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Age-related Trends in Red Spruce Needle Anatomy and their Relationship to Declining Productivity

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AGE-RELATED TRENDS IN RED SPRUCE NEEDLE ANATOMY AND THEIR RELATIONSHIP TO DECLINING PRODUCTIVITY

By

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AGE-RELATED TRENDS IN RED SPRUCE NEEDLE ANATOMY AND THEIR RELATIONSHIP TO DECLINING PRODUCTIVITY

By Margaret H. Ward

Thesis Advisor: Dr. Michael S. Greenwood

An Abstract of the Thesis Presented
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Many species of trees undergo predictable age-related changes in foliar morphology and physiology. Age-related declines in photosynthetic rates, which may lead to decreases in productivity, have been described for numerous species. However, the physiological basis for these phenomena is unclear, as are linkages between age-related trends in morphology/anatomy and physiology. Photosynthetic capacity in red spruce (*Picea rubens* Sarg.) may result from increased mesophyll resistance to CO_2 uptake in older trees. Additional studies with other species imply that the foliage of older trees may have a lower ratio of photosynthetic to non-photosynthetic tissue and a larger proportion of xylem to leaf area to compensate for increased water stress.

To better understand these linkages, we investigated the age-related trends in foliar anatomy for juvenile, mid-age, and old (mean ages 3-, ~12, ~53, and ~127-year-old) red spruce trees growing in a multi-cohort stand in Maine. In addition, a series of reciprocal grafts between age classes were made in order to distinguish between the influences of internal aging factors and external or environmental factors while minimizing the confounding effects of tree size and complexity. For natural foliage, six

trees of each age class were randomly selected and photosynthesis measurements were taken on three shoots per tree. One needle from each shoot was collected and sectioned for anatomical observation. Needle cross-sectional area, width, height, perimeter, vascular bundle area, xylem and phloem area, and tracheid lumen diameters were described for all age classes. Internal air space was determined by a displacement technique for three separate shoots from each tree.

Needles from older red spruce were wider and more massive. Relative to juvenile trees, needle cross-sectional area was roughly 2x greater for mid-age and old trees, and the vascular bundle cross-sectional area was approximately 3x larger. Although mass-based photosynthetic rates declined with tree age, the proportion of photosynthetically active mesophyll tissue to non-photosynthetic tissue, such as vascular tissue and resin canals, increased from juvenile to old trees. However, the proportion of internal air space in needles decreased significantly with increasing age, with approximately a 20% decrease from juvenile to old trees. The combined influences of cross-sectional area and the smaller proportion of internal needle air space for old trees, indicating a more compact mesophyll layer, may contribute to increased gas exchange resistance in older trees. Also, a greater proportion of cross-sectional xylem area to perimeter (a surrogate for leaf area) was found in old trees and could be a mechanism for hydraulic compensation.

The grafting study showed that both external and intrinsic genetic factors appeared to be influencing age-related changes in needle anatomy with increasing age in red spruce. While needle width increased with age regardless of rootstock age,

photosynthetic rates were a function of the rootstock used. Many traits appeared to be regulated by a combination of extrinsic and intrinsic factors.

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Introduction

Most forest species including red spruce (*Picea rubens* Sarg.) show a decline in growth efficiency (biomass production per unit foliar mass) before reaching old age. There is a phase in early tree growth when growth efficiency increases, and the end of this phase coincides with the development of a maximum leaf area index. After mid-age, growth efficiency tends to decline gradually (Seymour and Kenefic 2002, Ryan et al. 1997). Although this decline is well documented for many species, a number of possible causes are still being investigated. Understanding the physiological processes behind the growth decline associated with aging stands is important for improving forest growth models and strengthening our understanding of the basic biology of aging in trees.

The decline in tree productivity with stand age has been correlated with a decline in photosynthesis (Ryan et al. 1997, and Bond 2000, Day et al. 2001, Kull and Kopel 1987, Yoder et al. 1994, Hubbard et al. 1999, and Richardson et al. 2000). However McDowell et al. (2002) found that Douglas-fir *(Pseudotsuga menziesii* (Mirb.) Franco) showed a decline in growth efficiency associated with increased tree height but taller (older) trees did not exhibit differences in photosynthesis. Age-related decreases in photosynthesis have been linked to a decline in stomatal conductance in ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) (Hubbard et al. 1999), and lodgepole pine (*Pinus contorta* var. *latifolia* Englem.) (Yoder et al. 1994). However, Day et al. (2001) found that age-related declines in photosynthetic rates appear to be unrelated to stomatal limitations in *P. rubens*.

Changes in whole-tree growth habits and morphology occur in conifers as they age. For example, height and diameter growth decline with age. This is apparent when comparing different aged loblolly pine (*Pinus taeda* L.) and eastern larch (*Larix laricina* [Du Roi] K. Koch) scions grafted onto juvenile rootstock (Greenwood and Hutchison 1993, Greenwood et al. 1989). Juvenile *P. taeda* and *L. laricina* scions had more branches per unit stem length and exhibited more orthotropic growth than scions from mature trees (Greenwood and Hutchison 1993, Greenwood et al. 1989). As trees reach a certain age/height, height growth decreases, and in some species including *P. rubens*, crowns flatten out, primary branches thicken and small branches take on a more bent and twisted appearance (Ryan and Yoder 1997).

Changes in needle morphology occur with increasing age for various conifers including hybrid Engelmann x white x Sitka spruce (*Picea engelmanni* Parry x *Picea glauca* (Moench) Voss x *Picea sitchensis* (Bong.) Carr) (Richardson et al., 2000), Norway spruce (*Picea abies* (L.) Karst) (Kull and Koppel 1987), Sitka spruce (*Picea sitchensis* (Bong.) Carr.) (Steele et al. 1989), Scotch pine (*Pinus sylvestris L.)* (Niinemets 2002), *P. menziesii* (Apple et al. 2002, Bond 2000), *P. Taeda* (Greenwood 1984), and *L. laricina* (Greenwood et al. 1989). A common tendency of conifers is for needles to become more massive with tree age, and this can result in decreased specific leaf area (SLA; total leaf surface area/leaf dry mass) with age. This has been shown for *P. rubens* and *L. laricina* (Day et al. 2001, Hutchison et al. 1990). In *P. rubens*, needle width and the width/length ratio increased in older trees (Day et al. 2001). These changes in *P. rubens* occurred regardless of whether the foliage being examined was sun- or shade-adapted (Day et al. 2001). For grafted *P. rubens*, Rebbeck et al. (1993) found no

statistically significant differences in specific leaf area, but needles from mature scions were 40% wider as well as 14% thicker than those from juvenile grafts.

A lower SLA in needles of taller trees may enhance internal water status because there is less leaf evaporative surface area per unit leaf mass. Therefore, needles from taller trees may be more efficient at conserving water that would otherwise be lost to cuticular transpiration (Richardson et al. 2000). Related to a decreased SLA, Mediavilla et al. (2001) suggested that an increased mesophyll thickness, and therefore a greater cell wall area available for CO_2 diffusion, will contribute to a decrease in CO_2 liquid-phase resistance in old trees. However, a negative aspect of a thicker leaf is that CO_2 resistance through intercellular air spaces to carboxylation sites of chloroplasts may increase due to a longer diffusion pathlength from the stomata to the surface of mesophyll cells (Niinemets 2002, Parkhurst 1986, Syvertsen et al. 1995). A thicker needle is not always associated with a lower proportion of internal air space. Mediavilla et al. (2001) found an increased leaf thickness was most often correlated with increased percent leaf air space in six sun-exposed woody deciduous and evergreen species. A higher proportion of internal air space could compensate for an increased resistance to gaseous CO_2 diffusion (Mediavilla et al. 2001). It could also result in the exposure of more mesophyll surface area to internal air space, thus decreasing liquid phase CO_2 resistance.

Previous studies have demonstrated that changes in internal leaf anatomy are associated with declines in photosynthesis with age for several tree species. In hybrid Engelmann × white × Sitka spruce (*Picea engelmannii* Parry x *Picea glauca* (Moench) Voss x *Picea sitchensis* (Bong.) Carr), foliage of the older trees had thicker epidermal walls, larger vascular cylinder cross sectional area, a thicker cuticle, and greater

interstomatal distances (Richardson et al. 2000). All of these changes were associated with lower photosynthetic rates; however, whether or not they are the cause of the decline in photosynthesis is unknown. Apple et al. (2002) found a higher percentage of photosynthetic mesophyll area in foliage from *P. menziesii* saplings, due to a smaller volume of hypodermal cells, astrosclerids, and vascular cylinders, as well as differences in needle shape compared to needles from older trees. Apple et al. (2002) suggest that a reduction in the proportion of photosynthetic mesophyll tissue in old *P. menziesii* trees could contribute to decreased photosynthetic efficiency with age. This study investigates whether or not this is shown for *P. rubens*.

Leaf-specific hydraulic conductivity may decline with increasing tree height (Bond 2000, Ryan and Yoder 1997, and Yoder et al. 1994) and as a result, compensation mechanisms may arise to improve leaf-specific gas exchange (McDowell et al. 2002). Older hybrid Engelmann × white × Sitka spruce had a larger ratio of vascular cylinder to needle cross-sectional area (Richardson et al. 2000). Similarly, Apple et al. (2002) proposed that proportionally larger vascular cylinders found in old-growth *P. menziesii* may increase water transport efficiency.

This study examined age-related trends in anatomy and morphology of *P. rubens* needles and their relationship to changes in foliar physiology. An initial phase of the study described leaf anatomy in relation to tree age for *P. rubens*, which has not been previously described. In addition, several specific hypotheses based on leaf anatomy were tested: 1.) The proportion of internal air space to total needle volume is greater in needles from old trees, compensating for a thicker leaf and allowing for more efficient internal CO_2 diffusion; 2.) As with other conifers, old trees have a lower proportion of

photosynthetic to non-photosynthetic leaf tissue which can be related to increased photosynthesis; 3.) The production of a larger proportion of vascular bundle to mesophyll area in the needles of old *P. rubens* trees may result as a mechanism for hydraulic compensation.

Materials and Methods

Study site

The study site, (described in greater detail in Day et al. 2001 and Seymour and Kenefic 2002), is located in the Penobscot Experimental Forest (PEF) in Bradley, Maine and consists of a multi-species, multi-cohort stand with a large component of *P. rubens*. It has been managed under a selection system by the USDA Forest Service since the early 1950s using a 5-year cutting cycle (Seymour and Kenefic 2002). The upper canopy is comprised primarily of two distinct mature cohorts: mid-age (averaged at 53 years) and old (averaged at 127 years) (Table 1.1).

Open-growing juvenile trees (~12 years old) (Table 1.1) were selected from a shelterwood-regenerated stand adjacent to the multi-cohort stand described above. These individuals were in close proximity (approximately 200 meters) to the *P. rubens* from the multi-cohort stand but, unlike the selection stand, juveniles were exposed to direct sun for a substantial portion of the day making their foliage more comparable to that of the mid-age and old trees. A younger group of juvenile trees (3 years old) extablished from seed collected from mature trees at the PEF study site (Table 1.1) were grown in pots at the University of Maine campus in Orono, ME. They were maintained at ± 4° C in a greenhouse during the winter and under 30% interception shade cloth during the summer.

The soil medium was composed of 50% peat moss, 25% perlite, 25% vermiculite, and supplied with Osmocote fertilizer (N-P-K of 18-6-12) at a rate of 4 kg m^{-3}.

Table 1.1 - Mean ± standard deviation for age, diameter at breast height (DBH) and diameter at 10 cm from base for juvenile trees, and height measurements for pot-grown juvenile, juvenile, mid-age and old trees.

Age class	Age (years)	DBH(cm)	Height (m)
old	127 ± 26	40 ± 3	17 ± 2
mid-age	53 ± 8	19 ± 2	10 ± 1
juvenile	12 ± 1	1.7 ± 0.4	1 ± 0.1
pot-grown juvenile	3	~1	~0.5

Gas exchange measurements

Gas exchange measurements on current-year, fully expanded foliage were made in late July and August in the upper third of the tree crowns between 8am and 1pm using a Li-Cor 6400 portable photosynthesis system with a 2 x 3 cm^2 cuvette. Shoots were exposed to a saturating irradiance of 1200 µmol m^{-2} s^{-1} using a LI6400B red/blue LED light source. Needles were kept at temperatures between 22-27 °C and the vapor pressure deficit was maintained under 2.0 kPa. Both the temperature and the vapor pressure deficit were below the range which would restrict photosynthesis in *P. rubens* (Day 2000). Once the conditions within the cuvette and photosynthetic rates stabilized, three gas exchange measurements were taken approximately five seconds apart and measurements were averaged. All shoots measured for photosynthesis were subsequently collected and transported to the laboratory in a cooler with moist paper towels or

sphagnum moss to keep them cool and hydrated. Gas exchange measurements including photosynthesis, stomatal conductance, and internal CO_2 concentration were taken on six trees of each age class (Table 1.1).

Three shoots per tree were selected from three different areas on the canopy, all of which were exposed to full sunlight. Gas exchange measurements for each age class were done on separate days so that comparisons between age classes would not be confounded by diurnal variation in light and temperature. Because ambient temperatures were not recorded during the measurement period, ambient temperatures were taken from the climate center at the Bangor airport in Bangor, Maine. Temperatures averaged over the sampling period for 3-, 12-, 53-, and 127-year-old trees were 20 °C, 27 °C, 23 °C and 24 °C respectively. Because Bangor is approximately 10 km away from the study site, there is an unknown microclimate effect that is not accounted for here.

Morphology and anatomy

Needles used for gas exchange measurements were carefully removed from the shoots in the laboratory. The total projected area of these needles was estimated using a high-resolution scanner with WinSeedle™ software (Regent Instruments Quebec, QC, Canada), and photosynthetic rates were calculated using area measurements. Needle dry mass was recorded (after dessication at 60 °C for two days), and specific leaf area (projected leaf area/leaf dry mass) was calculated.

A subsample of needles was immediately killed and fixed in formalin-acetic acid-alcohol (FAA) as described by Berlyn and Miksche 1976. The ends of the needles were removed using a razor blade under FAA solution in order to eliminate any morphological

or anatomical variation associated with the extreme ends of the needles, and to allow the fixative to penetrate more readily. Needles in FAA were placed in a vacuum dessicator chamber for approximately ten minutes to remove air, and then stored in vials of FAA at room temperature.

The needles were dehydrated through a graded ethyl alcohol and tertiary butyl alcohol series and embedded in paraffin as described by Berlyn and Miksche (1976). After exposing the end of the needles, paraffin blocks were soaked overnight in a solution of water, glycerol, DMSO, and liquid detergent (88:10:1:1) at 35 °C to soften the cell walls for improved sectioning (Richardson et al. 2000). Needle cross-sections were cut at 10 µm on a rotary microtome and ribbons were mounted on slides using Haupt's adhesive (Berlyn and Miksche 1976). Slides were rehydrated and stained with Safranin O and Fast Green and cover slips attached using Permount® mounting medium.

Digital images of cross-sections were made at 25, 100, and 400x using a SPOT RT color digital camera and a Zeiss Axioscop microscope. Measurements were made for needle cross-sectional area, needle height and width, cross-sectional area of the vascular bundles, area of xylem and phloem within the vascular bundles, resin canal area, and the perimeter of the entire needle cross section. This was performed using the public domain Scion Image program (U.S. National Institutes of Health; http://rsb.info.nih.gov/nih-image/). Percent mesophyll area was calculated by subtracting the area of the vascular bundle and the area of the resin canals from the total cross-sectional area. The proportions of xylem area to mesophyll area and phloem area to mesophyll area, as well as the ratios of xylem area and phloem area to perimeter were measured. The proportion

of mesophyll was adjusted for internal air space; mean air space for age-class (discussed below). Tracheid lumen diameters and the ratio of the tracheid lumen area/tracheid cell wall area were also measured.

Internal air space

Internal air space of red spruce needles was determined in late July and August 2003 on current year needles of 7-10 cm long shoots collected from the same trees that had been used for gas exchange measurements. The total volume per needle was calculated by placing the shoot in a 10 ml graduated cylinder, which was filled to 10 ml with water. The shoot was then removed and its volume estimated from the displaced volume of water. Next, all needles were carefully removed from the shoot and the same method was used to find the volume of the twig from which the needle volume was determined. The difference of these two values was calculated to determine the volume of the needles. Fresh and dry mass of the needles were measured as described above.

Internal air space of the needles was estimated using a formula described by Yokoi and Kishida (1985) and Koike (1988) for an evergreen woody plant, *Aucuba japonica* Thunb.:

$$V_a=V_f-(w_f-w_d)/r_w-w_d/r_d$$

where V_a(ml) = air space in needles ; V_f(ml) = volume of fresh needles; w_f (g) = fresh mass; w_d (g) = dry mass; r_w (g ml^{-1}) = density of water; r_d = density of dry matter (g ml^{-1}). Yokoi and Kishida (1985) determined from their study that the density of foliar dry matter ranges from 1.4 to 1.5. They averaged these values to produce 1.45 g and this is

what was used for the density of dry matter (Yokoi and Kishida 1985, Koike 1988). The percent air space was determined by multiplying the ratio of air space in the needles/volume of fresh needles by 100.

Statistical analysis

Three separate cross-sections per slide from a single needle from each shoot were measured and the means used for analysis. This was done for all anatomical parameters except tracheid lumen diameter and cell wall thickness. A subsample of four representative tracheids from each cross section was selected for measurements.

All statistical analyses were carried out using the SAS System for Windows (version 9.0, SAS institute, Inc. Cary, NC). Using the SAS GLM procedure, single-factor analysis of variance (ANOVA) was used to test the effect of age class on the physiological, morphological and anatomical variables. Means were separated using the Tukey's Studentized Range (HSD) Test, and a Levines test was used to assess homogeneity of variances. To meet the assumptions of the ANOVA, log transformed data were used for needle cross section, width, xylem, and the ratio of phloem area to mesophyll area, and square root transformations were used for percent mesophyll area and the ratio of xylem to mesophyll.

Results

Photosynthesis

On both an area and mass basis, photosynthesis declined with tree age in accordance with the findings of Day et al. (2001) (Table 1.2). Both of the younger age groups (3- and 12-year-old trees) had higher photosynthesis per unit leaf area than the two older age classes (53- and 127-years old). However, differences in means were not statistically significant between 3- and 12-year-old trees, nor were they significant between 53- and 127-year-old trees. Net photosynthesis per unit dry mass declined (61%) between 3- and 53-year-old trees, but did not differ between 53- and 127-year-old trees. Stomatal conductance (g_s) also declined by 43% between 3- and 127-year-old trees. Internal CO_2 concentration (C_i) did not show an age-related trend (Table 1.2).

Table 1.2 - Mean ± SE for photosynthesis, stomatal conductance, and internal CO_2 concentration from non-grafted material of different aged trees (3, ~12, ~53, and ~127 years old). For each variable, means not followed by the same letter are significantly different ($P < 0.05$). P values were obtained by an ANOVA with N = 72

Measurement	**3 years**	**~12 years**	**~53 years**	**~127 years**	***P***
Net photosynthesis ($\mu mol\ CO_2\ m^{-2}s^{-1}$)	13.72 ± 0.79 a	12.99 ± 0.46 a	7.64 ± 0.51 b	8.59 ± 0.60 b	<.0001
Net photosynthesis ($\mu mol\ CO_2\ kg^{-1}s^{-1}$)	66.15 ± 4.18 a	55.79 ± 1.56 b	25.85 ± 2.19 c	23.69 ± 1.74 c	<.0001
Stomatal conductance ($mol\ H_2O\ m^{-2}\ s^{-1}$)	0.247 ± 0.0194 a	0.189 ± 0.024 ab	0.110 ± 0.012 c	0.140 ± 1.5 bc	<.0001
Internal CO^2 concentration ($\mu mol\ CO_2\ mol^{-1}$)	229 ± 6.90 bc	265 ± 4.87 a	208 ± 8.14 c	233 ± 4.74 b	<.0001

Morphology

Needle height, width, perimeter, and total cross-sectional area increased with tree age (Table 1.3; also see Figure 1.1). Needle width and cross-section area showed the most dramatic increases, 100% and 160% respectively, between 3- and 127-year-old trees. These characteristics showed the greatest increase during the 12 to 53-year-old transition. A similar but less pronounced relationship was found for the cross-section height and perimeter, which increased by 38% and 69% respectively with age. Specific leaf area (SLA) declined with tree age (Table 1.3), and the sharpest decline was again between 12- and 53-year-old trees. There was a statistically significant decreasing trend (8%) between 3- and 127-year-old trees in the proportion of internal air space of needles from juvenile to old trees. This attribute occurred as a continuum with adjacent age classes not exhibiting significant differences (Table 1.3).

