THE EFFECTS OF *ARMILLARIA* ROOT ROT DISEASE ON THE GROWTH OF *PICEA MARIANA* TREES IN THE BOREAL PLAINS OF WESTERN MANITOBA

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ABSTRACT

Armillaria spp. are a complex of fungal pathogens affecting Manitoba populations of upland black spruce (*Picea mariana* (Mill.) B.S.P.). In Duck Mountain Provincial Forest and Porcupine Provincial Forest, stands are severely infested with *Armillaria* root disease. In 2007-2008, dead and living trees in 6 infection centres per region were sampled for growth and mortality analyses. In 2009, a subset of 5 infected dead and 5 uninfected living trees from two sites per region were selected for stem analysis. To determine if *Armilla ria* caused a tree-level impact on growth, dendroecological techniques were used to examine mortality patterns and growth changes prior to mortality. The onset of mortality in affected stands occurred quasi-synchronously across the sampling regions, though differences existed among individual sites within each region. Testing of incremental growth ratios indicated that infected trees experienced a sustained decline in basal area and volume increment 5-15 years prior to death, as compared to uninfected trees. This significant decline in growth was however not expressed in overall tree productivity. Comparing logistic regression curves of cumulative basal area, height, and volume growth did not reveal significant differences between coefficients associated with dead and living trees, except for the inflection points (height and volume). Infected trees reached half of their total height and volume growth at a significantly younger age than uninfected trees and it is speculated that their increased vigour and larger root systems may have predisposed them for infection. Mortality in all black spruce stands occurred at an average of 96-99 years, which is beyond the current rotation age (75 years) for these stands. These findings suggest that this rotation age is sufficient in the presence of infection in upland black spruce.

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1. INTRODUCTION

1.1 Forestry and *Armillaria*

In order to effectively manage forests for timber production as well as for ecosystem services and wildlife values, it is essential to predict and understand causes of tree mortality (Antos et al. 2007). Members of the fungal genus *Armillaria spp.* have a circumboreal distribution, and are present in all forest regions of Canada (Morrison and Mallett 1996) and are an important contributor to tree mortality in the boreal forest. This has resulted in significant economic losses (Bendel and Rigling 2008).

In Canada, *Armillaria* root disease contributes to tree decay that impacts timber resources. Brandt (1995) estimated losses in forest resources in Alberta and Saskatchewan of 3% from 1988-1992, amounting to annual volume losses due to mortality of 60 000 m^3 and 116 000 m^3, respectively. In Manitoba, decay affected 3% of the province's forest resources, causing a volume loss of 144 000 m^3 per year. The average onset of decay for all three provinces was a stand age of 80 years (Brandt 1995). British Columbia experiences losses in managed forests from *Armillaria* alone of 2-3 million m^3 (Morrison and Mallet 1996). Incidences of *Armillaria* infection are becoming more common across Canada in recent years, and are thought to be favoured by any forest management activity that creates stumps (Morrison and Mallett 1995). In order to effectively address this growing problem, it is important to understand the impacts of the disease at both the stand level and the tree level.

In Manitoba, Duck Mountain Provincial Forest (DMPF) and Porcupine Provincial Forest (PPF) contain extensive *Armillaria* root rot infestation in upland black spruce (*Picea mariana* (B.S.P.) Mill) stands (Knowles 2004; Knowles 2007; Epp et al. 2009). In the DMPF, it was estimated that 2% of potential merchantable volume has been lost in the Clearwater Creek operating area due to tree mortality, and that the area of infestation may double over the next 15 years (Knowles 2007). However, another estimate places merchantable volume losses in DMPF much higher, at 14-45% (Pines *in* Epp et al. 2009). In the PPF, root disease in the Schade Lake operating area has caused volume loss estimated at 102 m^3, or 8% of total potential volume (Knowles 2004). Fifteen-year projections suggested that the infected area may more than triple in size. In Ontario, older black spruce (median age of 70) have exhibited an 8.4% reduction in merchantable volume due to mortality caused by *Armillaria* (Whitney 1978).

Infection by *Armillaria ostoyae* is thought to be associated with a gradual decline in accumulation of volume and basal area. Antos et al. (2007) found that the basal area increment (BAI) of living control trees showed a continual increase through time, whereas the BAI of deceased trees only increased until ~40 years prior to death. Research on lodgepole pine (*Pinus contortata* Dougl. ex Loud. var. *latifolia* Engelm.) by Mallett and Volney (1999) found no significant differences in total height or DBH between infected and uninfected trees. However, losses were found in annual volume increment (43%), specific volume increment (32%), and height increment (23%) (Mallett and Volney 1999). The taper of tree stems increased after infection, as *Armillaria* had a greater effect on radial growth than height growth (Mallett and Volney 1999).

1.2 Biology of *Armillaria*

Armillaria root rot is caused by fungal pathogens in the phylum Basidiomycotina, order Agaricales (Mallett 1990). The most common isolate in Manitoba is *Armillaria ostoyae* (Romagn.) Herink (86.3%), followed by *A. sinapina* Bérubé & Dessureault (12.4%) and *A. calvescens* Bérubé & Dessureault (1.3%). The preferred hosts of *Armillaria* are conifers, though broad-leaved trees and shrubs are also infected (Morrison and Mallett 1996). *Armillaria* spreads in expansive mycelial clones that move across a landscape, which can range in size from 1-5 hectares in some areas, and 20-965 hectares in others, with the largest ever recorded being 965 hectares and spanning 5.6km (Hagle 1996). Symptoms of infection in trees include growth reduction, yellowish to reddish discolouration of foliage (chlorosis), thinning crowns, stringy yellow root rot and butt rot, the presence of white mycelial fans, as well as resinosis around the root collar (Hiratsuka et al. 1995; Omdal et al. 2004; Hagle 2006). Symptoms can vary across climatic regions (Bendel and Rigling 2008). Much of the root system can also be infected or killed by the pathogen before any visible symptoms arise (Omdal et al. 2004). The most useful indicator of infection is the presence of mycelial fans (Bendel and Rigling 2008).

Armillaria infection spreads primarily through root grafts and rhizomorphs (dark-to-orange fungal strands) which form on decayed wood and extend throughout the soil up to 10m beyond the host plant (Hiratsuka et al. 1995). The pathogen penetrates the root through the use of mechanical pressure and enzymatic action, initiating infection (Fuller and James 1986). *Armillaria* is considered a 'disease of the site', as established mycelia are essentially permanent (Hagle 2006). *Armillaria* naturally exists in the soil, but usually exists in a host-disease

equilibrium whereby *Armillaria* is rarely a primary mortality agent, but functions to decompose dead woody tissues in the soil. When the equilibrium between hosts and disease is compromised, it results in the formation of active disease centers that kill trees much more readily (Morrison and Mallett 1996). Site characteristics play a role, including soil type and species present, in the incidence and spread of *Armillaria* root disease through effects on both the fungus and host (Wiensczyk et al. 1997).

The life cycle of *Armillaria* has three critical components (Morrison and Mallett 1996). First, the fungus infects a host by contacting healthy roots with underground rhizomorphs or root contacts with infected trees. It colonizes the roots of the hosts, and mycelia establish themselves within the root cells. Once the host dies either from infection or other mortality agents, the fungus persists in the deceased host until a new host becomes available. Finally, *Armillaria* spreads to the new host and the cycle begins again (Morrison and Mallett 1996). When active, the disease tends to occur in disease centres, where spread of the fungus causes concentric rings of dead and dying trees (Hagle 2006). Under certain conditions, *Armillaria* may form edible fruiting bodies known as honey mushrooms, which distribute spores over larger areas and may aid in disease dispersal (Fuller and James 1986).

Armillaria disturbs the water availability and nutrition of trees by blocking upward movement through the xylem, causing growth loss that may end in death (Cherubini et al. 2002). It can also kill bark, cambium, and wood cells that it encounters (Shigo and Tippett 1981). Conifer roots defend themselves by producing phenolic compounds to inhibit fungal invasion in

resin ducts, additional xylem rays, and axial parenchyma (Tippett and Shigo 1980). Resin production plugs canals and adjacent tracheids to present a temporary physical and chemical barrier, as it takes additional time and energy for fungal hyphae to penetrate resinous tissues (Cruickshank et al. 2006; Hagle 2006). Invaded and adjacent cells deposit secondary metabolites and present an organized periderm to contain the infection (Hagle 2006). The presence of traumatic resin canals has been used the roots and lower stem of Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco var. glauca (Beissn)) to date *Armillaria* infection (Cruickshank et al. 2006).

When death occurs in a sector of the root system, the host experiences cambial mortality in the part of the cambium connected with those roots (Cherubini et al. 2002). It has been found that infected mountain pine (*Pinus mugo* Terra) often live well beyond the date of partial cambial mortality, likely by using old xylem conduits that are still functional (Cherubini et al. 2002). When acting as a primary pathogen, *Armillaria* kills vigorous trees. In active disease centres, it tends to kill trees between the ages of five and 20 in just a few years. It kills 80-100 year old trees in 10-20 years (Mallett 1992; Morrison and Mallett 1996). As an opportunistic secondary pathogen, it often attacks trees that are stressed, weakened, or wounded by adverse environmental conditions, insects, or diseases (Hiratsuka et al. 1995; Piercey-Normore and Bérubé 2000; Cherubini et al. 2002). Interactions of *Armillaria* with other stressors increases the risk of mortality (Antos et al. 2007).

1.3 Dendroecology and tree mortality

Tree-ring analysis provides information regarding the growth and life history of trees (Cherubini et al. 2002, Rubino and McCarthy 2004), Dendroecology allows for the investigation of growth prior to mortality, which may provide insight into causal factors of death. Periods of growth suppression, or other anomalies (eg. frost rings, light rings), can be determined from tree-ring patterns (Antos et al. 2007). Tree-ring analysis aids in assessment of reactions of trees to environmental stressors and disturbances (Cherubini et al. 2002). Tracing the life histories of infected trees as compared to those of uninfected trees can give insight into *Armillaria*'s impact on tree growth.

