis

The first step in image analysis entailed capturing digital images of the 32 rings of interest at 10X magnification using a Nikon DS-FI1 digital camera attached to a Nikon Eclipse 200 microscope connected to a computer. A green filter was used to increase the contrast between tracheid walls and the lumen. The digital images included the previous year's latewood, the earlywood and latewood of the ring of interest and the next year earlywood from left to right respectively (Appendix B). Adobe Photoshop 5.0 LE (Adobe Systems Inc. 1998) was used to merge images when necessary. Each image was then imported into the WinCELL Pro V 2009a (Régent Instruments Inc. 2009) computer program for image correction and analysis (Figure 4; Appendix B). Correction of images involved the use of a tablet and pen to visually repair damage to the tracheid cell walls that resulted from thin sectioning, as well as to increase contrast between the tracheid lumen and cell wall boundaries (Figure 4). For each image, tracheid dimensions were measured along three radial files selected from the upper, middle, and lower portion of the image, respectively. The selection criteria for the three radial files required that they be at least six radial files apart and be relatively undamaged from slide preparation (Figure 4). Tracheids measured along the radial files were divided into an earlywood and latewood component because WinCELL calculates a cell wall/lumen ratio and classifies latewood as tracheids with a wall/lumen ratio >0.25 (Ko Heinrichs et al. 2007). The tracheid dimensions that were measured included tracheid lumen area (LA), lumen diameter (LD), tracheid diameter (TD), and cell wall thickness (CWT), which for an individual tracheid was calculated as half the shared cell wall with the tracheid preceding and following the tracheid being measured along the radial file.

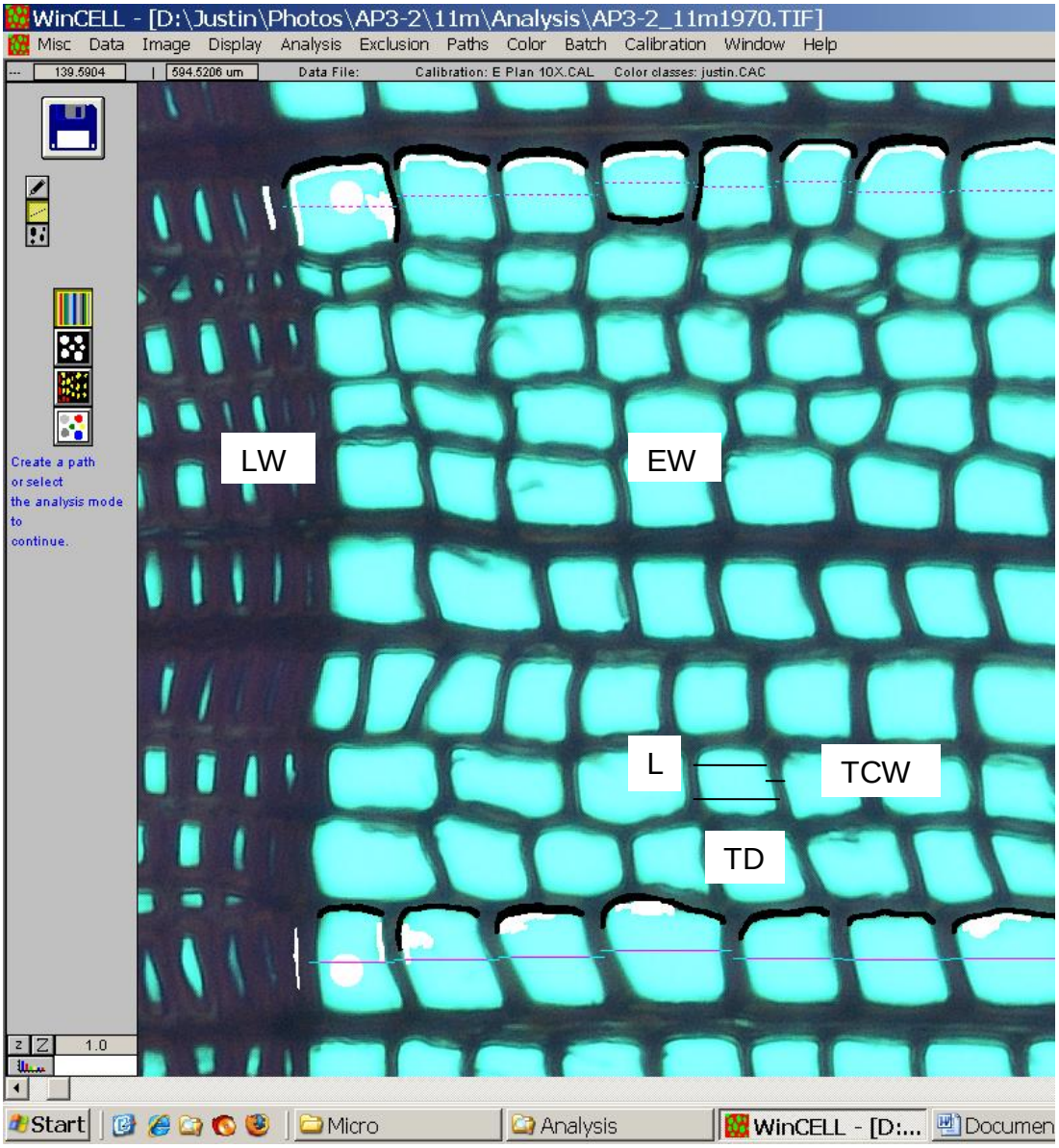

Figure 4: Digital image of a tree-ring sample (partial image) from a black spruce tree showing earlywood tracheids. Indicated in the image are the latewood (LW) in the year t-1 and earlywood (EW) in the year t. Also shown is the lumen diameter (LD) and tracheid diameter (TD) of an individual tracheid. The cell wall is shared between two tracheids, each contributing half of the diameter of the cell wall thickness (CWT). The top and bottom lines indicate two radial files that were measured.

In addition to the 4 parameters describing tracheid dimensions, the number of tracheids (NT) and their cumulative width (CW) were measured. This resulted in 6 parameters to examine WER tracheids in the earlywood (EW), latewood (LW) and total ring (RW). For each sample, the averages of the 3 radial files were calculated for the earlywood, latewood and total ring for use in statistical analysis. Averages were also obtained for the first 8 tracheids from the three radial files of each sample so that the earlywood of the WER and NWER could be compared.

2.4 Statistical analysis

All statistical analyses were undertaken using the student version of Systat 11 (Systat Software Inc. 2004) computer program. The data was first tested for differences between the WER and NWER to assess whether initial macroscopic differences were confirmed at the microscopic level. The same strategy was applied to the control rings. In the absence of any statistically significant differences, pooling of the samples could occur, resulting in an increased sample size from n=8 in each of four groups (WER, NWER, WERC, NWERC) to n=16 in each of two groups (WER, WERC). The same analyses were conducted for the data generated from the first 8 tracheids. Next, the selection of parametric or non-parametric testing required that normality and homogeneity of variance within groups be determined and was assessed using Shapiro-Wilk's test and Levene's test, respectively. Assumptions of normality and homogeneity of variance were violated so non parametric tests were applied. Finally, the groups were tested for differences for LA, LD, CWT, TD, NT, and CW in EW, LW and RW.

