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Physiological and morphological acclimation to height in cupressoid leaves of 100-year-old *Chamaecypris obtusa*

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Abstract

Many species of Cupressceae have cupressoid (scale-like) leaves, which are morphologically and functionally intermediate between branches and leaves. While past studies on height acclimation of cupressoid leaves have focused on acclimation to the vertical light gradient, the relationship between morphology and hydraulic function remains unexplored. Here, we compared physiological and morphological characteristics between treetop and lower-crown leaves of 100-year-old *Chamaecyparis obtusa* trees (ca. 25 m tall) focusing on height-acclimation of hydraulic properties. Treetop leaves were physiologically acclimated to water stress: lower osmotic potential at turgor loss and at saturation, higher cell-wall elasticity compared to lower-crown leaves. Leaf hydraulic conductance, however, was not affected by height. Treetop leaves showed acclimation to high light availability: greater leaf mass per area, greater leaf area per shoot, higher branching frequency, and higher photosynthetic rate compared to lower-crown leaves. We inferred that transfusion tissue flanking the leaf vein, which were more developed in the treetop leaves, contributed to maintenance of leaf hydraulic conductance by facilitating efficient water transport from xylem to surrounding mesophyll. This may be an anatomical adaptation to overcome hydraulic limitation of photosynthetic production with increasing height.

Keywords: hydraulic conductance, hydraulic limitation, light acclimation, photosynthesis, transfusion tissue

Introduction

Many species of Cupressceae have cupressoid (scale-like) leaves, which are morphologically and functionally intermediate between branches and leaves (De Laubenfels 1953, Eckenwalder 1976, Harlow et al. 2005, Little 2006). The bifurcating growth pattern of cupressoid leaves produces fronds or fern-like foliage clusters (Parker and Johnson 1987), whose structure is analogous to the branching pattern of shoot axes as well as to the venation pattern of broadleaves. The morphology of fronds, especially their branching pattern is an important component of morphological plasticity contributing to height- and light-acclimation of foliage within the crown of Cupressceae species such as *Chamaecyparis obtusa* Endl. (Utsugi 1999) and *Thuja plicata* J. Donn. ex D. Don. (Edelstein and Ford 2003).

In tall trees, the light environment of leaves changes markedly within the crown such that treetop and lower-crown leaves express highly different physiological and morphological characteristics (e.g., Ishii et al. 2002, Ishii et al. 2008, Mullin et al. 2009, Cavaleri et al. 2010). While light availability for photosynthesis is high near the treetop, hydraulic constraints incur constant water stress, while the opposite conditions prevail in the lower crown. Morphological acclimation to the vertical environmental gradient is well documented for gymnosperms having pinoid (needle-like) leaves (e.g., Brooks et al. 1996, Stenberg et al. 1998, Niinemets et al. 2002, Ishii et al. 2007). In comparison, there are only a few studies conducted on species having cupressoid leaves (Utsugi 1999, Edelstein and Ford 2003, Harlow et al. 2005).

Leaf morphological characteristics are important determinants of hydraulic properties both among (Zwieniecki et al. 2004, Brodribb et al. 2005) and within species (Oldham et al. 2010, Coble and Cavaleri 2015; Chin and Sillett 2016). For example, hydraulic conductance of broadleaved angiosperms increases with increasing venation because water can be transported more efficiently to terminal cells within the leaf (Brodribb et al. 2007, Scoffini et al. 2011, Sack and Scoffini 2013). In comparison, single-veined gymnosperm leaves lack vessels for efficient water transport and thus have lower leaf hydraulic conductance (Brodribb et al. 2005). At the branch-level, on the other hand, bifurcations form constrictions in the hydraulic pathway such that hydraulic conductivity decreases with increasing branching frequency (Tyree and Ewers 1991).

While past studies on height acclimation of cupressoid leaves have focused on acclimation to the vertical light gradient, the relationship between leaf morphology and hydraulic function remains unexplored. In tall *T. plicata* trees (42−45 m in height), foliage concentration within fronds increases from lower to upper crown as a result of increasing branching frequency (Edelstein and Ford 2003). While such morphological acclimation may be adaptive for increasing leaf area for light interception, the effects on hydraulic function have not been investigated.