Dendroecological research on *Armillaria* has been completed outside of the Canadian prairie provinces. Antos et al. (2007) proposed that different pathogens caused different growth patterns in hosts prior to death. They observed that infected trees exhibited either a gradual decline, an abrupt decline, or no decline in radial growth prior to mortality. Cherubini et al. (2002) studied the impacts of two fungal pathogens on tree ring formation. They found that tree-ring patterns associated with *Armillaria*-infected mountain pine trees in Sweden were significantly different then those associated with *Heterobasium annosum* (Fr.) Bref. The *Armillaria*-infected mountain pine showed a slow decline in radial growth, indicating several decades of suppression, though they were unable to determine if the effect was caused by the pathogen alone, competition from neighbouring trees, or a combination of the two. On the other hand, trees infected by *Heterobasium annosum* appeared to have died over a very short period of time. The authors suggested that on their study site, *Armillaria* functioned as a secondary

pathogen (Cherubini et al. 2002). In British Columbia, Antos et al. (2007) studied subalpine fir (*Abies lasiocarpa* (Hooker) Nuttall) infected with *Armillaria*, and suggested that the mortality of canopy trees was a gradual process. This process was related to a long-term decline in basal area increment, partially caused by the pathogen.

1.4 Objectives of study

Though estimates of the impact of *Armillaria* on growth loss due to mortality at the stand level have been documented (Mallett, 1990; Mallett and Volney 1999; Knowles 2007), little is known about the overall effects of disease on productivity over the life span of the tree. Though *Armillaria* has a circumboreal distribution, its tree-level impacts are not known for a majority of tree species. The objectives of this study were twofold. First, the synchronicity of the onset of mortality in black spruce trees was determined across 12 sampling sites distributed in two regions. Second, the impact of *Armillaria* infection on the growth of black spruce trees was assessed using stem analysis. More specifically, the growth (basal area, height and volume) of both infected and uninfected black spruce trees was compared between two regions. It was hypothesized that mortality would be synchronous within each region, as well as between the two regions. It was also hypothesized that infected trees would show a reduction in basal area, height and volume increment as compared to uninfected trees. It was predicted that this decline would result in a decrease in the overall productivity of the infected trees as expressed by cumulative basal area, height, and volume growth curves.

2. METHODOLOGY

2.1 Study area

The study area encompasses two regions of western Manitoba; the Duck Mountain Provincial Forest (DMPF, located at 51°41' N, 101°01' W) and Porcupine Provincial Forest (PPF, located at 52°40' N, 101°22' W), in western Manitoba (Figure 1). Both provincial forests are situated on the Manitoba Escarpment, a geological formation which forms the boundary between the Manitoba Lowlands and the Saskatchewan Plateau (Nicholson 1987). More specifically, sampling sites fall within the Clearwater Creek operating area in the DMPF, and the Rice Creek operating area in the PPF (Knowles 2009, personal communication).

Sampling locations are all within the boreal plains ecozone, which is a transition area between northern boreal forest and southern aspen parkland (Scott 1995; Smith et al. 1998). The transects are located within the mid-boreal uplands ecoregion (Shay et al. 1999). In each sampled transect (Figure 1), sites heavily infested with *Armillaria* were identified by Manitoba Conservation (Knowles 2002). These corresponded to upland black spruce stands with dense bryophyte layers and mesic plant species as verified in the field. It was noted in field observations that the stands were dense, with evidence of windthrow and downed wood. Balsam fir (*Abies balsamea* (L.) P. Mill.) occurred in the understory, as well as some scattered paper birch (*Betula papyrifera* Marsh.), jack pine (*Pinus banksiana* Lamb.), and beaked hazel (*Corylus cornuta* Marsh). Undergrowth observations consisted of carpet of sphagnum and feather mosses, with common herbaceous species including *Rubus idaeus* Blanco, *Lycopodium* spp., *Ledum*

groenlandicum Oeder, *Viburnum spp*., *Cornus canadensis* L., *Vaccinium spp*., and *Equisetum* spp.

The DMPF covers 376 000 ha, enclosing Duck Mountain Provincial Park, which covers 142 200 ha (Epp et al. 2009). Located to the north of the DMPF, the PPF covers 209 000 ha (Government of Manitoba 2000). Altitude varies widely, with the eastern escarpment of the DMPF rising 300-400m in elevation. Upland areas consist of mixed-wood deciduous/coniferous forest. The fire cycle in the DMPF was reported to have lengthened in the 20th century (Tardif 2004). In pre-settlement times, much of the DMPF was characterized by a fire cycle of 50-60 years, however it currently has extended to ~200 years, and greater than 450 years in some areas (Tardif 2004; Epp et al. 2009). This is likely due to active fire suppression and forest fragmentation caused by human development, and to climatic trends. Much of the upland area experienced large fires in the 1880s (Tardif 2004; Epp et al. 2009), and it is thought that upland black spruce stands originated after these fires based on estimates of tree age.

The climate of the region is mid-boreal with short, cool summers and cold winters (Girardin and Tardif 2005). The nearest weather station is located in Swan River (52°03' N, 101°13' W, elevation of 347m), which is situated between the PPF and DMPF. The area features warm summer and cold winters. For the reference period 1971-2000, the average temperature was -18.2°C in January and the average temperature was 18.1°C in July. Overall yearly average temperature was 1.6°C, and average yearly precipitation was 530.3mm, of which 394.1 mm fell as rain (Environment Canada 2009).

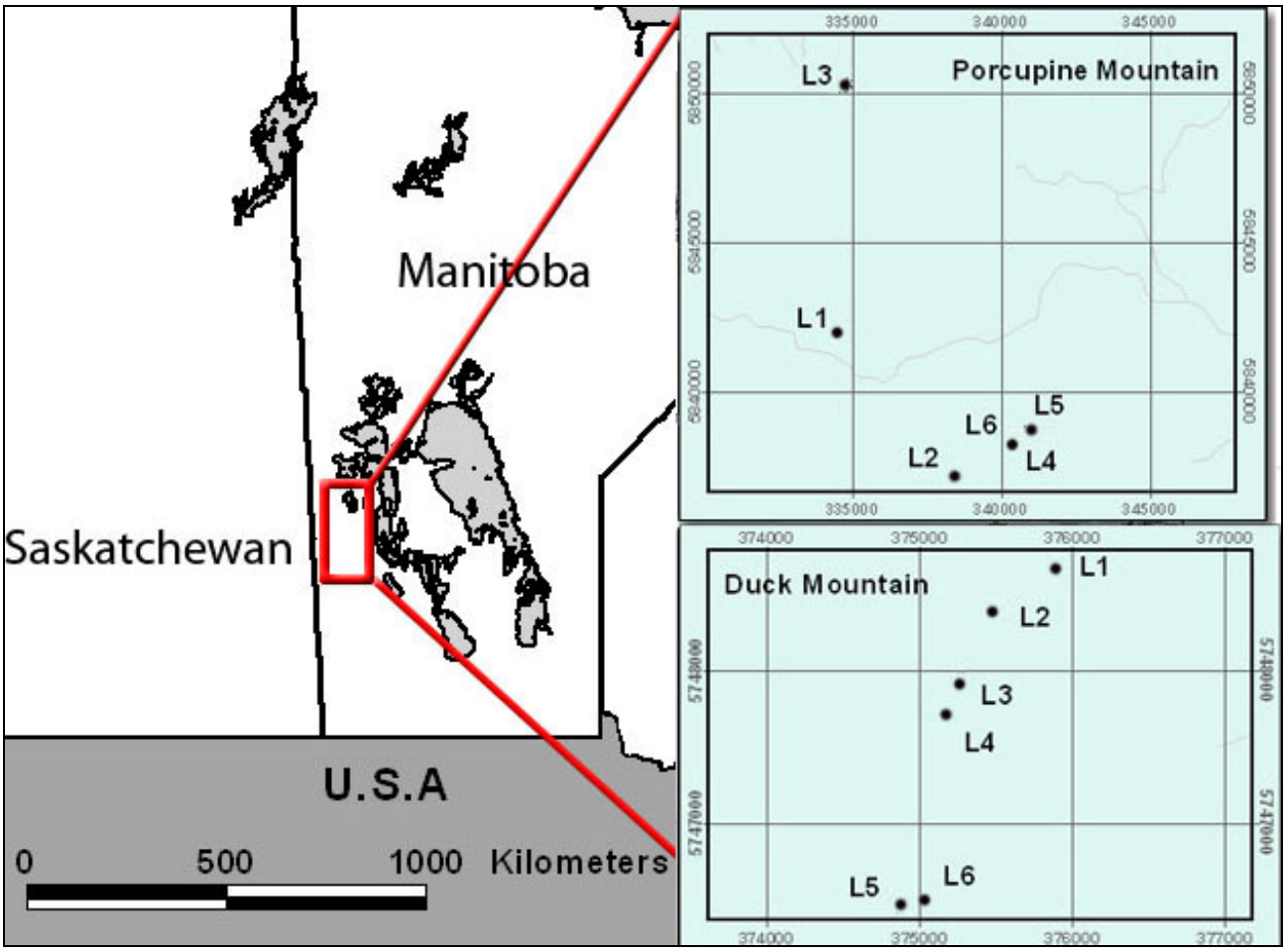

Figure 1: Location of the study area. Rectangle indicates the general area enclosing both the Duck Mountain Provincial Forest and Porcupine Provincial Forest. Right insets specify sampling sites (transects) in each region. Of the six disease centres in both regions located on the map, L2 and L3 indicate locations where stem analysis was conducted.

2.2 Field methods

Initial sampling was conducted in the DMPF in 2007 and in the PPF in 2008 by Manitoba Conservation. In DMPF, six linear transects (5m x 100m) were established in the Clearwater Creek operating area (Figure 1). The transects were located along known root disease centres, as identified by an inventory cruise carried out five years prior as well as from aerial reconnaissance flights (Knowles 2004; Knowles 2007). The same protocol (Knowles, personal communication 2009) was used for the PPF Rice Creek operating area where six transects were also sampled (Figure 1). Along each transect, two increments cores at diameter at breast height (DBH) were removed from every 10^{th} living black spruce for a total of 145 cores in the DMPF and 138 in the PPF. Two cross-sections were also removed from the stem (one at the base and one at DBH) of each dead tree (standing or fallen) encountered along the transect, for a total of 392 cross sections in the DMPF and 331 in the PPF. Data were recorded concerning the status and condition of the dead trees. All of the sampled dead trees were infected with *Armillaria*, and exhibited evidence of root and butt rot.

In 2009, two transects in each region were revisited (Figure 1), and data concerning site characteristics and species present were recorded. Transects 2 and 3 from each region were selected based on accessibility, average age, and absence of evidence of other disturbances (such as windthrow). Along each transect, five living and five dead trees were selected for stem analysis. The transects were divided into five 20m segments, with one dead and one living tree selected from each zone. Dead trees were chosen from stems that had been previously sampled by Manitoba Conservation in 2007/2008, as observed by spray-painted identification numbers.

