

Full Research Paper

Changes in Spectral Properties, Chlorophyll Content and Internal Mesophyll Structure of Senescing *Populus balsamifera* and *Populus tremuloides* Leaves

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Abstract: In this paper we compare leaf traits and spectral reflectance for sunlit and shaded leaves of *Populus tremuloides* and *Populus balsamifera* during autumn senescence using information derived from an Analytical Spectral Device (ASD) Full Range spectrometer. The modified simple ratio (mSR₇₀₅) and modified normalized difference index (mND₇₀₅) were effective in describing changes in chlorophyll content over this period. Highly significant ($P < 0.01$) correlation coefficients were found between the chlorophyll indices (mSR₇₀₅, mND₇₀₅) and chlorophyll a, b, total chlorophyll and chlorophyll a/b. Changes in mesophyll structure were better described by the plant senescence reflectance index (PSRI) than by near-infrared wavebands. Overall, *P. balsamifera* exhibited lower total chlorophyll and earlier senescence than *P. tremuloides*. Leaves of *P. balsamifera* were also thicker, had a higher proportion of intercellular space in the spongy mesophyll, and higher reflectance at 800 nm. Further research, using larger sample sizes over a broader range of sites will extend our understanding of the spectral and temporal dynamics of senescence in *P. tremuloides* and *P. balsamifera* and will be particularly useful if species differences are detectable at the crown level using remotely sensed imagery.

Keywords: plant senescence reflectance index, hyperspectral.

1. Introduction

Autumn foliar senescence in deciduous temperate tree species is typically induced by shortening of the photoperiod [1]. Within a leaf, a sequence of programmed changes take place during senescence, among them chlorophyll loss [2-5], decreased chlorophyll a/b ratio [6-8], declining photosynthetic ability [9-11], transition from nutrient assimilation to nutrient remobilization [11,12], collapse of cell walls [13] and changes on plant hormone balance (polyamines and ethylene) [45]. Some of these changes may be tracked with concomitant alterations in leaf optical properties. In general, visible reflectance (400-700 nm) increases in response to chlorophyll degradation [14,15]. Near-infrared reflectance (700-1300 nm) initially rises as the number of scattering interfaces increases as cells split and cell contents shrink away from cell walls, and eventually decreases in accordance with further mesophyll breakdown [16]. More specifically, wavelengths near 550 nm and 700 nm have been shown to be particularly sensitive to changes in chlorophyll content during leaf senescence [17], while correlations of specific wavebands with the demise of mesophyll structure during senescence have not been explored. The plant senescing reflectance index ($PSRI=(R_{678}-R_{500})/R_{750}$) was developed by [18] for the sole purpose of describing the dynamics of senescence processes in leaves and fruits, and is sensitive to the carotenoid/chlorophyll ratio.

While the series of physiological and, to a lesser extent, anatomical events leading to leaf death have been described for numerous plant species, it remains unknown if they are equivalent for both sun and shade-acclimated leaves of the same plant. Sun-exposed and shaded leaves have been shown to exhibit considerable physiological and morphological differences. Although the degree of within-tree plasticity of leaf morphology and physiology varies among species, related to light tolerance and successional status [19-21], it is apparent that some species are capable of acclimating their foliage to the light environment [19-22]. Within a single crown, changes in leaf anatomy tend to reflect changes in light availability during development [23]. In comparison with shade-acclimated leaves, sun-acclimated leaves may exhibit some or all of the following features: smaller leaf surface area [24,22], greater leaf width [24,25,22], lower chlorophyll concentration (on a per mass basis) [25], higher amounts of the photoprotective xanthophyll pigment [26], thicker and/or multi-layered palisade mesophyll [24,22], denser mesophyll structure [26], and higher light-saturated photosynthetic rates (Gamage *et al.* 2003). Chlorophyll a/b ratio, besides decreasing during senescence, also tends to decrease with decreasing light availability [27-29]. The influence of these differences (due to light acclimation) on leaf optical properties is not well defined to date. Among species of sun and shade-adapted rainforest plants, similar optical properties were found in the visible region (400-700 nm) despite differing anatomical features and photosynthetic efficiencies [30]. The same conclusion was drawn for 26 temperate species from understory, intermediate, and open light environments [31].

Within a single species, however, [25] observed greater reflectance of orange (*Citrus sinensis* (L.) Osbeck) sun leaves than shade leaves in the visible region of the electromagnetic spectrum, which was consistent with a lower chlorophyll concentration (on a per mass basis). [32] confirmed this for the case of three tree species (oak (*Quercus* spp.), beech (*Fagus sylvatica* L.), hornbeam (*Carpinus betulus* L.)), although his results were not significant. However, both visible and near-infrared transmittance of sun leaves were significantly less ($p=0.05$) than that of shade leaves. In the NIR (700-1300 nm), lower transmittance of sun leaves was attributed to their high quantity of intercepting leaf tissues, including

cellular constituents and intercellular spaces. Similarly, increased NIR reflectance and decreased transmittance correlate with increases in leaf thickness [31], a trend that should hold for sun versus shade leaves since sun leaves are the thicker of the two.