Here, we studied the cupressoid leaves of *C. obtusa* in a 100-year-old plantation (canopy height = 25m). We compared physiological and morphological characteristics between treetop and lower-crown leaves focusing on height-acclimation of hydraulic properties. *C. obtusa* is a commercially important plantation species in Japan and many physiological studies have been conducted on seedlings, saplings and young trees (e.g., Yokota and Hagihara 1996, Nagakura et al. 2004, Han and Chiba 2009, Araki et al. 2010, Miyamoto et al. 2013). Old-growth *C. obtusa* forests in Japan, however, contain individuals ca. 300 years old (Hoshino et al. 2001) and individual life-span may exceed 1000 years. In addition, plantations exceeding harvesting age (40−60 years) are increasing due to recent decline of the domestic timber market. Thus, there is increasing demand for scientific knowledge regarding the physiological characteristics of large, old *C. obtusa* trees.

Materials and Methods

Study site and sampling

The study was conducted in a 100-year-old *C. obtusa* plantation (ca. 1 ha) owned by Enryakuji Temple on Mt. Hiei, Ohtsu City, Shiga Prefecture, Japan (35°5′28″N, 135°50′5″E, 720 m ASL). According to the Japan Meteorological Agency database (www.data.jma.go.jp), annual mean temperature and total precipitation in the region during 1986−2015 were 15˚C and 1579 mm, respectively. The plantation is located in the Jishugongen area of the sacred mountain. Stand density was 300 trees ha^{-1}. Mean diameter at breast height (DBH, 1.3 m above average ground level) and tree height (measured in 2004) of the *C. obtusa* trees were 37.2 cm and 22.2 m, respectively (Suzuki et al. 2007).

We selected three *C. obtusa* trees in the plantation for sampling (Table 1). We climbed the trees using single-rope technique and sampled foliated branches ca. 30 cm long (Fig. 1a) from the treetop and the lowest branch in the crown of each tree. The samples were brought to the ground, recut underwater, sealed in black plastic bags, and transported to the laboratory for measurement.

To quantify the light environment for each sample branch, hemispherical photographs were taken above each sampling location using a fisheye lens (FC-E8, Nikon Inc., Tokyo, Japan) attached to a digital camera (Coolpix 990, Nikon Inc., Tokyo, Japan).

The scale-like leaves of *C. obtusa* occur in pairs at right angles to each other and the adaxial surface is appressed to the shoot axis. This results in a bifurcating branching pattern as the shoot elongates. Due to their indeterminate growth pattern, leaf age cannot be determined accurately. Because our objective was to compare functionally equivalent growth units, we chose to sample similar-sized terminal ends of each branch consisting of small foliated sections ca. 5 cm in length (Fig. 1a, hereafter: sample shoots).

Leaf physiology

To compare water-relations characteristics between upper- and lower-crown leaves, we obtained the pressure-volume curve of three sample shoots from each branch, using the bench-drying approach to the pressure-volume technique (Tyree and Hammel 1972, Schulte and Hinckley 1985). Using a pressure chamber (Model 1000, PMS Instruments, Corvallis, USA), we repeatedly measured bulk leaf water potential (Ψ_L, MPa) and fresh weight (g) of the sample shoots (repeat pressurization method, Hinckley et al. 1980, Ritchie and Roden 1985, Parker and Colombo 1995). Care was taken to increase and decrease the pressure in the chamber very slowly (less than 0.01 MPa s^{-1}) so as not to damage the sample shoots.

We measured leaf hydraulic conductance (K_{leaf}, mmol m^{-2} s^{-1} MPa) following methods described in Brodribb and Holbrook (2003). Based on data obtained from the pressure-volume curves, the sample branches were bench dried in darkness until bulk leaf water potential reached approximately half of turgor loss point ($\Psi_{0,\ \mathrm{bulk}}$ leaf water potential before rehydration). This was confirmed by regularly sampling and measuring bulk leaf water potential of small shoots adjacent to the sample shoot to be sampled. Then four sample shoots were detached underwater and rehydrated by allowing them to absorb water from the cut end. After taking note of the time required for rehydration, we measured bulk leaf water potential after rehydration (Ψ_t) and calculated K_{leaf} as:

$$K_{\mathrm{leaf}} = C \ln (\Psi_0/\Psi_t) / t \qquad [1]$$

Where C is leaf capacitance obtained from the pressure-volume curve (Brodribb and Holbrook 2003).