Anatomy

The proportion of the cross-sectional needle area occupied by mesophyll decreased from 90% to 87% with increasing tree age (Table 1.3, Figure 1.2). The proportion of mesophyll adjusted for internal air space was slightly higher in older trees than in young trees (Table 1.3). The cross-sectional area of the vascular bundle increased by 262% between 3- and 127-year-old trees; phloem area increased 132%, and xylem area 229%. In addition, the ratios of xylem area to mesophyll area, the xylem area to mesophyll area adjusted for air space, of xylem area to needle cross-sectional area and xylem area to perimeter also increased with increasing tree age (Table 1.3). The greatest

increase (94%) was shown for the ratio of xylem area to perimeter, where all age classes were significantly different from one another except for the 3- and 12-year-old juveniles. In addition, tracheid lumen diameter increased by 19% with increasing tree age (Table 1.3; also see Figure 1.3). The ratio of lumen area to tracheid cell wall area was smaller in old trees, but total lumen area still showed a 177% increase from 3- to 127-year-old trees.

The ratio of phloem area to perimeter also increased with age, but not to the same degree as the xylem to perimeter ratio (Table 1.3). The ratio of phloem to mesophyll did not differ significantly between age classes, and no patterns were apparent. When the ratio of phloem area to mesophyll area was adjusted for air space, statistical significance was found between age classes; however there were no age-related trends. There was a decrease from 3- to 12-year-old trees (20%), but the transition to older trees shows slight increases. Lastly, changes in the ratio of phloem area to cross sectional area were statistically significant between age classes according to the ANOVA (P=0.0505) but no age-related trends were present.

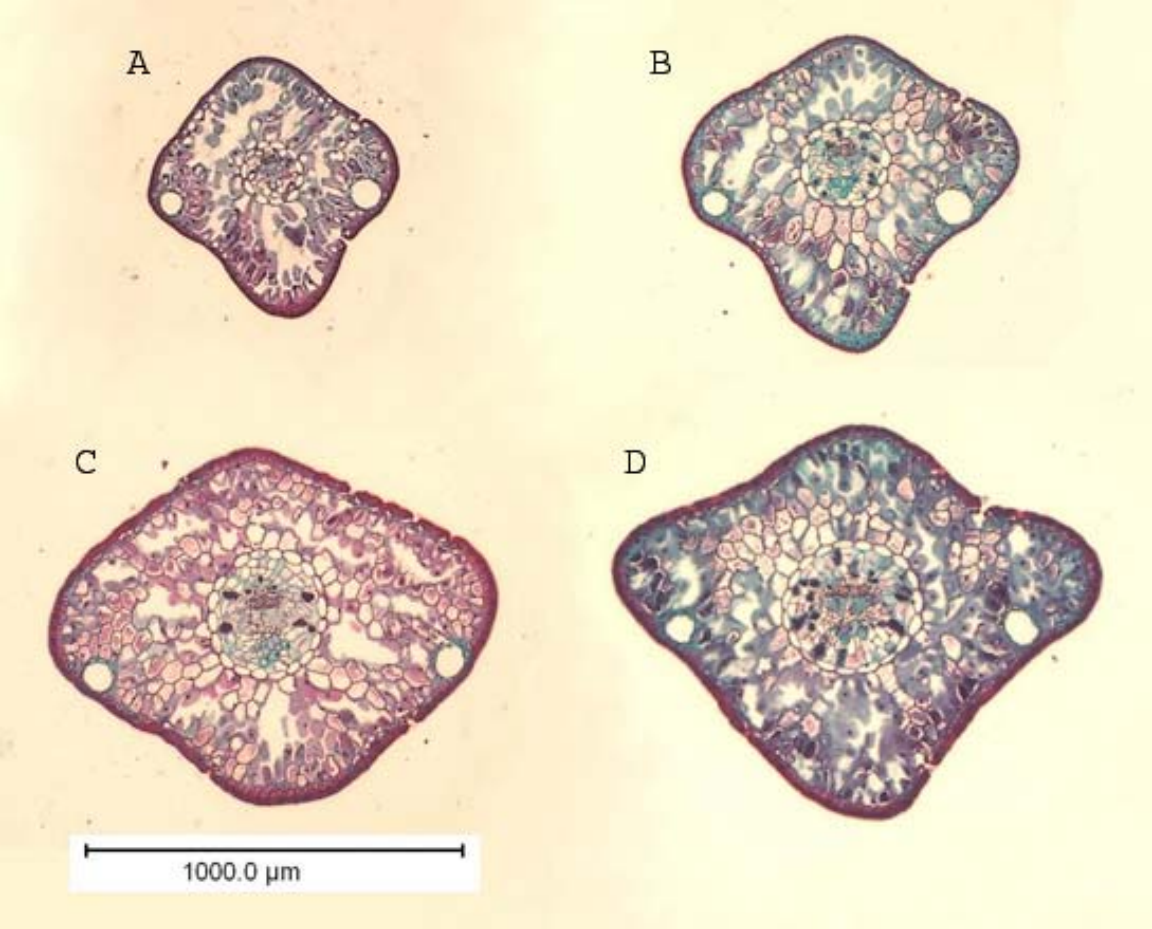

Figure 1.1 – Needle cross-sections representative of mean needle widths for A.) 3-year old B.) 12-year old C.) 53-year old and D.) 127-year old *P. rubens* trees.

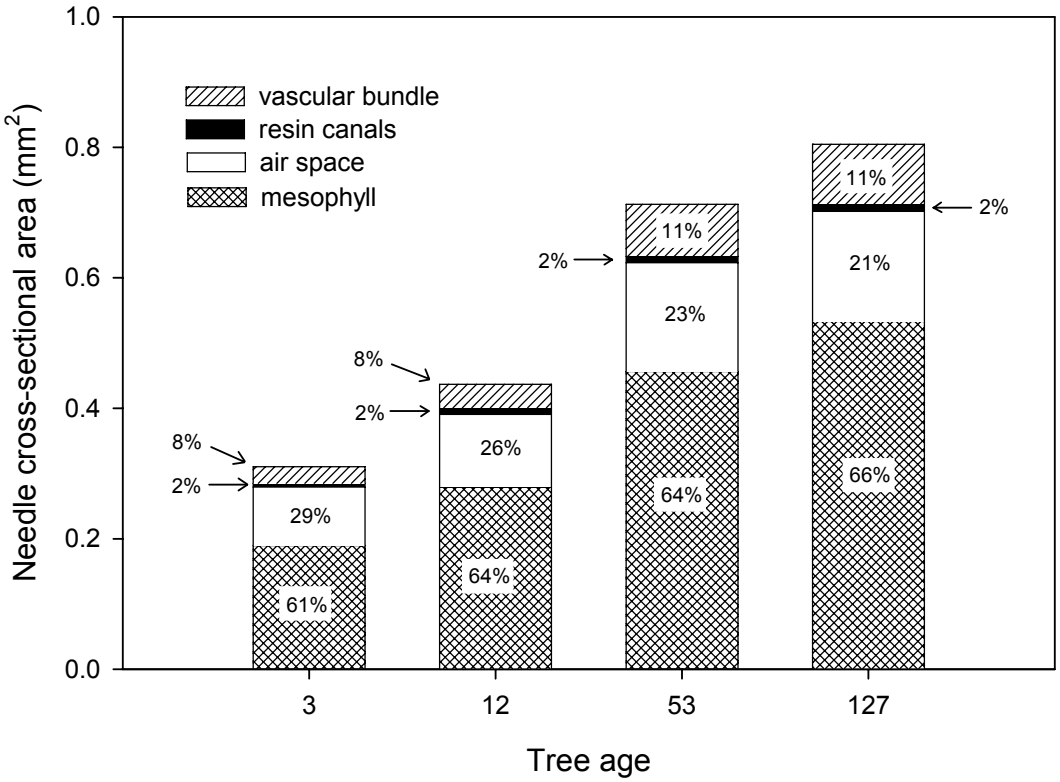

Figure 1.2 – Total needle cross-sectional areas and areas occupied by vascular bundle area, resin canal area, air space, and mesophyll area for different aged *P. rubens* trees (averaged at 3, ~12, ~53 and ~127 years old).

Table 1.3 - Mean ± SE for measurements of red spruce needle cross sections from current-year needles of different aged trees (averaged at 3, ~12, ~53 and ~127 years old). For each variable, means not followed by the same letter are significantly different ($P < 0.05$).

Measurement	**3 years**	**~12 years**	**~53 years**	**~127 years**
Specific leaf area	48.11 ± 0.89 a	43.35 ± 1.00 b	33.29 ± 0.63 c	27.52 ± 0.44 d
% Internal air space	28.81 ± 1.15 a	25.72 ± 1.31 ab	23.32 ± 0.86 bc	20.94 ± 0.69 c
Width (μm)	663.2 ± 10.2 d	804.6 ± 23.4 c	1207.1 ± 25.6 b	1327.9 ± 33.1 a
Height (μm)	724.5 ± 14.2 c	851.5 ± 24.6 b	952.3 ± 22.9 a	996.5 ± 28.8 a
Cross section (μm^2)	310841 ± 7619 c	435460 ± 19637 b	713306 ± 19902 a	807433 ± 26490 a
Perimeter (μm)	2082.5 ± 25.0 d	2496.0 ± 56.0 c	3248.3 ± 50.5 b	3520.4 ± 63.5 a
Phloem (μm^2)	1566.7 ± 111.1 b	1761.3 ± 121.1 b	3176.1 ± 145.8 a	3636.4 ± 174.4 a
Xylem (μm^2)	1042.2 ± 45.2 d	1401.5 ± 80.7 c	2820.7 ± 101.7 b	3426.2 ± 160.3 a
Vascular bundle (μm^2)	25340 ± 769.9 d	35912 ± 1546.4 c	79875 ± 2568.0 b	91612 ± 4379.1 a
Proportion Mesophyll*	0.90 ± 0.003 a	0.89 ± 0.002 a	0.87 ± 0.002 b	0.87 ± 0.004 b
Proportion Mesophyll▲	0.61± 0.009 b	0.64 ± 0.011 ab	0.64 ± 0.008 ab	0.66 ± 0.008 a
Xylem/mesophyll	0.0037 ± 0.0001 b	0.0036 ± 0.0001 b	0.0045 ± 0.0001 a	0.0049 ± 0.0002 a
Xylem/mesophyll♣	0.0055 ± 0.0002 bc	0.0050 ± 0.0002 c	0.0062 ± 0.0002 ab	0.0064 ± 0.0003 a
Xylem/cross section	0.0034 ± 0.0001 b	0.0032 ± 0.0001 b	0.0040 ± 0.0001 a	0.0043 ± 0.0002 a
Xylem/perimeter	0.50 ± 0.02 c	0.56 ± 0.02 c	0.87 ± 0.02 b	0.97 ± 0.04 a
Phloem/mesophyll	0.0057 ± 0.0004 a	0.0045 ± 0.0002 b	0.0051 ± 0.0002 ab	0.0052 ± 0.0002 ab
Phloem/mesophyll†	0.0085 ± 0.0007 a	0.0062 ± 0.0003 b	0.0069 ± 0.0003 ab	0.0068 ± 0.0003 ab
Phloem/cross section	0.0051 ± 0.0004 a	0.0040 ± 0.0002 b	0.0045 ± 0.0002 ab	0.0045 ± 0.0002 ab
Phloem/perimeter	0.76 ± 0.05 b	0.70 ± 0.04 b	0.97 ± 0.04 a	1.03 ± 0.04 a
Tracheid lum. diam. (μm)	4.18 ± 0.13 c	4.77 ± 0.08 b	5.30 ± 0.11 a	4.99 ± 0.09 ab
Lumen/cell wall	0.60 ± 0.02 a	0.63 ± 0.01 a	0.56 ± 0.02 b	0.51 ± 0.01 c
Lumen area (μm^2)♦	632 ± 39 b	875 ± 49 b	1584 ± 65 a	1750 ± 110 a

∗ = proportion of mesoplyll to total cross-sectional area
▲= proportion of mesophyll to total cross-sectional area (mesophyll is adjusted for airspace)
♣ =mesophyll is adjusted for air space
†= mesophyll is adjusted for air space
♦= (Lumen/cell wall) × xylem area (μm^2)

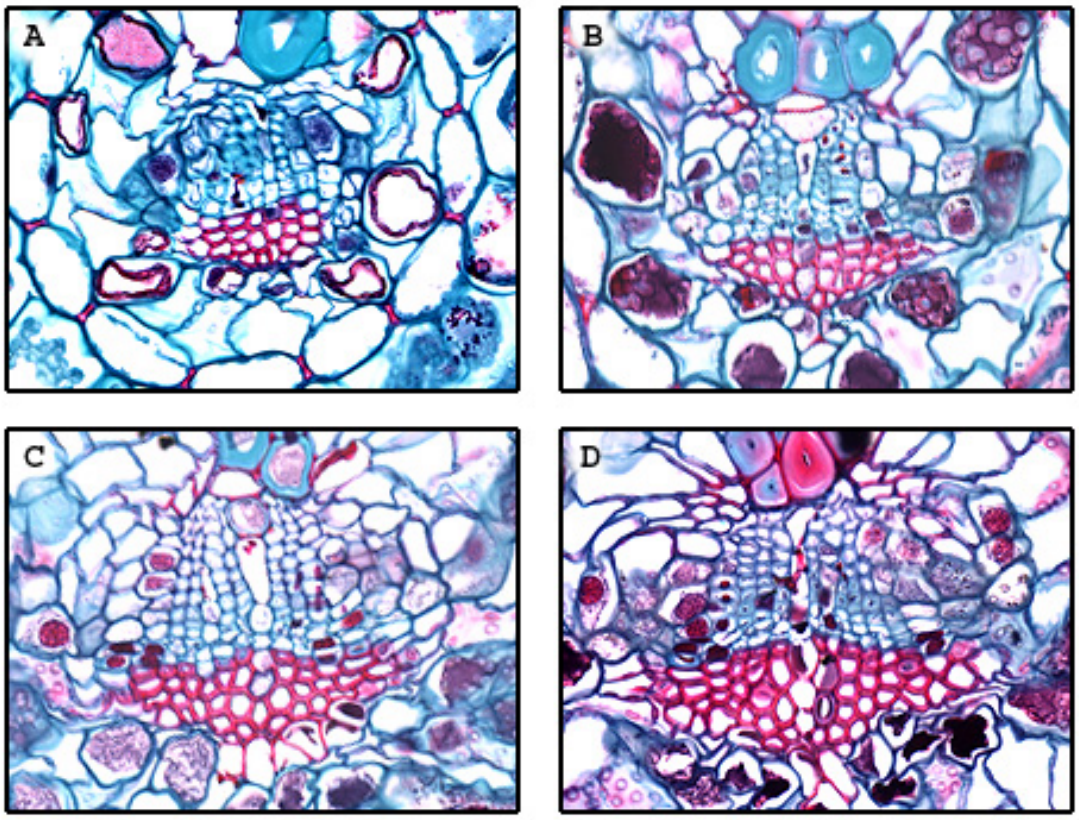

Figure 1.3 – Needle cross sections showing xylem (red) and phloem (green). A, B, C, and D represent needles from 3-, 12-, 53- and 127-year-old trees respectively.

Discussion

As trees age and grow taller they are exposed to changing external conditions such as increased intensity of sunlight and wind (Day et al. 2002), internal attributes such as changing carbohydrate and hormone availability (Greenwood 1984), variation in pest and pathogen influences and changing belowground competition dynamics for nutrients and water (Day et al. 2002). Trees may have evolved age-related changes in morphology in response to these changes in their environment, and these trends might be initiated by changes in environmental conditions that occur with increasing tree age. For example, Niinemets (2002) suggests that greater water limitations are responsible for the smaller leaves which are common on old, tall trees. In redwoods (*Sequoia sempervirens*) and *P. menziesii*, Koch et al. (2004) and Woodruff et al. (2004) provide evidence that the reduction in turgor with tree height is responsible for the smaller, less expanded foliage in the tops of trees compared to the lower crown. Turgor pressure in leaves is important to leaf cell expansion (Dale 1988). Cell expansion is affected more than photosynthesis by a reduced tissue water potential (Luxmoore 1991, Lambers et al. 1998). Therefore, although anatomy and morphology can affect water status in a leaf to alter photosynthetic rates, the reverse relationship may also occur; changes in leaf anatomy and morphology found with increasing tree height may result from a lower leaf water potential.

Although turgor appears to have a biological effect on different aged *S. sempervirens* and *P. menziesii*, the trend may not occur between different *P. rubens* age classes examined in this study since no significant difference in twig xylem water potential was found between 60- and 120-year-old *P. rubens* at dawn or midday from the same site in the summer of 1995 (Day et al. 2001). Also, the small difference in water

potential that occurred between 60- and 120-year-old trees can be accounted for by the difference in gravitational potential. The gravitational potential difference is about 0.1 MPa between 60- and 120-year-old *P. rubens* and about 0.2 MPa between 3- and 120-year-old *P. rubens* given that gravity exerts 0.1 MPa of pressure for every 10 meters of tree height. With the small differences in gravitational potential between *P. rubens* age classes, age (height)-related changes in turgor would not be as important for *P. rubens* as for *Sequoia sempervirens* and *P. menziesii* trees which grow about four times as tall as P. rubens and therefore have a much larger difference in gravitational potential between age classes.

Age-related differences in internal air space

With increasing tree age, *P. rubens* needles showed a decrease in SLA. This could have an adaptive advantage, as a thicker denser needle may stand up to harsh conditions such as snow, ice and wind more effectively. However, it could also have a negative effect on photosynthetic efficiency. A low SLA reflects either thick leaves or leaves with a high biomass density (Poorter et al. 1990, Witkowski and Lamont 1991, Niinemets 1999). The thicker, denser leaves found on old trees may exhibit more internal shading, which might lead to decreased photosynthetic rates. In other words, as the ratio of mesophyll cell wall area to leaf area increases, and assuming that the amount of chlorophyll along the mesophyll cell walls does not change, an increased amount of light will probably be required for light saturation of photosynthesis (Nobel 1977). Niinemets (1999) in a meta-analysis of woody species concluded that, although a greater leaf thickness and greater density indicates the presence of more available photosynthetic

material per unit leaf area, the greater resistance to CO_2 diffusion in thicker, denser leaves can limit assimilation rates. Similarly, when studying citrus, peach and Macadamia trees, Syvertsen et al. (1995) found that internal leaf conductance decreased with increasing leaf thickness, decreasing SLA, and an increased volume of mesophyll cells per unit leaf area. Consideration of the proportion of internal air space is important when explaining differences in CO_2 diffusion within leaves.

For CO_2 assimilation to occur, gaseous CO_2 must first enter the leaf through stomata. Then it must travel in a gaseous form through the intercellular air space within the leaf until it reaches the surface of the photosynthetic mesophyll cells. It dissolves in liquid when crossing the cell wall and plasma membrane and entering the cytosol and the choroplasts (Lloyd et al. 1992; Evans and von Caemmerer 1996). The overall resistance for CO_2 traveling through a leaf (R_{leaf}) can be described as:

$$R_{leaf} = R_s + R_a + R_l$$

Where R_s is resistance caused by transport through stomatal pores, R_a is the resistance caused by the CO_2 diffusion pathway through the substomatal cavity and the internal air spaces of the leaf, and R_l is the liquid phase resistance that occurs across the cell wall, cell membrane and into the chloroplasts. The first part of the pathway, stomatal conductance ($1/ R_s$), exhibited a general decreasing trend with increasing tree age in *P. rubens* (Table 1.2). The R_a and R_l are also important components of the pathway which will be discussed.

A smaller proportion of internal air space in leaves could contribute to increased internal gas-phase CO_2 resistance (R_a) because there is less space available for CO_2 diffusion (Mediavilla et al. 2001). The first hypothesis stated that needles from old trees

would have a larger proportion of internal air space to total needle volume. This was not true, demonstrated by the increased proportion of internal air space to total needle volume found in young *P. rubens* (Table 1.3). Parkhurst and Mott (1990) studied 13 broad-leaved species with high levels of stomatal conductance and photosynthesis and showed that diffusion of gaseous CO_2 in the intercellular air spaces can limit CO_2 assimilation. This was most pronounced in hypostomatous leaves (those with stomata on their lower surface), and the same trend was observed in amphistomatous leaves (leaves with stomata on both sides) but the effect was smaller. If *P. rubens* needles are compared to these amphistomatous leaves, it seems as though the intercellular gas phase resistance would not be important to total internal CO_2 conductance. However, it is difficult to make conclusions based on a comparison between a stress-tolerating conifer and highly productive broad-leaved species which have different structural and physiological adaptations. Also, it is logical to propose that the higher stomatal conductance found in the amphistomatous leaves (Parkhurst and Mott 1990) is what caused gas-phase CO_2 resistance to have such a minor effect on CO_2 assimilation when compared to the hypostomatous species. From this standpoint, *P. rubens,* which has much lower levels of stomatal conductance (~2×) and photosynthesis (~3×) than even the broad-leaved hypostomatous species, may be affected by gas-phase CO_2 resistance even to a greater extent than the hypostomatous leaves.