In the present study, the two species of interest are common to our location in central Alberta, Canada. Aspen (*Populus tremuloides* Michx.), first of all, is considered the most widely distributed tree species in North America [33,34], while balsam poplar (*Populus balsamifera* L.), secondly, prefers riparian sites [35,36]. The congeneric species are shade-intolerant pioneers, often among the first recolonizers of open areas following disturbance [35]. Both have the capacity to reproduce vegetatively. Aspens may become extensively clonal [37] and balsam poplars sprout from roots and stumps [35]. The clones may differ in morphological characteristics as well as in timing of leaf flush, flowering and leaf fall, which can lead to differences in autumn leaf colours in adjacent clones [38]. While a few studies have focused on autumn foliar senescence of trembling aspen (Dean *et al.* 1993, [4] (also note that [8] addressed senescence of *P. sargentii*, and [38.1] did the same for European aspen, *P. tremula* L.), equivalent studies on balsam poplar are nonexistent. [7] and [33] noted contrasting trends in chlorophyll a/b ratio during senescence of aspen leaves. Whereas [7] observed fairly uniform chlorophyll a/b during the summer, and a drop during senescence, [33] observed striking increases in chlorophyll a/b for five progressive stages of senescence of aspen leaves, a phenomenon not previously recorded among deciduous tree species, and possibly linked to earlier structural loss of grana lamellae compared to stroma lamellae. The reasons behind the incongruent results remain without clarification to date.

Our research aims to expand current knowledge of the dynamics of fall senescence of *P. tremuloides* and *P. balsamifera* in our region as well as potential differences in these dynamics for sun and shade leaves of each species. We do this by exploring what we term the ‘spectro-temporal’ changes (changes in spectral characteristics over time) in leaf optical properties, chlorophyll content (total chlorophyll, chlorophyll *a* and *b*) and internal anatomical features (palisade and spongy mesophyll thickness, total leaf thickness, percentage intercellular space within the spongy mesophyll). The specific objectives are three-fold: 1) To compare the two species, both which play important ecological roles in the boreal forest of Canada, 2) To compare sun and shade leaves, since light acclimation can lead to important differences in the morphology and physiology of leaves, and 3) To explore differences in mature and senescing leaves with respect to chlorophyll content and internal anatomy. We also consider our results in light of future remote sensing applications.

2. Methods

2.1 Leaf spectral reflectance and chlorophyll content

Leaf samples of *P. tremuloides* (aspen) and *P. balsamifera* (balsam poplar) were collected over 16 sampling dates (every week) during August to October 2002, the period of autumn senescence. Leaf spectral measurements and chlorophyll analysis were performed on the 2002 samples. In 2003, additional samples were collected over seven dates during the same time period. The primary purpose of the 2003 samples was leaf thin section analysis in support of the 2002 data. Leaf spectral reflectance and SPAD readings (chlorophyll content) also accompany the 2003 samples. The trees were situated

near the top of a ravine bordering the North Saskatchewan River in south central Edmonton, Alberta, Canada. At each collection, three leaves each were harvested from the sunlit and shaded portions of the trees. Hereafter, the following acronyms will be used to refer to leaf samples: aspen sun (ASU), aspen shade (ASH), poplar sun (PSU) and poplar shade (PSH). After labelling, the petioles of the leaves were wrapped in a moist paper towel. Leaves were then packed into a black plastic bag inside a cooler containing ice and taken for analysis.

2.2 Instruments used in this study

Two spectrometers were used over the course of the study for spectral reflectance measurements. The FieldSpec Pro FR spectroradiometer (Analytical Spectral Devices, Boulder, CO, USA) (model FSP350-2500P), first of all, has three photodiode arrays and measures a spectrum from 350-2500 nm. The spectral resolution is 3 nm wide in the VNIR and 10 nm in the SWIR. The sampling interval of 1.4 nm in the visible and near-infrared (VNIR) and 2 nm in the short wave infrared (SWIR) is interpolated to a finer sampling interval of 1 nm for a total of 2151 contiguous channels. The bare fibre-optic has a FOV of 25 degrees. The FieldSpec Pro FR was used for all samples collected in 2002.

The UniSpec Spectral Analysis System VIS/NIR (PP Systems, Amesbury, MA, USA), secondly, has a 256-element photodiode array and a spectral range of 350-1100 nm. Spectral resolution is <10 nm and sampling interval is 3.3 nm. The UniSpec has a bifurcated fibre-optic: one branch delivers light from an internal 7.0 W halogen lamp while the other returns the reflected light to the detector. For measurements, a leaf clip was attached to the fore-optic, which limits the FOV to a constant 2.3 mm and prevents ambient light entry. The UniSpec was used only for samples obtained in 2003 for leaf thin section analysis (section 2.2).

Lastly, the SPAD 502 (Minolta Camera Co., Osaka, Japan) is a hand-held chlorophyll absorbance meter. When the meter is clamped over leafy tissue, an indexed chlorophyll content (0-99.9) reading is given, based on absorbance at 650 and 940 nm.

2.3 SPAD measurements and chlorophyll content analysis (samples collected during 2002)

Six SPAD measurements were recorded per leaf, three per each side of the leaf blade, avoiding the main vein. A 16 mm-diameter sample was cored from each leaf, from which an additional SPAD measurement was taken. The cored samples were placed in small plastic vials, wrapped in tinfoil and frozen.

Of the over 250 leaf cores accumulated over the 16 sampling dates, 100 were selected for chlorophyll analysis. The chosen samples provided adequate representation of the four leaf types (sun and shade leaves of the two species) over a wide range of SPAD values. Total chlorophyll, chlorophyll a, and chlorophyll b content were determined using the dimethyl sulphoxide (DMSO) extraction method described by [44]. For each species and illumination category (sun or shade), second-degree polynomial SPAD calibration curves were developed for the estimation of chlorophyll a, chlorophyll b and total chlorophyll ($0.79 \leq r^2 \leq 0.98$) and used to estimate chlorophyll content for the remainder of the leaf cores.