We measured light-saturated maximum net photosynthetic rate ($P_{\max}$) of terminal foliage on each sample branch using a portable gas exchange system fitted with an LED light source (LI-

6400, Li-Cor Inc., Lincoln, NE, USA). Air temperature and CO_2 concentration in the cuvette were maintained at 25°C and 380 ppm, respectively. We adjusted the light intensity inside the cuvette to 500 μmol m^{-2} s^{-1} and this was increased gradually to 1600 μmol m^{-2} s^{-1} to obtain P_{max}. Following the measurement, the leaves inside the cuvette (2×3 cm) were carefully cut out and scanned using the flatbed scanner for measurement of leaf area and then oven dried to constant weight to determine leaf mass.

Leaf morphology and anatomy

We measured the morphology of all sample shoots used for water-relations measurement. All sample shoots from each branch were photographed on a slide-viewer to obtain the silhouette image for measurement of the shoot silhouette area (Fig. 2). Then the shoots were divided into small sections, laid without overlap on the slide-viewer and photographed to obtain the projected (one-sided) leaf area. Finally, the leaves were oven-dried to constant weight to obtain leaf dry mass (M_L, g).

To observe leaf anatomy, terminal leaves were sampled from each sample branch, fixed in FAA (formalin, acetic acid, 50% ethyl alcohol; 5:5:90 v/v), and washed under tap water overnight. Leaf samples were then transversely sectioned to 26 μm thickness using a sliding microtome (REM-710, Yamato Kohki Industrial Co., LTD., Japan). In every sample, leaf sections were taken consistently from third-order axes (Strahler ordering, Fig. 1b). Transverse sections comprising the median leaf and two lateral leaves (Fig. 1c) were made and double stained with safranin-fast green to differentiate lignified cells and living cells. Our objective was to observe two anatomical features related to leaf hydraulics, xylem and transfusion tissue. In a preliminary study, we made several continuous cross-sections and found that this was the section of the leaf where the leaf vein of the two lateral leaves could be clearly distinguished as having bifurcated from the vascular bundle of the shoot axis and xylem cells and transfusion tissue could be clearly observed. Five to eight leaf sections were taken from each sampled branch and observed under an optical microscope (Nikon, Eclipse 80i, Nikon, Tokyo) and photographed with a digital camera (E-620, Olympus, Tokyo).

Data analysis

The hemispherical photographs were analyzed using Gap Light Analyzer ver. 3.0 (Simon Fraser University, Burnaby, BC, Canada) to obtain canopy openness (%), a measure of light availability.

The pressure-volume curves were analyzed to calculate osmotic potential at saturation (Ψ_{sat}), osmotic potential at turgor loss (Ψ_{tlp}), relative water content at turgor loss (RWC_{tlp}), and elastic modulus (ε) at the bulk leaf level following methods described in Tyree and Hammel (1972) and Schulte and Hinckley (1985).

The silhouette images of the sample shoots were analyzed using Image-J (ver. 1.48, National Institute of Health, USA) to calculate shoot silhouette area (A_S). The scanned images of the non-overlapping shoot sections were used to calculate projected leaf area (A_L), leaf perimeter (P_S), and branching frequency (F_b, total number of bifurcating junctions within the shoot). Using these measurements, we defined the following variables of leaf/shoot morphology:

$\mathrm{LMA} = M_L / A_L$

$\mathrm{SPAR} = A_S / A_L$

P_S and F_b were divided by A_L to standardize measurements for comparison of leaf concentration (sensu Edelstein and Ford 2003) among different-sized shoots.

Photographed images of the leaf sections were analyzed to quantify the transverse-sectional area of xylem (A_X, µm^2) and of transfusion tissue (A_{TT}, µm^2) of the two lateral leaves using Adobe Photoshop software (ver. Adobe Systems Inc., San Jose, CA, USA).

All variables were compared in a two-way ANOVA with crown position (treetop/lower-crown) as the main effect and sample tree as a random effect. Individual differences were tested using Tukey's HSD test. A_X and A_{TT} were highly variable among samples and not normally distributed. Therefore, we used non-parametric analysis (Wilcoxon rank sum test) to compare treetop and lower-crown leaf anatomy by pooling the data for all three trees after confirming there was no significant tree effect (Kruskal-Wallis test). All statistical analyses were done using JMP 10J (SAS Institute Japan, Tokyo, Japan).

Results

Light availability at treetop was much higher than at lower-crown, as indicated by greater canopy openness (64.4 ± 15.0 and 6.7 ± 2.7%, respectively, mean ± s.d., $F = 45.89$, $P = 0.0025$).