Liquid-phase mesophyll resistance, the diffusion resistance for CO_2 between the leaf intercellular air space and the carboxylation sites in the chloroplasts, is another important part of the CO_2 pathway that may be greater in taller trees, thus contributing to a decrease in photosynthesis (Nobel 1977). Larger trees with denser leaves usually have

thicker cell walls with higher lignin content (Niinemets 2002). This will result in a decreased ability for water and CO_2 to permeate the cell walls (Brett and Waldron 1996). Increased liquid-phase CO_2 resistance will also occur if less mesophyll surface area is exposed to intercellular air spaces (Syvertsen et al. 1995; Nobel 1977; Koike 1988). Syvertsen et al. (1995) proposed that CO_2 liquid phase resistance may be a more influential constraint on internal CO_2 diffusion than gas phase resistance because the molecular diffusion coefficient for CO_2 is about three times greater in the gaseous phase than in the liquid phase (Nobel 1977, Niinemets 1999) so it diffuses 10,000 times slower in water than in air (Evans and von Caemmerer 1996). However, since the distance for CO_2 travel across cell membranes is much shorter than the path from stomata to the cell walls, this concept may be difficult to support with confidence. Niinemets (1999) and Nobel (1977) proposed that needle thickness is not as important for determining the upper limits for photosynthesis as needle density and the ratio of mesophyll to the projected leaf surface area, which reflects the surface area of chloroplasts exposed to the intercellular air space. The decreased proportion of internal air space found in needles of old *P. rubens* suggests a greater liquid-phase mesophyll resistance for old trees. This is assuming that air space is, for the most part, distributed evenly within needles from old trees as it is for needles of young trees.

To summarize, an increased proportion of air space to total needle volume could allow more efficient gaseous diffusion within the needle. Also, more internal air space likely means more mesophyll cell wall surface area is exposed to CO_2 and this will increase rates of CO_2 liquid phase diffusion. Therefore, it is likely that the greater proportion of internal air space to total needle volume found in needles of juvenile *P.*

rubens could cause an overall increase in internal CO_2 diffusion and, therefore, increased CO_2 assimilation rates.

Do needles of old trees have proportionally less mesophyll?

The second hypothesis proposed that there is a greater proportion of photosynthetic to non-photosynthetic tissue in needles of young *P. rubens* based on the 10% decrease in percent mesophyll found between 10- and 450-year-old *P. menziesii* (Apple et al. 2002). Part of this decline in mesophyll area with age in *P. menziesii* was due to the presence of astrosclerids which took up space in needles from old trees that would otherwise have been taken up by photosynthetic mesophyll cells. Astrosclerids also blocked mesophyll cell surface area that is needed for gas exchange (Apple et al. 2002). Nothing resembling astrosclerids was found within needles of *P. rubens*. In addition, older *P. rubens* trees showed a slightly larger proportion of mesophyll area than young trees (8% increase between 3- and 127-year-old trees), due to their smaller proportion of internal air space. Therefore, the decline in photosynthesis found with increasing tree age was not caused by a decrease in proportion of mesophyll/total leaf tissue.

Hydraulic compensation at the leaf-level

According to Darcy's law, water flux through a tree is proportional to the difference in water potential that occurs with increasing tree height and the hydraulic conductance of the tree (Tyree and Ewers 1991). The resistance (inverse of hydraulic

conductance) to water transport in a tree (R_{tree}) is the sum of a series of resistances and can be expressed by the following equation derived from the Ohm's law analogy (Tyree and Ewers 1991):

$$R_{tree}=R_{root} + R_{stem} + R_{needle} +R_{stomatal} + R_{boundary\ layer}$$

This study reveals information regarding leaf (needle) resistance, a small but potentially very important component of the resistance pathway for water flow from the soil to the atmosphere.

The "hydraulic limitation" hypothesis states that conductivity is decreased in tall, mature trees due to the combination of gravity and a longer, more complex pathway for the water to travel (Bond 2000, Ryan and Yoder 1997, Yoder et al. 1994). A combination of decreased hydraulic conductivity and a greater gravitational potential can lead to increased water stress in the tops of tall trees. In order to prevent xylem cavitation, stomatal conductance may decrease, leading to increased resistance to gas exchange and the CO_2 uptake required for photosynthesis (Ryan and Yoder 1997). Therefore, a greater hydraulic resistance may contribute to the decline in photosynthetic efficiency and limited carbon uptake that occurs with tree age in older trees (Ryan et al. 2000; Hubbard et al. 1999; but see McDowell et al. 2002; Yang and Tyree 1994).

However, as trees age various compensation mechanisms may counteract these potential constraints on hydraulic conductivity (McDowell et al. 2002, Becker et al. 2000). Possible compensation mechanisms include increased root surface area, increased water storage, a greater water potential difference between the soil and leaf, an increased ratio of sapwood to leaf area, and increased specific conductivity of sapwood (McDowell et al. 2002). McDowell et al. (2002) found evidence for hydraulic compensation in *P.*

menziesii, however they concluded it was not sufficient to fully compensate for decreased hydraulic conductivity and stomatal conductance in tall individuals.

Producing a greater proportion of xylem area to needle surface area could be a strategy for compensating for the less sufficient water supply associated with leaves of taller trees. The proportionally larger vascular cylinders in needles of old-growth Douglas-fir could result in more efficient water transport (Apple et al. 2002). In *P. rubens*, the ratio of vascular bundle area/needle cross-sectional area was larger in older trees and, more specifically, the ratio of cross-sectional xylem area to cross-sectional perimeter (a surrogate for leaf area) increased by 94% between 3- and 127-year-old trees. In addition, the mean tracheid lumen diameter for *P. rubens* increased by 19% with increasing tree age. The increased tracheid lumen diameter of older *P. rubens* foliage may substantially increase leaf-level hydraulic conductivity which is proportional to the lumen diameter raised to the fourth power (Tyree and Ewers 1991).

The proportion of tracheid lumen area to tracheid cell wall area decreased significantly with tree age in *P. rubens*. In other words, although the overall xylem areas and lumen diameters were proportionally larger in older trees, the proportion of lumen to cell wall was lower. However, when calculating the total lumen area based on the total xylem area minus a calculated cell wall area, there was a 177% increase between 3- and 127-year-old trees. This study clearly supports the idea of hydraulic compensation in needles of old trees; however, age-related change in the balance of the hydraulic system of the whole tree has not been studied.

Sink/source dynamics

A larger proportion of phloem to mesophyll could lead to the inference that leaves have the capacity to export more photosynthate per unit leaf mesophyll area regardless of whether they are producing more photosynthate. For *P. rubens*, there was a moderate (20%) decrease in phloem/mesophyll adjusted for air space from 3 to 12-year-old trees but no significant changes occurred between the other age classes. It may be important to consider that the 3-year-old trees were grown in different conditions (raised and maintained in pots) than the other age classes which were growing in the PEF. This could explain the difference in the youngest age-class. If possible confounding factors were absent, we could suggest that needles from the 3-year-old trees are capable of exporting more photosynthate or could be accumulating more photosynthate, thus requiring more phloem for export. This would make sense based on their higher photosynthetic rates, but the available evidence is not strong enough to support this idea. Also, in wheat plants, if half the amount of phloem is taken away from within the peduncle of the plant, grain growth is not affected (Wardlaw & Moncur, 1976). This suggests that at least in wheat, an angiosperm grass, phloem conductivity was well in excess of required capacities. If this is also the case in *P. rubens*, the ratio of phloem to mesophyll would not explain the variation in photosynthesis even if there was a significant age-related trend.

It is important to consider an additional perspective in relation to the reduction in productivity with tree age, that of sink/source relationships. The majority of this discussion has focused on how leaf anatomy and morphology could cause changes in

photosynthesis with age, which therefore influences growth rates. There has been sufficient evidence to support this perspective; higher photosynthetic rates will allow greater sink activity under certain conditions (Luxmoore 1991). However, it is equally important to consider the reverse situation; it is possible that photosynthetic rates are determined by growth rates, or sink capacity (Luxmoore 1991). If other factors besides photosynthesis are causing reductions in growth with age in *P. rubens*, such as external environmental stresses including water and nutrients, the resulting smaller carbon sinks could allow unused photosynthate to accumulate, therefore resulting in a feedback inhibition of photosynthesis (Day et al. 2001, Yoder et al. 1994, Luxmoore 1991).

In conclusion, this study has provided an in-depth description of the internal leaf anatomy in relation to tree age for *P. rubens*. This information has led to the conclusion that there are, in fact, statistically significant relationships that occur between leaf anatomy and photosynthesis with tree age. The first hypothesis which stated that internal air space should be more plentiful in needles of old trees was not supported. A greater proportion of internal air space to needle volume was found in juvenile trees, and this could aid in more efficient CO_2 diffusion and thus higher photosynthetic rates. The results show that the proportion of mesophyll tissue was not significantly higher in needles from young trees, leading to the conclusion that it is not mesophyll area alone that is responsible for the decline in photosynthesis. Lastly, I found that a larger proportion of xylem and phloem was present in the needles of old trees. Although no substantial conclusions could be made in reference to the phloem, I propose that the higher proportion of xylem found in needles of old trees could be a mechanism for hydraulic compensation. The changes in physiology in trees with age could also be due

in part to additional anatomical characteristics that I did not explore. These include stomatal frequency and interstomatal distance, differences in thickness of epicuticular wax, and lignin content of needles.

Chapter Two: Photosynthesis and anatomy of needles from year-old reciprocal grafts on juvenile, mature, and old *Picea rubens*

Introduction

The previous chapter discussed the relationship between age-related changes in the physiology and anatomy of needles from red spruce (*Picea rubens*), but the developmental mechanisms responsible for these age-related changes were not examined. An age-related change in the behavior pattern of meristems results from a physiological process known as maturation (Greenwood 1995). The process consists of changes in morphology and physiology of trees with increasing age that in some cases are irreversible (Ritchie and Keeley 1994). To date, we do not have enough information to decipher to what degree characteristic age- or size-related trends are under genetic control, or determined by a plastic response to external environmental factors (Day et al. 2002, Greenwood 1995). Part of the challenge is due to the long life cycle of trees, which makes it very difficult to follow changes in gene expression within individual trees as they occur with tree age (Poethig 1990). In addition, tree size, age and the environment of meristems change in concert, and this can be confounding. However, by grafting scions from different aged trees onto a common-aged rootstock, it is possible to observe the effects of maturation of the apical meristem, without these confounding effects (Greenwood & Hutchison 1993; Day et al. 2002).

Age-related changes in *P. rubens* may be a product of the changing environment, in other words factors that are extrinsic to the shoot apical meristem of a plant (Poethig 1990). For example, tree-tops in older, taller trees are usually exposed to more intense sunlight and wind; also, taller trees have to transport nutrients and water much further

from root to shoot (Day et al. 2002). Carbohydrate and hormone availability can change with increasing tree size (Greenwood 1984), as can the influence of pests and pathogens, and competition dynamics for below-ground resources (Day et al. 2002). It is possible to cause variation in the vigor of a shoot by altering its environment (Greenwood and Hutchison 1993; Poethig 1990). For example, the reduced vigor most often associated with old plants can be reversed either by a change in nutrition, by grafting a shoot from an old tree onto young rootstock (Hackett 1985, Poethig 1990, Greenwood et al. 1989), or by rooting a shoot from an old tree (Hackett 1985). Huang et al. (1992) was able to restore juvenile traits in adult redwood (*Sequoia sempervirens*) by repeatedly grafting onto rooted juvenile cuttings. Some of the juvenile traits shown were increased rooting, more stem elongation and stem branching, the appearance of juvenile leaves, and a reduction in the mortality of explants. The rejuvenated state persisted for more than three years. Huang et al. (1992) also found different types of proteins in the rejuvenated shoots compared to natural shoots from adult trees providing further evidence that extrinsic factors can influence maturation.

Age-related changes are not necessarily a direct result of increased size and complexity (Greenwood 1984), but could be determined by genetic influences. Grafting studies have shown the most convincing evidence for genetic regulation of development with age (Day et al. 2002). Mature characteristics of mature eastern larch (*Larix laricina)* scions grafted onto juvenile rootstock persisted for two growing seasons, suggesting that a genetic change in the plant meristem plays a role in regulating maturation because, contrary to the findings of Huang et al. (1992), nutrients or other signals such as plant growth regulators coming from the rootstock didn't change mature characteristics back to

juvenile characteristics (Greenwood et al. 1989). Similarly, loblolly pine (*Pinus taeda* L) scions from 1, 4, 8, and 12-year old trees grafted onto 2-year old rootstock maintained their age-related growth characteristics for at least 2-3 years (Greenwood 1984), suggesting that the rootstock or extrinsic environment did not have a substantial effect on them. Robinson and Wareing (1969) proposed that maturation takes place after meristems have gone through a certain number of cell divisions, a process that is independent of external environmental conditions. According to Hackett (1985), the periphery of a tree can be ontogenetically older based on characteristics such as decreased rooting potential, while a greater rooting potential (a juvenile characteristic) is preserved at the ontogenetically younger (but chronologically older) basal part of the plant. This suggests that maturation only occurs in the apical meristem, the part of the plant that undergoes cell divisions (Robinson and Wareing 1969).

Quantitative changes in gene expression occur during aging in conifers, and this could also be evidence for intrinsically-regulated change (Greenwood and Hutchison 1993). There is increased expression of sequences for a chlorophyll a/b binding protein (cab) in juvenile in comparison with mature *L. laricina* (Hutchison et al. 1990), and the same difference was found for English ivy (Woo et al. 1994). Changes in gene expression can be caused by DNA methylation. Fraga et al. (2002) found that DNA methylation increases with age in meristematic areas of *Pinus radiata*. However, Greenwood et al. (1989) found no difference in DNA methylation between juvenile and mature scions of *L. laricina*, although the methods did not determine results for separate genes, so some differences could have been hidden. Baurens et al. (2004) also found no difference in DNA methylation between juvenile and reproductively mature material for

Acacia mangium. However, they found that, regardless of the age of the donor plant, microshoots with juvenile leaf morphology had more DNA methylation than microshoots with mature leaf morphology (Baurens et al. 2004).

According to Greenwood and Hutchison (1993), the observed loss of vigor with tree age may be due to size-related developmental changes, maturation, or a combination of both. As mentioned before, Greenwood et al. (1989) found that the revitalization of different aged scions may occur with grafting. However, since some characteristics of old trees can persist after grafting, the increased vigor associated with grafting onto juvenile rootstock does not necessarily indicate a reversal of the maturation process, and perhaps intrinsic factors are at work as well. Gene expression of a plant is both influenced by its environment and will determine how it responds to its environment, and these responses seem to change with increasing plant age and size (Greenwood and Hutchison 1993), thus providing evidence for both extrinsic and intrinsic influences. Changes in gene expression may not be the cause of maturation but rather just an effect (Greenwood 1995).

Changes in production, distribution of, and sensitivity to growth hormones such as gibberellins and auxin as trees age can affect meristem behavior. Gibberellins can promote flowering and also cause rejuvenation in English Ivy (Greenwood 1995, Eysteinsson and Greenwood 1993). In contrast to the rooting response in *P. taeda*, Eysteinsson and Greenwood (1993) found that the flowering response to gibberellins actually increased with ortet (donor tree) age in *L. laricina*. This suggests that the response to these hormones (extrinsic influences) is associated with changes in gene expression within the plant (Greenwood et al. 1989, Greenwood 1995). Diaz-Sala et al.

(1996) observed a reduction in rooting competence by epicotyl cuttings of *P. taeda* in comparison to hypocotyl cuttings. The development of epicotyls (ontogenetically older than hypocotyls) from the embryonic shoot meristem results in a marked reduction in auxin sensitivity and rooting response to auxin relative to hypocotyls (Diaz-Sala et al. 1996). Although adventitious root formation depends on auxin transport, the lack of rooting in epicotyls is not associated with a decline in polar auxin transport (Diaz-Sala et al. 1996) and could therefore be explained by a combination of extrinsic (auxin availability) and intrinsic (ability to respond to auxin) factors.

In this study, scions from juvenile, mid-age and old (mean ages: 3-, 54- and 127-year-old) trees were grafted onto juvenile, mid-aged and old rootstock. Age-related trends in physiology, anatomy and morphology of *P. rubens* needles from these reciprocal graft combinations were examined in order to test four hypotheses initially proposed by Day et al. (2002). The first hypothesis, the "stimulus- response model," proposes that fully developed foliage will exhibit changes in behavior in response to the changing environment. The second hypothesis is based on an 'extrinsic' model in which the plant meristem produces leaf primordia that are controlled by their external environment and exhibit phenotypic plasticity as they expand and differentiate. Thirdly, the 'intrinsic' hypothesis is that permanent genetic changes in meristems take place as a result of a pre-determined genetic aging program, regardless of the environment. The fourth hypothesis predicts that 'intrinsic' changes result from 'extrinsic' factors and determine differences of foliar characteristics with tree age (Day et al. 2002). If the original age-related characteristics of a scion change after grafting to take on characteristics of the rootstock to which it is grafted, this would support the 'extrinsic'

model. Alternatively, if age-related traits of scions are unaltered after grafting onto a different aged rootstock, we might conclude that maturation is regulated by 'intrinsic' factors.

Materials and Methods

Grafting

During April 2002, a reciprocal series of cleft grafts were made between different aged *P. rubens* scions and rootstock. All scions were collected from and grafts were made in the top third of the crown. This was done to minimize topophytic maturation effects related to tree height (Greenwood et al. 1989). All possible scion-rootstock age combinations were produced using juvenile (~3 y), mid-age (~ 54 y), and old (~ 127 y) trees. Trees from the Penobscot Experimental Forest (PEF) were used for the scions and rootstock for mid-age and old trees. Outdoor potted trees were used for juvenile rootstock because there were not enough specimens in the PEF to utilize for grafting, and survival of grafts on juvenile trees in the field could have been poor due to potential animal grazing and competition. For juvenile grafts on mid-age and old trees at the PEF, scions were taken directly from open-grown juveniles (averaged at 12 years old) from the PEF. Juvenile scions grafted onto juvenile rootstock were taken from the same potted rootstock due to limited availability from field-grown trees.

Five mid-age and five old trees were selected from the PEF for grafting. Eighteen grafts, six grafts of each age class, were made per tree, and dispersed over the top third of the canopy. For the pot-grown juveniles, only one scion was grafted onto the leader of

each tree because crown size was limiting. A total of 90 juvenile trees were grafted including 30 trees for each scion age, and an additional 30 trees were left without grafts as a control group.

Gas exchange measurements

Gas exchange measurements were made in mid-late August 2003 on reciprocal grafts using the same technique and equipment as for ungrafted material (Chapter 1). Measurements were taken on completely expanded current year foliage between 8 am and 12 pm. Five of each graft age class combinations were randomly selected for these measurements. Because ambient temperatures were not directly recorded during the measurement period, ambient temperatures were taken from the climate center at the Bangor airport in Bangor, Maine. Temperatures averaged over the sampling period for 3-, 54-, and 127-year-old rootstock were 27°C, 24°C and 23°C respectively. Because Bangor is ~10 km away from the study site, there is an unknown microclimate effect that is not accounted for here.

Morphology and anatomy

Out of the surviving grafts, one of each of the three age combinations per tree was randomly selected from five mid-age trees, five old trees, and fifteen potted juvenile trees. This was done in August 2003 after the scions had flushed a second time since grafting in the spring of 2002, and shoots exhibited fully expanded current year needles.

Shoots were collected, cross-sections were prepared, and anatomical measurements were taken using the same methods described for the non-grafted material (see Chapter One). Internal air space was not measured for grafted material due to the limited availability of needles from the grafts.

Statistical analysis

All statistical analyses were carried out using the SAS System for Windows (version 9.0, SAS Institute, Inc. Cary, NC). An ANOVA was run using the GLM procedure for the effect of scion age and rootstock age on morphology and physiology of *P. rubens* grafts, as well as the interactions between scion age and rootstock age. The means were separated using the Tukey's Studentized Range (HSD) Test and the Levines test was used to assess homogeneity of variances. A least significant difference (protected LSD) test was used for means separations of SLA for 3-year old rootstock. To meet the assumptions of ANOVA, log transformed data was used for width, perimeter, xylem area, and phloem/mesophyll when looking at differences between various scion ages within individual rootstock age classes (Table 2.13, and Appendix B Tables 4 and 5). To meet the assumptions of ANOVA for scion age, tree age, and scion age by tree age interactions, log transformed data was used for width, cross section, perimeter, xylem, vascular bundle, xylem/perimeter, and phloem/perimeter (Table 2.7). In addition, log transformed data was used for stomatal conductance (Table 2.4) and for needle width, photosynthesis, and SLA (Figures 2.1, 2.3, and 2.4). Square root transformed data was used for xylem/perimeter (Figure 2.2).

Results

Photosynthesis

The results for all scion and rootstock ages showed that both rootstock and scion age affected photosynthesis (Table 2.1, Figure 2.1). Photosynthesis per needle area, photosynthesis per needle mass, stomatal conductance, and internal CO_2 concentration varied with rootstock age (Tables 2.1 and 2.3) but only photosynthesis per needle mass varied with scion age (Tables 2.1 and 2.2). Specific leaf area also varied with rootstock age but not with scion age (Table 2.1, Figure 2.2). Moreover, when a characteristic varied both with rootstock and scion age, rootstock age had a larger effect (Tables 2.2 and 2.3). For example net photosynthesis expressed on the basis of needle mass ranged from 33.9 to 59.4 μmol CO_2 kg^{-1} s^{-1}, a 75% change based on rootstock age but only from 40.2 to 47.2, a 17% change based on scion age (Tables 2.2 and 2.3). The age of the rootstock had an effect on the five photosynthesis characteristics (at least the 3-year-old age class is different from the others) when considering 3-year-old and ~54-year-old scions (Tables 2.4 and 2.5), but for ~127-year-old scions the age of the rootstock only had an effect on two variables; internal CO_2 concentration and SLA (Table 2.6). There was no interaction between rootstock and scion age except for net photosynthesis when expressed on the basis of needle mass (Table 2.1).