2.4 Leaf spectral reflectance measurements (samples collected during 2002)

The FieldSpec Pro FR bare fibre-optic was fit through a mounting gun attached to a tripod, and adjusted to a nadir viewing position above a black, non-reflecting (<2%) surface on which each leaf was placed. Distance between the fibre-optic and the sample was 23 mm, which resulted in a field of view of approximately 10 mm in diameter. Illumination was provided using an external 50 W halogen, at an angle of 45 degrees. FieldSpec Pro FR optimization was performed at the same time as each white reference (initially and after groups of 9 measurements). Instrument configuration was adjusted to average 20 scans per white reference and 25 per dark current reading. Twenty scans were averaged per recorded leaf spectrum. Reflectance spectra were recorded as the ratio of sample data to white reference (99% reflectance Spectralon panel) data under the same illumination and viewing conditions. Three spectral measurements, from the side of the leaf opposite where the core was extracted, were recorded per leaf, and later averaged.

2.5 Histology (samples collected during 2003)

2.5.1 Sample collection

The following autumn (August-October 2003), sun and shade leaves from the same trees were sampled for histological analysis. For each sample, three SPAD values were recorded from one side of the leaf blade. Similarly, three UniSpec leaf spectral measurements were recorded from within the same area. For UniSpec measurements, a dark current reading and white reference (using barium sulfate), both averages of 10 scans, were performed after every other leaf. Sample reflectance was obtained by comparing leaf reflectance (also an average of 10 scans) to reflectance of the barium sulfate standard.

2.5.2 Leaf fixation and thin section analysis

Thin sections were made for study of changes in internal mesophyll structure over the period of fall senescence. Initially, small pieces were cut from the leaf blade (approximately 10 mm long and 2-3 mm wide, from the same side as the SPAD and reflectance measurements), avoiding the midrib. Fixation followed, in which the leaf pieces were subject to vacuum for two weeks in a formalin acetoalcohol (FAA) solution. After fixation, samples were run through an ethanol processing centre (Fisher Histomatic 166) and embedded in paraffin molds. Using a microtome, thin sections (5 µm) were then cut from the embedded samples. Slides were mounted and stained (using two stains: Harris' haematoxylin stain and Safranin O and fast green, for comparison), and viewed and photographed under a Leica DM RXM light microscope at 20X magnification.

2.6 Data analysis

2.6.1 Indices

Three spectral vegetation indices were computed over the time course of senescence, 1.) the modified simple ratio, mSR_{705} [42], 2.) the modified normalized difference index, mND_{705} [42], and 3.) the plant senescence reflectance index, PSRI [18]:

$$mSR_{705} = \frac{(R_{750} - R_{445})}{(R_{705} - R_{445})} \quad (1)$$

$$mND_{705} = \frac{(R_{750} - R_{705})}{(R_{750} + R_{705} - 2R_{445})} \quad (2)$$

$$PSRI = \frac{(R_{678} - R_{500})}{R_{750}} \quad (3)$$

Indices were plotted over both time and total chlorophyll content.

2.6.2 Histological analysis

Total width and palisade and spongy mesophyll width were determined using the 100 μm scale provided in each photo, from which a new ruler was constructed with 10 μm increments. To determine the proportion of airspace versus cells in the spongy mesophyll, we determined the percentage of pixels in an area of interest within the range of colour that typified air space using the Matlab Image Processing Toolbox. One to three areas of interest were delineated per image, to encompass as much of the spongy mesophyll as possible while avoiding veins. There were 114 images in total for the various species/light regime combinations (ASU, 34, ASH, 23, PSU, 26, PSH, 31) covering the seven sampling dates in 2003. Per sampling date, there were 2-5 images per species/light regime combination.

2.6.3 Correlation analysis

Correlation analysis was performed to determine the strength of relationships between chlorophyll content (total chlorophyll, chlorophyll *a* and *b*) and spectral indices (mSR_{705} , mND_{705} , PSRI) as well as anatomical features (total, palisade mesophyll and spongy mesophyll width, percent intercellular space) and spectral wavebands (445.9, 499, 548.5, 676.7, 706.2, 748.6, 800.7, 849.4, 901.2, and 949.6 nm) or indices. The wavebands listed provide a representation throughout the visible and near-infrared regions and several are also used in computation of indices (Eq. 1-3). Correlation analyses involving chlorophyll content were performed using 2002 data, for which we had a greater number of sampling

dates; those involving histological features were restricted to the 2003 dataset. All samples were tested for normality before the t-tests and we consider an equal variance between sample groups.

3. Results

3.1 Results

In autumn 2002, for neighbouring *P. tremuloides* and *P. balsamifera* trees along the south side of the North Saskatchewan River, visible differences in the timing of senescence events were apparent between species. *P. balsamifera* was the first to show signs of leaf yellowing and the first to undergo total leaf drop. By October 15 (day 288), 99% of *P. balsamifera* leaves had fallen. For *P. tremuloides*, all leaves had abscised by October 26 (day 299).

3.1.1 Indices and chlorophyll content

Over the time course of autumn senescence, several key differences may be observed between species and/or illumination category (Figure 1). When plotted according to date, mSR₇₀₅ and mND₇₀₅ for ASH leaves are consistently higher than for ASU leaves. For *P. balsamifera*, sun leaves generally exhibit higher values of both indices until near the end of the study period. At day 281, this phenomenon is reversed, and at day 285, values are near equal for PSU and PSH leaves. Over both species and illumination categories, ASU leaves exhibit the highest mSR₇₀₅ and mND₇₀₅ values; PSH leaves generally (with exceptions) show the least. When plotted against chlorophyll content, *P. balsamifera* leaves tend to have slightly higher mSR₇₀₅ and mND₇₀₅ values than *P. tremuloides*, but little distinction may be made between sun and shade leaves.