Climate data were obtained from the Swan River climate station to examine possible relationships between June temperature and WER and FR formation. June temperature was selected as June corresponds to the period when most tracheid development has been reported to take place (Ko Heinrichs et al. 2007; Rossi et al. 2008). To examine this relationship, the frequency of WER and FR were recorded for each year and height along the 4 radii in each of the 4 sites (Appendix C). The results obtained were for individual years observed at multiple heights. In each site, the results for each year in the ten trees were summed together to represent the frequency observed for the entire site. As the sums for the year in each region were for multiple heights (i.e. 0m up to 18m), the height with the highest frequency of WER or FR was selected to represent frequency for that particular year. The regional data were then converted to relative frequency (%) by dividing the sum of WER and FR per year by 80 (maximum radii for 20 trees) and multiplying the result by 100. This procedure underestimates the WER and FR severity at the top and bottom of the stem where 80 radii were not necessarily present. For each year, the relative frequency of WER and FR along the radii was transformed into three classes (absent: 0%, weak: <5%, and strong: ≥5% of the radii) to test for differences in temperature. For non-parametric testing, the Kruskal-Wallis (KW) test was used to compare the three groups. Results from the KW test that were significantly different were followed by pairwise Mann-Whitney U test between groups.

3.0 Results

3.1 Occurrence of macroscopically determined WER and FR in black spruce stems

Both FR and WER were observed in the cross sections collected from the four sites, with higher frequency of FR observed in Duck Mountain Provincial Park and higher frequency of WER observed in Porcupine Provincial Forest (Figure 5). In many instances, a FR occurred in the year prior to WER formation (Figure 5). In the Duck Mountains, the highest frequency of FR was observed in 1908 at 0.5 meters, occurring in 70 percent of the radii (Figure 5a). The highest frequency of WER was observed in 1916 at 1.3 meters, occurring in 20 percent of the radii. In the Porcupine Provincial Forest, the highest frequency of FR was observed in 1915 at 2.5 meters, occurring in 49% of the radii (Figure 5b). Additionally, the 1942 and 1969 FR were observed in 25% of the radii at 8 and 9 meters and 10% of the radii at 16 meters, respectively. The highest frequency of WER occurred in 1916 at 0.5 meters, occurring in 60% of the radii. Additionally, the 1943 and 1970 WER were observed in 24% of the radii at 2, 2.5 and 3 meters, and 36% of the radii at 11 meters, respectively (Figure 5b).

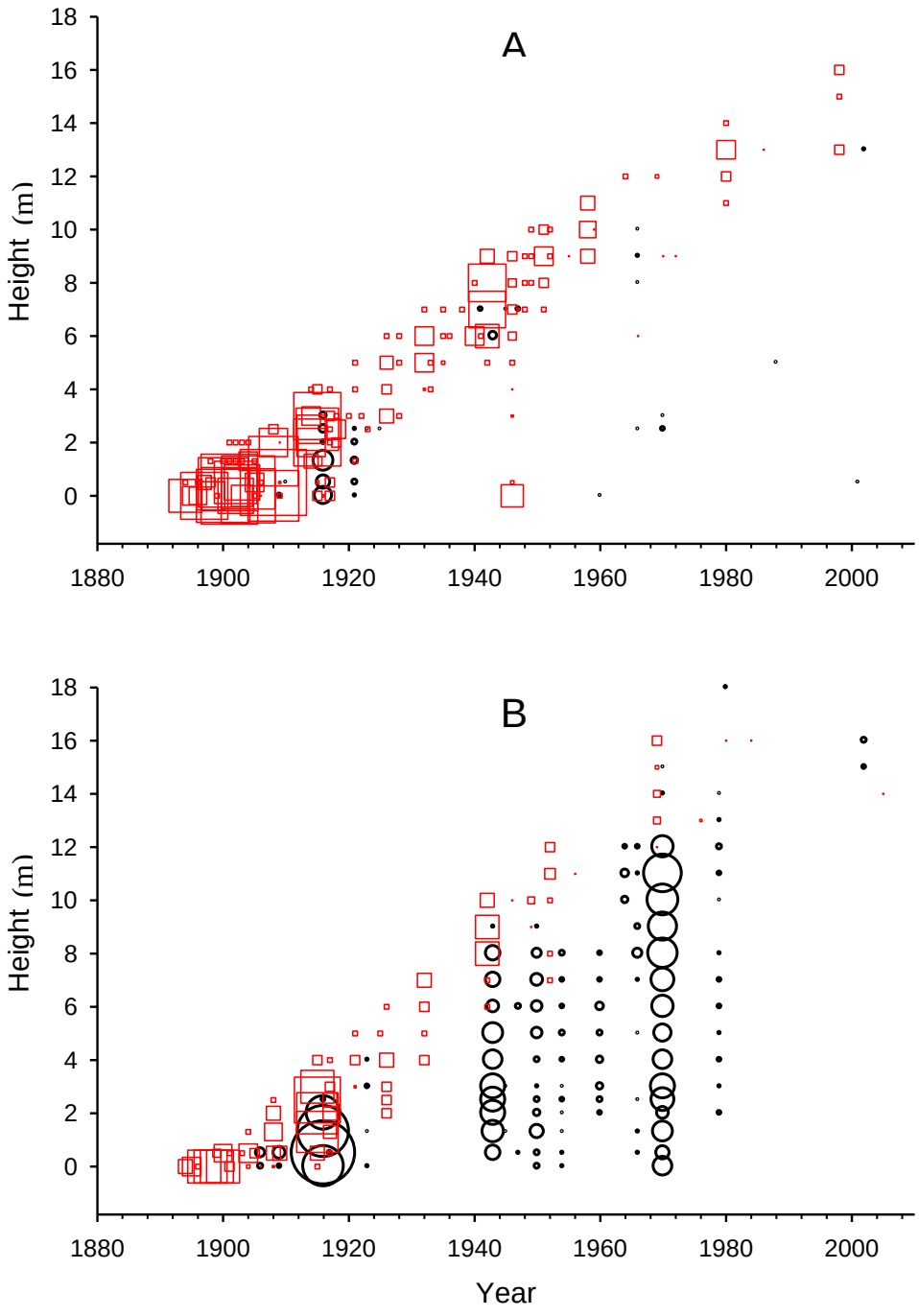

Figure 5: Distribution of white earlywood rings and frost rings in the twenty black spruce stems from Duck Mountain Provincial Park (A) and the twenty stems from Porcupine Provincial Forest (B). Black circles indicate years with white earlywood rings and red squares indicate years with frost rings. The sizes indicate the relative abundance of WER and FR at a given year/height. The results for the two regions were all divided by three to reduce the size of the symbols in the plot and a graph of WER and FR was made for each of the two study areas.

The distribution of WER in the black spruce stems was observed to extend from the base into the upper portion of the tree and was generally not observed in the upper few meters of the stem (Figure 5). Further, the frequency for a given year was fairly uniform throughout the stem. The formation of FR in the black spruce stems occurred regularly in both regions with similar distributions observed within black spruce stems (Figure 5). In general, FR formation was most frequent and intense when tree height was below 4 metres and decreased with height. The diagonal distribution of FR indicated that they occurred higher in the stem as the tree grew taller, up to a maximum of 16 meters.

The spatial distribution of the 1916, 1943 and 1970 WER occurring in black spruce and jack pine samples from across Manitoba was plotted (Figure 6). The 1916 WER was more frequent in black spruce and occurs in both species in the area labelled 63K (Figure 6a). The 1916 WER in black spruce and jack pine occurred over a large area of northern Manitoba and higher elevation areas of western Manitoba (Figure 6a). The 1943 WER has been observed in four sites sampled thus far, and also have a wide distribution in northern and western Manitoba (Figure 6b). The 1970 WER was observed in black spruce and occurred over a large area of northern and western Manitoba (Figure 6c).