The thickness of the samples taken from 2007/2008 was measured so as to more accurately reconstruct the height of the dead trees sample in 2009. After a short survey, the best preserved dead trees were selected (excluding those with missing sections of stem, or that were too rotten). The living control trees sampled within the same zone were required to have a DBH within 1 cm of that of the dead tree to minimize size variation between living and dead trees. Selected living trees were at least 10.5m from the transect (though the majority were over 20m away), and were visually evaluated for the presence of *Armillaria*. Visual assessment was completed by examining the tree for crown dieback, chlorosis, cracking and freeflowing sap in the bark near the base, white mycelial fans (under the bark both above, at, and below the root collar), as well as the presence of stringy orange mycorrhizae in the surrounding soil. Only living trees showing no visible signs of infection were sampled.

Stem analysis was conducted in accordance with Epp et al. (2009). Trees were felled and marked lengthwise with paint to indicate an orientation line. Perpendicular marks in a different colour were made along the trunk at the base (0m), 0.5 m, 1.3 m, 2 m, and each subsequent 0.5m until stem diameter was less than 2 cm. Total tree height was recorded and a cross-section disk removed at each marker. Site, tree number, and disk height were recorded on the upper side of the cross-section, as well as an orientation line corresponding with the upper side of the felled tree.

2.3 Laboratory analysis

In the laboratory, all 2007 and 2008 samples were prepared and analyzed using standard dendrochronological procedures. Stem disks and cores were dried, sanded by laboratory assistants and dated and cross-dated by an experienced dendrochronologist. The pointer year method of cross-dating was used (Yamaguchi 1991) as well as chronologies previously developed for the region (Tardif 2004, Girardin and Tardif 2005). New tree-ring chronologies were developed from living trees. The radial growth of all living trees as well as a sub-sample of dead trees (15 disks per transect) was also measured at DBH on a Velmex measuring stage at a precision of 0.001 mm.

As part of the 2009 sampling, all disks were prepared for stem analysis using the same procedures as above. All disks collected from 0m to 3m were analyzed, as well as disks in 1m increments above this (eg. 4m, 5m, 6m, etc.). Disks were also dated and cross-dated along four radii by an experienced technician to allow for a minimization of error. Given time constraints, only one of the two sites from each region sampled in 2009 was selected for the purpose of the thesis. Disks taken from sites AP3 and AD3 were scanned at a resolution of 800 dpi on a high-resolution flatbed scanner, and saved digitally in '*.tiff' format. The width of each ring was measured electronically using the program WinDENDRO™ v. 2009a (Régent Instruments Inc., 2009). Measurements were conducted along four radial paths separated by right angles (Appendix 1). The radial paths were anchored along the orientation line established at time of tree felling. Where a knot, excessive rot, or other obstruction prevented a radius from being measured 90° from an adjacent path, the path was manually moved. If path reorientation

occurred at or within 45° of an adjacent radius, that path was discarded and no measurements were made. Several trees could not be measured digitally due to extensive decay or suppression, and were measured on a Velmex measuring stage . All measurements, both digital and manual, were independently validated by an experienced dendrochronologist to reduce errors.

2.3.1 Statistical analysis of mortality data

The origin and mortality data from the 2007 and 2008 samples, taken from living tree cores and dead tree disks at DBH, were used to generate longevity histograms for each transect. Of the two cores taken per tree, the core with the lower age was excluded. An additional eighteen samples were excluded from each region due to poor sample conditions. The mortality and origin dates of the samples were compiled into a spreadsheet, and converted into a measure of longevity by subtracting origin date from mortality date (the last observed year). The resulting data set was tested for normality and homogeneity of variances. It was found that the longevity distribution of living and dead 2007 samples from the DMPF was normal, but was variances were not homogenous. In the case of the PPF, the distribution was not normal but variances were homogenous. As a result, the differences in the origin date, mortality date, and longevity of trees were tested between regions non-parametrically using a Mann-Whitney U-Test. Kruskal-Wallis tests were used to compare the origin dates, mortality dates, and longevities among the six transects within each region. If the Kruskall-Wallis revealed a significant difference, Mann-Whitney U-tests were conducted to compare each individual transect pairwise in that region. All statistical testing was conducted using SPSS Statistics 17.0 (SPSS Inc. 2008).

2.3.2 Variation in mortality date along the stem

Following the dating and cross-dating of the samples collected from dead trees in 2009, it was noted that the year of the last observed ring differed along the stem. To assess the variation in the year of the last ring produced in the stem, the year of the last produced ring was recorded for 19 trees (one was excluded due to extensive rot) at 0M, 0.5M, and 1.3M. Additionally, the "true" year of death (the year of the last ring produced along the entire length of the stem) of each tree was recorded, as well as the lowest height at which true year of death was observed. This data was plotted, and scatterplots and boxplots were used to visualize the variation in year of the last observed ring along the stem.

2.3.3 Testing for differences in basal area, height, and volume growth

In order to asses if samples from the living and dead trees in each region (DMPF and PPF) could be pooled into two groups (living and dead), field data on longevity, height, and DBH were compared. Mann-Whitney U-tests were conducted to compare this data. It was determined that the data could be pooled into two groups; living trees (N = 10) and dead trees (N = 10) following the absence of any statistical differences ($p<0.05$) between the regions (Appendix 2).

Ring-width measurements generated for the 10 living and 10 dead trees sampled for stem analysis were converted into annual measurements of basal area, height, and volume increment using the Microsoft Excel macro XLStem™ 1.3a (Régent Instruments Inc., 1999). The procedure followed that used in Epp and Tardif (2004). First, mean radial growth was calculated

from the average of the four paths using the quadratic mean method. The mean radius was then used to calculate basal area increment using the formula for the area of a circle. For each of the three growth variables, XLStem™ 1.3a was used to calculate mean annual increment, with the linear interpolation method being selected for height. Mean annual increments were used to calculate moving growth ratios for individual trees starting in the year 1985. This year was selected on the basis of exploratory analysis, as well as the fact that mortality usually occurs 10-20 years after infection (Mallett 1992; Morrison and Mallett 1996). The rationale behind comparing growth ratio was that in absence of infestation, both groups of trees should have experienced similar ratios, suggesting that climate and geometric growth trends may be the main factors influencing growth. Infection by *Armillaria* would be expected to cause the growth ratio of the two groups to diverge.

The moving growth ratio for each year for each tree was calculated using the formula:

$$MR_j = \frac{\sum g_{f-j}}{\sum g_{a-e}}$$

Where MR_j is the moving ratio for year j (ex: 1985), g_{f-j} is the average incremental growth from year f to year j (1981-1985), and g_{a-e} is the average incremental growth from year a to e (1976-1980). A moving ratio value was calculated for each tree, for every year from 1985 to the end of the tree's life. The moving ratios for the 10 dead trees and the 10 living trees were then tested for each year for significant differences using a Mann-Whitney U-test ($\alpha = 0.05$). This was repeated for each year until 2003, when the number of observations became too low to conduct the test. The same procedure was used for basal area, height, and volume ratios.

To determine if the overall cumulative growth (the summation of annual increments over time) for basal area, height, and volume differed among dead and living trees, a 3-parameter logistic regression model was used:

$$y = \frac{a}{1+(x/x_0)^b}$$

Where y is the value of the growth variable, x is the year of increment, x_0 is the inflection point of the curve (indicating the year at which 50% of growth is reached), a is the asymptotic level of growth, and b is the growth constant (slope).

Regression curves for basal area, height, and volume were calculated by SigmaPlot 9.01 (Systat Software, Inc. 2004), using the average cumulative growth for each year. A model was calculated for the living trees (N = 10) as well as the dead trees (N = 10). Following this, 3-parameter sigmoidal regression models were generated for basal area, height, and volume, for each individual tree. From these individual models (a total of 60), the coefficients a, b, and x_0 for the living trees were tested against the coefficients of the dead trees with Mann-Whitney U-tests ($\alpha = 0.05$) for statistical differences.

3. RESULTS

3.1 Black spruce origin, mortality, and longevity

Origin dates (as measured at DBH) were not significantly different overall between the DMPF and PPF (Mann-Whitney U-test: $p = 0.818$, n = 309), with the average for each region being 1902. Within the DMPF, origin dates did not significantly differ among the 6 transects (Kruskal-Wallis Test: $p = 0.066$, n = 169). Origin dates did significantly differ among the 6 transects in the PPF (Kruskal-Wallis Test: $p < 0.001$, n = 140) (Table 1), with a number of differences emerging. The transect of earliest origin was transect 5 (average origin date of 1896) and the transect of latest origin was transect 3 (average origin date of 1906) (Appendix 3).

Mortality dates did significantly differ between the DMPF and PPF (Mann-Whitney U-test $p = 0.041$, n = 309), with mortality occurring an average of two years later in the DMPF. Mortality dates significantly differed within the DMPF (Kruskal-Wallis Test: $p = 0.003$, n = n = 169), as transect 6 had an average tree mortality date three years later than the mean for the region. They also differed among the PPF (Kruskal-Wallis Test: $p = 0.012$, n = 140) due to transect 4, in which mortality occurred an average of four years earlier than the other transects (Table 1 and Appendix 3).

There was no statistical difference in tree longevity (time between tree origin and death) observed between the DMPF and PPF (Mann-Whitney U-Test: $p = 0.240$, n = 309). However, significant differences were observed among transects within the individual regions (Kruskal Wallis: $p = 0.003$, n = 169 for the DMPF, and Kruskal-Wallis: $p = 0.000$, n = 140 for the PPF).

In the DMPF, trees from transect 6 lived significantly longer than in the other transects (five years longer than the regional average), which was due to the significantly different mortality date. In the PPF, a number of significant differences in tree longevity emerged (Table 1). Many of these differences were associated with trees from transects 4 and 5. Trees in transect 4 lived six years longer than the average for the region due to later significantly later onset of mortality. Transect 5 was five years older than the average for the region, which was due to the earlier origin of the stand (Appendix 3).

3.2 Variation in mortality date along the stem

The analysis of dead black spruce trees revealed that in many cases, the year of the last ring produced at 0m, 0.5m, and 1.3m did not reflect that of the last year observed along the entire stem (taken to be the true year of death). As a result, mortality dates derived from lower portions in the stem would be underestimated (Figure 2 and Appendix 4). This indicated that the black spruce trees stopped producing rings lower along the stem, though the tree remained living and produced rings in higher portions of the stem. On average, the mortality date derived at 0m (Figure 2a) and 0.5m (not presented) was less reflective of true mortality date than 1.3m (Figure 2b), though there was variation between trees. Determining the date of mortality at the base of the tree yielded an average underestimation of 1.7 years, determining it at 0.5m yielded an average underestimation of 1.0 years, and determining it at 1.3m yielded an average underestimation of 0.8 years compared to the mortality date obtained higher on the stem.