For mature leaves to early senescent leaves, PSRI for *P. balsamifera* leaves are slightly positive whereas for *P. tremuloides* leaves are slightly negative. The slightly positive PSRI values for mature *P. balsamifera* leaves are of note, as other documented species with dark-green plant tissues and high chlorophyll content have all exhibited slightly negative PSRI values (Merzlyak *et al.* 1999). Within *P. tremuloides*, ASH PSRI values are higher than for ASU until after day 274. Thus, in later stages of senescence, higher PSRI is observed for ASU leaves. PSU and PSH PSRI values are similar in mature to early senescing leaves, but in late senescence (days 281 and 285), PSRI is higher for PSU leaves. When comparing the two species, PSRI increases sooner for *P. balsamifera* (~day 272) than for *P. tremuloides* (day 285). When plotted against chlorophyll content, PSRI curves are similar for both species and illumination category, although there are no PSRI values for *P. tremuloides* above 0.07.

Prior to chlorophyll breakdown, shade leaves of both *P. balsamifera* and *P. tremuloides* have higher total chlorophyll and chlorophyll *a* contents (Figure 2). Similarly, *P. balsamifera* shade leaves have higher chlorophyll *b* contents. Chlorophyll *b* for *P. tremuloides* leaves, prior to chlorophyll breakdown, is similar for sun and shade leaves, but higher in shade leaves once chlorophyll content declines. Comparing the two species, *P. tremuloides* had higher initial total chlorophyll and chlorophyll *b* contents than *P. balsamifera*. For both trees, declines in chlorophyll content in 2002 began approximately day 255 (Sept 12). For *P. tremuloides*, the decline was faster for sun leaves than for shade leaves. *P. balsamifera* leaves reversed this role in that total chlorophyll and chlorophyll *a*

contents decreased sharply for shade leaves during mid-senescence (from approximately day 255 to day 270). Overall, declines in chlorophyll *a*, *b* and total chlorophyll were sharper and more variable for *P. balsamifera* than for *P. tremuloides*.

Figure 1. Spectral indices (modified simple ratio, mSR705; modified normalized difference index, mND705; plant senescence reflectance index, PSRI) plotted according to time (left) and total chlorophyll content (right) for *P. tremuloides* sun (ASU) and shade (ASH) leaves and *P. balsamifera* sun (PSU) and shade (PSH) leaves. Each symbol represents an average from three leaves.