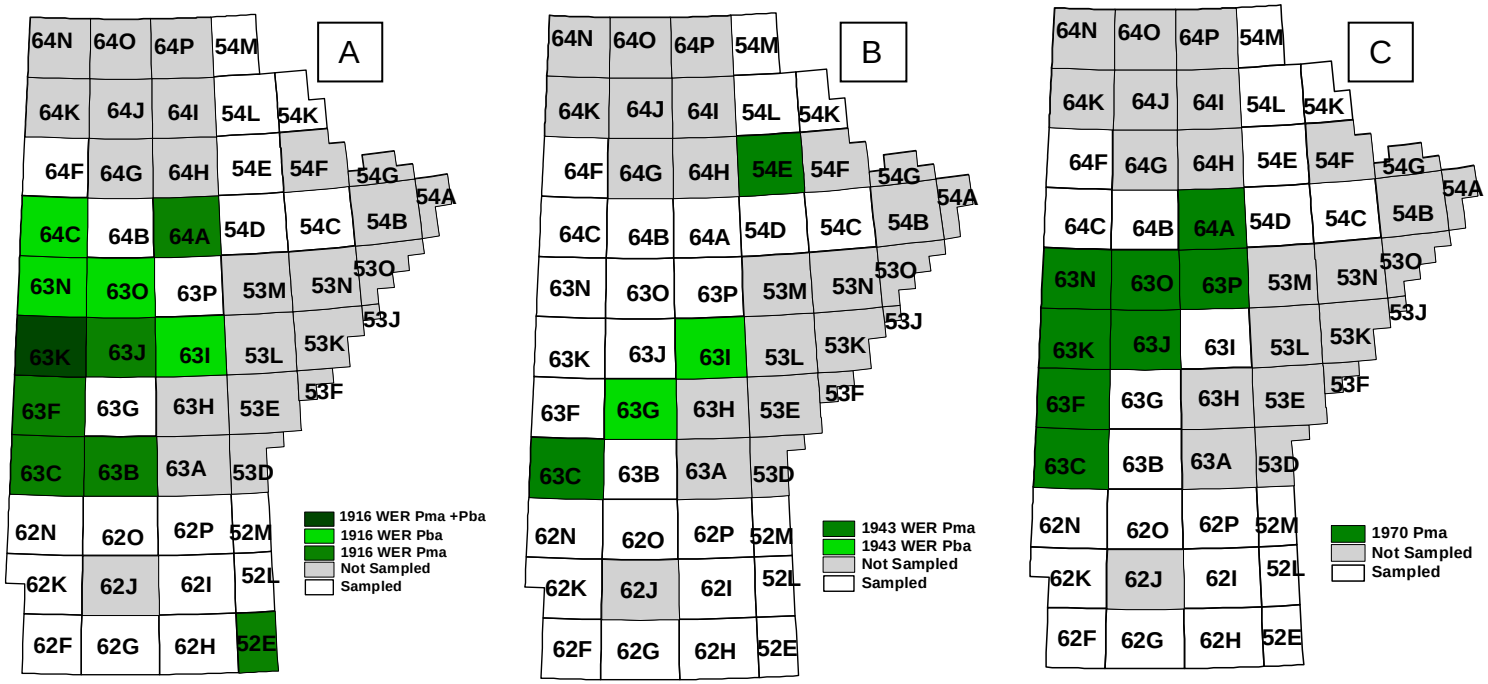

Figure 6: Distribution of white earlywood rings in black spruce and jack pine in Manitoba. The years shown are 1916 (A), 1943 (B) and 1970 (C). The light green colour represents jack pine, the intermediate green represents black spruce, and the dark green represents where white earlywood rings were observed in both species.

The results from the Kruskal-Wallis test indicated that June minimum temperature in at least one of the three FR classes in Duck Mountain Provincial Park differed significantly from the others (Table 1). The Mann-Whitney U pairwise comparisons indicated that the $x \geq 5$ class had a June minimum temperature that was significantly cooler than the $x=0\%$ ($p=0.001$) and $0\%<x<5\%$ classes ($p=0.010$) and the $x=0\%$ and $0\%<x<5\%$ classes were not significantly different ($p=0.484$).

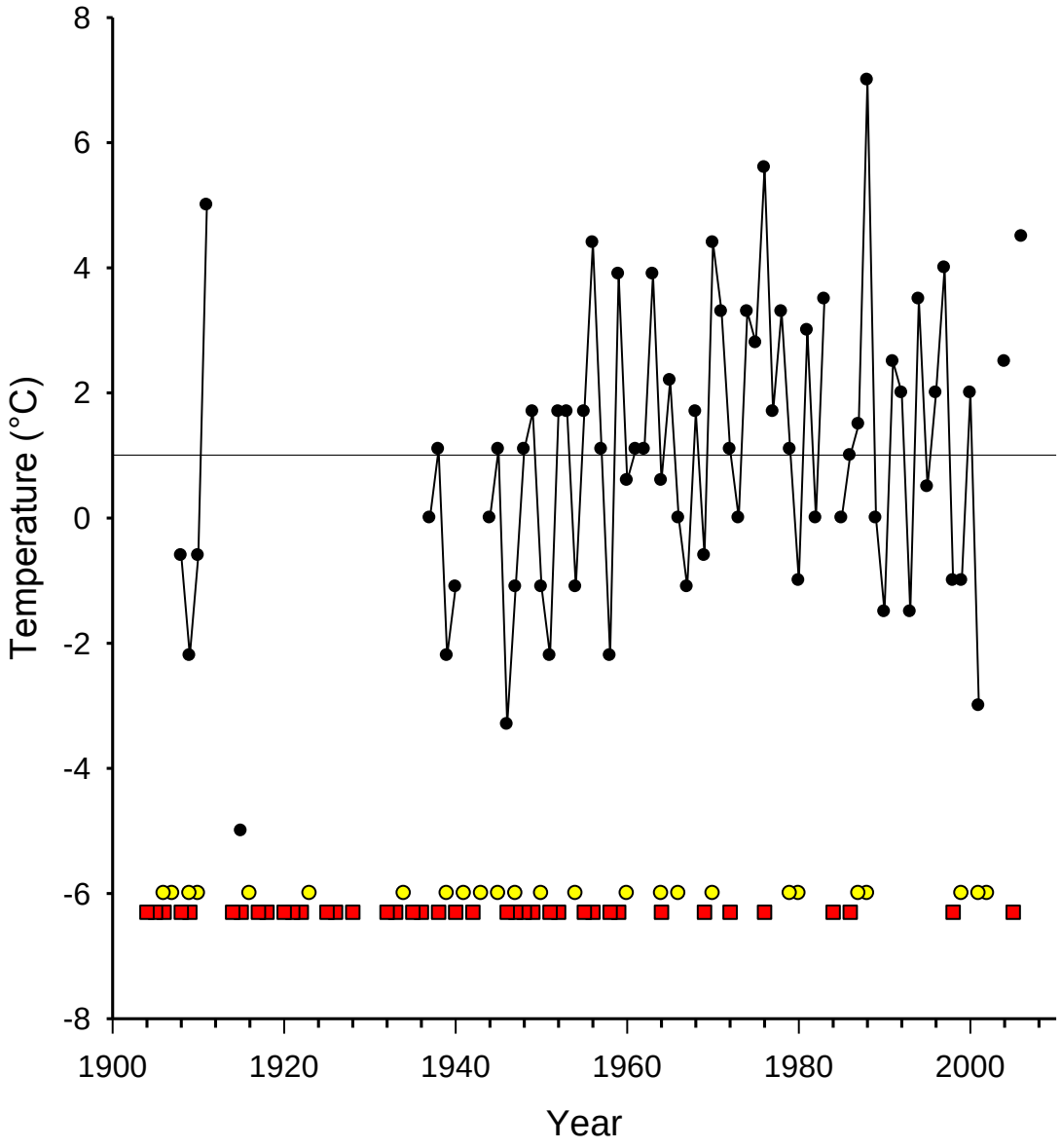

Figure 7: June minimum temperature recorded at the Swan River climate station. Yellow circles represent the years in which white earlywood ring was observed in at least one radius and red squares represent frost ring occurrence in at least one radius, for the overall study region. The mean of the June minimum temperature is also shown as a horizontal line at 1°C.