Table 1: Summary statistics of dead tree data collected from diameter at breast height in 2007-2008. Different letters (*a* and *b*) indicates a significance difference between the group of that letter and the group of another letter, based on a systematic pairwise series of Mann-Whitney U-tests ($\alpha < 0.05$). Pairwise comparisons were completed among transects 1-6 within each region.

		Origin Date		Mortality Date		Longevity	
Transect	N	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
Duck Mountain							
Transect 1	30	1902*a*	2	2000*a*	1	100*a*	5
Transect 2	16	1903*a*	2	2001*a*	2	98*a*	9
Transect 3	45	1901*a*	<1	2000*a*	1	99*a*	6
Transect 4	33	1902*a*	1	1999*a*	1	96*a*	8
Transect 5	29	1903*a*	1	2000*a*	1	97*a*	7
Transect 6	16	1901*a*	<1	2004*b*	1	104*b*	4
All Transects	6	1902	1	2001	2	99	7
Porcupine Mountain							
Transect 1	20	1903*b*	1	1998*a*	2	95*ab*	8
Transect 2	29	1901*a*	<1	1997*a*	1	95*ab*	8
Transect 3	33	1906*c*	<1	1997*a*	1	91*a*	8
Transect 4	20	1901*a*	1	2003*b*	1	102*c*	6
Transect 5	19	1896*ab*	3	1998*a*	2	101*bc*	15
Transect 6	19	1902*ab*	1	1999*a*	2	96*b*	8
All Transects	6	1902	3	1999	2	96	10

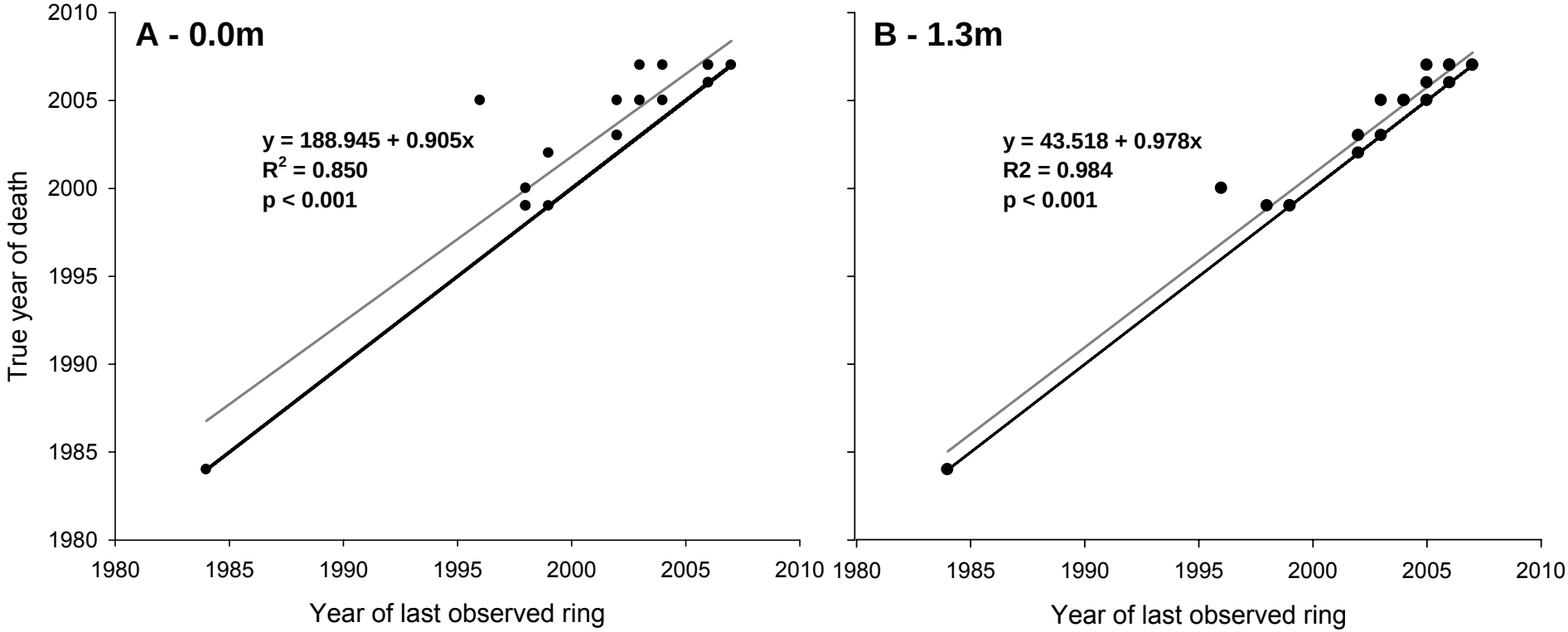

Figure 2: The relationship between year of death determined at a given height as compared to the true year of death (year of the last ring observed over the entire stem) for 0.0m (A) and 1.3m (B). Plotted values indicate the observed year of death at that height as compared to true mortality date. The gray line represents linear regression of observed values for illustrative purposes, whereas the black line represents a 1:1 relationship, which would occur if observed year of death was identical to true year of death.

3.3 Comparison of growth ratios between dead and living trees

Incremental measurements for basal area, height and volume revealed large variability among trees (Appendix 5). Despite this variability, decline in growth prior to mortality can be observed in dead trees as was also emphasized by the analysis of the increment growth ratios from 1985-onward (Figure 3). For basal area, there were numerous periods in which the growth ratios were statistically different (Figure 3a). Except for the years 1987 and 1988, when the basal area ratio of the dead trees was significantly above that of the living trees, a systematic trend with the dead trees exhibiting a significantly lower growth ratio than the living trees was observed from 1997 onward except for year 1999 and 2003. The average basal area increment growth ratio of the dead trees was on average 25.3% less than that of the living trees from 1997-2003. This indicated a decline in growth in the infected dead trees as compared to their living counterparts. No significant differences were observed between the growth ratios of living and dead trees in height increment (Figure 3b).

Volume increment growth ratios also yielded a significant difference in the early period (1988) with dead trees having lower a ratio than living ones (Figure 3c). As with basal area, the volume ratio of dead trees was significantly lower than that of living trees from 1997 onward. The volume increment growth ratio of dead trees was on average 30% less than that of the living trees from 1997-2003.

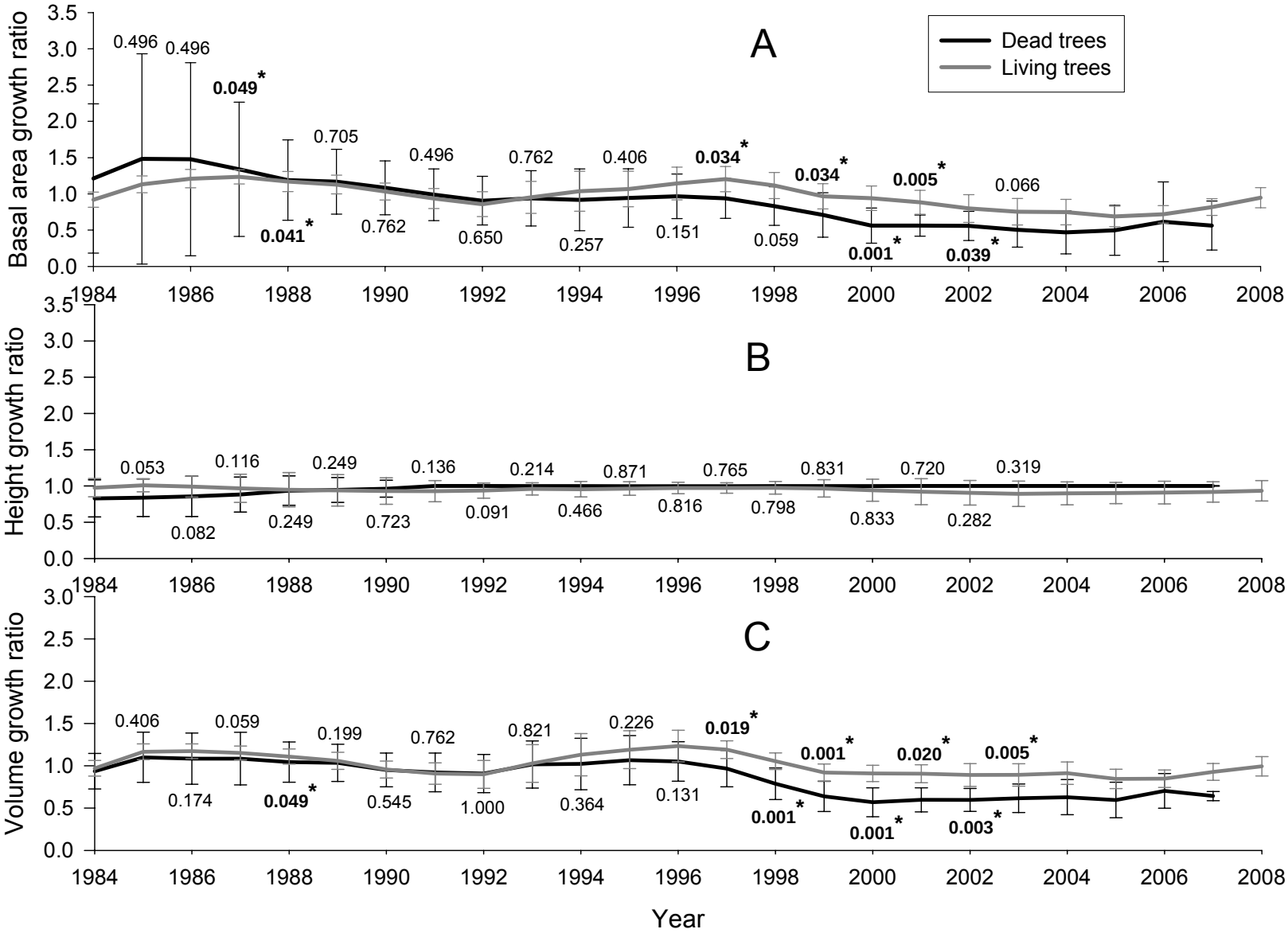

Figure 3: Increment growth ratios of dead (black) and living (gray) trees over time where A) basal area B) height and C) volume. Each year represents the last year of the ten year testing period. Numbers represent Mann-Whiney U-test *P* values, where the bold values and symbol * indicates a significant difference ($\alpha < 0.05$). Error bars represent one standard error from the mean.