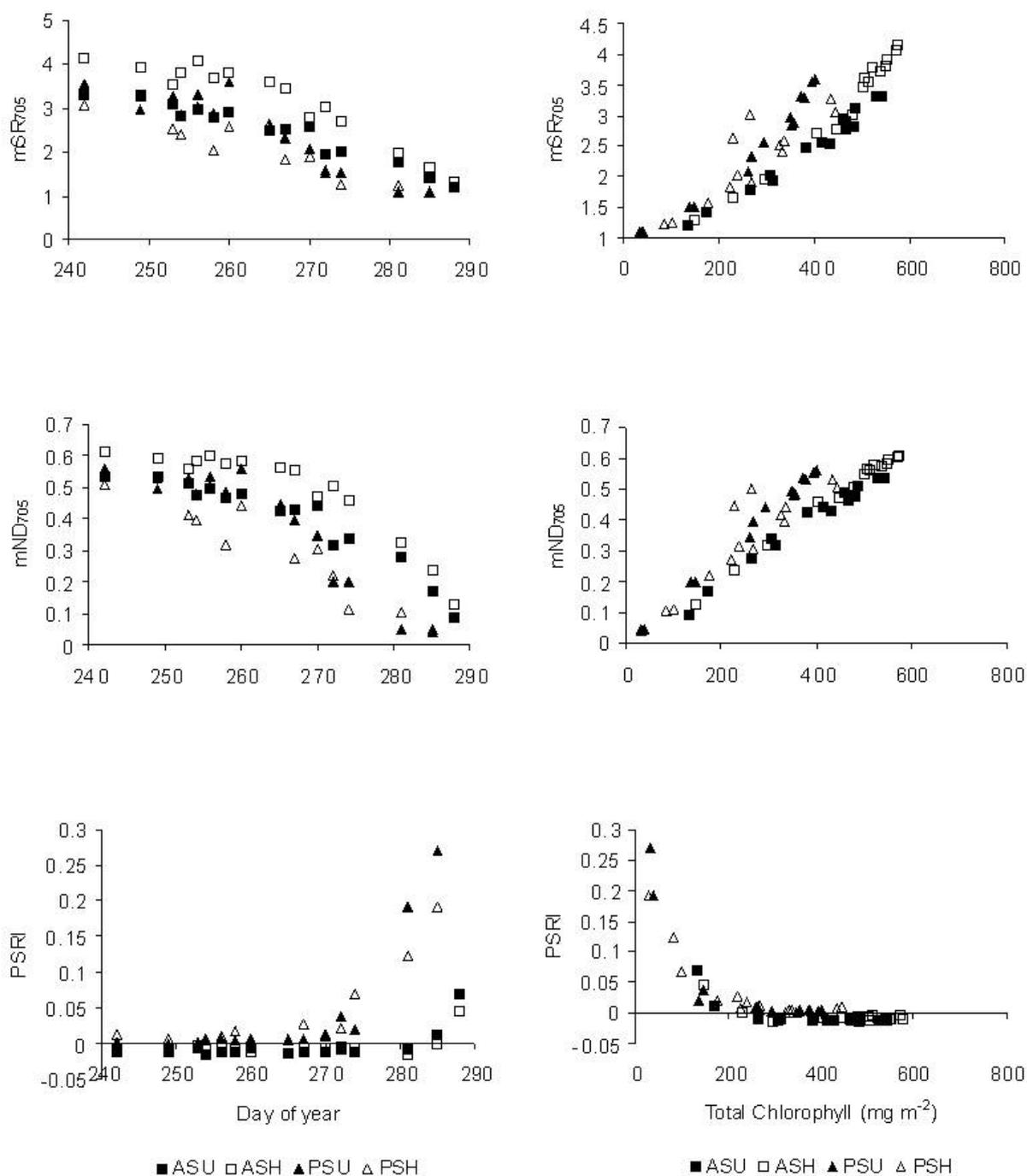
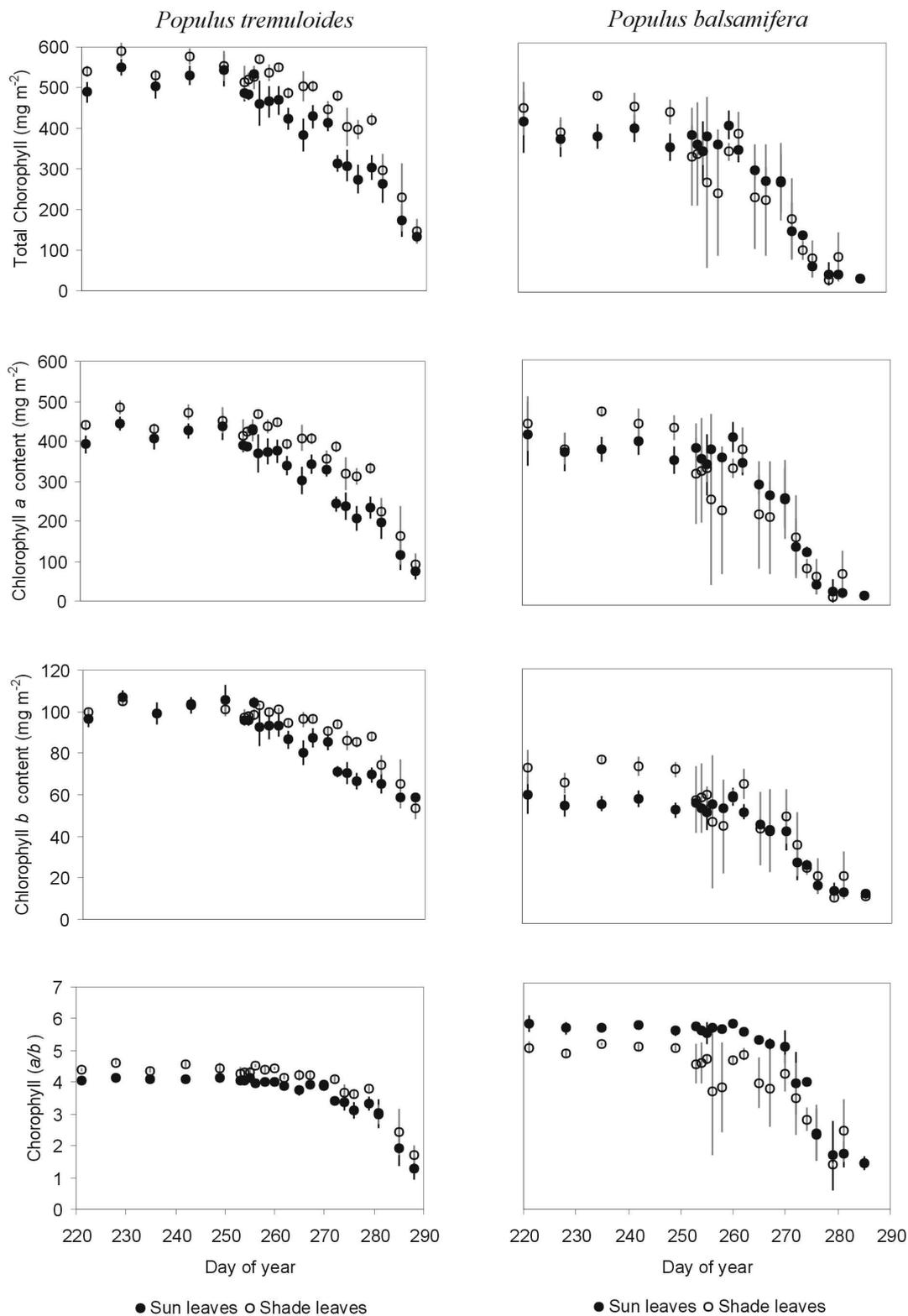


Figure 2. Changes in total chlorophyll, chlorophyll *a* and *b* and chlorophyll *a/b* during senescence of *P. tremuloides* and *P. balsamifera* sun and shade leaves. Each symbol represents the mean \pm 1 SD from three leaves, where chlorophyll content is based on regressions based on SPAD curves. Leaf drop occurred by day 288 for *P. balsamifera* and day 299 for *P. tremuloides*.



P. balsamifera and *P. tremuloides* differ markedly in the behaviour of the chlorophyll a/b prior to and during senescence. *P. tremuloides* has lower initial chlorophyll a/b but its decline started later than in *P. balsamifera* (approximately day 262 for *P. tremuloides* versus day 254 for *P. balsamifera*). Throughout the entire period, chlorophyll a/b for *P. tremuloides* shade leaves is greater than for sun leaves whereas the reverse is observed in *P. balsamifera* (also observed for the 2003 dataset with the exception of two dates (day 268, Sept 25 and day 274, Oct 1) for *P. balsamifera*, data not shown).