In each tree, treetop leaves had lower Ψ_{sat} and Ψ_{tlp}, and higher ε than lower-crown leaves (Fig. 3, $F = 7.93$, $P = 0.0119$; $F = 12.80$, $P = 0.0023$; $F = 63.44$, $P < 0.0001$, respectively). While RWC_{tlp} was generally higher for the treetop leaves ($F = 7.93$, $P = 0.0119$, within each tree, the effect of crown position was not statistically significant ($P = 0.1293$, 0.3235, 0.0886 for tree 701, 706, and 750, respectively). By contrast, K_L was not affected by height ($F = 0.21$, $P = 0.651$). In each tree, P_{max} of treetop leaves was higher than that of lower-crown leaves ($F = 16.59$, $P = 0.0152$).

Treetop leaves had greater LMA, smaller SPAR, higher branching frequency, and longer leaf perimeter than lower-crown leaves (Fig. 4, $F = 173.84$, $P < 0.0001$; $F = 6.27$, $P = 0.0150$; $F = 67.91$, $P < 0.0001$; $F = 16.60$, $P < 0.0001$, respectively), although the difference in SPAR was statistically significant for tree 750 only.

Transverse leaf cross sections comprised the vascular bundle of the shoot axis subtended by two lateral leaves (Fig. 5a). Transfusion tissue occurred on both sides of the leaf vein of the lateral leaves and was more developed in the treetop leaves than in the lower-crown leaves (Fig.

5b,c). In each tree, treetop leaves had larger transverse-sectional areas of both xylem and transfusion tissue (Fig. 6, $Z = 2.149$, $P = 0.0305$ and $Z = 3.020$, $P = 0.0024$, respectively). We divided A_{TT} by A_{X} to compare transfusion tissue area relative to xylem area and found that this value was also larger for treetop leaves ($Z = 2.542$, $P = 0.0106$).

Discussion

In addition to leaf-level acclimation to the vertical light gradient, leaf physiology and morphology change in response to the vertical gradient of water potential in tall trees (Woodruff et al. 2009, Cavaleri et al. 2010, Oldham et al. 2010, Azuma et al. 2016). In this study, treetop leaves of *C. obtusa* exhibited both physiological and morphological acclimation to water stress. Lower Ψ_{sat} reflects osmotic adjustment for maintaining turgor under low leaf water potentials, while lower Ψ_{tlp} reflects greater tolerance to water stress and higher ε reflects more rigid cell walls, which enable cells to retain water when leaf water potential decreases (Nardini et al. 1999, Pallardy 2007, Ishida et al. 2008). Such adjustments in water-relations characteristics of treetop leaves likely contributed to relative water contents at turgor loss similar or higher than that of lower-crown leaves, so that physiological function is maintained despite lower leaf water potentials.

Greater LMA and smaller SPAR of treetop leaves compared to lower-crown leaves reflected higher leaf concentration (i.e., greater leaf mass and leaf area per shoot), which increases light interception in the well-illuminated treetop (Edelstein and Ford 2003, Utsugi et al. 2006). In terms of leaf water relations, greater LMA means less evaporative surface area per dry mass, which prevents decreases in leaf-specific hydraulic conductivity under water-stressed conditions (Niinemets et al. 2002, Marshall and Monserud 2003). At the shoot level, high leaf concentration of treetop shoots was realized by high branching frequency. These leaf/shoot characteristics could also be associated with increasing mesophyll resistance to water transport within leaves and increasing number of terminal leaves to which water must be delivered, as well as increasing hydraulic resistance along the water transport pathway within shoots. Leaf hydraulic conductance of treetop leaves, however, was similar to that of lower-crown leaves, suggesting that water supply to leaves was maintained by some compensating mechanism.