3.4 Comparison of cumulative growth between dead and living trees

The logistic regression used to model basal area cumulative increment of both living and dead trees were highly significant ($p < 0.001$) with respective R-square values of 0.67 and 0.69 (Figure 4a, Appendix 6). None of the three parameters of logistic regression (growth rate, theoretical maximum, and inflection point) differed significantly ($p < 0.05$) between living and dead trees, however the inflection point in dead trees was reached earlier than in living trees at the 90% significance level (Table 2). Regression of cumulative height increment yielded a model that explained most of the variation in both living ($R^2 = 0.95$, $p < 0.001$) and dead ($R^2 = 0.97$, $p < 0.001$) trees (Figure 4b). The growth rate coefficient and theoretic maximum coefficient did not significantly differ between the groups of trees. The inflection point was however significantly different (Table 2) and the dead trees reached half of their maximum height 9.11 years earlier than the living trees. Cumulative volume regression curves (Figure 4c) were high significant for both living ($R^2 = 0.70$, $p < 0.001$) and dead ($R^2 = 0.68$, $p < 0.001$) trees. Growth rate and theoretic maximum did not significantly differ, but the inflection point was significantly different as the dead trees reached half of their maximum volume 5.7 years earlier than the living trees (Table 2).

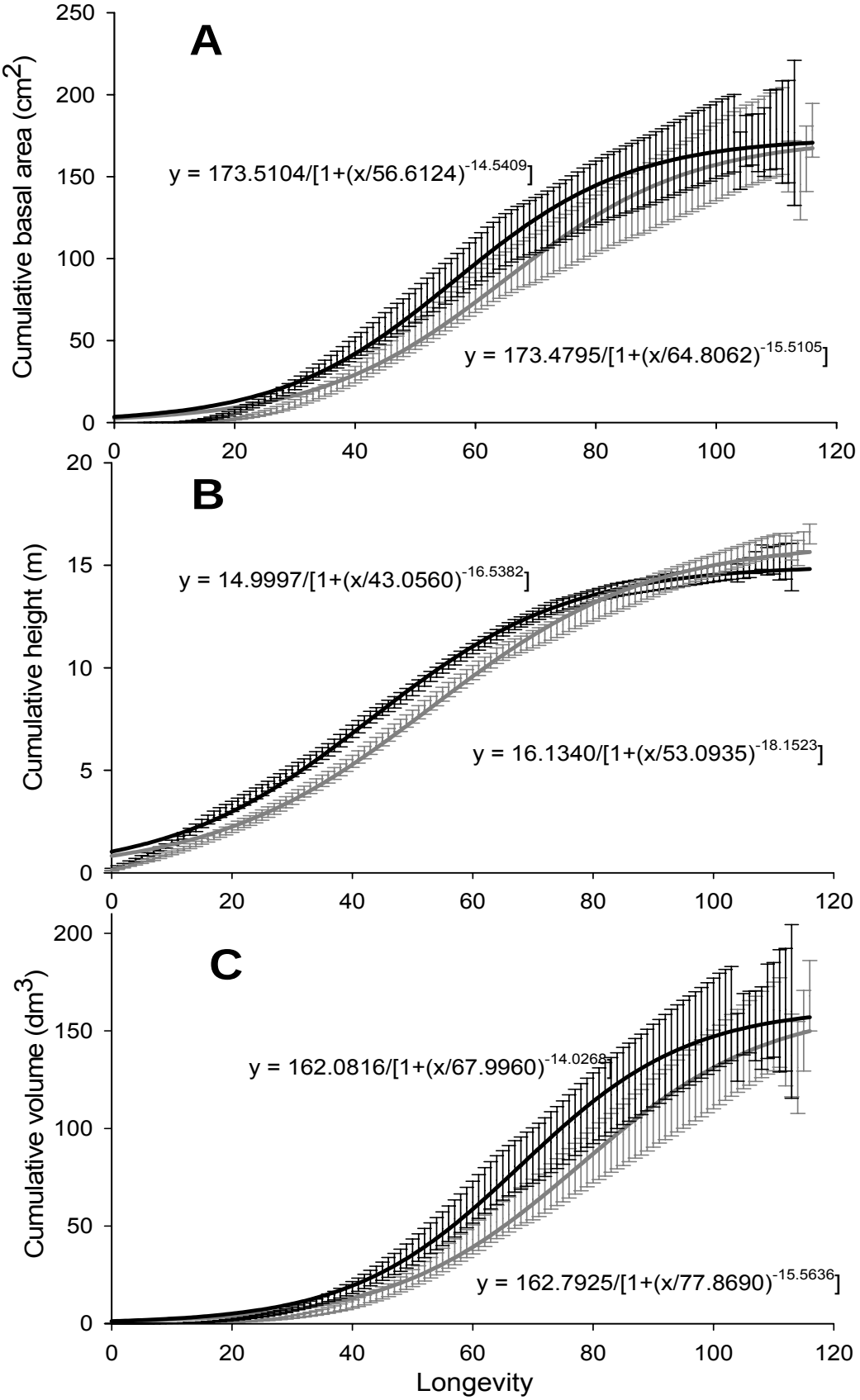

Figure 4: Three-parameter logistic regression showing cumulative growth of all dead (black) and living (gray) trees in the Duck Mountain Provincial Forest and Porcupine Provincial Forest, where: A) basal area B) height and C) volume. Error bars represent standard error of the mean.

Table 2: Mean and standard deviation of three sets of logistic growth parameters derived from each individual models fitted to the 10 living and 10 dead trees. Predicted growth parameters of the logistic curve are *a* (theoretical maximum), *b* (growth), and x_0 (inflection point). The symbol * and bold font indicates a significant result according to Mann-Whitney U-Tests ($p < 0.05$).

		Living trees					Dead trees					
		N	min	max	Mean	Standard deviation	N	min	max	Mean	Standard deviation	P
BA (cm^2)	*a*	10	70.64	376.94	182.07	84.21	10	71.32	328.25	181.08	72.16	0.971
	b	10	12.03	20.68	15.76	2.29	10	12.29	19.20	15.31	2.46	0.579
	X_0	10	53.52	79.65	65.85	7.92	10	41.40	66.96	58.16	7.29	0.063
Height (m)	*a*	10	16.23	18.21	12.29	1.78	10	15.10	16.80	12.72	1.39	0.143
	b	10	18.21	22.06	14.45	2.34	10	16.58	19.86	12.80	2.42	0.315
	X_0	10	53.34	59.43	45.91	4.20	10	43.32	48.71	36.80	3.91	**0.000***
Volume (dm^3)	*a*	10	175.80	323.85	44.25	77.43	10	175.61	333.01	57.62	78.47	0.912
	b	10	18.06	31.19	12.80	5.27	10	15.12	18.37	10.74	2.25	0.190
	X_0	10	82.90	107.86	62.46	12.99	10	70.78	82.40	56.76	6.90	**0.011***

4. DISCUSSION

4.1 Black spruce mortality and *Armillaria*

Results have shown that mean mortality date was significantly different between the DMPF and PPF, but this difference was only two years. Given that *Armillaria* usually kills older trees in 10-20 years (Mallett 1992; Morrison and Mallett 1996), it is likely that the onset of the disease occurred in a similar time period (likely 10-20 years before death). This is consistent with Brandt (1995), who found that the average onset of tree decay in the prairie provinces was at 80 years of age. Given that the trees in the DMPF died at an average of 99 years and in the PPF at an average age of 96, infection likely occurred at roughly 80 years of age. Given the average mortality dates (2001 in the DMPF and 1999 in the PPF), infection likely occurred sometime after 1980. This is supported by the decline in growth ratios that is first observed in the 1980s, becoming significant in 1997 for the variables of basal area and volume. It is likely that the infected trees sampled in our study lived for some time with symptoms of rot and reduced growth that preceded their eventual mortality, as is consistent with infection in older stands (Mallett 1992; Hagle 2006)

Our results indicated that longevity differed among some transects in the DMPF and PPF, explained by corresponding differences in origin date and mortality date. Differences in mortality dates among transects within each region could have been due to later onset of the disease, or greater resistance of trees in that line. Origin date was not significantly different between the two regions, and the only differences within regions were in the PPF. Thus, trees in the DMPF are likely from the same cohort, which is confirmed by fire-reconstruction data for the

DMPF (Tardif 2004). Trees from the PPF are likely from a similar cohort, given that the earliest average origin date and the latest average origin date were only a decade apart. An exception exists in transect 5, which seems to exhibit two separate cohorts (Appendix 3). There is a systematic bias in that the origin date of all trees is underestimated, as attaining accurate origin dates for black spruce should be done from samples taken below the adventitious roots, as the root collar of mature trees may be located below ground in regions where organic matter has built up on the soil surface (DesRochers and Gagnon 1997). Though this can add 3-19 years to age estimates compared to those at ground level, this may not be possible in studies on *Armillaria* due to decay. As the bias is systematically distributed, it is not considered to be problematic.

In older stands, *Armillaria* operates as an opportunistic secondary pathogen that tends to attack stressed or weakened trees (Hiratsuka et al. 1995; Piercey-Normore and Bérubé 2000; Cherubini et al. 2002). Once *Armillaria* was introduced into these sites, either by spore or underground rhizomorphs, it is likely that the relatively old black spruce trees had relatively low levels of resistance to infection. Though traumatic resin canals have been used to date onset of infection in Douglas Fir (Cruickshank et al. 2006), their presence was not systematically assessed in our black spruce samples. Since the infection year could not be determined, it cannot be associated with specific disturbance events (eg. climatic anomalies or insect outbreaks). It is unknown if susceptibility to infection was due to a disturbance that affected both the DMPF and PPF, or simply associated with tree age.

It is not possible to determine if mortality was directly caused by *Armillaria*. It is likely, however, that infection by the fungus was a contributing factor. Gradual declines in growth often result from a combination of factors (Antos et al. 2007). Though infected trees experienced stress and growth decline caused by the disease, mortality may ultimately have been caused by another factor, whether major or minor. One likely cause of mortality is windthrow, which entails stem snapping or uprooting from the force of wind. The risk of windthrow in black spruce increases with incidences of stem and root rot (Bergeron et al. 2009). Soil conditions are not thought to impact the incidence of uprooting in black spruce (Elie and Ruel 2005). It is possible that once *Armillaria*-infected trees began to die, gap creation could have resulted in additional mortality due to windthrow. It has been previously observed that death by blow-down of adjacent trees predisposed black spruce to windthrow (Taylor and MacLean 2007). Windthrow was noted in the transects revisited in 2009, with the damage so extensive in DMPF Transect 1 that it was deemed unsuitable for sampling. Trees weakened by *Armillaria* may have been more susceptible to windthrow than healthy trees.