In the near-infrared, reflectance at 800 nm was significantly higher for *P. balsamifera* (mean \pm 1SD = 51.68 \pm 5.25%) than for *P. tremuloides* (mean \pm 1SD = 47.50 \pm 2.86%) (based on a two-tailed t-test, $P < 0.001$). Significant differences were not observed between sun and shade leaves for either species, however ($P > 0.05$). Over time, reflectance at 800 nm for both species remained nearly constant (data not shown).

3.1.2 Histological analysis

Analysis of leaf thin sections also revealed a number of important differences between *P. tremuloides* and *P. balsamifera* and their sun-lit and shaded leaves during senescence. Statistical tests were not performed due to the limited number of samples in some categories, and one of the limitations of the histological analysis is a lack of *P. tremuloides* shade leaves at low chlorophyll contents ($< 250 \text{ mg m}^{-2}$) (Table 1). Key findings from Table 1 and visual observations include the following:

Comparisons between *P. tremuloides* and *P. balsamifera*

P. tremuloides leaves are thinner than *P. balsamifera* leaves

P. tremuloides leaves have lower percent intercellular space in the spongy mesophyll than *P. balsamifera* leaves

Comparisons between sun and shade leaves

Sun leaves are thicker than shade leaves (Figure 3)

For sun leaves, the palisade mesophyll is thicker than the spongy mesophyll (Figure 3)

For *P. tremuloides* shade leaves, the palisade mesophyll is thicker than the spongy mesophyll but for *P. balsamifera* shade leaves, the spongy mesophyll is thicker than the palisade mesophyll (Figure 3)

Both sun and shade leaves of *P. tremuloides* and *P. balsamifera* had two palisade cell layers

For mature leaves, percent intercellular space was greater in shade leaves than for sun leaves, notably for *P. tremuloides*

Comparisons between mature and senescing leaves

For sun leaves, percent intercellular space is higher in senescing leaves

Cell disintegration in the spongy mesophyll was evident in late stages of senescence (Figure 4).

Table 1. Comparison of anatomical features and near-infrared reflectance at 800.7 nm for mature and senescing *P. tremuloides* and *P. balsamifera* leaves.

Variable	Mature (Total Chl>400mgm ⁻²)		Senescing (Total Chl<250mgm ⁻²)	
	ASU (n=4)	ASH (n=8)	ASU (n=3)	ASH
Total thickness (µm)	88.43(1.42)	68.09(7.09)	84.73(16.52)	NA
Thickness palisade (µm)	49.35(2.61)	31.02(4.76)	48.13(12.24)	NA
Thickness spongy (µm)	29.90(0.58)	27.19(3.47)	26.88(4.88)	NA
Intercellular space (%)	28.35(4.00)	37.06(5.94)	41.28(10.97)	NA
R _{800.7}	56.07(6.87)	54.46(4.06)	52.63(3.27)	NA
	PSU (n=2)	PSH (n=4)	PSU (n=5)	PSH (n=3)
Total thickness (µm)	100.70(21.07)	80.24(8.05)	109.24(18.62)	77.83(13.61)
Thickness palisade (µm)	49.50(9.90)	28.47(0.66)	44.12(8.59)	35.27(13.74)
Thickness spongy (µm)	42.60(10.39)	42.25(7.89)	55.12(10.43)	39.50(11.36)
Intercellular space (%)	46.62(6.55)	50.66(5.46)	51.08(9.85)	48.22(12.78)
R _{800.7}	58.71(0.10)	55.59(3.65)	56.41(2.43)	55.03(1.55)

Note: NA=not available. No ASH samples with total chlorophyll< 250 mg m⁻² from the 2003 dataset were gathered. R_{subscript} is reflectance at the indicated waveband. Sample sizes indicate number of thin sections used to compute means. For thickness, five measurements were made and averaged per thin section. *P. tremuloides* sun (ASU) and shade (ASH), *P. balsamifera* sun (PSU) and shade (PSH).

Figure 3. Mean palisade and spongy mesophyll thickness (± 1 SD) for mature *P. tremuloides* sun (ASU) and shade (ASH) leaves and *P. balsamifera* sun (PSU) and shade (PSH) leaves, based on measurements from August 8, 2003 thin sections (n=2-5).