Table 2.1 - P values for photosynthesis, stomatal conductance, internal CO_2 concentration, and SLA (specific leaf area) from grafted material of different aged trees (3, ~54, ~127). Significance is shown by scion age, rootstock age, and scion × rootstock age interaction. N=5

Measurement	Scion age	Rootstock age	Scion × Rootstock
Net photosynthesis (μmol CO_2 m^{-2} s^{-1})	0.7631	0.0001	0.5019
Net photosynthesis (μmol CO_2 kg^{-1} s^{-1})	0.0364	<.0001	<.0001
Stomatal conductance (mol H_2O m^{-2} s^{-1})	0.2591	<.0001	0.2880
Internal CO_2 concentration (μmol CO_2 mol^{-1})	0.1357	<.0001	0.6006
Specific leaf area (cm^2/g)	0.0065	<.0001	0.0925

Table 2.2 - Mean ± SE for photosynthesis and SLA (specific leaf area) measurements of grafted material based on scion age (3-, ~54- and ~127- years old). For each variable, means not followed by the same letter are significantly different ($\alpha = 0.05$). N=5

Measurement	3 year old	54 year old	127 year old
Net photosynthesis (μmol CO_2 m^{-2} s^{-1})	11.29 ± 0.70 a	11.45 ± 0.40 a	11.80 ± 0.69 a
Net photosynthesis (μmol CO_2 kg^{-1} s^{-1})	47.15 ± 5.25 a	42.05 ± 2.82 ab	40.24 ± 2.34 b
Stomatal conductance (mol H_2O m^{-2} s^{-1})	0.23 ± 0.03 a	0.20 ± 0.02 a	0.19 ± 0.02 a
Internal CO_2 concentration (μmol CO_2 mol^{-1})	295 ± 17.32 a	275 ± 17.88 a	264 ± 13.45 a
Specific leaf area (cm^2/g)	38.89 ± 2.80 a	34.55 ± 1.99 b	34.67 ± 1.81 b

Table 2.3 - Mean ± SE for photosynthesis and SLA (specific leaf area) measurements of grafted material based on rootstock age (3-, ~54- and ~127- years old). For each variable, means not followed by the same letter are significantly different ($\alpha = 0.05$). N=5

Measurement	3 year old	54 year old	127 year old
Net photosynthesis (μmol CO_2 m^{-2} s^{-1})	13.38 ± 0.67 a	11.16 ± 0.27 b	10.01 ± 0.45 b
Net photosynthesis (μmol CO_2 kg^{-1} s^{-1})	59.39 ± 3.94 a	38.74 ± 1.34 b	33.87 ± 2.04 b
Stomatal conductance (mol H_2O m^{-2} s^{-1})	0.30 ± 0.03 a	0.17 ± 0.01 b	0.16 ± 0.01 b
Internal CO_2 concentration (μmol CO_2 mol^{-1})	345 ± 16.78 a	241 ± 4.63 b	248 ± 6.78 b
Specific leaf area (cm^2/g)	46.31 ± 1.76 a	32.58 ± 0.87 b	29.22 ± 0.61 b

Table 2.4 – Mean ± SE, and P values (*=P<0.05) for photosynthesis measurements of scions from 3-year-old trees grafted onto different aged (3-, ~54-, ~127-year-old) rootstock. For each variable, means followed by different letters are significantly different ($\alpha = 0.05$). N=5

Measurement	3 year old	54 year old	127 year old	*P*
Net photosynthesis (μmol CO_2 m^{-2} s^{-1})	14.16 ± 1.1 a	10.51 ± 0.5 b	9.20 ± 0.63 b	0.0020
Net photosynthesis (μmol CO_2 kg^{-1} s^{-1})	73.36 ± 3.4 a	38.31 ± 2.4 b	29.77 ± 2.25 b	<.0001
Stomatal conductance (mol H_2O m^{-2} s^{-1})	0.36 ± 0.06 a	0.17 ± 0.02 b	0.17 ± 0.02 b	0.0018
Internal CO_2 concentration (μmol CO_2 mol^{-1})	369.9 ± 26.8 a	248.1± 11.2 b	265.7 ± 12.1 b	0.0009
Specific leaf area (cm^2/g)	52.4 ± 2.5 a	34.8 ± 1.6 b	29.5 ± 0.9 b	<.0001

Table 2.5 – Mean ± SE, and P values (*=P<0.05) for photosynthesis measurements of scions from 54-year-old trees grafted onto different aged (3-, ~54-, ~127-year-old) rootstock. For each variable, means followed by different letters are significantly different ($\alpha = 0.05$). N=5

Measurement	3 year old	54 year old	127 year old	*P*
Net photosynthesis (μmol CO_2 m^{-2} s^{-1})	12.57 ± 0.8 a	11.59 ± 0.3 ab	10.20 ± 0.49 b	0.0403
Net photosynthesis (μmol CO_2 kg^{-1} s^{-1})	54.38 ± 3.8 a	38.26 ± 1.4 b	33.52 ± 2.81 b	.0006
Stomatal conductance (mol H_2O m^{-2} s^{-1})	0.29 ± 0.05 a	0.16 ± 0.01 b	0.16 ± 0.02 b	.0063
Internal CO_2 concentration (μmol CO_2 mol^{-1})	351.3 ± 31.0 a	228.6 ± 2.8 b	244.5 ± 12.9 b	.0015
Specific leaf area (cm^2/g)	43.3 ± 0.8 a	31.7 ± 1.5 b	28.7 ± 1.3 b	<.0001

Table 2.6 – Mean ± SE, and P values (*=P<0.05) for photosynthesis measurements of scions from 127-year-old trees grafted onto different aged (3-, ~54-, ~127-year-old) rootstock. For each variable, means followed by different letters are significantly different ($\alpha = 0.05$). N=5

Measurement	3 year old	54 year old	127 year old	*P*
Net photosynthesis (μmol CO_2 m^{-2} s^{-1})	13.41 ± 1.6 a	11.37 ± 0.5 a	10.63 ± 1.11 a	.2512
Net photosynthesis (μmol CO_2 kg^{-1} s^{-1})	44.45 ± 4.82 a	39.65 ± 3.2 a	38.30 ± 4.64 a	.6370
Stomatal conductance (mol H_2O m^{-2} s^{-1})	0.24 ± 0.04 a	0.18 ± 0.03 a	0.15 ± 0.02 a	.1185
Internal CO_2 concentration (μmol CO_2 mol^{-1})	313.9 ± 29.6 a	244.9 ± 6.2 b	233.3 ± 6.0 b	.0155
Specific leaf area (cm^2/g)	43.2 ± 3.4 a	31.3 ± 1.1 b	29.5 ± 1.1 b	.0013

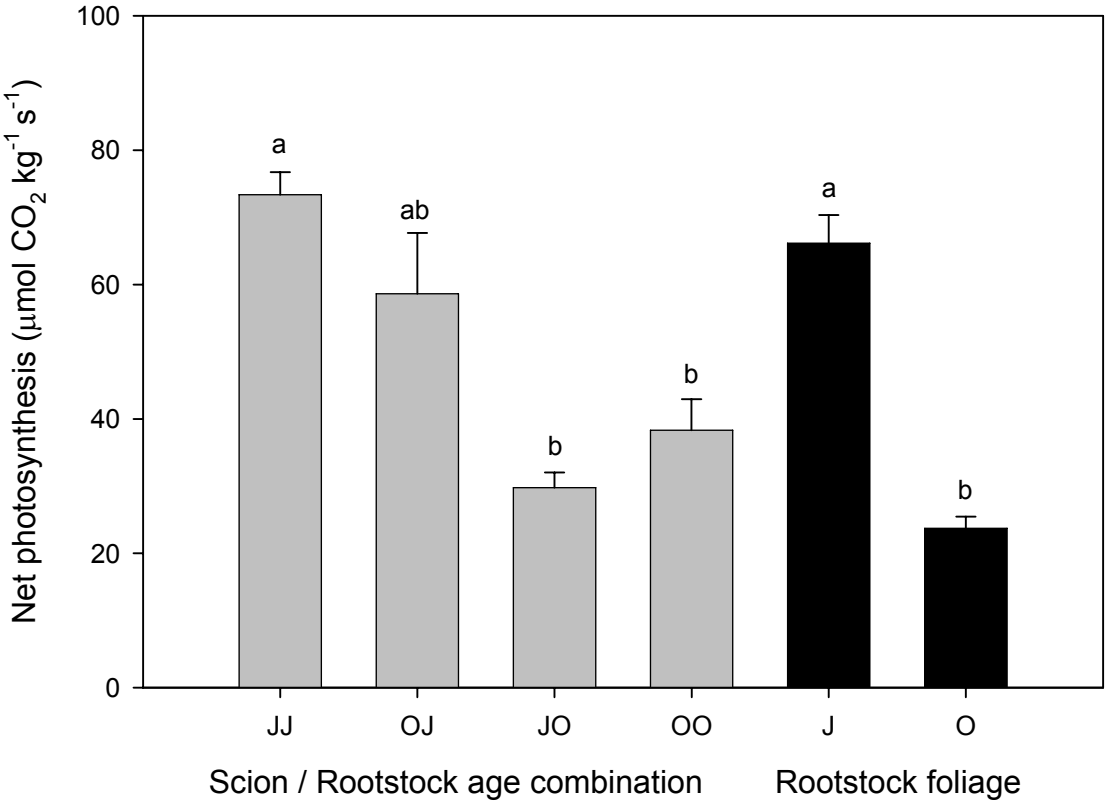

Figure 2.1 - Mean ± SE for net photosynthesis of *P. rubens* needles from different aged graft combinations. Juvenile (J; 3-years-old) and old (O; 127 years old) rootstock/scion age combinations are represented by the grey bars. Solid black bars represent non-grafted material. Bars labeled with different letters are significantly different ($\alpha = 0.05$). N=5 for graft material and N=18 for rootstock material.

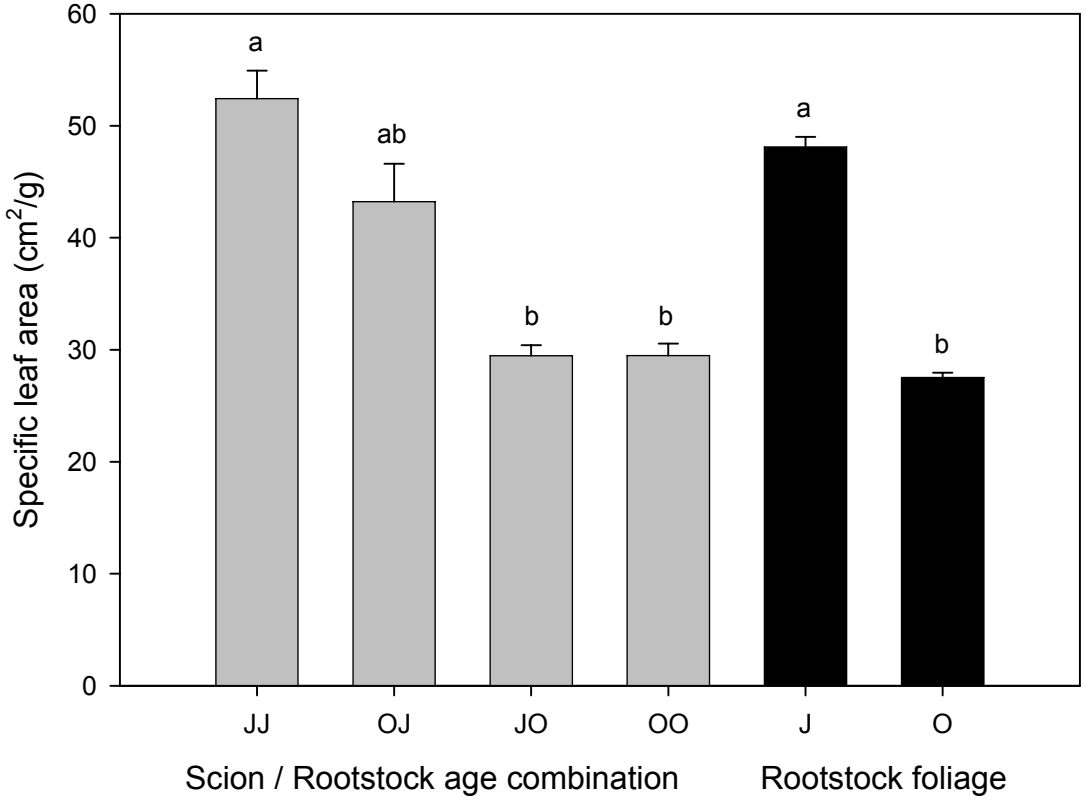

Figure 2.2 - Mean ± SE for specific leaf area of *P. rubens* needles from different aged graft combinations. Juvenile (J; 3-years-old) and old (O; 127 years old) rootstock/scion age combinations are represented by the grey bars. Solid black bars represent non-grafted material. Bars labeled with different letters are significantly different ($\alpha = 0.05$). N=5 for graft material and N=18 for rootstock material.

Morphology and anatomy

Both rootstock and scion age affected the anatomical characteristics measured in this study (Table 2.7). Twelve characteristics varied with rootstock age (Tables 2.7 and 2.9) and nine characteristics varied with scion age (Tables 2.7 and 2.8). For characteristics that varied both by rootstock and scion age, the rootstock age appeared to have a greater effect. For example, needle width changed from 745 to 1267 μm for 3- to ~127-year-old rootstock (a 70% increase, Table 2.9, Figure 2.3), but only from 896 to

1231 μm for 3- to ~127-year-old scions (a 37% increase, Table 2.8, Figure 2.3). Likewise, the ratio of xylem/perimeter (Figure 2.4) changed from 0.70 to 1.03 μm for 3- to ~127-year-old rootstock (a 47% increase) and from .74 to 1.03 for 3- to ~127-year-old scions (a 39% increase).

Table 2.7 - P values for needle cross section measurements from the grafted material. N=5

Measurement	**Scion age**	**Rootstock age**	**Scion × Rootstock age**
Width (μm)	<.0001	<.0001	0.0647
Height (μm)	<.0001	<.0001	0.4742
Cross section (μm^2)	<.0001	<.0001	0.0647
Perimeter (μm)	<.0001	<.0001	0.0850
Phloem (μm^2)	<.0001	<.0001	0.2192
Xylem (μm^2)	<.0001	<.0001	0.0580
Vascular bundle (μm^2)	<.0001	<.0001	0.5405
% Mesophyll	0.1065	0.0024	0.6467
Xylem/mesophyll	0.0720	0.4607	0.3654
Xylem/cross section	0.0739	0.6206	0.3290
Xylem/perimeter	<.0001	<.0001	0.1055
Phloem/mesophyll	0.3852	0.0062	0.1362
Phloem/cross section	0.3691	0.0015	0.1410
Phloem/perimeter	<.0001	0.0125	0.3182

Table 2.8 - Mean ± SE for measurements of needle cross sections of grafted material based on scion age (juvenile, mid-age and old). For each variable, means not followed by the same letter are significantly different ($\alpha = 0.05$).

Measurement	**3 year old**	**60 year old**	**120 year old**
Width (μm)	896.2 ± 71.9 b	1172.53 ± 70.3 a	1230.7 ± 81.4 a
Height (μm)	818.9 ± 45.9 b	975.8 ± 35.0 a	1018.7 ± 34.9 a
Cross section (μm)	485281 ± 53451.6 b	730536 ± 62274.6 a	766464 ± 65185.6 a
Perimeter (μm)	2578.4 ± 165.5 b	3223.7 ± 157.3 a	3383.2 ± 174.0 a
Phloem (μm^2)	1885.4 ± 162.9 b	2973.3 ± 239.8 a	3353.8 ± 262.7 a
Xylem (μm^2)	2005.8 ± 239.4 b	3323.4 ± 332.7 a	3595.4 ± 352.3 a
Vascular bundle (μm^2)	45829.1 ± 5557.0 b	83641.7 ± 8767.5 a	89459.5 ± 9058.6 a
% mesophyll	0.89 ± 0.0039 a	0.88 ± 0.0042 a	0.88 ± 0.0037 a
Xylem/mesophyll	0.0046 ± 0.0002 a	0.0052 ± 0.0002 a	0.0053 ± 0.0003 a
Xylem/cross section	0.0041 ± 0.0001 a	0.0045 ± 0.0002 a	0.0047 ± 0.0002 a
Xylem/perimeter	0.74 ± 0.05 b	1.00 ± 0.06 a	1.03 ± 0.07 a
Phloem/mesophyll	0.0047 ± 0.0003 a	0.0047 ± 0.0002 a	0.0051 ± 0.0002 a
Phloem/cross section	0.0042 ± 0.0003 a	0.0042 ± 0.0001 a	0.0045 ± 0.0002 a
Phloem/perimeter	0.72 ± 0.03 b	0.91 ± 0.04 a	0.97 ± 0.05 a

Table 2.9 - Mean ± SE for measurements of needle cross sections of grafted material based on rootstock age (juvenile, mid-age and old). For each variable, means not followed by the same letter are significantly different ($\alpha = 0.05$). N=5

Measurement	**3 year old**	**60 year old**	**120 year old**
Width (μm)	744.9 ± 48.9 b	1287.6 ± 57.69 a	1267.0 ± 45.5 a
Height (μm)	776.5 ± 38.5 b	1034.8 ± 26.2 a	1002.08 ± 33.9 a
Cross section (μm)	382835.0 ± 36997.4 b	810839.9 ± 51363.4 a	788605.8 ± 45045.2 a
Perimeter (μm)	2270.8 ± 119.5 b	3492.2 ± 122.3 a	3422.4 ± 109.1 a
Phloem (μm^2)	1822.4 ± 174.4 b	3307.1 ± 249.2 a	3082.9 ± 228.0 a
Xylem (μm^2)	1665.0 ± 178.9 b	3671.3 ± 292.1 a	3588.3 ± 306.5 a
Vascular bundle (μm^2)	38671.38 ± 4452.8 b	91838.9 ± 7963.4 a	88420.1 ± 7760.5 a
% mesophyll	0.89 ± 0.0033 a	0.87 ± 0.0036 b	0.87 ± 0.0038 b
Xylem/mesophyll	0.0048 ± 0.0002 a	0.0051 ± 0.0002 a	0.0052 ± 0.0003 a
Xylem/cross section	0.0043 ± 0.0002 a	0.0045 ± 0.0001 a	0.0045 ± 0.0003 a
Xylem/perimeter	0.70 ± 0.05 b	1.03 ± 0.05 a	1.03 ± 0.07 a
Phloem/mesophyll	0.0054 ± 0.0002 a	0.0046 ± 0.0001 b	0.0045 ± 0.0003 b
Phloem/cross section	0.0048 ± 0.0002 a	0.0041 ± 0.0001 b	0.0039 ± 0.0002 b
Phloem/perimeter	0.78 ± 0.04 b	0.93 ± 0.04 a	0.89 ± 0.05 ab

All anatomical characteristics of 3-year-old and ~54-year-old scions varied with rootstock age except for mesophyll (%), xylem/mesophyll, xylem/cross section, and phloem/perimeter (Table 2.10 and 2.11). The differences were found only for the 3-year-old rootstock in comparison to the other age classes. The ~127-year-old scions on different aged rootstock, however, exhibited differences for all characteristics except for xylem/mesophyll, xylem/cross-section, xylem/perimeter, phloem/mesophyll, phloem/cross-section, and phloem/perimeter (Table 2.12).

Considering different aged grafts on 3-year old rootstock only, for comparison with many previous experiments (Greenwood et al. 1989, Greenwood 1984, Day et al. 2001), we can see that width, height, cross section, perimeter, xylem, xylem/perimeter and vascular bundle all increased with increasing scion age (Table 2.13). For all of these variables, the 3-year old age class was grouped separately from the older age classes. This trend was also found for phloem even though it was not statistically significant.