4.2 Impact of *Armillaria* on the growth of black spruce trees

Our results indicate that trees infected by *Armillaria* registered a continuous decline in basal area and volume growth prior to their death, though this was not expressed in overall tree productivity. On the contrary, infected trees have shown to be more vigorous at an earlier age as compared to non-infected trees. The greater impact of Armillaria on basal area increment compared to height increment in black spruce trees concurs with observation made by Mallett and Volney (1999) in lodgepole pine. Antos et al. (2007) also noted a decline in growth in

subalpine fir, though it was more gradual and occurred over a longer time period (~40 years) than that which occurred in our study. Cherubini et al. (2002) found that *Armillaria*-infected trees experienced a long, slow decline in growth over several decades prior to death, where the fungus functioned as a secondary pathogen on trees that may already have been suppressed. Bloomberg and Morrison (1989) noted that losses in tree growth were substantial, and cumulative over a prolonged period.

The growth decline in our study was accompanied by the interruption of radial growth at lower heights in the stem as observed by cross-dating samples across all heights. It was not possible to ascertain the true mortality at heights of 0m, 0.5m, and 1.3m with complete accuracy due to the absence of rings. The last dated ring at these heights was, in most cases, an underestimation of the true age of death. Had only samples taken at DBH been used, both the date of mortality as well as the decline in growth would have been underestimated. An underestimation in mortality date would also lead to an underestimation of tree longevity as well as the length of the period of growth decline. If this phenomenon is applicable to other species, sampling only at or below DBH may have implications for other studies. In order to determine the true year of death in infected black spruce, samples should also be taken in upper portions of the stem. Alternatively, a correction factor could be applied, given that on average, sampling at 0m resulted in an average underestimation of 1.7 years, sampling at 0.5 resulted in an average underestimation of 1.0 years, and sampling at 1.3m yielded an average underestimation of 0.8 years.

This discrepancy between last observed years (outmost ring formed) at different heights in the stem has not been documented in surveyed literature studying the effects of *Armillaria* (Cherubini et al. 2002; Omdal et al. 2004; Cruickshank et al. 2006; Antos et al. 2007; Bendel and Rigling 2008). It is also not known if this phenomenon is confined to black spruce or occurs in other species infected with *Armillaria*. In black spruce, *Armillaria* is known to cause a reduction in radial growth in the area immediately over the infected root (Cruickshank 2002), which extends up to 3m above the soil line. This reduction is localized to the regions above infected roots, and do not seem to extend over the entire length of the tree. This phenomenon appears to be contributing to the inability to detect the true year of death in the lower sections of many infected stems, as well as to growth loss. Stressed trees tend to exhibit discontinuous or missing rings (Cherubini et. al 2002), and disease-related stress may have resulted in the lack of deposition of rings in the lower part of the stem towards the end of the trees' life-spans.

Discontinuous rings or locally absent rings are a result of locally inactive cambia, often during reduced growth (Schweingruber 2007). They are common in trees that are dying due to minimally available resources, reduced ability of the crown to assimilate nutrients, or local cambial death. Growth reduction leading to cambial death tends to result in discontinuous and narrow rings preceding death. *Armillaria mellea* is known to surround the stem with hyphal strands that advance at different speeds (Schweingruber 2007). Thus, it is likely that the absence of ring formation at lower heights in our study is due to local cambial death caused by stem invasion by fungal hyphae (Shigo and Tippett 1981), whereas upper tree growth is maintained through the use of older tracheid conduits that are still functional (Cherubini et al. 2002).

Despite their declining growth in the last 5-15 years prior to death, the infected trees were shown to have been initially more vigorous than non-infected trees. This was supported by the fact that the infected trees reached half of their maximum height and volume growth at a significantly earlier time than the living trees. They were growing more quickly at a younger age. It is possible that that increased tree vigour could have been a predisposing factor for infection. Bloomberg and Morrison (1989) speculated that vigorous trees were likely to become infected sooner and more extensively due to their larger root systems. Cruickshank (2000) also observed that the incidence of infection increased with tree size. Garbelotto et al. (1997) suggested that factors such as the frequency of root contacts/grafts, root architecture, and root size may affect the efficiency of fungal spread in a similar fungus (*Heterobasidion annosum*). Since infection is related to the spatial distribution of inoculum (Rosso 1994), trees with larger root systems presumably have more surface area exposed, and thus a higher chance of encountering fungal rhizomorphs or grafting roots with infected trees. Therefore it is likely that in the present study, the vigour in infected trees predisposed them for infection and eventual mortality associated with *Armillaria*.

4.3 *Armillaria* and forest dynamics

In boreal forests dominated by black spruce, stand establishment often occurs after a stand-replacing fire (Caners and Kenkel 2003). Once established, not all stands persist to the old-growth stage under a natural fire regime, and the understory usually does not entirely replace the canopy prior to the reoccurrence of fire (Johnson 1996). Where fire cycles are longer, gap dynamics prevail as the dominant disturbance due to windthrow and insect outbreaks (Aakala et

al. 2007). The life span of black spruce varies regionally, but average maximum age is reported at 200 years (Viereck and Johnston 1990). In the DMPF and PPF, even-aged upland black spruce stands were historically influenced by the cyclic occurrence of fire. This is no longer the case due to active fire suppression, anthropogenic forest fragmentation, and possibly climatic trends (Epp et al. 2009). The fire cycle in the DMPF has extended to greater than 450 years in some areas.

Where stands escape fire long enough to reach the old-growth stage, the death of the post-fire cohort of trees creates gaps that allow for replacement by understory stems (St-Denis et al. 2010). Stand breakup in black spruce typically occurs in a clustered arrangement (Lussier et al. 2002), which is often attributed to the contagious properties of biotic mortality agents (Aakala et al. 2007). This eventually leads to an uneven-age structure with an open canopy (St-Denis et al. 2010). It is possible that in the absence of fire, later successional stages of the black spruce stands in the DMPF and PPF would undergo senescence and come to exhibit irregular structure, and species composition may change (Aakala et al. 2007). The creation of active *Armillaria* disease centres in the DMPF and PPF is accelerating this process by causing canopy gaps (Epp et al. 2009).

The black spruce stands in this study are in an area of active management, and mortality due to rot has negative economic implications (Bendel and Rigling 2008). Natural disturbance-based forest management (NDBM) may be a desirable management scheme for allowing the maintenance of an age-class distribution similar to that found under pre-fire suppression

conditions (Epp et al. 2009). Varying rotation ages (the stand age at which harvesting can commence) in the DMPF would allow for stands to enter second and third cohorts, providing for natural variability on the landscape as well as inputs of coarse woody debris that are not possible with traditional silviculture (Epp et al. 2009). Under a mean-time-since-fire management model for the DMPF, 49% of the forest should be in the first cohort, 25% in the second cohort, and 26% in the third cohort (Epp et al. 2009), which is not currently met. Stands dominated by black spruce constitute 14% of the area of the DMPF (Epp et al. 2009), and approximately 60% of the entire DMPF is in the first cohort stage (Epp et al. 2009).

Currently, the average commercial rotation age of black spruce is 75 years (Epp et al. 2009). Hrib et al. (1983) recommended that harvesting of *Armillaria*-infected norway spruce (*Picea abies* (L.) Karst) occur at 70-80 years of age, before significant growth decline and mortality. This would be suitable for the stands in this study, as mortality occurred at roughly 80-100 years of age with a prior growth decline. It is important to note, though, that since the infected trees were initially more vigorous, they may have continued to grow at a greater rate than living trees in the absence of infection. Theoretical growth in the absence of infection could be modelled, which may be a subject for future study. At present, results suggest commercial rotation age is suitable so as to harvest trees before growth loss, death, and decay occurs. It is important to consider that cutting often occurs well after rotation age, and that wood supply modelling considers that these forests will be available for future use (Knowles, personal communication). The black spruce stands in our study will likely experience ongoing mortality, resulting in an overestimation in the amount of available wood if *Armillaria*-related mortality is

not factored into the modelling process.

However, a 75-year rotation age may not be ideal for NDBM. Tardif (2004) and Epp et al. (2009) suggested that a time-since-fire of 110 years be used to set rotation ages in the DMPF, which would allow a certain population of black spruce stands to pass into second and third cohort stages. The black spruce stands in this study are part of a post-fire cohort that is slowly breaking up due to natural processes of mortality and succession that are being accelerated by *Armillaria*. Allowing these stands to persist would aid in the provision of variable forest habitats that would be useful in managing for biodiversity conservation in the area. Additionally, harvesting may not be the most pragmatic option for these stands. *Armillaria* is considered a 'disease of the site' (Hagle 2006), since established mycelia are essentially permanent, even if stump and root removal is undertaken (DeLong et al. 2005; Vasaitis et al. 2008). General forest management operations seem to favour the fungus (Morrison and Mallet 1996; Cruickshank 2000), and planted seedlings are highly susceptible to infection (Piercey-Normore and Bérubé 2000). As such, replanting of harvested forests to restore upland black spruce stands may be unsuccessful due to infection and death of seedlings, and natural regeneration may become infected. Increasing vigour of existing or replanted trees is not an effective strategy to combat the disease (Rosso 1994; Hagle 2006).

It is perhaps advisable to allow the infected stands to persist, as the presence of older forests allows for the development of subsequent cohorts and structural variability across the landscape (Epp et al. 2009). This may especially valuable in the DMPF, where 60% of the area is

in the first cohort stage, with post-fire recruitment dominating the canopy. Allowing some areas to transition into the second-cohort stage would allow for enhanced forest variability across the landscape. Advanced regeneration is limited in the black spruce stands of this study, and further studies would be needed to determine their likely future structure and type to determine if they are suitable for retention as part of NDBM on a landscape scale.

5. CONCLUSIONS

Results indicated that the stand origin and longevity in black spruce stands infected with *Armillaria* root disease was similar between the DMPF and PPF. Though mortality date differed between the regions, the average difference was only two years. Although differences of origin, longevity, and mortality occurred among transects within regions, it is likely that disease onset occurred at a similar time period across both the DMPF and PPF. The date of mortality was underestimated when measured between the base and 1.3m, suggesting that mortality date cannot be ascertained at these heights with complete accuracy, and the conventional sampling height for dendroecological studies (1.3m) is not reflective of the true year of death in *Armillaria*-infected black spruce. Underestimating mortality date could inaccurate esitmations of growth losses.