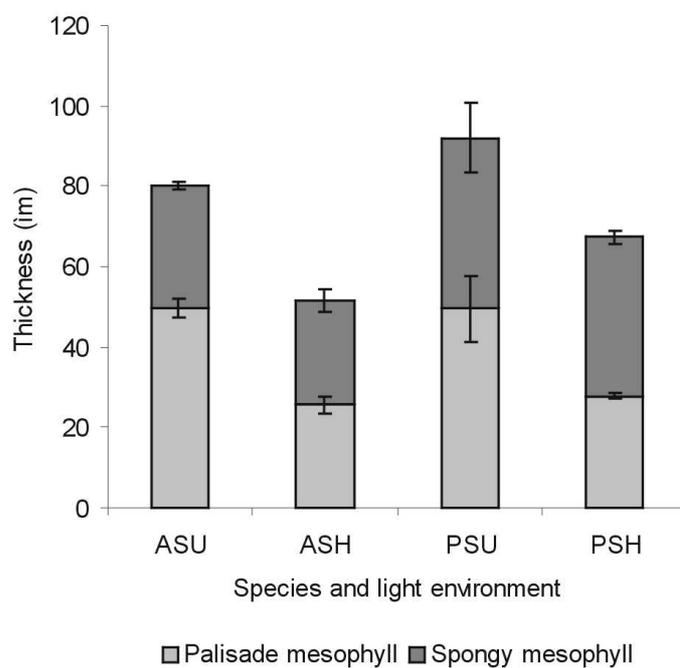
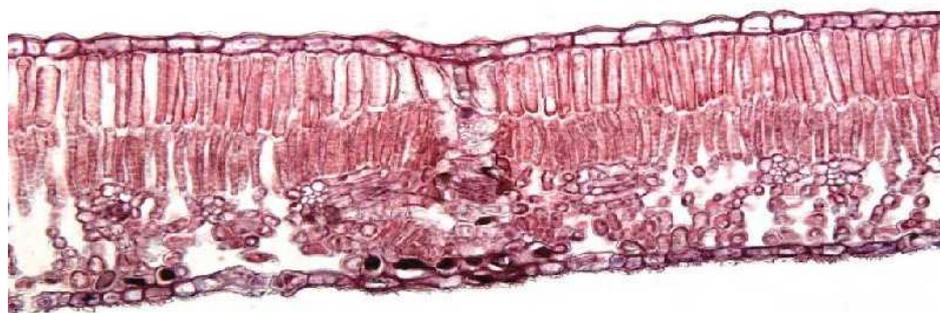
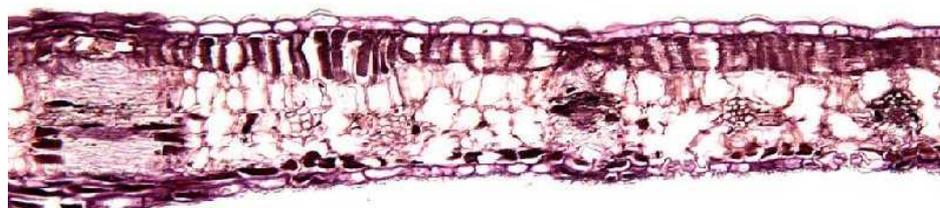


Figure 4. Thin sections of mature (a) and senescing (b) *P. tremuloides* sun leaves.



(a)



(b)

100 µm



3.1.3 Correlation analyses

Highly significant ($P < 0.01$) correlation coefficients were found between the chlorophyll indices (mSR₇₀₅, mND₇₀₅) and chlorophyll a, b, total chlorophyll and chlorophyll a/b (exception: ASU mSR₇₀₅ with chlorophyll a/b, significant at $P < 0.05$) (Table 2). PSRI was not included in the correlation analysis with chlorophyll because the relationship was non-linear (Figure 1).

There were fewer significant relationships between spectral bands and leaf anatomical features (total thickness, palisade mesophyll thickness, spongy mesophyll thickness, percent intercellular space). Of particular interest, no significant correlations were found between any of these leaf traits and wavebands in the near-infrared. This is consistent with our finding that reflectance at 800.7 nm was fairly stable over a large range of chlorophyll contents within each species. However, a number of significant relationships were detected between leaf traits and wavebands in the chlorophyll absorption wells (445.9 nm, 676.7 nm) as well as with PSRI. This was the case for ASU leaves, in which all leaf traits (total thickness, palisade mesophyll thickness, spongy mesophyll thickness, percent intercellular space) were significantly ($P < 0.05$) correlated with reflectance at 445.9 nm, 676.7 nm, as well as with PSRI. For *P. tremuloides* shade leaves, the only significant correlations found were between palisade mesophyll thickness and PSRI and between percent intercellular space and PSRI. For *P. balsamifera* sun leaves, palisade mesophyll thickness was correlated ($P < 0.05$) with R_{445.9}, R_{676.7} and PSRI. No significant relationships existed between *P. balsamifera* shade leaves and any of the leaf anatomical traits.

Table 2: Correlation coefficients (r) for relationships between spectral vegetation indices and leaf chlorophyll content (2003 data)

Leaf type	Index	Chl a	Chl b	Chl a/b	Total Chl
ASU	mS	0.91	0.96	0.71	0.930*
	R ₇₀₅	9**	1**	1*	*
	mN	0.92	0.91	0.79	0.929*
	D ₇₀₅	5**	0**	0**	*
ASH	mS	0.98	0.98	0.97	0.985*
	R ₇₀₅	5**	2**	1**	*
	mN	0.98	0.98	0.98	0.980*
	D ₇₀₅	0**	1**	1**	*
PSU	mS	0.99	0.99	0.91	0.992*
	R ₇₀₅	2**	0**	1**	*
	mN	0.98	0.98	0.96	0.982*
	D ₇₀₅	2**	5**	2**	*
PSH	mS	0.97	0.96	0.85	0.972*
	R ₇₀₅	4**	0**	9**	*
	mN	0.98	0.97	0.90	0.982*
	D ₇₀₅	2**	7**	8**	*

Note. * $P \leq 0.05$; ** $P \leq 0.01$. *P. tremuloides* sun (ASU) and shade (ASH), *P. balsamifera* sun (PSU) and shade (PSH).

4. Discussion

4.1 Comparison of species

Several key differences in leaf spectral reflectance and leaf traits between *P. tremuloides* and *P. balsamifera* have been identified in this study. Overall, the earlier onset of senescence in *P. balsamifera* as compared to *P. tremuloides* was reflected in an earlier rise in PSRI, an index designed for this purpose (Figure 1). Two additional indices, mSR₇₀₅ and mND₇₀₅, which tightly correlate with chlorophyll content, were useful in monitoring trends in chlorophyll content over the progress of senescence (Table 2). Values of these indices were generally higher for *P. tremuloides*, with the higher total chlorophyll content. *P. balsamifera*, on the other hand, exhibited higher chlorophyll a/b. Leaf thickness and percentage intercellular space were also greater in *P. balsamifera* (Table 1), which corresponds well with its significantly higher mean reflectance at 800.7 nm ($P < 0.001$).