Table 2.10 - Mean ± SE, and P values (*=P<0.05) for measurements of needle cross sections of 3-year-old grafts on 3-, ~54-, and ~127-year-old rootstock. For each variable, means not followed by the same letter are significantly different ($\alpha = 0.05$). N=5

Measurement	**3 year old**	**54 year old**	**127 year old**	***P***
Width (μm)	529 ± 12 b	1068 ± 54 a	1092 ± 24 a	<.0001
Height (μm)	608 ± 21 b	934 ± 23 a	915 ± 66 a	0.0002
Cross section (μm^2)	219770 ± 6393 b	604012 ± 40409 a	632060 ± 42322 a	<.0001
Perimeter (μm)	1728 ± 34 b	3001 ± 95 a	3006 ± 78 a	<.0001
Phloem (μm^2)	1127 ± 66 b	2299 ± 117 a	2230 ± 211 a	<.0001
Xylem (μm^2)	856 ± 52 b	2472 ± 184 a	2690 ± 252 a	<.0001
Vascular bundle (μm^2)	19863 ± 1785 b	59966 ± 6874 a	57658 ± 4506 a	<.0001
Mesophyll (%)	89 ± 0.7 a	88 ± 0.6 a	89 ± 0.7 a	0.4840
Xylem/mesophyll	0.0044 ± 0.0003 a	0.0047 ± 0.0002 a	0.0049 ± 0.0004 a	0.5301
Xylem/cross section	0.0039 ± 0.0002 a	0.0041 ± 0.0002 a	0.0043 ± 0.0003 a	0.6136
Xylem/perimeter	0.49 ± 0.03 b	0.82 ± 0.05 a	0.89 ± 0.08 a	0.0005
Phloem/mesophyll	0.0058 ± 0.0005 a	0.0044 ± 0.0002 ab	0.0040 ± 0.0004 b	0.0204
Phloem/cross section	0.0052 ± 0.0004 a	0.0038 ± 0.0002 b	0.0036 ± 0.0003 b	0.0125
Phloem/perimeter	0.66 ± 0.05 a	0.76 ± 0.03 a	0.74 ± 0.07 a	0.3521

Table 2.11 - Mean ± SE, and P values (*=P<0.05) for measurements of needle cross sections of ~54-year-old grafts on 3-, ~54, and ~127-year-old rootstock. For each variable, means not followed by the same letter are significantly different ($\alpha = 0.05$). N=5

Measurement	**3 year old**	**54 year old**	**127 year old**	***P***
Width (μm)	860 ± 31 b	1380 ± 93 a	1278 ± 66 a	0.0004
Height (μm)	842 ± 23 b	1048 ± 37 a	1038 ± 65 a	0.0115
Cross section (μm^2)	463723 ± 22616 b	894587 ± 73538 a	833297 ± 87382 a	0.0014
Perimeter (μm)	2523 ± 62 b	3663 ± 182 a	3485 ± 187 a	0.0004
Phloem (μm^2)	2152 ± 172 b	3763 ± 454 a	3004 ± 209 ab	0.0097
Xylem (μm^2)	2068 ±155 b	4394 ± 439 a	3509 ± 506 ab	0.0045
Vascular bundle (μm^2)	47433 ± 2057 b	104959 ± 9834 a	98533 ± 14470 a	0.0032
Mesophyll (%)	89 ± 0.5 a	87 ± 0.7 a	87 ± 0.7 a	0.0497
Xylem/mesophyll	0.0050 ± 0.0003 a	0.0056 ± 0.0003 a	0.0048 ± 0.0004 a	0.2977
Xylem/cross section	0.0044 ± 0.0003 a	0.0049 ± 0.0003 a	0.0042 ± 0.0004 a	0.2739
Xylem/perimeter	0.82 ± 0.05 b	1.19 ± 0.08 a	0.99 ± 0.10 ab	0.02
Phloem/mesophyll	0.0052 ± 0.0002 a	0.0048 ± 0.0002 ab	0.0042 ± 0.0002 b	0.0258
Phloem/cross section	0.0046 ± 0.0002 a	0.0042 ± 0.0002 ab	0.0037 ± 0.0002 b	0.0178
Phloem/perimeter	0.85 ± 0.05 a	1.01 ± 0.08 a	0.86 ± 0.02 a	0.1007

Table 2.12 - Mean ± SE, and P values (*=P<0.05) for measurements of needle cross sections of ~127-year-old grafts on 3-, ~54-, and ~127-year-old rootstock. For each variable, means not followed by the same letter are significantly different ($\alpha = 0.05$). N=5

Measurement	**3 year old**	**54 year old**	**127 year old**	***P***
Width (μm)	846 ± 81 b	1414 ± 73 a	1432 ± 48 a	<.0001
Height (μm)	880 ± 61 b	1122 ± 29 a	1054 ± 22 a	0.0037
Cross section (μm^2)	465011 ± 62037 b	933922 ± 66523 a	900459 ± 46094 a	0.0002
Perimeter (μm)	2562 ± 185 b	3812 ± 147 a	3776 ± 92 a	<.0001
Phloem (μm^2)	2188 ± 323 b	3859 ± 225 a	4015 ± 239 a	0.0006
Xylem (μm^2)	2072 ± 253 b	4148 ± 349 a	4566 ± 475 a	0.0010
Vascular bundle (μm^2)	48718 ± 8241 b	110592 ± 11704 a	109069 ± 5827 a	0.0005
Mesophyll (%)	89 ± 0.6 a	87 ± 0.6 ab	87 ± 0.2 b	0.0286
Xylem/mesophyll	0.0051 ± 0.0004 a	0.0051 ± 0.0001 a	0.0059 ± 0.0007 a	0.4145
Xylem/cross section	0.0045 ± 0.0004 a	0.0044 ± 0.0001 a	0.0051 ± 0.0006 a	0.4426
Xylem/perimeter	0.80 ± 0.07 a	1.08 ± 0.05 ab	1.21 ± 0.13 a	0.0170
Phloem/mesophyll	0.0052 ± 0.0004 a	0.0048 ± 0.0002 a	0.0052 ± 0.0005 a	0.6301
Phloem/cross section	0.0047 ± 0.0004 a	0.0042 ± 0.0001 a	0.0046 ± 0.0004 a	0.5535
Phloem/perimeter	0.8320 ± 0.09 a	1.01 ± 0.03 a	1.07 ± 0.08 a	0.0884

Table 2.13 - Mean ± SE, and P values (*=P<0.05) for measurements of needle cross sections of grafted scions (3, ~54, and ~127-year-old) on juvenile (3-year-old) rootstock. For each variable, means not followed by the same letter are significantly different (α = 0.05). N=5

Measurement	**3 year old**	**54 year old**	**127 year old**	***P***
Width (μm)	529 ± 12 b	860 ± 31 a	846 ± 81 a	0.0004
Height (μm)	608 ± 21 b	842 ± 23 a	880 ± 61 a	0.0007
Cross section (μm^2)	219770 ± 6393 b	463723 ± 22616 a	465011 ± 62037 a	0.0008
Perimeter (μm)	1728 ± 34 b	2523 ± 62 a	2562 ± 185 a	0.0001
Phloem (μm^2)	1127 ± 66 b	2152 ± 172 a	2188 ± 323 a	0.0650
Xylem (μm^2)	856 ± 52 b	2068 ±155 a	2072 ± 253 a	<.0001
Vascular bundle (μm^2)	19863 ± 1785 b	47433 ± 2057 a	48718 ± 8241 a	0.0022
Mesophyll (%)	89 ± 0.7 a	89 ± 0.5 a	89 ± 0.6 a	0.9656
Xylem/mesophyll	0.0044 ± 0.0003 a	0.0050 ± 0.0003 a	0.0051 ± 0.0004 a	0.2836
Xylem/cross section	0.0039 ± 0.0002 a	0.0044 ± 0.0003 a	0.0045 ± 0.0004 a	0.2958
Xylem/perimeter	0.49 ± 0.03 b	0.82 ± 0.05 a	0.80 ± 0.07 a	0.0009
Phloem/mesophyll	0.0058 ± 0.0005 a	0.0052 ± 0.0002 a	0.0052 ± 0.0004 a	0.5786
Phloem/cross section	0.0052 ± 0.0004 a	0.0046 ± 0.0002 a	0.0047 ± 0.0004 a	0.4430
Phloem/perimeter	0.66 ± 0.05 a	0.85 ± 0.05 a	0.8320 ± 0.09 a	0.1106

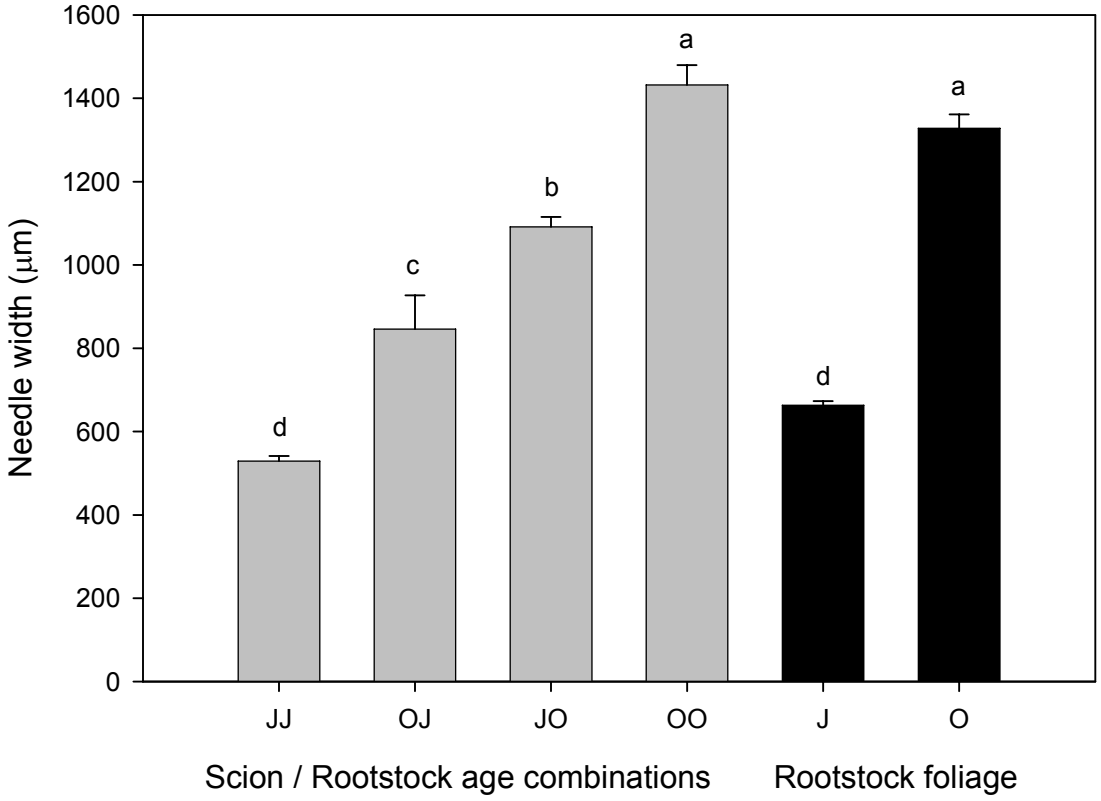

Figure 2.3 - Mean ± SE for width of *P. rubens* needles from different aged graft combinations. Juvenile (J; 3-years-old) and old (O; 127 years old) rootstock/scion age combinations are represented by the grey bars. Solid black bars represent non-grafted material. Bars labeled with different letters are significantly different ($\alpha = 0.05$). N=5 for graft material and N=18 for rootstock material.

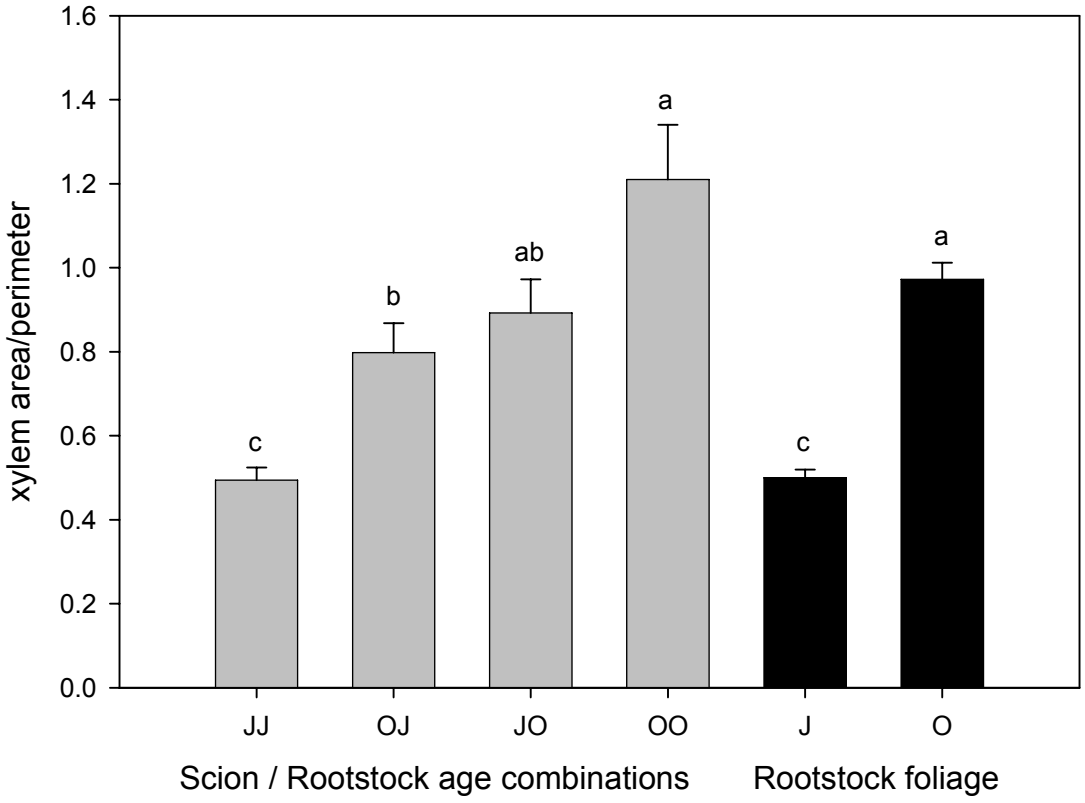

Figure 2.4 - Mean ± SE for the ratio of xylem area/perimeter of *P. rubens* needles from different aged graft combinations. Juvenile (J; 3-years-old) and old (O; 127 years old) rootstock/scion age combinations are represented by the grey bars. Solid black bars represent non-grafted material. Bars labeled with different letters are significantly different ($\alpha = 0.05$). N=5 for graft material and N=18 for rootstock material.

Discussion

Based on the previous chapter, the age-related characteristics that showed the strongest trends and are likely to apply to the grafted material are an increase in needle width, cross-sectional area, perimeter, xylem area, vascular bundle area, an increase in the ratio of xylem/perimeter, and a decrease in specific leaf area with increasing tree age. If the extrinsic hypothesis is correct, these changes will occur on common-aged scions on different aged rootstocks. In contrast, if changes occur between different aged scions on

a common aged rootstock, this would suggest intrinsic effects. If changes were to occur both across different aged scions and across different aged rootstock, this would imply a combined effect of the intrinsic and extrinsic factors.

There is also the possibility that the coupling of trends reflects how variation in one age-related characteristic may influence another. For example, the decline in net photosynthesis between 3- and ~127-year-old scions, and with 3- and ~127-year-old rootstock occurs in parallel with an increase in needle cross-sectional area (Tables 2.2, 2.3, 2.8, 2.9). As discussed in the previous chapter, an increase in needle cross-sectional area or needle thickness can have the potential to alter photosynthetic rates (assuming that leaf density is constant). If the increased cross-sectional area occurs due to a genetic influence, for example, and photosynthesis is determined by the resulting increase in needle thickness (which could be classified as an extrinsic factor), photosynthesis will ultimately be determined by a combination of intrinsic and extrinsic factors. Although it is not discussed in detail, changes in anatomy or physiology may occur due to complex interactions between intrinsic and extrinsic factors, and this pattern could occur for any characteristics mentioned in this study.

Trends found for characteristics of *P. rubens* needle morphology across different aged scions on juvenile rootstock are comparable to results of other similar grafting studies. Different aged *P. rubens* scions grafted onto 3-year-old rootstock exhibited an increase in needle width, height, cross-sectional area, perimeter, xylem area, vascular bundle area, and the ratio of xylem area/perimeter with increasing age, all of which were consistent with trends found for ungrafted material in Chapter 1. Similar trends in grafted material were found in a study by Rebbeck et al. (1993) where needle surface area

was greater and needles were wider (40%) and thicker (14%) in mature *P. rubens* scions compared to juvenile scions, both on juvenile rootstock. Day et al. (2001) also found greater needle width in scions from old trees compared to those from young trees after three growing seasons grafted onto juvenile rootstock. In this study, SLA declined significantly with increasing age of *P. rubens* scions grafted onto juvenile rootstock (Appendix B Table 1), contrary to the findings of Day et al. (2001). For *P. rubens*, Day et al. (2001) observed a decline in photosynthesis and stomatal conductance with increasing age of scions grafted onto a common aged juvenile rootstock. Similarly, Rebbeck et al. (1993) found that photosynthesis per leaf area was 25% greater and photosynthesis on a mass basis was 40% greater for juvenile scions in comparison to old-growth scions both grafted onto juvenile rootstock. A decrease (39 %) in photosynthesis on a mass basis with increasing scion age on juvenile rootstock was also found in our study. From these common rootstock studies we can infer that an 'intrinsic' genetic component is at work determining these age-related characteristics since traits associated with different aged scions were persistent even when exposed to a common environment and same sized rootstock.

Based on the trends shown for the photosynthetic and anatomical data, changes appear to occur with both increasing rootstock age and scion age, indicating growth is affected by both extrinsic and intrinsic influences. However, variation in anatomy and physiology was associated more frequently with changes in rootstock age compared to scion age, indicating a greater effect due to extrinsic factors, at least for the characteristics investigated in this study. Physiological data and specific leaf area show strong evidence for extrinsic effects, given that differences occurring across different

aged rootstock were statistically significant for specific leaf area, stomatal conductance, internal CO_2 concentration, and photosynthesis on an area and mass basis (Table 2.1 and 2.3). In contrast, changes occurring between different aged scions regardless of rootstock age were not statistically significant for photosynthesis on an area basis, stomatal conductance, or internal CO_2 concentration, indicating that intrinsic effects were not as influential as extrinsic effects for these characteristics (Table 2.1 and 2.2). In addition effects due to extrinsic factors were also predominant in the anatomical characteristics, where all but two (xylem/mesophyll and xylem/cross-sectional area) showed statistical significance with increasing rootstock age, while all but five (% mesophyll, xylem/mesophyll, xylem/cross-section, phloem/mesophyll and phloem/cross-sectional area) exhibited statistical significance with increasing scion age (Table 2.7). Taken together, many age-related changes occurring in needle anatomy and physiology across different scion and rootstock age combinations indicate evidence for intrinsic and extrinsic control, but extrinsic factors appear to have the most influence on the characteristics investigated in this experiment.

A potential source of variation in this experiment is that the ambient CO_2 was higher for the potted juveniles (a difference of 75 μmol CO_2 mol^{-1}), and this could have contributed to the higher internal CO_2 concentration in the 3-year-old age class (345 μmol CO_2 mol^{-1} as opposed to 241 and 248 μmol CO_2 mol^{-1} in the ~54- and ~127-year-old trees) (Table 2.3). Although needle anatomy probably has an effect on photosynthesis (Chapter One), elevated CO_2 concentrations could also contribute to differences in CO_2 assimilation. For example, two trembling aspen (*Populus tremuloides* Michx.) clones exposed to elevated CO_2 concentrations during the time from budbreak to leafdrop (560

p.p.m. compared to control trees which were under 360 p.p.m. during the day and 360-500 p.p.m. at night) showed increases in CO_2 assimilation of 33% and 38% (Noormets et al. 2001). The CO_2 for the 3-year-old trees could also have affected other characteristics in addition to the rate of photosynthesis. An elevated atmospheric CO_2 concentration of 700 compared to 350 $\mu l\ l^{-1}$ caused SLA to decrease in wheat (*Triticum aestivum*) and maize (*Zea mays*) (Rozema 1993). In the *P. rubens* grafting experiment, the ambient CO_2 concentation was recorded concurrently with photosynthesis measurements, so the day-length variation or seasonal changes in CO_2 concentration are unknown.

Greenwood (1984) found that differences in growth habit including increased male and female strobilus production, decreased branch and needle dry weight per graft, decreased branch length per graft and a decrease in the number of branches per graft were the most pronounced when comparing scions from 1- and 4-year old *P. taeda*. Differences were much smaller between scions from 4-, 8-, and 12- year old trees (Greenwood 1984). Moreover, the rate of most rapid change occurred between age 1 and 5 in *L. laricina* (Greenwood et al. 1989) between 1 and 4 years of age in Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) (Ritchie and Keeley 1994), and across age classes <40-years old in *P. rubens* (Day et al. 2001). Similarly, in our study, the greatest differences in needle cross-sectional width, height, cross-sectional area, perimeter, phloem area, xylem area, and vascular bundle area were found between ages 3 and ~54. This pattern is shown across scion age and across rootstock age (Tables 2.8 and 2.9). Therefore, for most of the anatomical characteristics investigated, a similar change between different aged trees is occuring due to extrinsic factors (indicated by rootstock age) as it is occurring due to intrinsic factors (indicated by scion age). This suggests that

both extrinsic and intrinsic age-related changes follow similar patterns with respect to life-stages.

Several sources of variation could confuse the results. These include the genetic variation that occurs between individual trees as well as differences in vigor between scions and rootstock of different aged trees. Although a difference in grafting success could occur between different aged trees, Greenwood et al. (1989) found that grafting success was not different for scions from different aged *L. laricina*. Hydraulic conductivity of the graft unions was not measured between different aged grafts in this experiment, but Day et al. (2001) showed that there was no difference in specific hydraulic conductivity between graft unions for different aged scions on juvenile rootstock in *P. rubens*. Additional evidence for a lack of grafting effects is based on a comparison of autografts (scions taken directly from a tree and grafted back onto the same tree) with the natural ungrafted foliage discussed in Chapter One. The 3-year-old autografts compared to ungrafted 3-year-old foliage showed the same SLA, photosynthesis, ratio of xylem area/perimeter, and needle width. A comparison of ~127-year-old autografts and ungrafted foliage revealed a similar pattern (Figures 2.1, 2.2, 2.3, 2.4).