In this study, we found that increasing amounts of transfusion tissue was associated with maintenance of leaf hydraulic conductance. Transfusion tissue is unique to gymnosperms (Hu and Yao 1981) and functions as connecting tissue between the leaf vein and mesophyll. They consist of transfusion tracheids, which transport water from the xylem, and transfusion parenchyma, which transport sugars to the phloem (Canny 1993). In *Podocarpus grayi* de Laub., for example, transfusion tracheids aligned perpendicular to the leaf vein, form an interconnected matrix that distributes water radially through the mesophyll (Brodribb and Holbrook 2005). For single-veined leaves, minimizing radial resistance to water transport form vein to mesophyll is an important determinant of their morphology (Zwieniecki et al. 2004). Theoretical models of leaf hydraulic properties indicate that high resistance to radial transport reduces the amount of leaf tissue surrounding the vein (Zwieniecki et al. 2004). Thus, for treetop leaves of *C. obtusa*, with high LMA, development of transfusion tissue would contribute to maintaining leaf hydraulic conductance by reducing radial resistance and increasing water transport efficiency. Transfusion tracheids have been documented in many gymnosperms including *Chamaecyparis* (Ivanescu et al. 2007, Dorken 2013). In *Sequoiadendron giganteum* (Lindl.), increasing amounts of transfusion tissue promotes hydraulic conductivity of treetop leaves (Chin and Sillett 2016). Our results suggest that, in treetop leaves of *C. obtusa*, they may facilitate water transport between the leaf vein and mesophyll to maintain leaf hydraulic conductance.

Recently, it was found that transfusion tissue also contribute to leaf water storage (hydraulic capacitance) in tall trees, mitigating negative effects of increasing water stress with increasing height (Ishii et al. 2014, Azuma et al. 2016). Transfusion tracheids have also been observed to collapse under water stress in *S. sempervirens* (Oldham et al. 2010), *Taxus baccata* (Zhang et al. 2014), and *C. japonica* (Azuma et al. 2016). Tracheid collapse is readily reversible suggesting they may function as a hydraulic buffer preventing xylem embolism (Brodribb and Holbrook 2005, Zhang et al. 2014, Azuma et al. 2016). In addition to their role in stress avoidance and hydraulic safety, our results suggest that transfusion tracheids may also contribute to hydraulic efficiency to maintain leaf hydraulic conductance.

Because leaves contribute a large proportion of whole-plant hydraulic resistance, they represent a potential hydraulic bottleneck in the water transport pathway (Woodruff et al. 2016). The efficiency of water conduction through leaves is critical to maintaining high photosynthetic rate, especially under water stressed conditions (Johnson et al. 2012). Leaf hydraulic conductance is positively correlated with maximum photosynthesis and stomatal conductance within and across species (Aasamaa et al. 2001, Brodribb et al. 2005, Brodribb et al. 2007). Anatomical features of the leaf related to leaf hydraulics are important drivers of maximum photosynthetic rate and ultimately of plant productivity (Brodribb et al. 2007). At the treetop, where light is readily available, water is the significant constraint on photosynthesis and maintenance leaf hydraulic conductance is critical to realizing high photosynthetic production. Because leaf hydraulic conductance plays a critical role in maintaining plant function (Brodribb et al. 2005), it is not surprising that tall trees exhibit acclimation responses to overcome hydraulic limitation of photosynthesis. Anatomical changes such as the increase in transfusion tissue in treetop leaves of *C. obtusa*, may represent adaptive strategies for overcoming hydraulic limitation.

Recently, Chin and Sillett (2016) proposed a heat-sink function of transfusion tissue, which may optimize leaf internal vapor flux to maintain stomatal aperture and increase hydraulic conductivity. Our observations and results show that spatial distribution of transfusion tissue within leaves of *C. obtusa* can be highly variable. Further studies are needed to elucidate their longitudinal distribution and its relationship with leaf hydraulic properties. Finally, it should also be noted that, because leaf photosynthetic rates and hydraulic conductance were measured in the laboratory, they represent potential values for the *C. obtusa* leaves in this study. Field measurements of leaf photosynthesis and transpiration are needed to elucidate whether the results observed here actually contribute to increasing photosynthetic production of treetop leaves in situ.

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Table 1. Diameter at breast height, crown height, and canopy openness (lower crown and treetop) of the three study trees of 100-year-old *Chamaecyparis obtusa*.

Tree	DBH (cm)	Height (m)	Canopy openness (%)	
			Low	Top
701	51.2	26.5	6.8	52.9
706	58.9	25.7	9.3	80.8
750	49.3	25.3	3.9	59.7

Figure captions

Figure 1. Branch (**a**), shoot (**b**), and leaves (**c**) of 100-year-old *C. obtusa*. Square in (**a**) shows the sampling unit (sample shoot). The dotted line in (**c**) shows the transverse section sampled for observation of leaf anatomy.

Figure 2. Silhouette images of treetop and lower-crown shoots of 100-year-old *C. obtusa* used for measurement of shoot silhouette area. The leaves were detached form the shoot and laid without overlap for measurement of projected leaf area.