Table 1: Mean and standard deviation of June minimum temperature for each of the three tree-ring anomaly classes. The number of observations in each class is also indicated. The prefix DMPP and PPF indicate Duck Mountain Provincial Park and Porcupine Provincial Forest, respectively. The suffix WER and FR indicate white earlywood ring and frost ring, respectively. Significant differences ($p<0.05$) obtained by the Kruskall-Wallis test are indicated in bold. Means in subcolumns followed by different letters (a, b) are significantly different (Mann-Whitney U test, $p<0.05$)

Attributes	Group	Mean (°C)	Standard deviation	N	K-W P-value
DMPPWER	0%	1.1	2.2	61	
	0%<x<5%	0.3	3.2	7	
	≥5%	NA	NA	NA	0.196
DMPPFR	0%	1.4a	2.2	52	
	0%<x<5%	0.9a	1.6	10	
	≥5%	-2.0b	1.6	6	**0.005**
PPFWER	0%	1.2	2.4	56	
	0%<x<5%	0	1.6	5	
	≥5%	0.2	2.2	7	0.235
PPFFR	0%	1.1	2.1	59	
	0%<x<5%	2.2	4.8	3	
	≥5%	-0.8	2.5	6	0.196

3.2 WER, NWER and control year pooling

As mentioned earlier, the WER/NWER and CWER/CNWER groups were tested to assess if this grouping, initially determined macroscopically, still existed at the microscopic level. Contrary to expectation, no statistically significant differences were observed between the WER and the NWER rings parameters measured (Table 2). The absence of significant differences was observed for measurements of the first 8 tracheids of the two groups (Table 2). These results indicated that macroscopic identification of WER compared to the microscopic identification provided an underestimation of their frequency. The same absence of significant differences was obtained when both control groups were compared for the entire ring and the first 8 tracheids (Table 2). Based on these results, it was decided to pool the WER and NWER into one group and to also pool the control groups into a single group.

Table 2: Mean and standard deviation for the tracheid parameters measured in the earlywood and latewood. Comparison tests were done between white earlywood ring (WER) and non-white earlywood ring (NWER) holding the year with WER constant, as well as between the WER control (WERC) and NWER control (NWERC), holding the year with NWER constant (A). Also shown are the results for the first eight earlywood tracheids treated alone (B). Significant differences ($p<0.05$) following Mann-Whitney U test are indicated in bold. LA=lumen area, LD=lumen diameter, CWT=cell wall thickness, TD=tracheid diameter, NT=number of tracheids, and W= width. The prefix EW, LW and RW indicate earlywood, latewood and ring width, respectively.

Attributes	WER n=8		NWER n=8			WERC n=8		NWERC n=8		
A) Entire ring										
	Mean	Standard Deviation	Mean	Standard Deviation	p-value	Mean	Standard Deviation	Mean	Standard Deviation	p-value
EWLA(μm^2)	464.37	133.20	458.91	121.93	0.916	441.62	116.35	433.31	90.45	0.752
EWLD(µm)	24.00	3.31	22.72	2.53	0.401	23.47	3.78	23.22	2.10	0.916
EWCWT(µm)	2.56	0.27	2.62	0.31	0.834	2.94	0.37	3.05	0.37	0.833
EWTD(µm)	29.12	3.44	27.95	3.02	0.529	29.34	4.06	29.31	2.80	0.833
EWNT	25.46	8.76	31.62	25.70	0.916	25.92	12.19	35.79	25.06	0.598
EWW(µm)	732.61	239.16	830.74	566.00	1.000	743.72	325.61	1019.82	660.24	0.461
LWLA(μm^2)	104.51	37.06	92.69	20.69	0.753	127.46	31.84	124.02	29.25	0.833
LWLD(µm)	7.13	1.70	6.63	1.19	0.600	8.97	1.66	8.77	1.30	0.833
LWCWT(µm)	4.18	0.72	4.30	0.47	0.600	4.05	0.41	4.10	0.52	0.674
LWTD(µm)	15.50	1.87	15.23	0.51	0.753	17.07	2.36	16.97	1.78	0.674
LWNT	10.54	5.95	13.29	10.89	0.491	14.00	6.93	14.46	5.94	0.673
LWW(µm)	168.36	106.10	205.13	173.33	0.674	245.90	142.30	249.10	109.40	0.598
B) 8 Tracheids										
EWLA (μm^2)	522.53	183.42	511.10	142.95	1.000	504.21	148.74	502.69	103.59	0.834
EWLD (µm)	26.58	4.80	24.68	3.69	0.345	25.91	4.42	26.19	2.33	0.753
EWCWT (µm)	2.33	0.44	2.27	0.35	0.528	2.66	0.39	2.78	0.39	0.875
EWTD (µm)	31.23	4.42	29.22	3.99	0.208	31.24	4.42	31.75	2.99	0.753

3.3 Statistical testing of WER and control group

The results from the six parameters tested for earlywood, indicate that tracheid wall thickness was significantly different between the WER and control group. The mean earlywood wall thickness of the WER group was found to be 13 percent thinner than the earlywood wall thickness of the control group (Table 3; Appendix D). In the latewood, significant differences were observed between the WER and control group for lumen area, lumen diameter, tracheid diameter, and latewood width (Table 3; Appendix E). Compared to the control group, the WER group was found to have reductions in latewood lumen area (23%), latewood lumen diameter (24%), latewood tracheid diameter (10%) and latewood width (25%). For the total ring, significant differences were observed for wall thickness with the WER wall thickness being about 8 percent thinner than that of the control group (Table 3; Appendix F). The first eight earlywood tracheids in the WER and control group were also tested for differences to examine the earlywood without the influence of the transition to latewood. Here, significant differences were observed for wall thickness with wall thickness in WER being about 15 percent thinner than that of the control group (Table 3).

Table 3: Mean and standard deviation for the tracheid parameters measured in the earlywood, latewood and total-ring for both the white earlywood (WER) and control rings (A). Also shown are the results for the first eight earlywood tracheids treated alone (B). Significant differences ($p<0.05$), following the Mann-Whitney U test, are highlighted in bold. LA=lumen area, LD=lumen diameter, CWT=cell wall thickness, TD=tracheid diameter, NT=number of tracheids and W=width. The prefix EW, LW and RW indicate earlywood, latewood, and ring width, respectively.