Sometimes the caliper of scions was unavoidably different between different aged trees. In a few instances, if a juvenile scion had a smaller caliper than most of the best grafting shoots on an old rootstock tree, these scions were grafted onto a smaller, less vigorous shoot on the old tree to ensure that the graft would knit properly. It is likely that a small shoot would be less vigorous and be a weaker sink for nutrients and water than larger shoots which often supported scions from older trees due to the more compatible

size between shoots and scions. In addition, although tracheid diameter was not measured for the grafted material, it is likely that juvenile scions have a smaller tracheid diameter based on the results from Chapter One. This could cause juvenile scions to be maladaptive on old rootstock and vice versa. Also, all measurements were taken after the grafts had been alive for two growing seasons (i.e. the buds had flushed twice, the first flush resulting from buds that had been preformed before grafting). Although the sampled foliage had been produced from buds developed after grafting, it seems likely that scions are still adjusting and changing according to their new environment and only time will reveal more concrete information regarding these processes.

Although grafts are still fairly young, we can plainly see that many morphological, anatomical and physiological characteristics changed in association with scion age, as well as rootstock age. This study, in combination with findings from similar previous work for different species, has provided even stronger evidence that the growth habits of apical meristems are affected by both an intrinsic component, and extrinsic environmental characteristics associated with tree size, location, or physiology. Furthermore, the present state of the grafts indicates that extrinsic factors may have a greater influence on needle anatomy and physiology than do intrinsic factors. The potential coupling of trends shown for anatomical or physiological age-related characteristics may also be at work in determining the relationship between intrinsic and extrinsic influences and should also be considered for these different aged graft combinations.

Chapter Three: The effect of abscisic acid on transpiration rates of red spruce (*Picea rubens*)

Introduction

The physiological age-related changes discussed in the previous chapters may occur partly because of changes in stomatal conductance with tree age. Stomata play an important role in plant growth and physiology, for they control the influx of CO_2 needed for photosynthetic carbon fixation, as well as the rate of transpirational water loss from the plant to the atmosphere (Schroeder et al. 2001 and Assmann and Shimazaki 1999). It is important for plants to be able to conserve water to prevent dessication, while also taking in enough CO_2 necessary for photosynthesis (Wilmer and Fricker, 1996). A younger, smaller tree with a less developed root system may be more susceptible to drought than a larger, more established tree. Therefore, a rapid response to dry soil, demonstrated by a decline in stomatal conductance and transpiration, could be necessary for a small tree to conserve enough water for survival.

Several hormones including cytokinins, auxins, gibberellins, ethylene, and abscisic acid can all affect stomatal aperture. However, most of the research on stomatal regulation by hormones has been dedicated specifically to the mechanisms involving abscisic acid (ABA) (Willmer and Fricker, 1996). There is strong evidence that ABA reduces transpiration rates in water-stressed trees (Wilkinson 1999). An exogenous supply of ABA decreased stomatal conductance and the rate of stomatal opening in leaves of deciduous trees (Aasamaa et al. 2002). In addition, the concentration of ABA in leaves and xylem declined with increasing leaf water potential in several species (Perks et al. 2002, Aasamaa et al. 2002). In response to dry soil, ABA is synthesized

and/or deposited in roots (S. Wilkinson 1999; Sauter et al. 2001) and translocated to leaves in the transpiration stream where it accumulates in the vicinity of stomata (S. Wilkinson, 1999). ABA can also be synthesized in leaves and transported to roots to be deposited or transported back to xylem vessels (Sauter et al. 2001).

Xylem sap pH and apoplastic pH are fairly acidic in well-watered plants. As a result, ABA arriving in a leaf is taken up by the symplast, away from the action of the guard cells (Sauter et al. 2001). Alternatively, an increase in pH in the apoplast of the leaf will occur as a result of water stress, and this will cause ABA to collect in the apoplast where it is concentrated around or inside guard cells. As a result, K^+, Cl^- and organic solutes are released from the guard cells into the external space, causing water release and stomatal closure. If an older tree is not conducting water as well as a young tree, it might exhibit a difference in its xylem pH. A lower xylem pH can ultimately lead to a reduction in stomatal conductance, and this may cause a reduction in photosynthesis. Wilkinson and Davies (1997) suggest from their findings that older plants may have a less sensitive response to pH signals in their xylem in comparison to younger plants.

A preliminary trial (unpublished data, Michael E. Day, Michael S. Greenwood) exposed excised shoots from juvenile and old red spruce (*Picea rubens*) trees to a 10^{-5} M ABA solution over 24 hours under constant light and temperature. Juvenile trees showed what was perceived as a greater response to the ABA as demonstrated by a greater decrease in transpiration in comparison to old trees. The purpose of the following study was to develop a procedure for replicating this experiment using a larger sample size, the ultimate goal being to compare the response of different aged trees to ABA to get a better understanding of the decline in photosynthesis and growth efficiency that occurs with

increasing tree age. Time did not allow for a comparison of different aged trees. However, a series of preliminary trials were run using various ABA treatments observed over time to gauge the transpiration of excised *P. rubens* shoots in relation to exogenously applied ± ABA. In addition, excised *P. rubens* shoots were exposed to control and ABA treatments adjusted to various pH levels to see if there was an effect of either or both of these factors on transpiration. I hypothesized that transpiration levels would decline in response to ABA, and that by increasing the concentrations of ABA (a dose response trial) an even greater decline in transpiration would result. Moreover, I proposed that the combined effect of an increased pH and the presence of exogenously supplied ABA would cause an even more pronounced decline in transpiration rates of excised *P. rubens* shoots.

Materials and Methods

Basic techniques used for all trials

A 10^{-2} M ABA stock solution was prepared by dissolving +/- ABA in 100% ethanol. The solution was vortexed to mix and transferred to a glass bottle and stored in the refrigerator. Shoots approximately 10 cm long were collected from trees and placed in beakers of water in a refrigerator for temporary storage (foliage kept out of water). All but the apical 2-3 cm of foliage was stripped off the shoots using a razor blade. The base of the shoots were cut under solution (either ABA or control) and placed in vials or beakers. For trials 1 and 2, each individual shoot was placed in a small vial of solution so that the foliage was outside of the vial and the stem was in the solution. Parafilm was stretched around the stem of the shoot and over the top of the viles to ensure that the only

possible water loss from the vials was due to transpiration from the shoot, not evaporation from the vials. For the subsequent trials, multiple shoots were placed in beakers of solution without parafilm, so all water loss was produced from a combination of evaporation and transpiration. Samples were kept under constant light and temperature in a growth chamber (product of Percival Scientific, Inc. (Boone, Iowa), Model E-54B). For all trials, water loss was measured by recording weight loss of the vials/beakers over time. For trials 1 and 2 the total projected area of these needles was found using a high-resolution scanner with WinSeedle^{TM} software. In the remaining trials, needles were dried in a 60º C oven for two days and weighed so that water loss could be calculated on a needle mass basis.

Trial 1: Time course of response to 10^{-4} ABA treatment using vials

The objective of this trial was to determine whether excised shoots from juvenile red spruce trees showed a response to an externally supplied solution of ABA. Shoots were collected from five trees, two shoots per tree. One shoot was used in the ABA treatment and the other in the control. This provided genetic consistency between the control and ABA treatments. In order to use a minimal amount of ABA solution, shoots were cut under water, and quickly placed into the vials of ABA solution and water. The purpose here was to measure the effect of ABA on red spruce shoots, while preventing embolisms by quickly transferring the shoots to the vials. A 10^{-4} M ABA solution was used, and the control consisted of a weak ethanol/distilled water solution. The control contained ethanol (the same amount as in the ABA solution) because it was important to keep everything consistent between the two treatments with the exception of ABA content. Vials were placed in the growth chamber under constant light and temperature

and the transpiration was measured for each shoot every ten minutes for four hours. Next, all needles were removed from shoots and the total projected needle areas were measured. Transpiration was calculated as the total ml of water lost per area of needles per hour for each sample.

As a repeat of this experiment, but to ensure that embolisms were avoided, two samples were taken from each of five trees growing in the shade house. One sample was used in the control solution, and one was placed in 10^{-4} M ABA solution. The samples were cut under solution and never transferred from this solution (unlike the first experiment) and then placed in the growth chamber. The weight of the vials was measured every hour for five hours. Again, the total projected needle area was measured, as well as the total ml of water lost per area of needles per hour.

Trial 2: Response of *Picea rubens* and *Abies balsamea* to 10^{-4} ABA

It is possible that the response to ABA may vary significantly between different species. The object of this trial was to test whether *P. rubens* showed a greater response to ABA when compared to balsam fir (*Abies balsamea*). Shoots were collected from the Penobscot Experimental Forest (PEF) in Penobscot County, Maine for both juvenile *P. rubens* and *A. balsamea*. Five *P. rubens* and five A. *balsamea* trees were sampled, two shoots taken from each tree for the control and 10^{-4} M ABA treatments. The experiment was conducted in the growth chamber with measurements taken every hour for five hours. The total projected needle area was measured and the total ml of water lost per area of needles per hour was calculated.

Trial 3: Time course of response to 10^{-4} ABA treatment using beakers

Similar to Trial 1, the following procedure was run in order to test whether excised shoots from juvenile *P. rubens* trees showed a response with time to ABA. The difference here is that groups of shoots were measured as opposed to individual shoots. Eight beakers were used, four containing 25 mL of control solution, and four beakers containing 25 mL of the 10^{-4} M ABA solution. Shoots from five one-year old trees were used. Each beaker contained five shoots, one from each of the five trees. Shoots were cut under the solution in the beakers, and beakers were placed in the growth chamber to allow for constant light and temperature conditions. Water loss was measured every half hour for four hours by weighing the beakers. Parafilm was not used in this experiment, therefore, water loss due to evaporation was permitted. It is important to note that water loss refers to the transpiration from the shoots as well as evaporation from the solution in the beaker. It is assumed that water loss due to evaporation is consistent between treatments. Calculations do not account for the two different components of "water loss" in this case, but the differences in transpiration are likely to account for most differences in water loss between various treatments. After all measurements were completed, shoots were placed in an oven for two days at 60º C and dry weights of needles per beaker were taken in order to calculate water loss on a mass basis (g/h×g needles).

Trial 4: Initial response to 10^{-4} M ABA

The purpose of this trial was to investigate how long it would take for *P. rubens* shoots to start showing a response to ABA. This was done by tracking the transpiration of the shoots immediately after the shoots were exposed to ABA. Shoots were first placed in water and measurements were recorded for two hours before ABA was added. Eight beakers were used in this experiment, four designated for ABA and four for a control. Each beaker contained five shoots from three-year-old trees, each beaker representing the same five individuals. Each beaker contained 25 mL of glass distilled water to begin with. At the two hour mark, 0.25 mL of 10^{-2} M ABA was added to four beakers to make a 10^{-4} M ABA solution (as close as possible). Ethanol (25 mL) was added to the other four beakers (Controls 1-4) without ABA. Some of the original 25 mL of water had probably evaporated/transpired prior to this point, which may have slightly thrown off the actual desired concentrations once ABA and ethanol were added after two hours, but probably not enough to have a negative effect on the results. Weight measurements were taken every half-hour for seven hours. Shoots were placed in envelopes and kept in a 60º C oven for two days. Dry weights of needles were also measured and water loss (g/h×g needles) was calculated.

Trial 5: Response to various doses of ABA

The following procedure was designed to find any differences in response of shoots to various concentrations of ABA. A control treatment and four concentrations of ABA were used (10^{-4} M, 5×10^{-5} M, 10^{-5} M, and 10^{-6} M). Four replications of each

treatment were used and were arranged into four blocks in the growth chamber. Each block consisted of one of each of the treatments in five beakers, each containing five shoots. The shoots were taken from the 1-2 year old seedlings. There was not enough material for each beaker to represent the same five individual trees. Therefore, ten trees were selected and ten shoots were collected from each tree. Shoots were randomly selected (grab bag technique) in order to chose five shoots for each beaker. Each 50 mL beaker was filled with 25 mL water or ABA solution. In this case the control was not supplemented with a small amount of ethanol as in other trials. Shoots were cut under the solution in the beakers to prevent embolisms. Once transpiration measurements were taken every half-hour for four hours, shoots were placed in small envelopes and placed in a 60º C oven for two days. Dry weights of needles were measured and water loss (g/h×g needles) was calculated.

Trial 6: Effect of pH on ABA response

The following procedure tested whether or not a change in the pH of an abscisic acid solution had any effect on the transpiration of red spruce shoots. Six treatments were administered:

1.) pH 6 + 5×10^{-5} M ABA
2.) pH 6 control
3.) pH 7 + 5×10^{-5} M ABA
4.) pH 7 control
5.) pH 8 + 5×10^{-5} M ABA
6.) pH 8 control

Two liters of artificial sap (AS) described by Wilkinson and Davies (1997) was mixed and used as the major component of the ABA and control solutions:

- 0.272 g KH_2PO_4
- 0.348 g K_2HPO_4
- 0.222 g $CaCl_2$
- 0.024 g $MgSO_4$
- 0.606 g KNO_3
- 0.030 g $MnSO_4$
- 2000 ml glass distilled water

This was used instead of distilled water as in previous experiments, because the buffering capabilities of the AS were desirable for adjusting the pH of the various treatments. The 5×10^{-5} M ABA solution was obtained by adding 5 ml of 10^{-2} M ABA stock solution to 1000 ml of AS. The control solution was obtained by adding 5 ml of 100% ethanol to 100 ml of AS to keep the ethanol concentration consistent between control and ABA solutions. The pH of each treatment was adjusted by adding small amounts of HCl or NaOH, and keeping track with a pH meter.

Five replications were used per treatment, and there were five shoots per beaker. Shoots were collected from three-year old trees from the greenhouse, and since the trees were not large enough to supply the amount of shoots needed in order for each beaker to represent the same individuals, ten shoots were collected late in the day from each of the fifteen trees. They were stored upright overnight in a refrigerator in beakers with water, then mixed up well in a large container and selected randomly (grab bag technique) one at a time and placed in beakers of solution. Beakers were placed on a bench in a greenhouse with direct sun exposure, and transpiration/evaporation measurements were taken every hour for five hours. Shoots were then placed in envelopes designated for each

individual beaker, and kept in a 60º C oven for three days. Dry weights of needles were taken and used to calculate water loss (g/h×g needles).

Statistical analysis

After analyzing the initial results, it became apparent that the conditions within the growth chamber were not uniform. Using data from trial 5 (Figure 3.6), transpiration rates were averaged among the different treatments for every block to show possible variation within the growth chamber. Although significance of block effects could not be tested (given only one experimental unit of each treatment per block), there are clearly difference conditions between the four locations within the chamber (Figure 3.1). Blocks 1 and 2 had a much higher rate of water loss in comparison to blocks 3 and 4. This may have resulted due to higher temperatures or light levels associated with one side of the chamber. To compensate for this effect, transpiration values for each treatment were standardized to the initial transpiration rates of that particular treatment (for all data shown, excluding trial 5 where data was standardized to the control treatment). So data were analyzed as a percentage of the initial water loss shown by each sample. All statistical analyses were carried out using the SAS System for Windows (version 9.0, SAS institute, Inc. Cary, NC). An ANOVA was run using the GLM procedure for the effects of treatment, time and treatment × time.

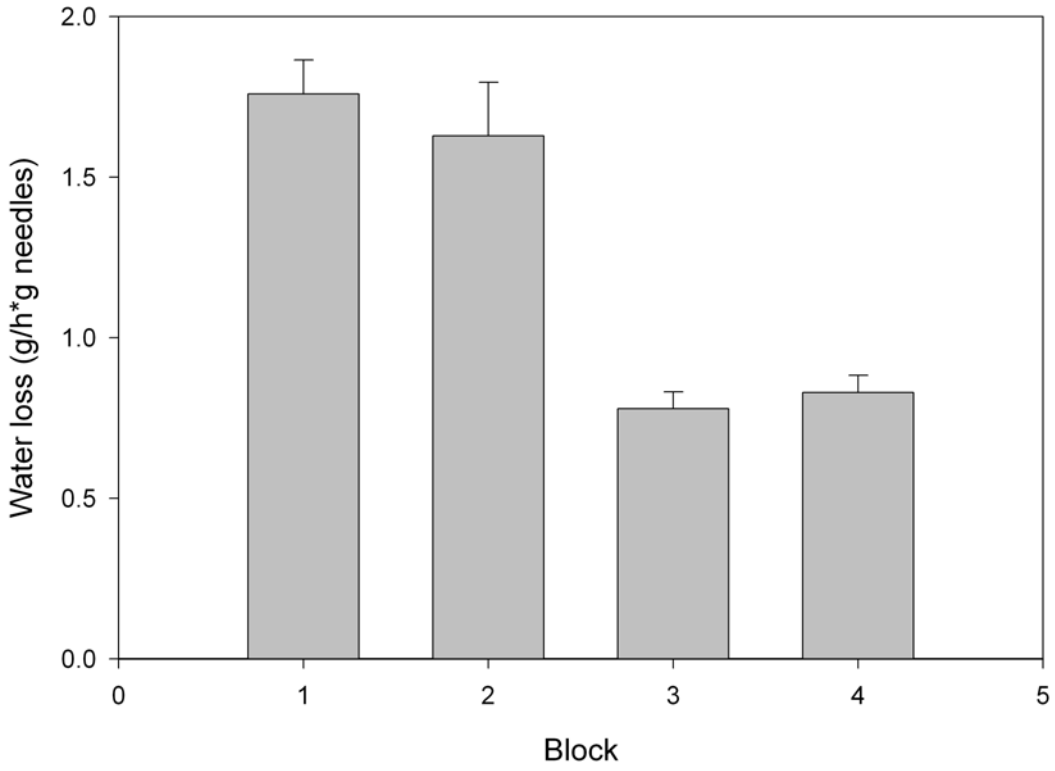

Figure 3.1 - Effects of variable conditions within the growth chamber. Values represent the averaged data for control, 10^{-4} M ABA, 10^{-5} M ABA, 5×10^{-5} M ABA and 10^{-6} M ABA treatments. Samples were divided into four different sections (blocks) within the growth chamber.

Results

Trial 1: Time course of response to 10^{-4} ABA treatments

There was a significant effect of treatment (control and ABA), time, and time by treatment interaction (Table 3.1) Transpiration of shoots in ABA continued to drop with time, while the transpiration of shoots in control solution remained fairly constant. Samples in a 10^{-4} M ABA solution exhibited less transpiration than the control treatments (P=0.0025) according to a t-test (Figure 3.2).

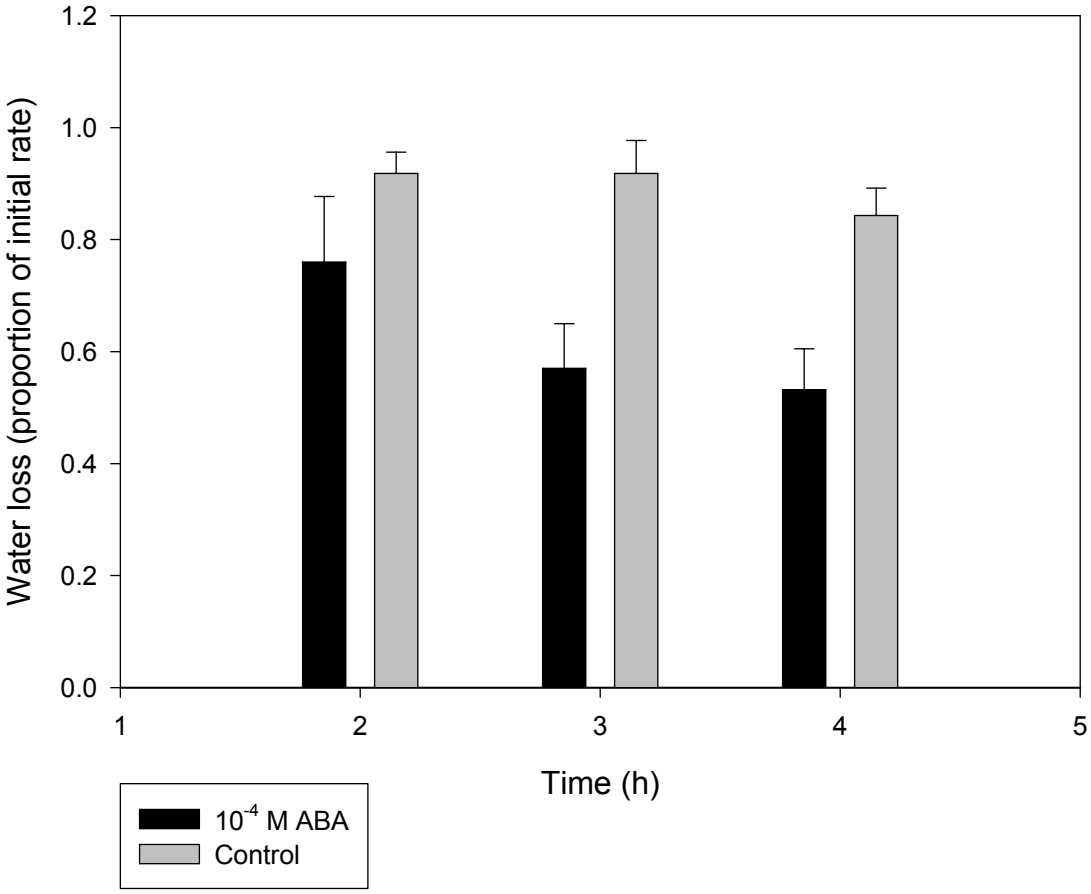

Figure 3.2 - Effect of a 10^{-4} M ABA solution on juvenile *P. rubens* shoots. Shoots were cut under water to prevent embolisms, then quickly transferred to ABA and water (control) treatments. Transpiration was measured as the rate of water loss (mL water lost/h*g needles), and is presented here as the proportion of the initial transpiration rates for each treatment.