Figure 3. Physiological characteristics of foliated shoots of 100-year-old *C. obtusa*: osmotic potentials at turgor loss (Ψ_{lp}) and at saturation (Ψ_{sat}), elastic modulus (ε), relative water content at turgor loss ($\mathrm{RWC}_{\mathrm{tlp}}$), leaf-area specific hydraulic conductivity (K_{L}), and leaf-area-based light-saturated photosynthetic rate ($P_{\mathrm{max}}/A_{\mathrm{L}}$) shown in relation to crown position for each study tree. Symbols and error bars show average and standard deviation respectively. Asterisks indicate significant difference between crown positions (*: $P< 0.05$, **: $P< 0.01$).

Figure 4. Morphological characteristics of foliated shoots of 100-year-old *C. obtusa*: leaf mass per area (LMA), shoot silhouette to projected leaf area ratio (SPAR), branching frequency per leaf area ($F_{\mathrm{b}}/A_{\mathrm{L}}$), and leaf perimeter per leaf area ($P_{\mathrm{L}}/A_{\mathrm{L}}$) shown in relation to crown position among trees. Symbols, error bars and asterisks are as in Fig. 3. (**: $P < 0.01$, ***: $P < 0.0001$)

Figure 5. Optical microscope image of the transverse-section of a treetop leaf of 100-year-old *C. obtusa* (**a**). Magnified image of rectangular region in (**a**) showing the transfusion tissue (tt) on both sides of the lateral leaf vein ($\mathrm{v_l}$), which includes the xylem (X) stained red (**b**). Transfusion tissue was less developed in the lower-crown leaves (**c**). v: vascular bundle.

Figure 6. Anatomical characteristics of leaves of 100-year-old *C. obtusa*: transverse-sectional area of xylem (A_{X}), transfusion tissue (A_{TT}) and transfusion tissue area relative to xylem area ($A_{\mathrm{TT}}/A_{\mathrm{X}}$) shown in relation to crown position. Boxes and lines indicate the upper and lower quartiles and the median. Error bars indicate the upper and lower second quartiles. Asterisks are as in Fig. 3