Armillaria caused a reduction in basal area and volume growth of black spruce from 5-15 years prior to mortality onward, but this decline did not translate into differences in overall productivity. Increased tree vigour early in life may have predisposed trees to infection by *Armillaria* due to the development of extensive root systems. The infected stands in the DMPF and PPF could be managed using a conventional rotation age, to minimize losses by harvesting before growth loss and death occurs, however, harvesting would not remove the pathogen from the site and it would continue to infect regeneration and/or planted trees. The infected DMPF and PPF stands could be allowed to persist on the landscape to provide age structure maintenance requirements for natural disturbance-based management. Further research would need to be done to determine the required proportions of old growth black spruce on the landscape as part of a large-scale natural disturbance-based management strategy.

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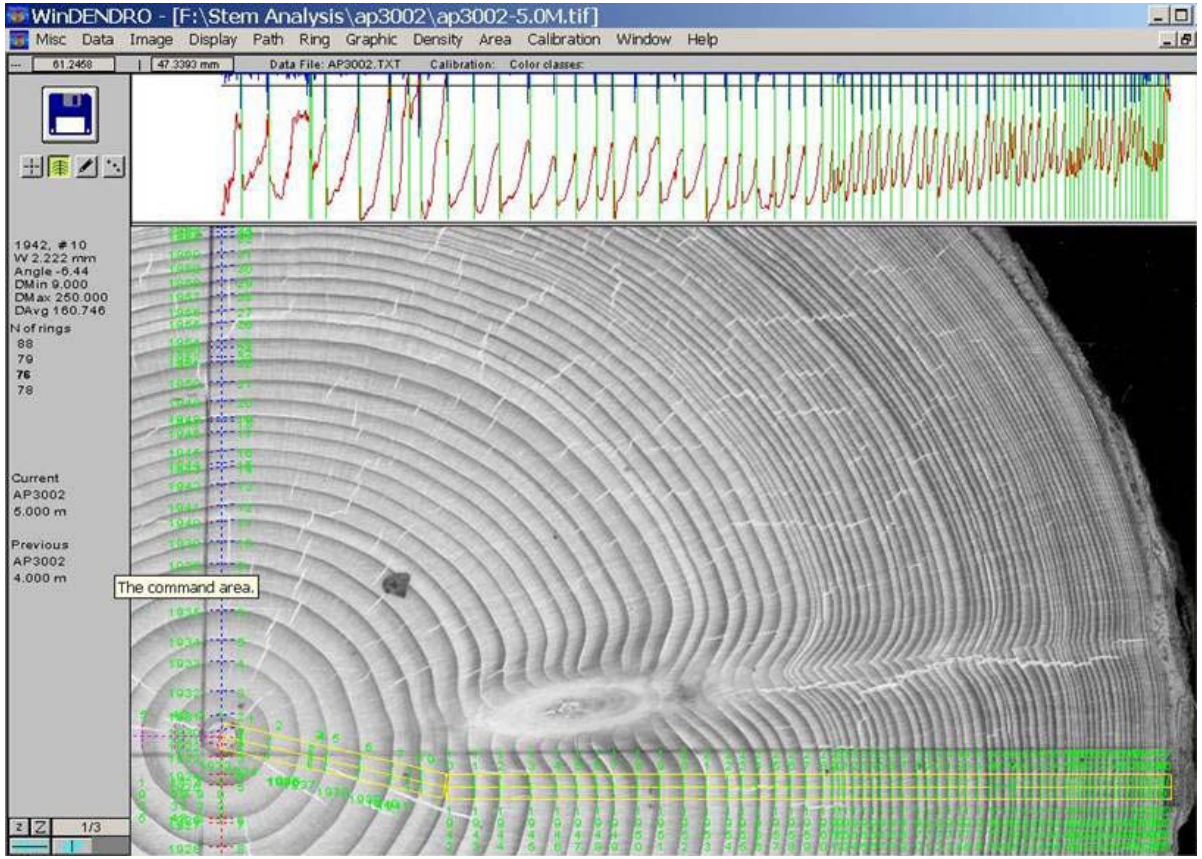
7. APPENDICES



Appendix 1: Screenshot of analysis window of WinDendro™ showing analysis paths. The path on the right has been manually moved to avoid a structural anomaly.

Appendix 2: Summary statistics (DBH, height, and age of living and deceased trees) of 2009 field data and results of comparisons made between regions with Mann-Whitney U-tests ($\alpha < 0.05$) on DBH, height, and age of living and deceased trees. *X* indicates mean, *s* indicates standard deviation, and *P* indicates given *P*-value for the test.

DBH (cm)

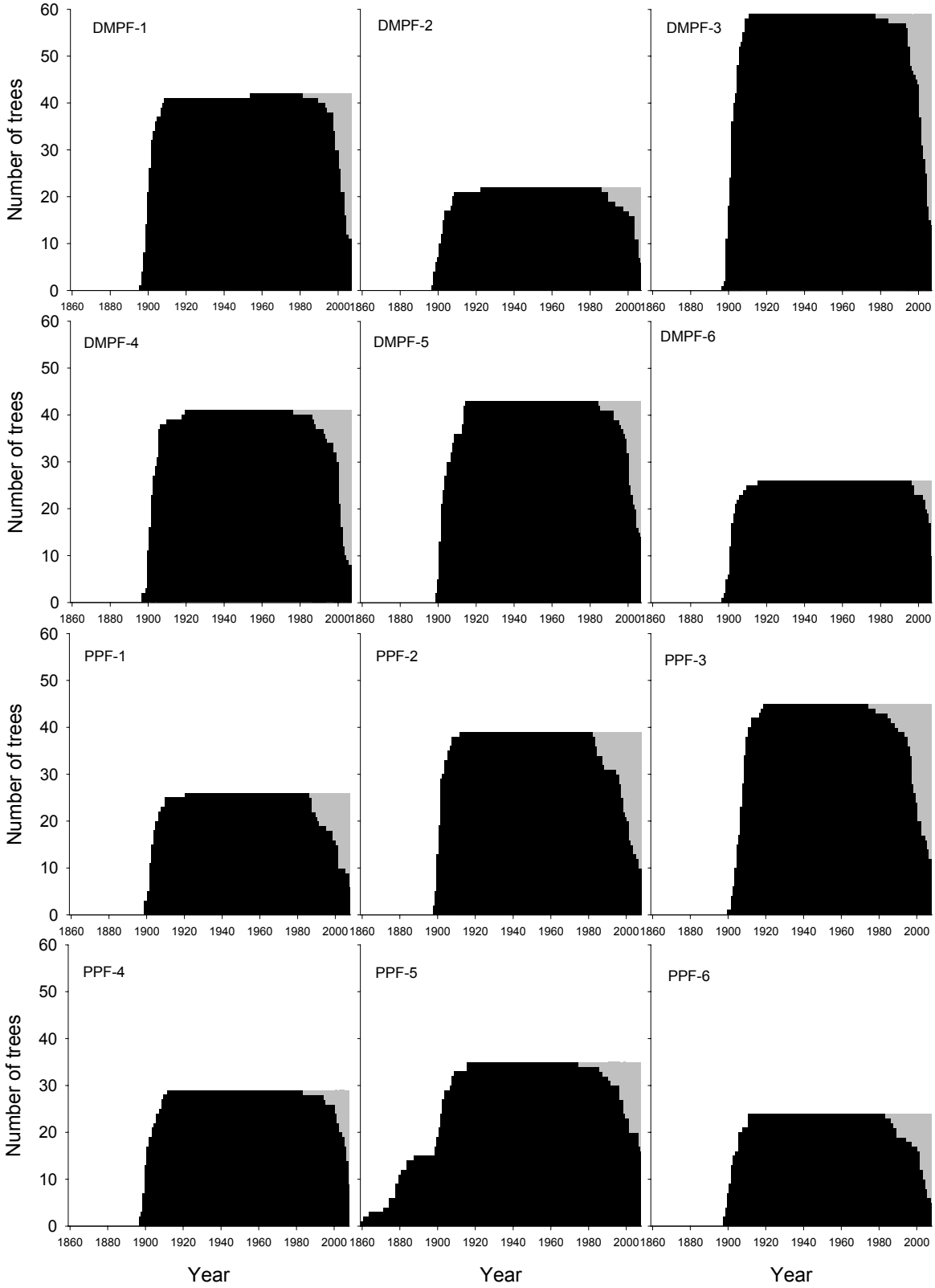
	DMPF-3					PPF-3					
	N	min	max	*mean*	*std*	N	min	max	*mean*	*std*	*P*
Living	5	11.2	18.8	16.2	3.0	5	12.8	23.2	17.3	3.8	0.917
Dead	5	11.3	18.4	16.2	2.9	5	12.4	24.2	18.1	4.2	0.347

Height (m)

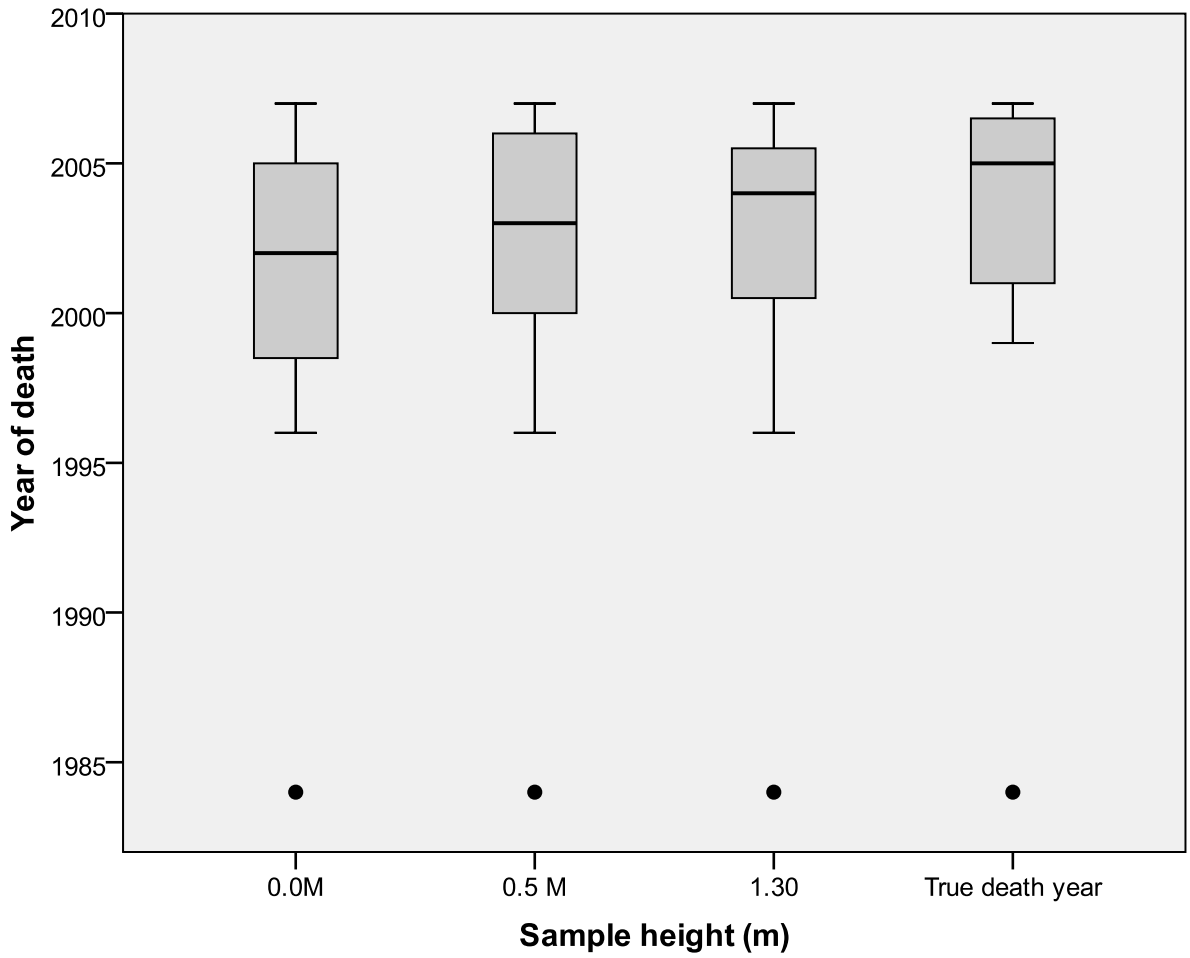
	DMPF-3					PPF-3					
	N	min	max	*mean*	*std*	N	min	max	*mean*	*std*	*P*
Living	5	13.2	17.6	16.0	1.7	5	15.1	18.3	16.6	1.5	0.754
Dead	5	11.3	18.4	16.2	2.9	5	12.7	16.1	14.5	1.5	0.209