Attributes	WER n=16		Control n=16		
A) Entire ring					
	Mean	Standard Deviation	Mean	Standard Deviation	p-value
EWLA (μm^2)	461.62	123.39	437.46	100.77	0.522
EWLD(µm)	23.36	2.92	23.34	2.96	0.940
EWWL(µm)	2.59	0.28	2.99	0.36	**0.002**
EWTD(µm)	28.54	3.18	29.33	3.37	0.451
EWNT	28.54	18.82	30.85	19.70	0.734
EWW(µm)	781.67	422.80	881.77	522.72	0.763
LWLA(µm)	98.60	29.63	125.74	29.59	**0.019**
LWLD(µm)	6.88	1.44	8.87	1.44	**0.001**
LWWL(µm)	4.24	0.59	4.07	0.45	0.546
LWTD(µm)	15.36	1.33	17.02	2.02	**0.021**
LWNT	11.92	8.59	14.23	6.24	0.104
LWW(µm)	186.75	140.12	247.50	122.63	**0.035**
B) 8 tracheids					
EWLA(μm^2)	516.82	158.97	503.45	123.83	0.880
EWLD(µm)	25.63	4.23	26.05	3.42	0.598
EWWL(µm)	2.30	0.34	2.72	0.39	**0.004**
EWTD(µm)	30.22	4.20	31.49	3.66	0.258

4.0 Discussion

4.1 Frost ring distribution

The distribution of FR in the black spruce stems was fairly similar between the two regions: though trees in the Duck Mountain Provincial Park had a higher frequency of FR than in Porcupine Provincial Forest. In many instances FR formation occurred in the same year for both regions, indicating that the climatic processes responsible were fairly widespread. However, FR formation was also site-specific, suggesting that FR formation may also be influenced by the micro-climate of individual sites (Schweingruber 2007). Frost ring formation was most intense for juvenile trees that were below 4 meters in height and decreased in visibility as the tree grew taller. Gurskaya and Shiyatov (2006) observed similar FR distributions in Siberian spruce (*Picea obocata* Ledeb.) and Siberian larch (*Larix sibirica* Ledeb.). They found that FR occurred in stems smaller than 5cm in diameter with a bark thickness below 3mm up to a maximum of 7 meters tall. Rhoads (1923), Fayle (1981) and Payette et al. (2010) have also reported the association of FR formation with small diameter, thin barked wood. The diagonal distribution of FR visually represents this association between FR and small diameter, thin barked wood as these conditions occur higher off the ground as the tree grows taller. However, in contrast to the maximum height observed by Gurskaya and Shiyatov (2006) the distribution of FR in our samples occurred up to 16m in the stem and similar heights for FR formation were observed by Fayle (1981) in red pine (*Pinus resinosa* Ait.).

Results from the climate comparison indicated that FR formation often occurred in years with June minimum temperature that were below freezing. This is supported by Payette et al. (2010) who reported that FR formation in black spruce requires a nocturnal temperature below 0°C for at least 6 hours, with a minimum of -6°C. Unfortunately, the temporal resolution of the data from the Swan River climate station is not detailed enough to observe temperature fluctuations for this short a time period. Results from the climate data statistics indicate that the relationship between strong FR formation (≥5%) and June minimum temperature was significant for the Duck Mountain region, but not for the Porcupine Forest region and may be the result of variable topography and air circulation expected within different sites. This is supported by Dy and Payette (2007) who reported that slight changes in elevation led to a heterogeneous distribution of frost damage.

4.2 White earlywood ring distribution

Contrary to the higher frequency of FR observed in the Duck Mountain region, the majority of WER were observed in the Porcupine Provincial Forest and mainly from site L3. The first hypothesis regarding WER distribution in the stem was partially supported because, while they were typically observed to have continuous distribution throughout the stem, they were largely absent in the upper few meters of the stem. Sutton and Tardif (2005) reported a similar distribution of white rings in trembling aspen and suggested that white rings were not observed in the top 30 percent of the tree due to the close proximity to photosynthate and hormone production in the crown. Close association with the crown suggests that, following incomplete defoliation, tracheid development near the top of the

tree may have sufficient availability of photosynthates and growth hormones to complete development and so may not be as affected by defoliation events. In addition, Lamontagne et al. (1998) have observed that recovery of photosynthetic function occurred faster in the upper crown than in the lower crown following artificial frost application to conifer branches. The results from statistical testing for pooling of the groups indicated that there was no significant difference between the WER and NWER group. This means that the macroscopic identification of WER underestimates the actual presence of WER. The visual identification of WER may also be underestimated in the lighter coloured sapwood as compared to the darker heartwood.

The distribution of WER observed by the dendroecology laboratory showed that they were observed synchronously across a large portion of northern and western Manitoba where sampling has been conducted. The occurrence of the same WER in multiple species of trees over a large area suggests that climatic events may be at the source of this anomaly. Climatic conditions are expected to be more severe in higher elevation areas, compared to lower elevation at the same latitude (Strahler and Strahler 2005). In our samples, WER like FR also varied in intensity and frequency within individual stems and between sites and may be the result of slight variation in micro-climates of individual trees (Gurskaya and Shiyatov 2006).

4.3 Frost damage and white earlywood rings

For WER of the same year occurring over a large area to be explained through frost influence, frost events must also have a large spatial distribution. Man et al. (2009) observed severe foliage damage in six conifer species in Ontario following a severe frost event. They reported that the conditions required to produce the type of damage observed was early loss of stem hardening followed by warm post-freeze temperatures and strong radiation. They observed the damage over a large area with affected trees up to 8m in height and estimated that over 70 percent of older needles and over 20 percent of buds were damaged. Horntvedt and Venn (1980) also reported a large frost event that affected conifers growing in southern Norway and Sweden. They reported that the conditions that caused the damage were late onset of stem hardening coupled with unfavourable temperatures in January and February. They observed foliage damage in several genera of conifers and reported that damage was highly variable within stands. However, in both studies, analysis of tree ring development in the affected conifers was not undertaken.

It is generally accepted that damage to foliage influences hormone and photosynthate production and has a negative impact on tissue development (Polge and Garros 1971; Sutton and Tardif 2005; Dy and Payette 2007; Schweingruber 2007). Damage to the foliage interferes with tissue development through alteration of the integrated carbon source/sink relationship in conifers. Hansen and Beck (1994) studied this relationship and observed that developing buds were supplied with carbohydrates from older needles, whereas tracheid development in the stem was dependent on reserve carbohydrates from

previous years growth. Further, Ericsson et al. (1980) studied the hierarchy of growth in conifers and found that foliage development was favoured at the expense of stem development. Therefore, if a tree has to replace foliage following defoliation, it does so by drawing on reserve carbohydrates that could have been used for tracheid wall development.

4.4 White earlywood tracheids

Our results indicated that cell wall thickness was reduced in the earlywood of WER compared to that of controls, which confirms our second hypothesis. Similar observations were made by Polge and Garros (1971) who reported the formation of a low density earlywood following artificial defoliation of maritime pine. Sutton and Tardif (2005) also observed similar results in fibres of trembling aspen following defoliation by forest tent caterpillar and explained this reduction in cell wall thickness as a result of redistribution of reserve carbohydrates to reflush the foliage that otherwise could have been used for secondary cell wall deposition. They also related the decreased cell wall thickness to hormone depletion, as several hormones necessary for growth, including auxins and gibberellins, are produced in the crown. Defoliation by frost events in the year prior to WER formation or during the winter prior to WER formation may have similar consequences for conifers through a reduction in carbohydrate availability.

Our results also indicated that several latewood characteristics, including latewood width, lumen area, and tracheid diameter were significantly reduced in WER years

compared to that of the control group. However, it is unclear whether differences observed for the latewood were related to the condition leading to WER formation. This uncertainty is shown by Wimmer and Grabner (2000) who observed that earlywood and latewood tracheid production were under different environmental controls, with latewood production correlated to low fall precipitation and warm temperatures. Deslauriers and Morin (2005) studied tracheid formation in balsam fir over several years and observed that the onset of latewood production was highly variable from year to year and was determined by climatic conditions. Similarly, Ko Heinrichs et al. (2007) observed that the timing and duration of latewood production was highly variable among species. As temperature has been reported to be largely responsible for latewood development, differences observed between the WER and control group could potentially be due to differing climate for the two years.