Table 3.1 - Analysis of variance: Time course of response to 10^{-4} ABA treatments (3 hrs)
DEP. VAR.: mL water loss/m^2 needles
N: 40

Source	Sum-of-squares	DF	Mean-square	F value	*P*
Trt	0.41689399	1	0.41689399	20.34	< 0.0001
Time	0.56125427	3	0.18708476	9.13	0.0002
Trt*Time	0.18970878	3	0.06323626	3.08	0.0411
Error	0.65597286	32	0.02049915		

For the second experiment, there were no statistically significant differences between the control and ABA treatments (P=0.14), and no significant treatment by time interaction (P=0.64) (Table 3.2). However, the trends shown here (Figure 3.3) are similar to those for the previous experiment (Figure 3.2); shoots exposed to a 10^{-4} M ABA exhibited lower transpiration rates than control shoots, and transpiration decreased over time. The difference in P values between the two experiments was probably due to greater variation within the second trial, because the 5-hour reading for the second experiment had a large standard error. It is possible that the cut shoots eventually reach a point in time where they react independently of the ABA treatment and are affected more by natural processes/ABA responses associated with the fact that they are detached from the tree. This may be supported because both the ABA and control treatments showed a decline in water loss (Figure 3.3).

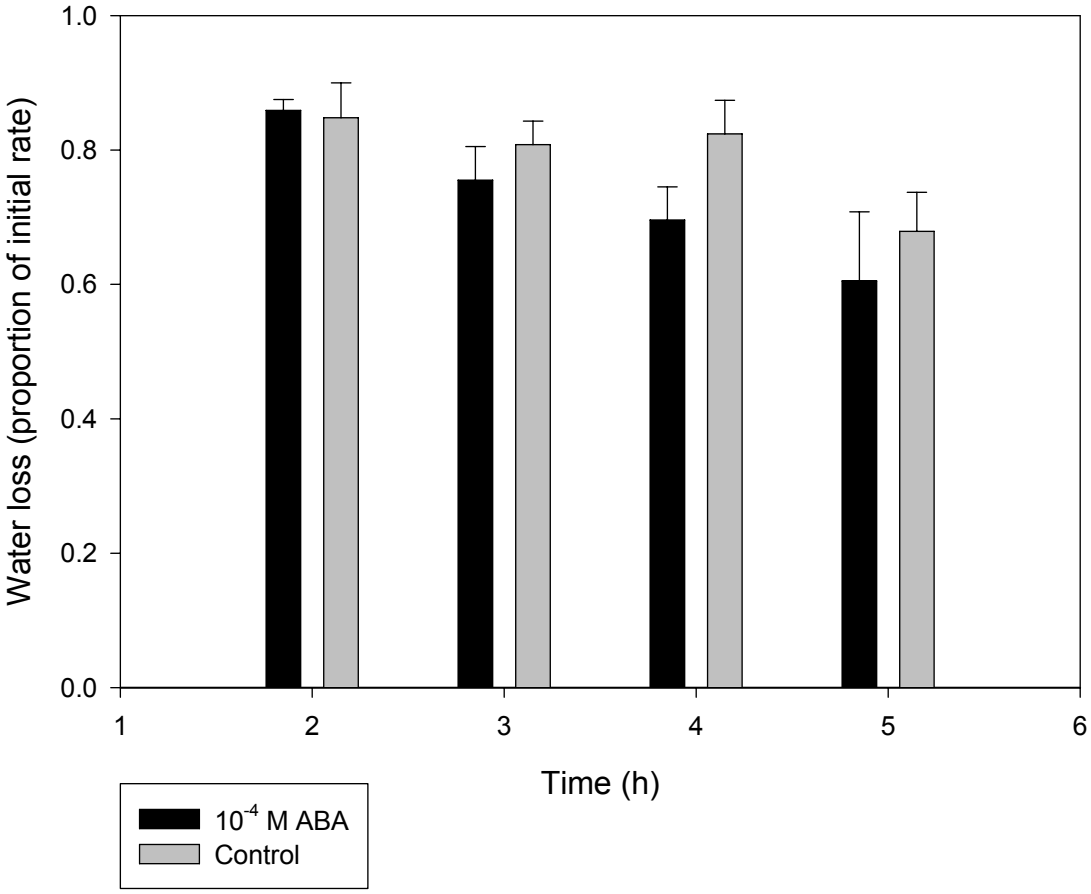

Figure 3.3 - The response of juvenile red spruce shoots to a 10^{-4} M ABA solution and control (water). Shoots were cut directly under solution and transpiration was recorded for 5 hours after exposure. Transpiration was measured as the rate of water loss (mL water lost/h*g needles), and is presented here as the proportion of the initial transpiration rates for each treatment.

Table 3.2 - Analysis of variance: Time course of response to 10^{-4} ABA treatments (4 hrs)
DEP VAR: mL water loss/m^2 needles N: 50

Source	Sum-of-squares	DF	Mean-square	F value	*P*
Trt	0.02965880	1	0.02965880	2.31	0.1360
Time	0.69366418	4	0.17341605	13.53	< 0.0001
Trt*Time	0.03233323	4	0.00808331	0.63	0.6434
Error	0.51257200	40	0.01281430		

Trial 2: Response of *Picea rubens* and *Abies balsamea* to 10^{-4} ABA

There was a statistically significant decline in transpiration associated with the ABA treatment (P=0.0014) (Table 3.3). The difference between ABA and control treatments is especially pronounced at hours 4 and 5 (Figure 3.4). However, no differences were found between the responses of *P. rubens* and *A. balsamea* (P=0.5839) to the different treatments.

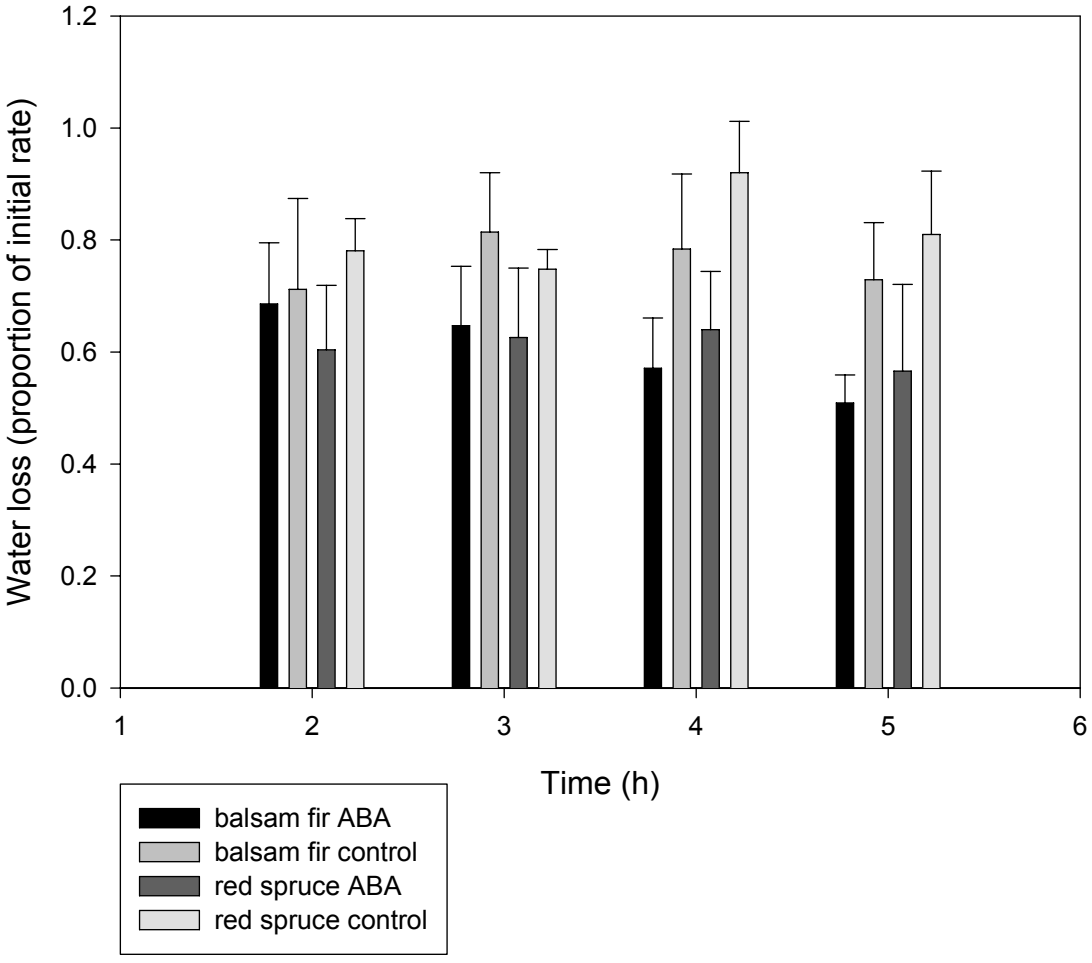

Figure 3.4 - Effect of 10^{-4} M ABA and control treatments on juvenile balsam fir (*Abies balsamea*) and red spruce (*Picea rubens*) shoots. Transpiration was measured as the rate of water loss (mL water lost/h*g needles), and is presented here as the proportion of the initial transpiration rates for each treatment.

Table 3.3 - Analysis of variance: Response of *Picea rubens* and *Abies balsamea* to ABA
DEP VAR: mL water loss/m^2 needles N: 100

Source	Sum-of-squares	DF	Mean-square	F value	*P*
Time	1.53292621	4	0.38323155	9.75	< 0.0001
Trt	0.52531554	1	0.52531554	10.90	0.0014
Species	0.01457885	1	0.01457885	0.30	0.5839
Time*Trt	0.20386458	4	0.05096615	1.06	0.3832
Time*Species	0.07108076	4	0.01777019	0.37	0.8303
Trt*Time	0.00959999	1	0.00959999	0.20	0.6566
Time*Trt*Sp	0.02785485	4	0.00696371	0.14	0.9649
Error	3.85594452	80	0.04819931		

Trial 3: Time course of response to 10^{-4} ABA treatment using beakers

When the whole data set was analyzed taking into account all time measurements, variances were equal for treatment but not time. When the dataset was analyzed from the time at hour 2 and up, however, variances were equal and the following data is based off this analysis. Transpiration of shoots increased significantly when exposed to the 10^{-4} M ABA treatment (Figure 3.5). Significant differences were found for treatment ($P< 0.0001$), and for treatment by time interactions ($P=0.0433$) (Table 3.4). The shoots in the control maintained fairly constant transpiration rates while shoots in ABA showed a significant decrease in transpiration over time. The most dramatic change for the samples in ABA occurred after about 2 hours (Figure 3.5).

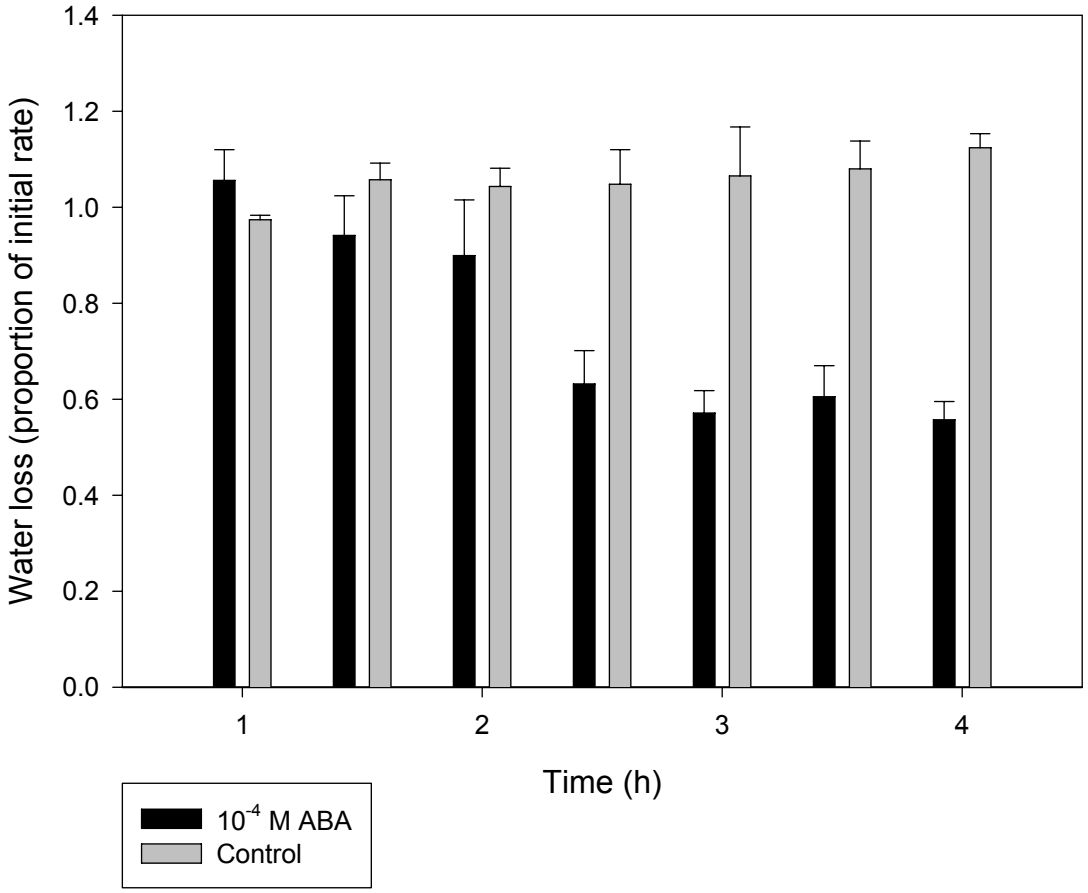

Figure 3.5 - Rate of water loss of juvenile red spruce shoots exposed to 10^{-4} M ABA and control treatments. Water loss accounts for transpiration, as well as water loss from beakers due to evaporation. The rate of water loss (mL water lost/h*g needles) is presented here as the proportion of the initial transpiration rates for each treatment.

Table 3.4 - Analysis of variance: Time course of response to ABA treatment (beakers)
DEP VAR: water loss (g/h*g needles) N: 40

Source	Sum-of-squares	DF	Mean-square	F value	*P*
Trt	1.75855989	1	1.75855989	93.06	< 0.0001
Time	0.12083432	4	0.03020858	1.60	0.2004
Trt*Time	0.21207176	4	0.05301794	2.81	0.0433
Error	0.56693308	39	0.01889777		

Trial 4: Initial response to 10^{-4} M ABA

Transpiration rates appeared to be unstable for the first 3 hours (Figure 3.6), so an ANOVA was run on hours 3.5-7 only, and results were as follows; There was a significant difference between treatments according to the t-test (P=0.04), but no significant interactions of treatment by time (P=0.76) (Table 3.5). In comparison to the above mentioned findings, when the whole data set was analyzed using all measurements and including all hours that the trial took place, there were no significant effects of treatment (P=0.72).

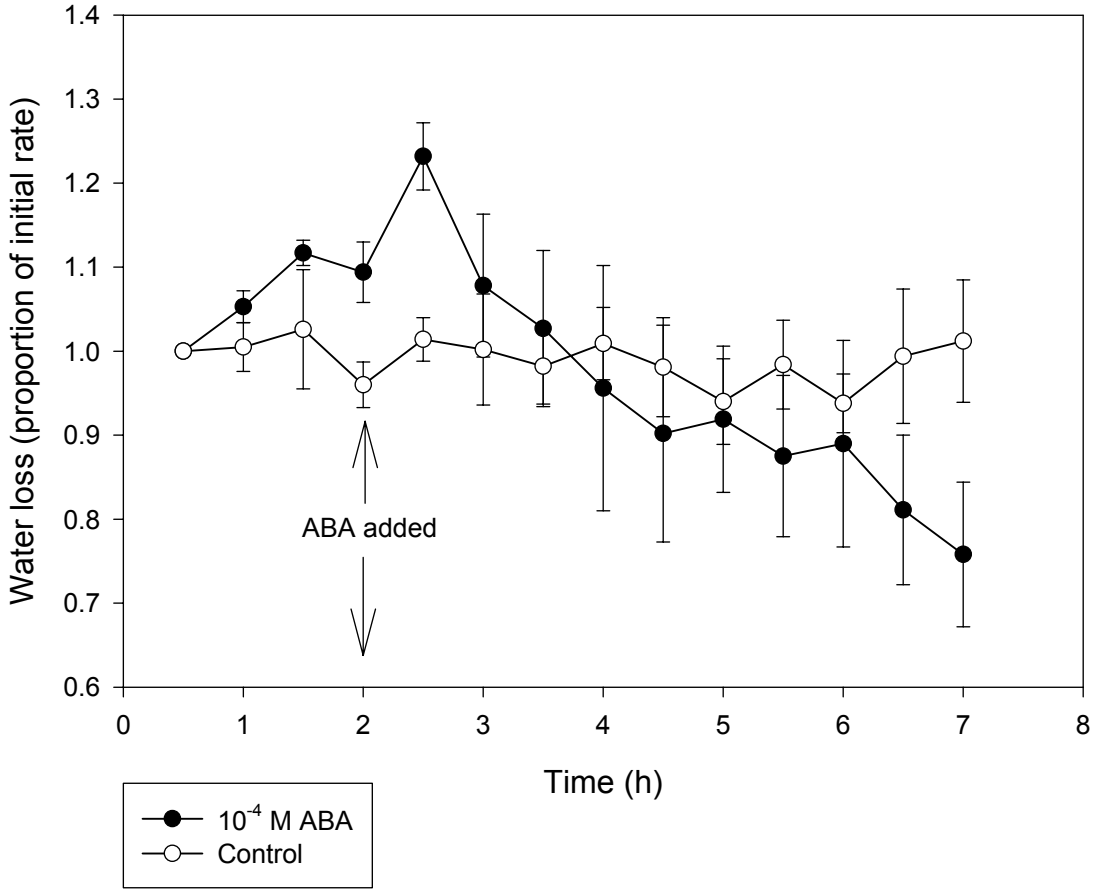

Figure 3.6 - Initial effect of 10^{-4} M ABA on juvenile red spruce shoots was tested by adding the ABA solution after two hours. Water loss accounts for transpiration, as well as water loss from beakers due to evaporation. Water loss (mL water lost/h*g needles) is presented here as the proportion of the initial transpiration rates for each treatment.

Table 3.5 - Analysis of variance: Initial response to 10^{-4} M ABA
DEP VAR: water loss (g/h*g needles) N: 64

Source	Sum-of-squares	DF	Mean-square	F value	*P*
Trt	0.12284263	1	0.12284263	4.11	0.0482
Time	0.08926276	7	0.01275182	0.43	0.8808
Trt*Time	0.12409744	7	0.01772821	0.59	0.7582
Error	1.43458829	48	0.02988726		

Trial 5: Response to various doses of ABA

All data were presented as a percentage of the control for each individual time measurement. This differed from the other trials in that the data for the different treatments were compared to the control itself instead of the initial values of that treatment. Also, this percentage was calculated separately for each time instead of being compared to the initial transpiration throughout the entire experiment. Transpiration of shoots increased significantly from 10^{-4} M to 10^{-6} M ABA (Table 3.6). However, a Tukeys test grouped the 10^{-6} M ABA treatment separately from all other treatments with no other statistically significant groupings.

As shown in Figures 3.3, 3.5 and 3.6, effects of ABA on shoots (shown by a decrease in transpiration) generally started to appear after about 2-2.5 hours. Therefore, data for this dose-response trial were analyzed a second time using transpiration rates occurring at hour 2.5 only. Although the ANOVA indicates that there are no differences between treatments at hour 2.5 (P=0.19), a trend showing increasing transpiration with decreasing ABA concentration are evident (Figure 3.7).