Age (years)

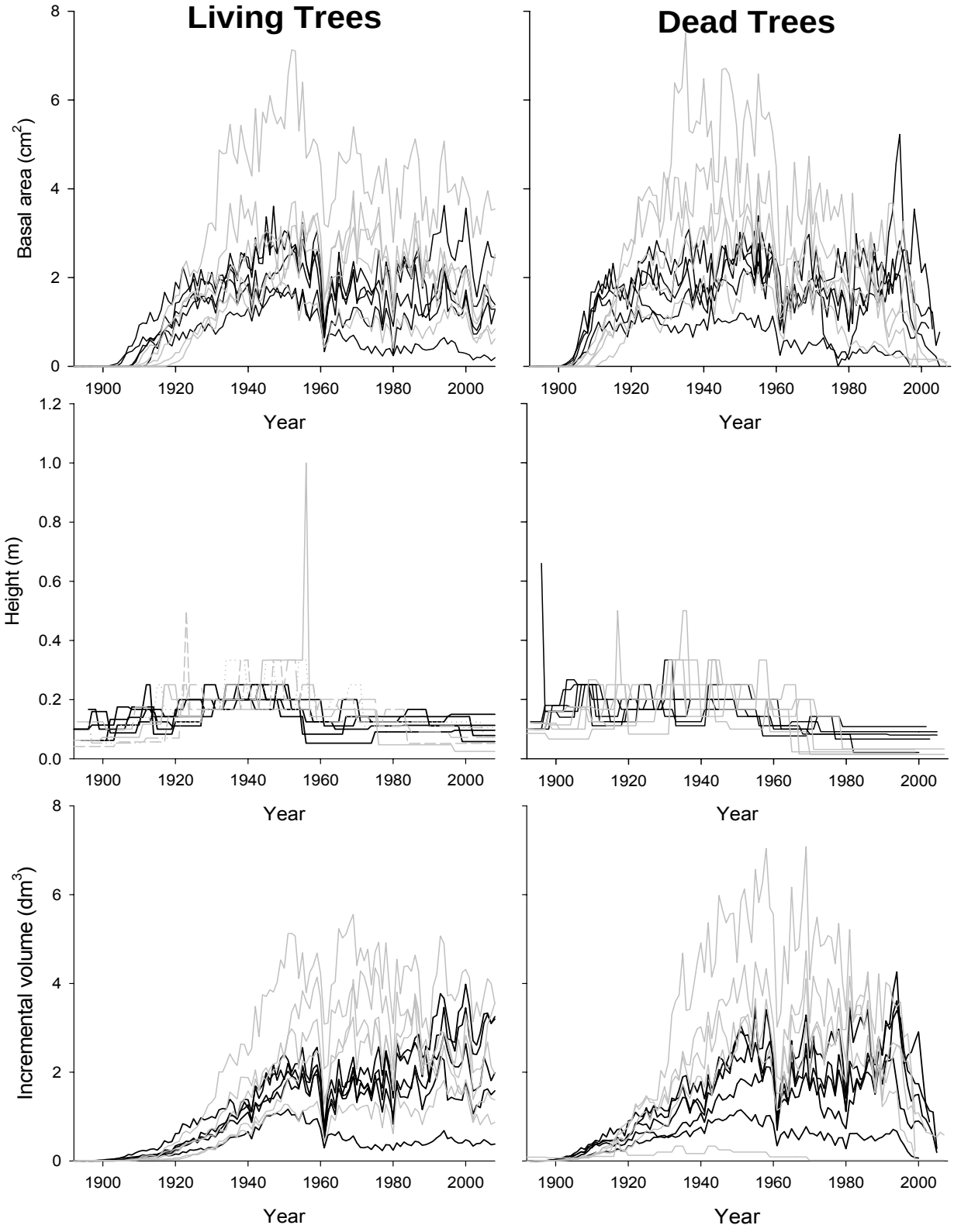
	DMPF-3					PPF-3					
	N	min	max	*mean*	*std*	N	min	max	*mean*	*std*	*P*
Living	5	112.0	116.0	114.0	2.0	5	111.0	116.0	114.2	2.2	0.914
Dead	5	113.0	114.0	109.4	3.6	5	103.0	116.0	109.0	5.3	0.834



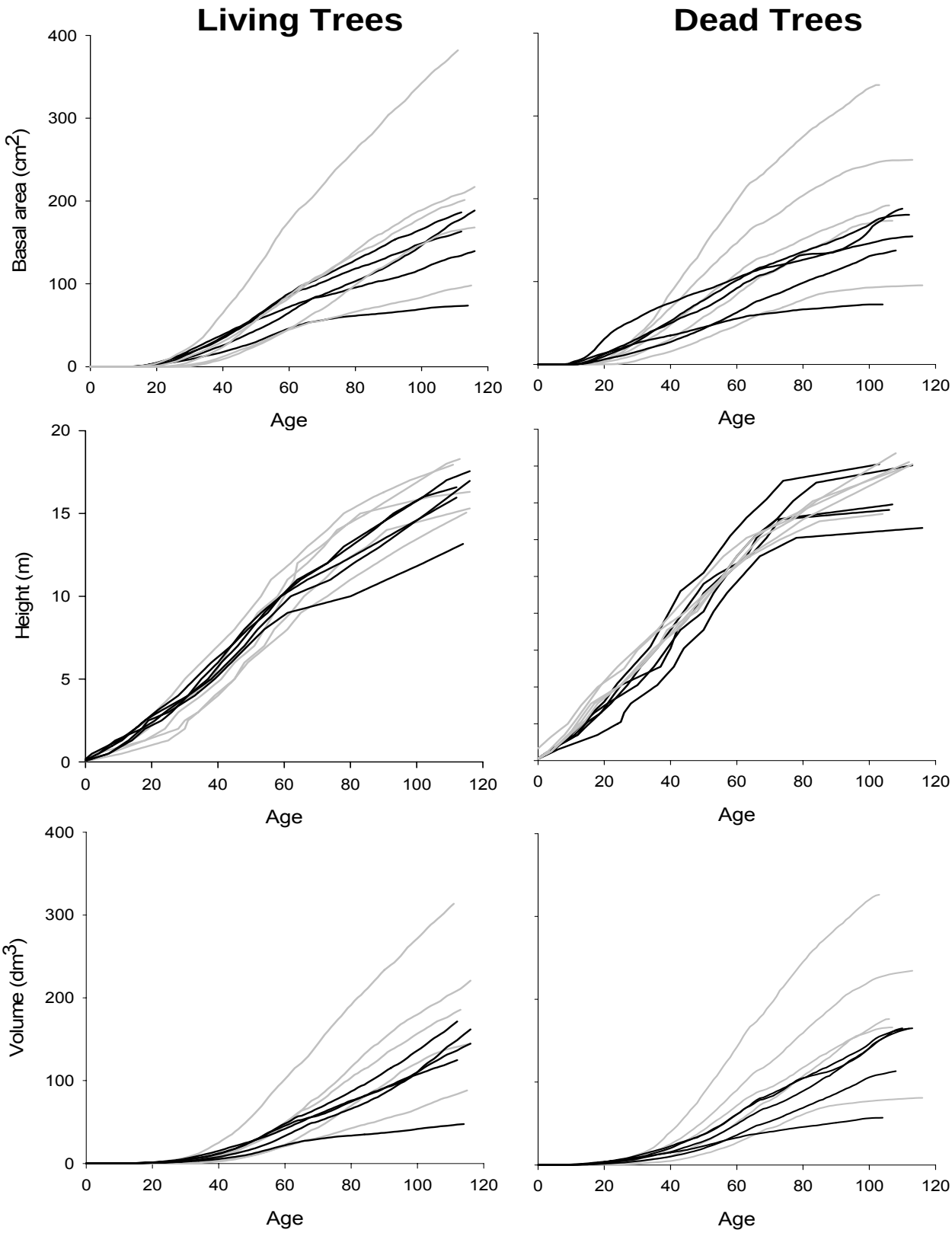
Appendix 3: Histograms showing the origin date and mortality date of living (gray) and dead (black) black spruce sampled along each transect in the Duck Mountain Provincial Forest and Porcupine Provincial Forest



Appendix 4: Boxplots of the year of death at different tree heights as identified by last ring, compared to true year of death (outermost ring along the stem).



Appendix 5: Incremental growth of five living and five dead trees in the Duck Mountain Provincial Forest (black lines) and Porcupine Provincial Forest (gray lines).



Appendix 6: Cumulative growth of five living and five dead trees in the Duck Mountain Provincial Forest (black lines) and Porcupine Provincial Forest (gray lines).