4.5 Implications and limitations of the study

The implications of this study for dendrochronology are twofold. First, effects of frost have been observed much higher in the stem (i.e. 16m) than was generally reported in the literature (i.e. 7m). There was some ambiguity in FR classification as frost events can be manifested as the characteristic bent rays and disfigured tracheids with the absence of a crushed xylem layer. The formation of FR were fairly common in upland black spruce, occurring in many of the rings. The weak association between WER formation and June minimum temperature was potentially because the climate data did not accurately reflect local site conditions. However, the wide distribution of WER in several

species suggested that climatic processes are at the source of their formation. In addition, the high frequency of FR means that the climate signal may have been obscured by frost events. Further work is needed to determine the effects that FR formation has on tree-ring development. Second, this study described a new anomaly that often has macroscopic manifestations that can be used along with other tree-ring anomalies to better represent past environmental conditions. However, the absence of significant differences between the WER and NWER group means that WER were underestimated in the samples. Further work is needed to clearly assess the cause of this anomaly, but a plausible hypothesis is their formation in response to foliage damage associated with environmental conditions during the dormant period or early in the growing season.

Conclusion

The presence of WER have been observed in several conifer species throughout western and northern Manitoba. They have been synchronously observed in black spruce stems in Duck Mountain Provincial Park and Porcupine Provincial Forest. This suggests that climatic processes may be at the source of this anomaly. In many of the samples, macroscopically identified WER were associated with a FR in the year prior to formation indicating a possible association between the two. Alternatively, WER may be the result of severe winter conditions prior to WER formation. Our results indicated that the formation of WER was continuous throughout the stem whereas the distribution of FR was associated with smaller diameter, thin barked portion of the tree. Further, WER were largely absent in the upper few meters of the stem, possibly resulting from close proximity to the crown or a masking effect by the relatively pale sapwood. Our results also indicated that macroscopic identification of WER underestimated their actual frequency observed at the microscopic level. At the tracheid level, results indicated that the earlywood cell walls of WER were reduced compared to the controls. This decrease in cell wall thickness has been observed in other studies and has been attributed to defoliation and a subsequent decrease of carbohydrate availability for cell wall deposition. Further research into WER formation in the different species should be conducted to obtain a better understanding of the spatial distribution of WER and degree of cell wall reduction in relation to climatic variables. A definitive cause of WER has yet to be determined.

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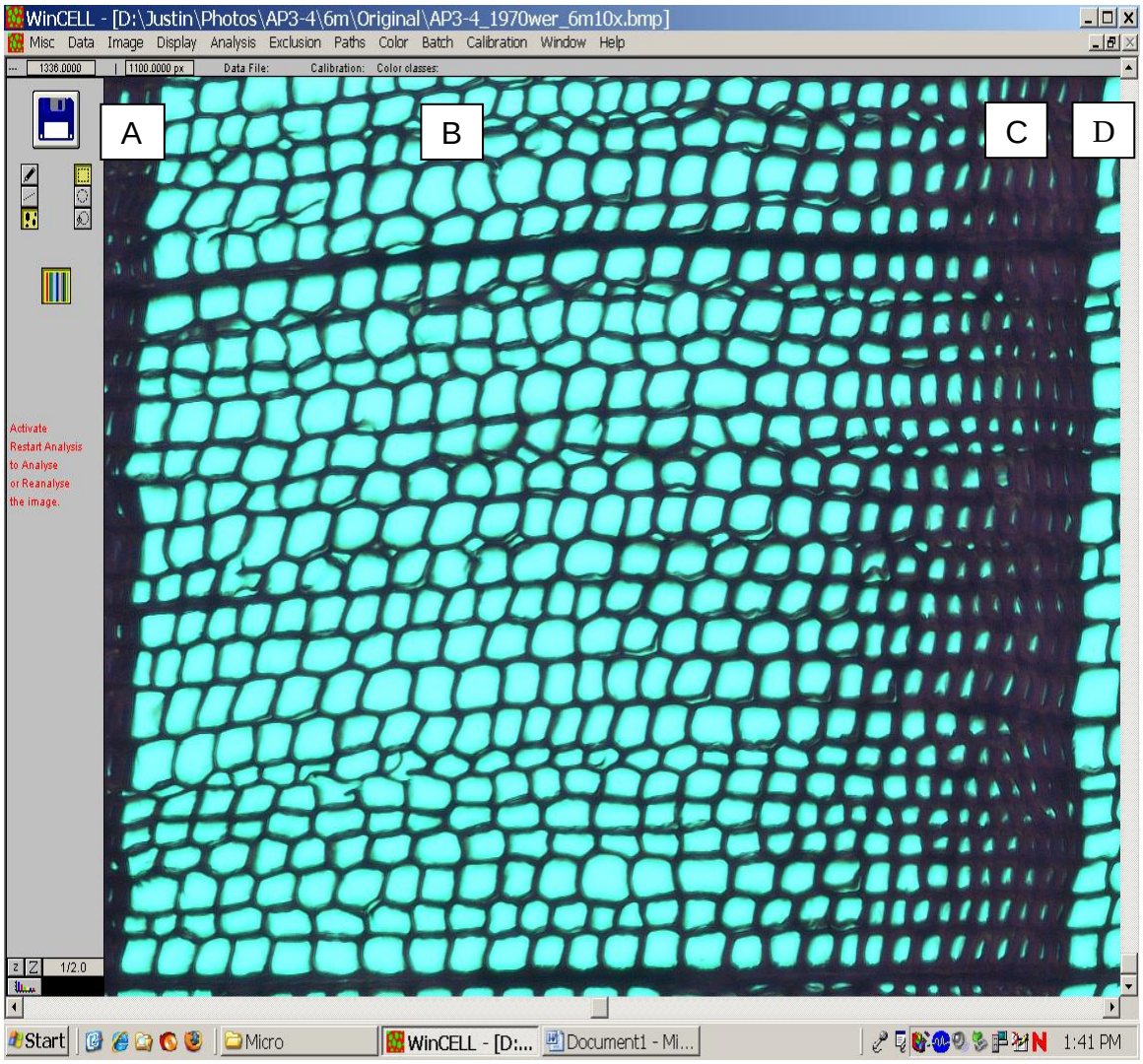
Appendices

Appendix A: Data for microtome sample selection. Numbers indicate living stems sampled and letters indicate dead stems sampled. The radii were chosen by random number generator where 1=A, 2=B, 3=C, 4=D.