Table 3.6 - Analysis of variance: Response to various doses of ABA
DEP VAR: water loss (g/h*g needles) N: 160

Source	Sum-of-squares	DF	Mean-square	F value	*P*
Trt	0.75026937	4	0.43756734	9.14	<0.0001
Time	0.56728316	7	0.08104045	1.69	0.1171
Trt*Time	0.99298112	28	0.03546361	0.74	0.8197
Error	5.74597602	120	0.04788313		

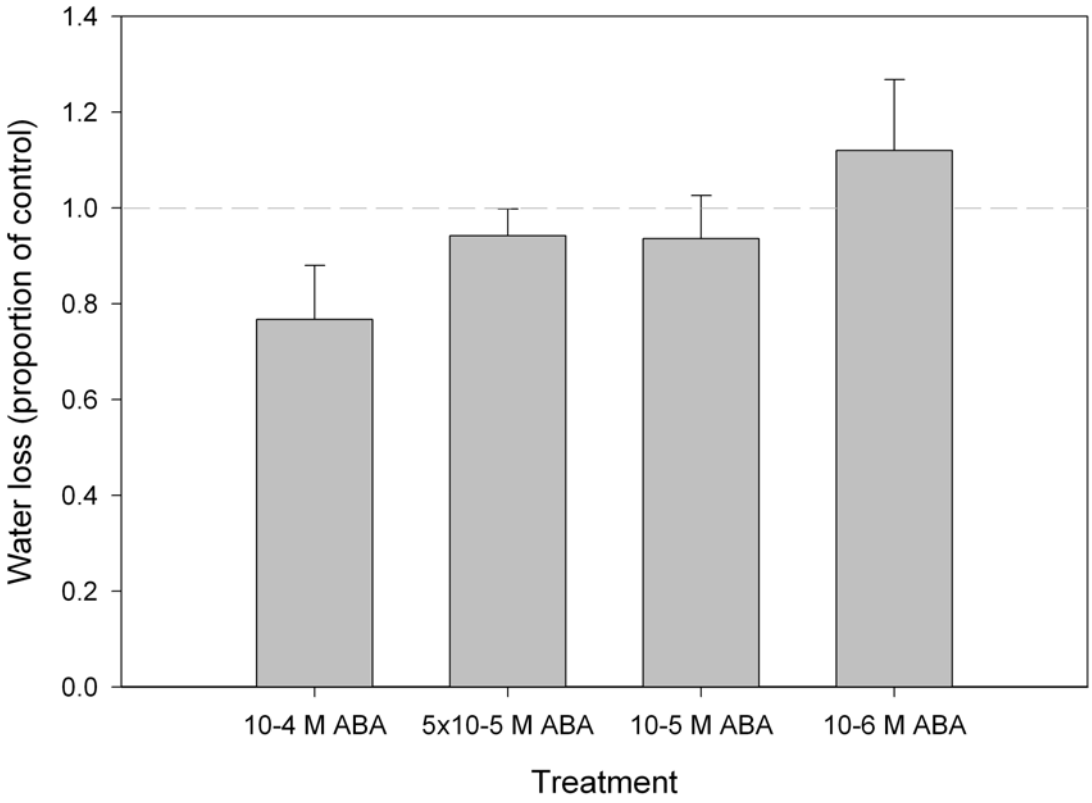

Figure 3.7 - Effect of four ABA doses on transpiration in juvenile red spruce shoots. Represents water loss after 2.5 hours of exposure to treatments. Water loss accounts for transpiration and evaporation from beakers. Water loss for each treatment is presented as a proportion of the control. Data is shown for hour 2.5 only.

Trial 6: Effect of pH on ABA response

No significant differences between treatments were found for this trial (Table 3.7). A t-test was run to compare ABA and control treatments (disregarding pH). Still, no significant difference was found (P=0.3365). Moreover, no significant correlations were found for the different levels of pH (Pearson correlation coefficient for pH*transpiration (water loss) = 0.02394 and P< 0.0001). Also, no significant correlations were found for time×transpiration (water loss) (Pearson correlation coefficient of -0.40371 and P=0.7728).

Table 3.7 - Analysis of variance: Effect of pH on ABA response
DEP VAR: water loss (g/h*g needles) N: 150

Source	Sum-of-squares	DF	Mean-square	F value	P
Trt	0.02679726	5	0.00535945	1.65	0.1522
Time	0.09906922	4	0.02476730	7.63	< 0.0001
Trt*Time	0.08144186	20	0.00407209	1.25	0.2246
Error	0.38326667	118	0.00324802		

Discussion

The application of 10^{-4} M ABA to juvenile red spruce shoots caused a decline in transpiration. This was found to be statistically significant in four of the experiments (Trial 1, 2, 3, 4). Even the trials that were not statistically significant did in fact reveal similar trends as trials 1, 2, 3, and 4. In Trial 3 and 4, this significance was found only when data from the first 2-3 hours of the experiment were excluded. This, in addition to visible trends in several of the figures above (Figures 3.3, 3.5, 3.6), indicate there may be a time delay from when shoots are exposed to ABA until they start to show a decline in transpiration. Figure 3.6, in particular, shows a response at about hour 3.5-4, which occurs 1.5-2 hours after the ABA was added to the shoots (hour 2). It is possible that guard cells need time to accumulate the appropriate amount of ABA from the xylem necessary to initiate measurable stomatal closure. Also, because shoots were exposed to an adequate water source, this may have an effect on the ABA response. High leaf water potentials have been shown to override ABA effects by causing ABA to be isolated from its site of action or by decreasing stomatal sensitivity to ABA, both of which could cause stomata to remain open (Aasamaa et al. 2002; but see Gowing et al. 1990).

In response to drought, an increase in xylem sap pH often occurs (Wilkinson et al. 1998) although the process by which this occurs is unknown. Xylem sap of *Helianthus*

annuus L. increased from a pH of 5.8-6.6 in well-watered conditions to 7.1 in soil water below 0.13 g g^{-1} (Gollan et al. 1992). In addition, Wilkinson and Davies (1997) found a 0.6 unit increase in pH of *Commelina communis* xylem sap after withholding water for 6 days. ABA is often held responsible for the majority of stomatal responses to drought, but it has been shown that stomata may close in response to this pH increase, even if a drought-induced increase in xylem sap ABA concentration has not yet occurred (Wilkinson and Davies, 1997). As long as the pH of the leaf allows ABA to contact the guard cells, even a low concentration of ABA can cause stomata to close (Davies et al. 2002). This is because the activity of ABA and the sensitivity of the ABA response is determined by pH. Our study, contrary to previous findings, did not reveal any significant differences between transpiration of shoots exposed to different pH levels. However, the importance of pH for contributing to the regulation of stomatal aperture should not be discounted for *P. rubens* based on this study. Although the pH experiment was conducted in a greenhouse with little distance between the beakers, variation in conditions between beakers could still have been present. Also, since the ABA concentrations used were higher than that which we expect to occur in red spruce, this may have had an influence on the effect of pH on transpiration that would not have occurred in natural foliage.

Another important aspect to consider is that the ABA concentration frequently used in this study (10^{-4} M) was probably stronger than that which would normally occur in *P. rubens* needles. For comparison to another conifer, Perks et al. (2002) found that ABA concentration of xylem sap of Scots pine (*Pinus Sylvestris* L.) was 2.5×10^{-7} M. In order to understand naturally occurring processes, it would be important to use only

naturally occurring ABA concentrations. However, a stronger concentration was necessary to trigger a detectable response in this case. It is possible that high concentrations of ABA were needed in these experiments to override some type of buffering inactivation mechanisms occurring within the shoots. Also, the correlation between ABA treatment levels and concentrations of active ABA in red spruce foliage is unknown. Just because the shoots were supplied with a high concentration of ABA does not necessarily mean that it was put to use. The pre-existing concentration of ABA in red spruce shoots was also not known and this could affect their response to the ABA supplied in these experiments.

There are many other variables that could be affecting the transpiration of these shoots, in addition to ABA concentration. The action of hormone receptors depends not only on the concentration of available hormones, but also on how sensitive the receptors are. Drought may cause changes in the ion composition of xylem sap, which could change the sensitivity of guard cells (Gollan et al. 1992). Also, leaf water potential will decline in response to drought conditions and this may increase sensitivity of the guard cells (Tardieu & Davies 1993 as cited in Jackson et al. 1995). In our experiments, the shoots were supplied with adequate water since their stems were submerged, and this could have affected the ABA response.

The experiments were sometimes conducted later in the day, when the trees may be shutting down for the night in a natural environment. For example, the first experiment in trial 1 was carried out until 4:40pm, wheras the second lasted until 7:16pm. Even though light was provided for the shoots in the growth chamber, they may have

closed their stomata as part of their natural rhythm. This may be a partial cause of the less significant results in the second experiment.

It is clear that excised red spruce shoots show a response when exposed to externally supplied abscisic acid, and this could be worth exploring further in the future using material from different aged shoots. In addition to replicating these trials, an important component would be to investigate the ABA concentrations that occur naturally within spruce needles to determine what the most reasonable hormone dosage is for monitoring the ABA response. The natural concentration of ABA within different aged trees could be investigated using GC-MS or immunoassay techniques. It may also be beneficial to look into alternate ways of introducing ABA into red spruce shoots.

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Appendices

Appendix A

CHAPTER ONE ANALYSIS OF VARIANCE BASED ON DATA FOR EACH DEPENDENT VARIABLE

Dependent variable: proportion internal air space N:24

Source	Sum-of-Squares	DF	Mean-square	F value	*P*
Age	2.04088303	3	0.68029434	11.07	0.0002

Dependent variable: vascular bundle N:72

Source	Sum-of-Squares	DF	Mean-square	F value	*P*
Age	56927271539	3	18975757180	146.64	<.0001

Dependent variable: Cross section N:72

Source	Sum-of-Squares	DF	Mean-square	F value	*P*
Age	1.99805879	3	0.66601960	172.80	<.0001

Dependent variable: Height N:72

Source	Sum-of-Squares	DF	Mean-square	F value	*P*
Age	788316.0179	3	262772.0060	27.00	<.0001

Dependent variable: Width N:72

Source	Sum-of-Squares	DF	Mean-square	F value	*P*
Age	1.10294565	3	0.36764855	203.01	<.0001

Dependent variable: Phloem N:72

Source	Sum-of-Squares	DF	Mean-square	F value	*P*
Age	56886120.04	3	18962040.01	53.55	<.0001

Dependent variable: Xylem N:72

Source	Sum-of-Squares	DF	Mean-square	F value	*P*
Age	3.27983820	3	1.09327940	140.83	<.0001

Dependent variable: Perimeter N:72

Source	Sum-of-Squares	DF	Mean-square	F value	*P*
Age	23792026.78	3	7930675.59	170.48	<.0001

Dependent variable: Proportion mesophyll N:72

Source	Sum-of-Squares	DF	Mean-square	F value	*P*
Age	0.00281684	3	0.00093895	26.09	<.0001

Dependent variable: Proportion mesophyll adjusted for air space N:72

Source	Sum-of-Squares	DF	Mean-square	F value	*P*
Age	0.02632222	3	.00877407	5.80	.0014

Dependent variable: Xylem/Perimeter N:72

Source	Sum-of-Squares	DF	Mean-square	F value	*P*
Age	2.87357083	3	0.95785694	70.65	<.0001

Dependent variable: Phloem/Perimeter N:72

Source	Sum-of-Squares	DF	Mean-square	F value	*P*
Age	1.42570000	3	0.47523333	14.26	<.0001

Dependent variable: Xylem/Cross section N:72

Source	Sum-of-Squares	DF	Mean-square	F value	*P*
Age	0.00001369	3	0.00000456	16.68	<.0001

Dependent variable: Phloem/Cross section N:72

Source	Sum-of-Squares	DF	Mean-square	F value	*P*
Age	0.07500556	3	0.02500185	2.73	0.0505

Dependent variable: Phloem/Mesophyll N:72

Source	Sum-of-Squares	DF	Mean-square	F value	*P*
Age	0.07230503	3	0.02410168	2.47	0.0692

Dependent variable: Phloem/Mesophyll adjusted for air space N:71

Source	Sum-of-Squares	DF	Mean-square	F value	*P*
Age	0.00004712	3	0.00001571	4.26	0.0082

Dependent variable: Xylem/Mesophyll N:72

Source	Sum-of-Squares	DF	Mean-square	F value	*P*
Age	0.00122756	3	0.00040919	17.98	<.0001

Dependent variable: Xylem/Mesophyll adjusted for air space N:71

Source	Sum-of-Squares	DF	Mean-square	F value	*P*
Age	0.00002153	3	0.00000718	8.02	0.0001

Dependent variable: Specific leaf area N:72

Source	Sum-of-Squares	DF	Mean-square	F value	*P*
Age	4731.367915	3	1577.122638	147.89	<.0001

Dependent variable: Tracheid lumen diameter N:72

Source	Sum-of-Squares	DF	Mean-square	F value	*P*
Age	12.03525972	3	4.01175324	21.06	<.0001

Dependent variable: Lumen/cell wall N:72

Source	Sum-of-Squares	DF	Mean-square	F value	*P*
Age	0.14855000	3	0.04951667	9.63	<.0001

Dependent variable: Lumen area N:72

Source	Sum-of-Squares	DF	Mean-square	F value	*P*
Age	15797473.94	3	5265824.65	58.08	<.0001

Appendix B

CHAPTER TWO ANALYSIS OF VARIANCE AND MEANS FOR PHOTOSYNTHETIC AND ANATOMICAL DATA OF GRAFTED MATERIAL

Table 1 - Mean ± SE, and P values (*=P<0.05) for photosynthesis and SLA (specific leaf area) measurements of grafted scions (~3, ~54, and ~127 year old) on *juvenile* (~3 year old) rootstock. For each variable, means not followed by the same letter are significantly different (P< 0.05). N=5

Measurement	**3 year old**	**60 year old**	**120 year old**	***P***
Net photosynthesis (μmol CO_2 m^{-2} s^{-1})	14.16 ± 1.1 a	12.57 ± 0.8 a	13.41 ± 1.6 a	0.65
Net photosynthesis (μmol CO_2 kg^{-1} s^{-1})	73.36 ± 3.4 a	54.38 ± 3.8 b	44.45 ± 4.82 bc	0.001
Stomatal conductance (mol H_2O m^{-2} s^{-1})	0.36 ± 0.06 a	0.29 ± 0.05 a	0.24 ± 0.04 a	0.64
Internal CO_2 concentration (μmol CO_2 mol^{-1})	369.9 ± 26.8 a	351.3 ± 31.0 a	313.9 ± 29.6 a	0.41
Specific leaf area (cm^2/g)	52.4 ± 2.5 a	43.3 ± 0.8 b	43.2 ± 3.4 b	0.03

Table 2 - Mean ± SE, and P values (*=P<0.05) for photosynthesis measurements of grafted scions (~3, ~54, and ~127 year old) on *mid-aged* (~54 year old) rootstock. For each variable, means not followed by the same letter are significantly different (P< 0.05).

Measurement	**3 year old**	**60 year old**	**120 year old**	***P***
Net photosynthesis (μmol CO_2 m^{-2} s^{-1})	10.51 ± 0.5 a	11.59 ± 0.3 a	11.37 ± 0.5 a	0.25
Net photosynthesis (μmol CO_2 kg^{-1} s^{-1})	38.31 ± 2.4 a	38.26 ± 1.4 a	39.65 ± 3.2 a	0.91
Stomatal conductance (mol H_2O m^{-2} s^{-1})	0.17 ± 0.02 a	0.16 ± 0.01 a	0.18 ± 0.03 a	0.65
Internal CO_2 concentration (μmol CO_2 mol^{-1})	248.1± 11.2 a	228.6 ± 2.8 a	244.9 ± 6.2 a	0.19
Specific leaf area (cm^2/g)	34.8 ± 1.6 a	31.7 ± 1.5 a	31.3 ± 1.1 a	0.21

Table 3 - Mean ± SE, and P values (*=P<0.05) for photosynthesis and SLA (specific leaf area) measurements of grafted scions (~3, ~54, and ~127 year old) on *old* (~127 year old) rootstock. For each variable, means not followed by the same letter are significantly different (P< 0.05).

Measurement	**3 year old**	**60 year old**	**120 year old**	***P***
Net photosynthesis ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$)	9.20 ± 0.63 a	10.20 ± 0.49 a	10.63 ± 1.11 a	0.45
Net photosynthesis ($\mu mol\ CO_2\ kg^{-1}\ s^{-1}$)	29.77 ± 2.25 a	33.52 ± 2.81 a	38.30 ± 4.64 a	0.24
Stomatal conductance ($mol\ H_2O\ m^{-2}\ s^{-1}$)	0.17 ± 0.02 a	0.16 ± 0.02 a	0.15 ± 0.02 a	0.66
Internal CO_2 concentration ($\mu mol\ CO_2\ mol^{-1}$)	265.7 ± 12.1 a	244.5 ± 12.9 a	233.3 ± 6.0 a	0.14
Specific leaf area (cm^2/g)	29.5 ± 0.9 a	28.7 ± 1.3 a	29.5 ± 1.1 a	0.87

Table 4 - Mean ± SE, and P values (*=P<0.05) for measurements of needle cross sections of grafted scions (3, ~54, and ~127-year-old) on mid-aged (~54-year-old) rootstock. For each variable, means not followed by the same letter are significantly different (P< 0.05). N=5

Measurement	**3 year old**	**54 year old**	**127 year old**	***P***
Width (μm)	1068 ± 54 b	1380 ± 93 a	1414 ± 73 a	0.0082
Height (μm)	934 ± 23 b	1048 ± 37 a	1122 ± 29 a	0.0028
Cross section (μm^2)	604012 ± 40409 b	894587 ± 73538 a	933922 ± 66523 a	0.0050
Perimeter (μm)	3001 ± 95 b	3663 ± 182 a	3812 ± 147 a	0.0033
Phloem (μm^2)	2299 ± 117 b	3763 ± 454 a	3859 ± 225 a	0.0050
Xylem (μm^2)	2472 ± 184 b	4394 ± 439 a	4148 ± 349 a	0.0020
Vascular bundle (μm^2)	59966 ± 6874 b	104959 ± 9834 a	110592 ± 11704 a	0.0056
Mesophyll (%)	88 ± 0.6 a	87 ± 0.7 a	87 ± 0.6 a	0.4323
Xylem/mesophyll	0.0047 ± 0.0002 b	0.0056 ± 0.0003 a	0.0051 ± 0.0001 ab	0.0408
Xylem/cross section	0.0041 ± 0.0002 b	0.0049 ± 0.0003 a	0.0044 ± 0.0001 ab	0.0426
Xylem/perimeter	0.82 ± 0.05 b	1.19 ± 0.08 a	1.08 ± 0.05 a	0.0037
Phloem/mesophyll	0.0044 ± 0.0002 a	0.0048 ± 0.0002 a	0.0048 ± 0.0002 a	0.3416
Phloem/cross section	0.0038 ± 0.0002 a	0.0042 ± 0.0002 a	0.0042 ± 0.0001 a	0.3775
Phloem/perimeter	0.76 ± 0.03 b	1.01 ± 0.08 a	1.01 ± 0.03 a	0.0069

Table 5 - Mean ± SE, and P values (*=P<0.05) for measurements of needle cross sections of grafted scions (3, ~54, and ~127-year-old) on old (~127-year-old) rootstock. For each variable, means not followed by the same letter are significantly different (P< 0.05). N=5

Measurement	**3 year old**	**54 year old**	**127 year old**	***P***
Width (µm)	1092 ± 24 b	1278 ± 66 a	1432 ± 48 a	0.0009
Height (µm)	915 ± 66 a	1038 ± 65 a	1054 ± 22 a	0.1923
Cross section (μm^2)	632060 ± 42322 b	833297 ± 87382 ab	900459 ± 46094 a	0.0254
Perimeter (µm)	3006 ± 78 b	3485 ± 187 a	3776 ± 92 a	0.0030
Phloem (μm^2)	2230 ± 211 b	3004 ± 209 b	4015 ± 239 a	0.0004
Xylem (μm^2)	2690 ± 252 b	3509 ± 506 ab	4566 ± 475 a	0.0258
Vascular bundle (μm^2)	57658 ± 4506 b	98533 ± 14470 a	109069 ± 5827 a	0.0053
Mesophyll (%)	89 ± 0.7 a	87 ± 0.7 a	87 ± 0.2 a	0.0926
Xylem/mesophyll	0.0049 ± 0.0004 a	0.0048 ± 0.0004 a	0.0059 ± 0.0007 a	0.3170
Xylem/cross section	0.0043 ± 0.0003 a	0.0042 ± 0.0004 a	0.0051 ± 0.0006 a	0.2888
Xylem/perimeter	0.89 ± 0.08 a	0.99 ± 0.10 a	1.21 ± 0.13 a	0.1201
Phloem/mesophyll	0.0040 ± 0.0004 a	0.0042 ± 0.0002 a	0.0052 ± 0.0005 a	0.1346
Phloem/cross section	0.0036 ± 0.0003 a	0.0037 ± 0.0002 a	0.0046 ± 0.0004 a	0.1227
Phloem/perimeter	0.74 ± 0.07 b	0.86 ± 0.02 ab	1.07 ± 0.08 a	0.0102

BIOGRAPHY OF THE AUTHOR

Margaret H. Ward was born in Nashua, New Hampshire. She graduated from Mascenic Regional High School in New Ipswich, NH in 1997 and continued her education at Ithaca College where she received a Bachelor's of Arts in Biology with a minor in art. After spending a year working and volunteering in New Hampshire, she enrolled in the graduate program in the Department of Forest Ecosystem Science at the University of Maine. She is a candidate for the Master of Science degree in Forestry from The University of Maine in May, 2005.