Differences in reflectance indices between species at key times of the year may have important implications for detecting species from a remote sensing perspective. This will be the case only if these differences are consistent between species and are transferred to the canopy level. Further study using hyperspectral airborne or space-borne imagery would be necessary to answer these types of questions. A significant complicating factor will be non-synchronous timing of leaf colour changes and leaf fall between clones, which has been known to occur within the same stand [38].

4.2 Comparison of sun and shade leaves

For both species, many of the expected differences between their sunlit and shaded leaves were observed, including greater total thickness and, in particular, greater palisade mesophyll thickness in sunlit leaves (Table 1). That mature shade leaves had greater percent intercellular space than mature sun leaves is consistent with [25,26]. Higher proportion of spongy mesophyll in shade leaves may serve to increase internal light scattering and absorptance under low light levels [39]. The two species, both shade-intolerant, therefore appear capable of some degree of morphological plasticity due to light environment, based on their variability in leaf histological features [20,23]. However, these differences did not result in significant differences ($\alpha = 0.05$) in reflectance at 800.7 nm between sun and shade leaves for either species. An earlier study by [31] concluded similarly, for which sun-adapted plants had similar optical properties in the near-infrared to shade-adapted plants.

Although total chlorophyll was initially higher for shade leaves for both *P. tremuloides* and *P. balsamifera*, this trend continued for *P. tremuloides* but destabilized in *P. balsamifera* with the onset of senescence (Figure 2). The most striking difference between sun and shade leaves of the two species was observed for chlorophyll a/b. For *P. tremuloides*, shade leaves consistently had higher chlorophyll a/b whereas for *P. balsamifera*, sun leaves almost always had the higher chlorophyll a/b. Since previous studies have shown a tendency for chlorophyll a/b to decrease with decreasing light availability [27,28,29], our results for *P. tremuloides* are contrary to the norm.

4.3 Comparison of mature and senescing leaves

Higher percent intercellular space for senescing ASU and PSU than for mature ASU and PSU (Table 1) are an indication of changes in the mesophyll such as cell wall breakdown, also evident in thin section photographs (Figure 4) (PSH leaves did not show this trend, however). Unfortunately, based on our data, it does not appear possible to track these types of changes, determined from simple leaf thin sections, using wavebands in the NIR. It is important to note here, however, that while large sample sizes were attained of reflectance spectra and for chlorophyll analyses, sample sizes for the histological analysis were limited due to the greater amount of time and detail required (see Methods). Larger sample sizes could have strengthened our conclusions based on Table 1.

Overall, correlations between internal anatomical features and wavebands in the NIR were poor ($P > 0.05$). Likewise, correlations between NIR and % intercellular space were weak in a study by [40], who used more labour-intensive oblique-paradermal sections to determine leaf section parameters. In their case, parameters highly correlated with NIR included the ratio of mesophyll cell surface area exposed to intercellular space per unit leaf area, leaf bicolouration, and the presence of a thick leaf cuticle. Their finding agrees with [15], who indicated that the number or total area of the air-cell wall interfaces may be a more important parameter for determining reflectance than the volume of air space. He also suggested that, with regard to internal light-scattering mechanisms, the palisade mesophyll may be just as important as the spongy mesophyll. On the other hand, we found significant correlation ($P < 0.05$) between transmittance at 800.7 nm and both percent intercellular space and spongy mesophyll width for nine tropical species with a much greater range for these three variables [41]. It appears that percent intercellular space and spongy mesophyll width may be more useful for prediction in the near-infrared in multiple species with dissimilar internal morphologies than for same-species samples covering the progress of senescence.

Chlorophyll a/b declined with time for *P. tremuloides* and *P. balsamifera* during autumn senescence (Figure 2). This decline is consistent with work by Sanger (1971), who also observed a decline in chlorophyll a/b in senescing *P. tremuloides* leaves. It does not correspond with findings by Dean *et al.* (1993), however, who observed an increase in chlorophyll a/b for this same species during senescence.

5. Conclusions

Throughout this study, we aimed to improve our understanding of the spectro-temporal dynamics of *P. tremuloides* and *P. balsamifera* leaves by characterizing changes in leaf traits in autumn and determining their correspondence with changes in leaf spectral characteristics. Since our results cover limited sample sizes over two autumn periods (2002 and 2003), we cannot make generalizations about their senescence behaviour over time nor space. Changes in chlorophyll content during this period, however, were well correlated with two indices, mSR_{705} and mND_{705} [42]. Deterioration of the mesophyll, however, while evident in thin section photographs, was not evident in a NIR response. This result was unexpected. In fact, [14] stated ‘collapse of the mesophyll’ as a common predictor for decreases of NIR. This predictor was not effective in explaining NIR levels in our data, and may not be useful over all species or leaf types. Transmittance in the NIR was not found to change with leaf

senescence either in a study by [43] on beans (*Phaseolus vulgaris* L.). On the contrary, several mesophyll features (palisade and spongy mesophyll thickness and percent intercellular space) were significantly correlated with PSRI, which incorporates wavebands from both the visible and NIR regions. This index should be explored to a greater extent for the purpose of tracking mesophyll breakdown in senescing leaves.

Sampling over multiple years, over a greater number of trees representing clones and over a broader range of sites would further extend our knowledge of the ecology of these species, particularly if coupled with study of satellite-borne hyperspectral imagery. Research of this type could indicate the potential for species discrimination and potentially clone discrimination from remotely sensed data, especially at critical times of the year such as autumn senescence. Extending the present study to cover the period of spring flush could expose additional key differences between these major hardwood components of the forest resource in western Canada.

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