Year	Visual I.D.	Sample number	Height (m)	Radii	Control	Radii
1916	WER	5	1.3	B	1914	B
	NWER	E	1.3	C	1919	C
1943	WER	E	1.3	C	1941	C
	NWER	3	1.3	B	1941	B
1970	WER	2	1.3	B	1968	B
	WER	5	1.3	B	1968	B
	NWER	4	1.3	B	1968	B
	NWER	A	1.3	B	1968	B
	WER	2	6	B	1968	B
	WER	B	6	B	1968	B
	NWER	4	6	B	1968	B
	NWER	5	6	B	1968	B
	WER	2	11	B	1968	B
	WER	C	11	A	1968	A
	NWER	1	11	B	1968	B
	NWER	E	11	C	1968	C

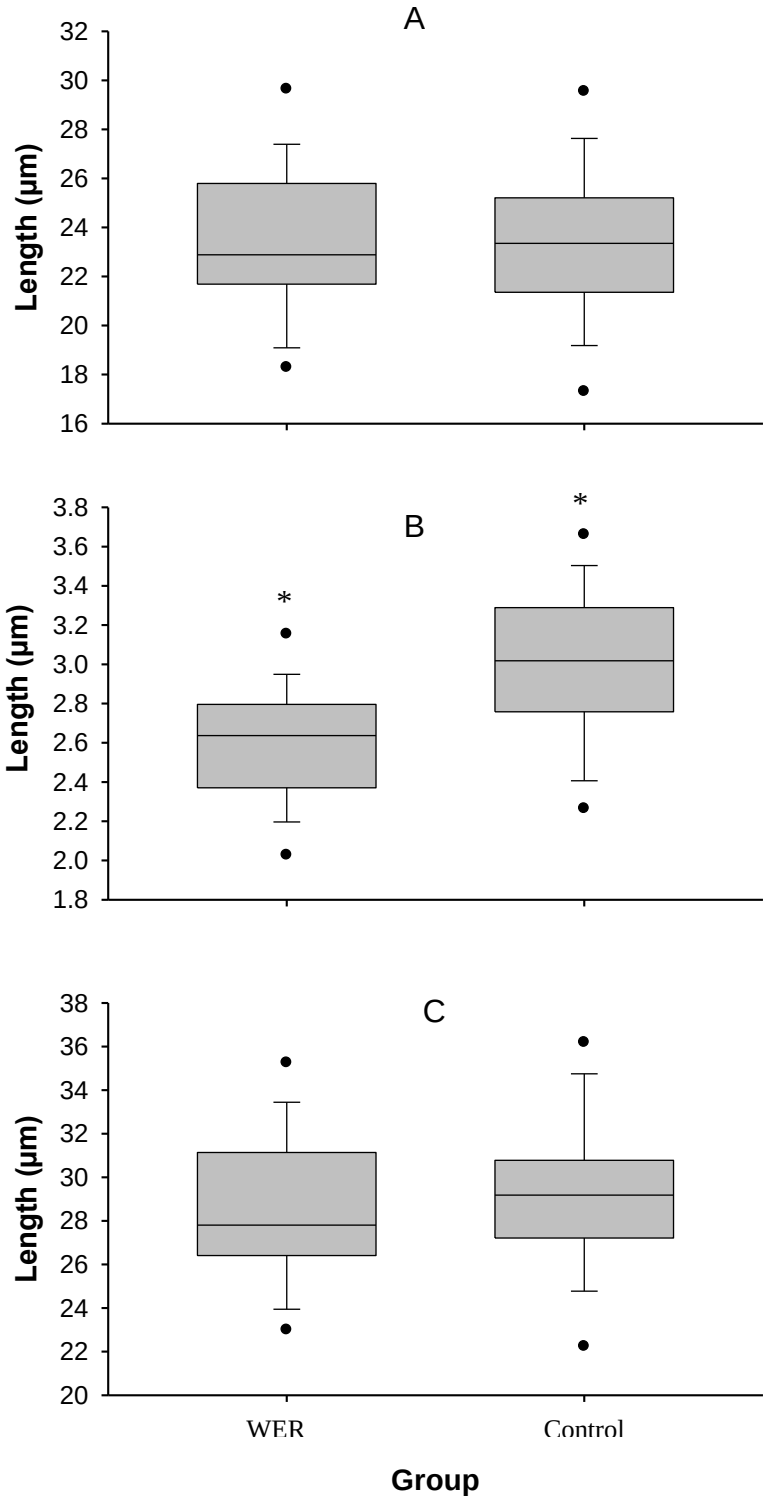
Appendix B: Digital image of a tree-ring sample from a black spruce tree showing latewood of the year t-1 (A), earlywood of the year t (B), latewood of the year t (C), and earlywood of year t+1 (D).



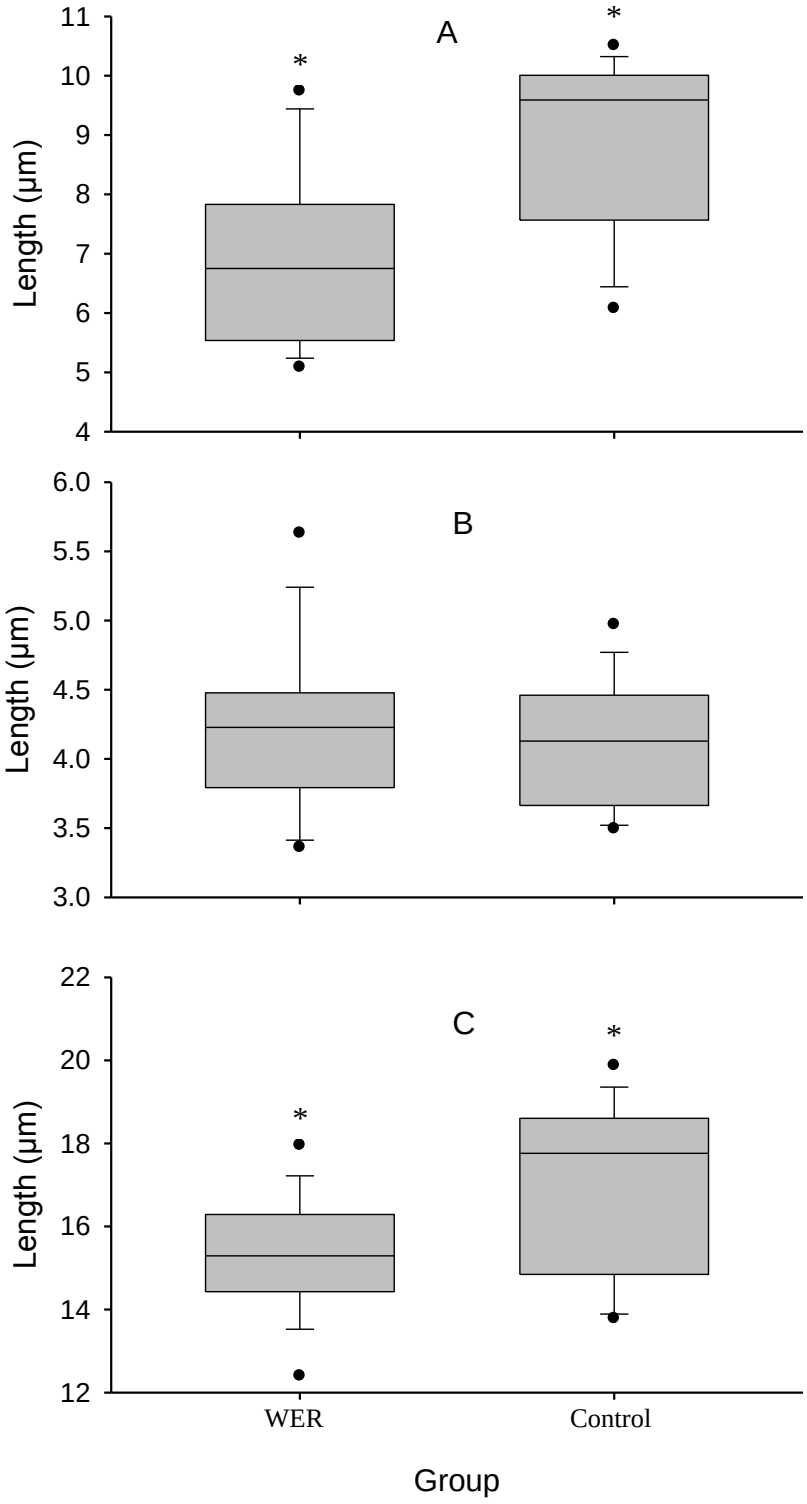
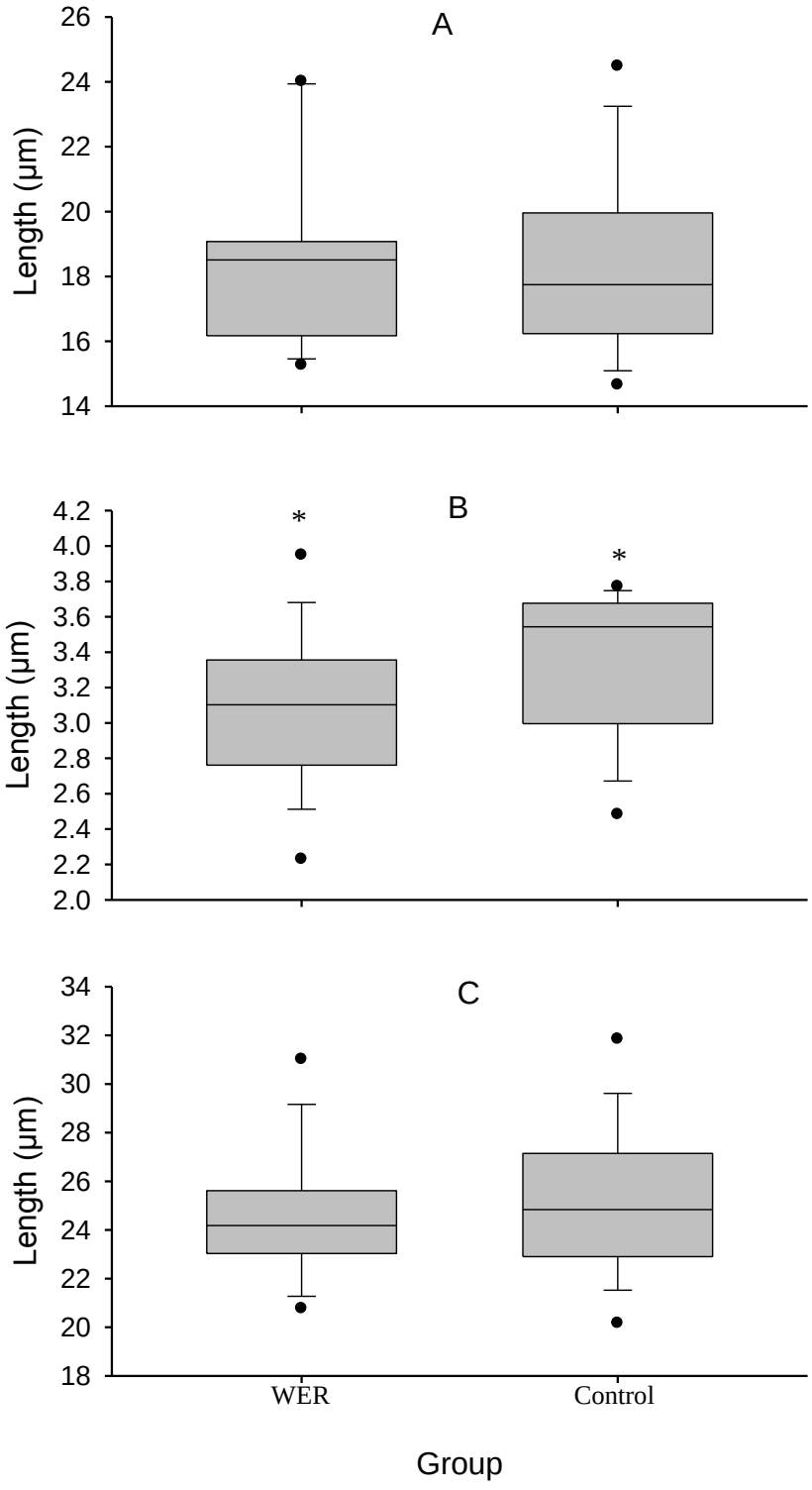
Appendix C: Date in which white earlywood rings (WER) and frost rings (FR) were observed in the two Duck Mountain and two Porcupine Forest sites. Sites that had the anomaly in the same year are indicated in bold.

FR				WER			
DM 1	DM 2	PM 1	PM 2	DM 1	DM 2	PM 1	PM 2
1894	1894	1894	**1904**	1910	1899	**1916**	1906
1895	1895	1895	**1909**	**1916**	1909	1923	1907
1896	1896	1896	**1915**	1919	**1916**	1934	1909
1897	1897	1897	**1917**	1921	1941	1939	1915
1898	1898	1898	1926	1923	**1966**	**1943**	**1916**
1899	1900	1899	**1932**	1925	**2002**	**1945**	1917
1900	1901	1900	**1942**	1935	-	**1947**	1920
1901	1902	1901	**1952**	1941	-	1950	**1943**
1902	1903	1903	-	**1943**	-	**1966**	**1945**
1903	**1904**	**1904**	-	**1945**	-	**1970**	**1947**
1904	1905	1905	-	**1947**	-	1979	1950
1905	1906	1906	-	1960	-	1980	1954
1906	1908	1908	-	**1966**	-	1988	1956
1908	**1909**	**1909**	-	**1970**	-	**2002**	1960
1909	1914	**1915**	-	1988	-	-	1964
1914	**1915**	**1917**	-	1998	-	-	**1966**
1915	1916	1921	-	1999	-	-	**1970**
1917	**1917**	1925	-	2001	-	-	1976
1932	1918	1926	-	**2002**	-	-	1979
1942	1920	**1932**	-	-	-	-	1987
1946	1921	**1942**	-	-	-	-	-
1952	1922	1946	-	-	-	-	-
1958	1923	1949	-	-	-	-	-
1970	1926	**1952**	-	-	-	-	-
1972	1928	1956	-	-	-	-	-
1980	**1932**	1969	-	-	-	-	-
1986	1933	1976	-	-	-	-	-
1998	1935	1980	-	-	-	-	-
-	1936	1984	-	-	-	-	-
-	1938	2005	-	-	-	-	-
-	1940	-	-	-	-	-	-
-	1941	-	-	-	-	-	-
-	**1942**	-	-	-	-	-	-
-	1946	-	-	-	-	-	-
-	1947	-	-	-	-	-	-
-	1948	-	-	-	-	-	-
-	1949	-	-	-	-	-	-
-	1951	-	-	-	-	-	-
-	**1952**	-	-	-	-	-	-
-	1955	-	-	-	-	-	-
-	1958	-	-	-	-	-	-
-	1959	-	-	-	-	-	-
-	1964	-	-	-	-	-	-
-	1966	-	-	-	-	-	-
-	1969	-	-	-	-	-	-
-	1980	-	-	-	-	-	-
-	1998	-	-	-	-	-	-

Appendix D: Box plots of earlywood tracheid dimensions for the white earlywood ring (WER) and control group. Box plots are for lumen diameter (A), cell wall thickness (B), and tracheid diameter (C). Dimensions with significant differences ($p<0.05$) following the Mann-Whitney U test are indicated by an asterisk (*).



Appendix E: Box plots of latewood tracheid dimensions for the white earlywood ring (WER) and control group. Box plots are for lumen diameter (A), cell wall thickness (B), and tracheid diameter (C). Dimensions with significant differences ($p<0.05$) following the Mann-Whitney U test are indicated by an asterisk (*).



Appendix F: Box plots of total ring tracheid dimensions for the white earlywood (WER) and control group. Box plots are for lumen diameter (A), cell wall thickness (B), and tracheid diameter (C). Dimensions with significant differences ($p<0.05$) following the Mann-Whitney U test are indicated by an asterisk (*).