Figure1

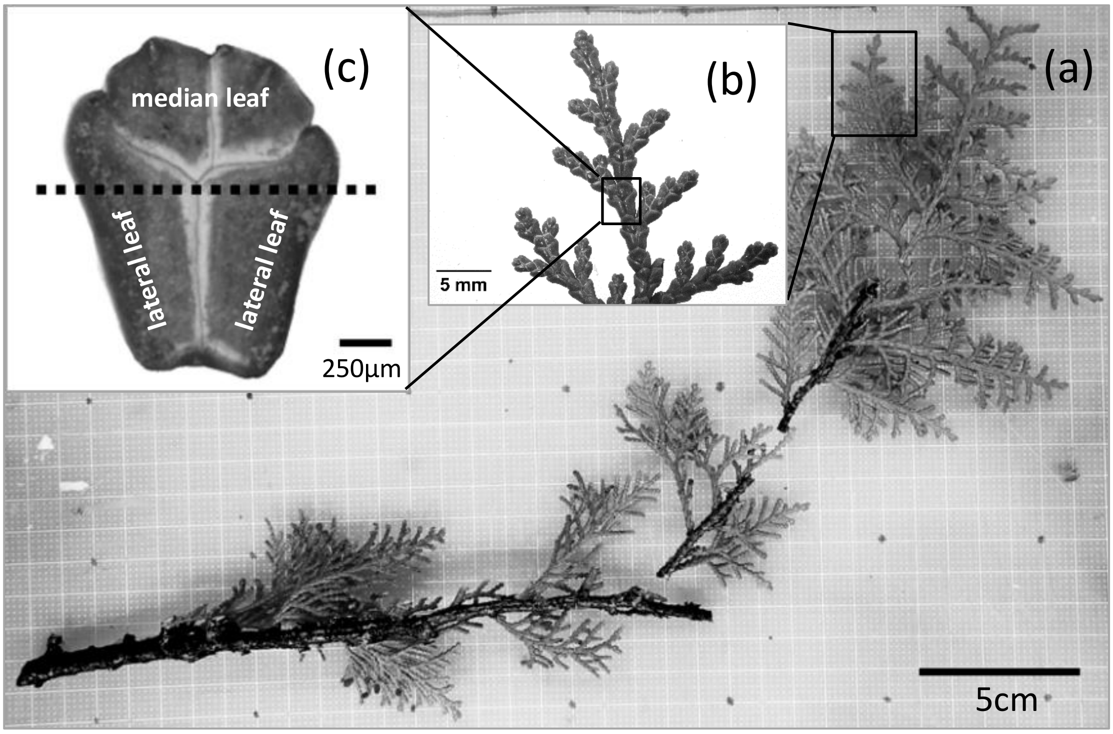


Figure2

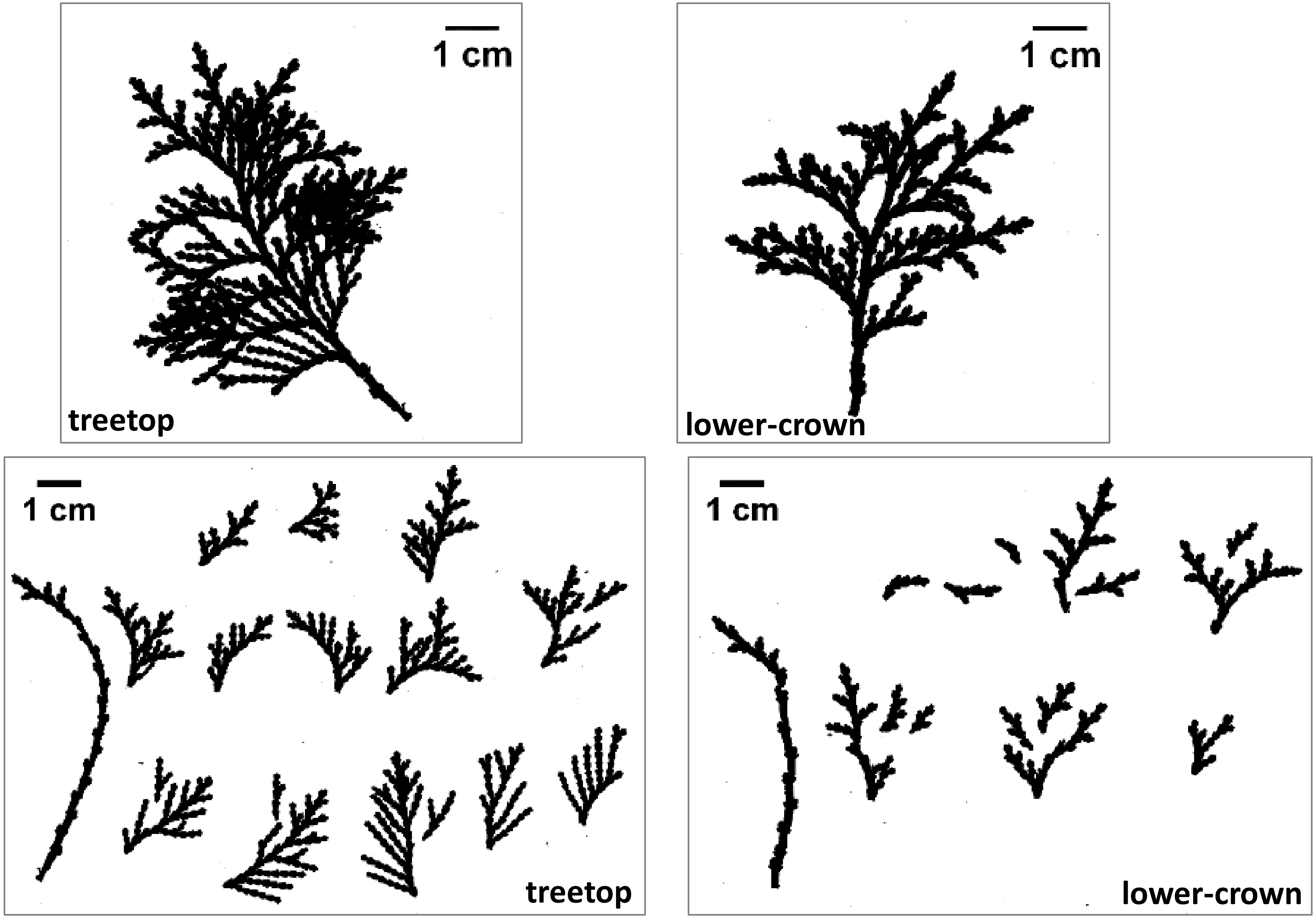


Figure3

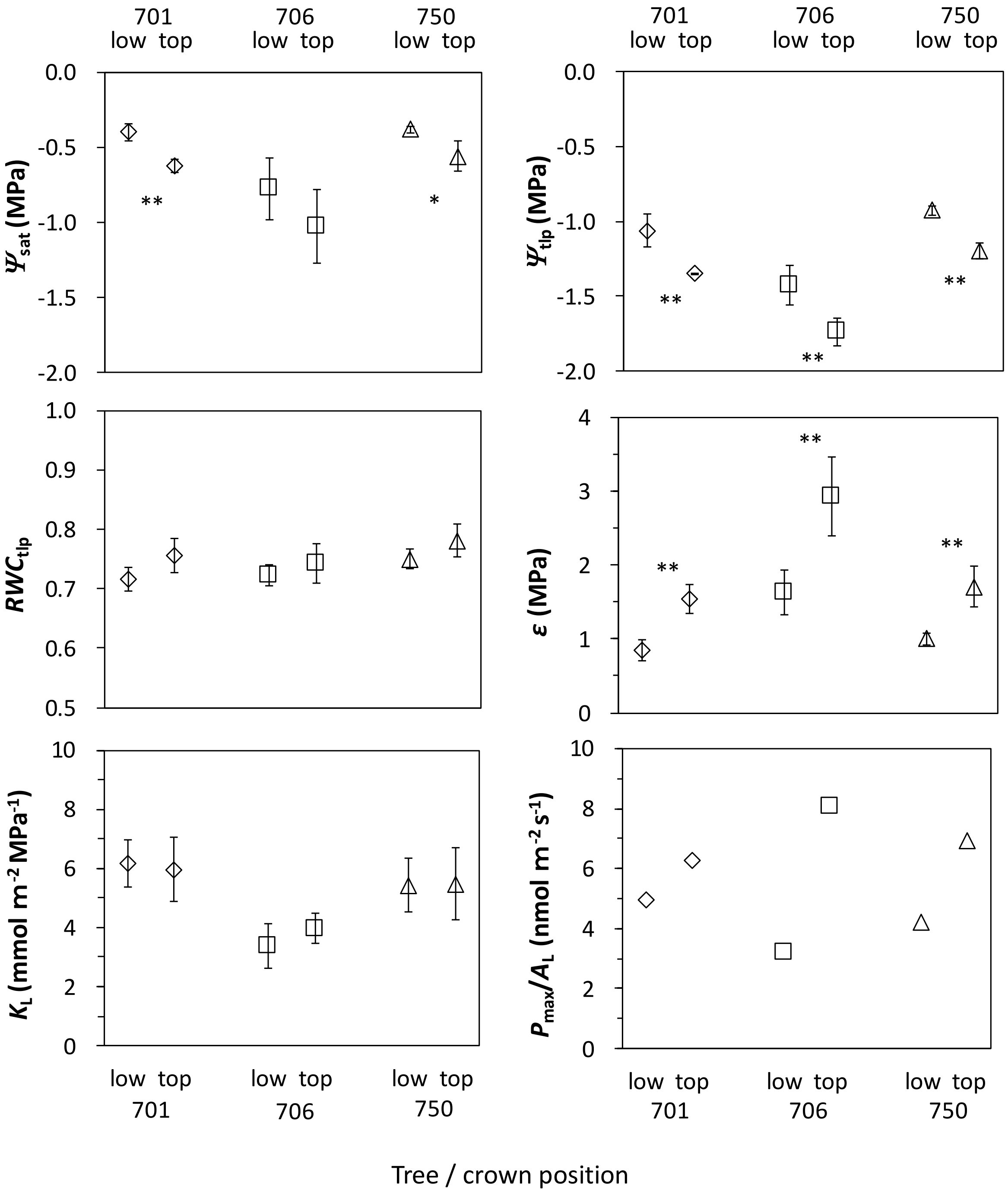

701
low top
706
low top
750
low top
0.0
-0.5
-1.0
-1.5
-2.0
Ψ_{sat} (MPa)
**
*
Ψ_{tlp} (MPa)
**
**
**
1.0
0.9
0.8
0.7
0.6
0.5
RWC_{tlp}
4
3
2
1
0
ε (MPa)
**
**
**
10
8
6
4
2
0
K_L (mmol m^{-2} MPa^{-1})
P_{max}/A_L (nmol m^{-2} s^{-1})
low top
701
low top
706
low top
750
Tree / crown position

Figure4

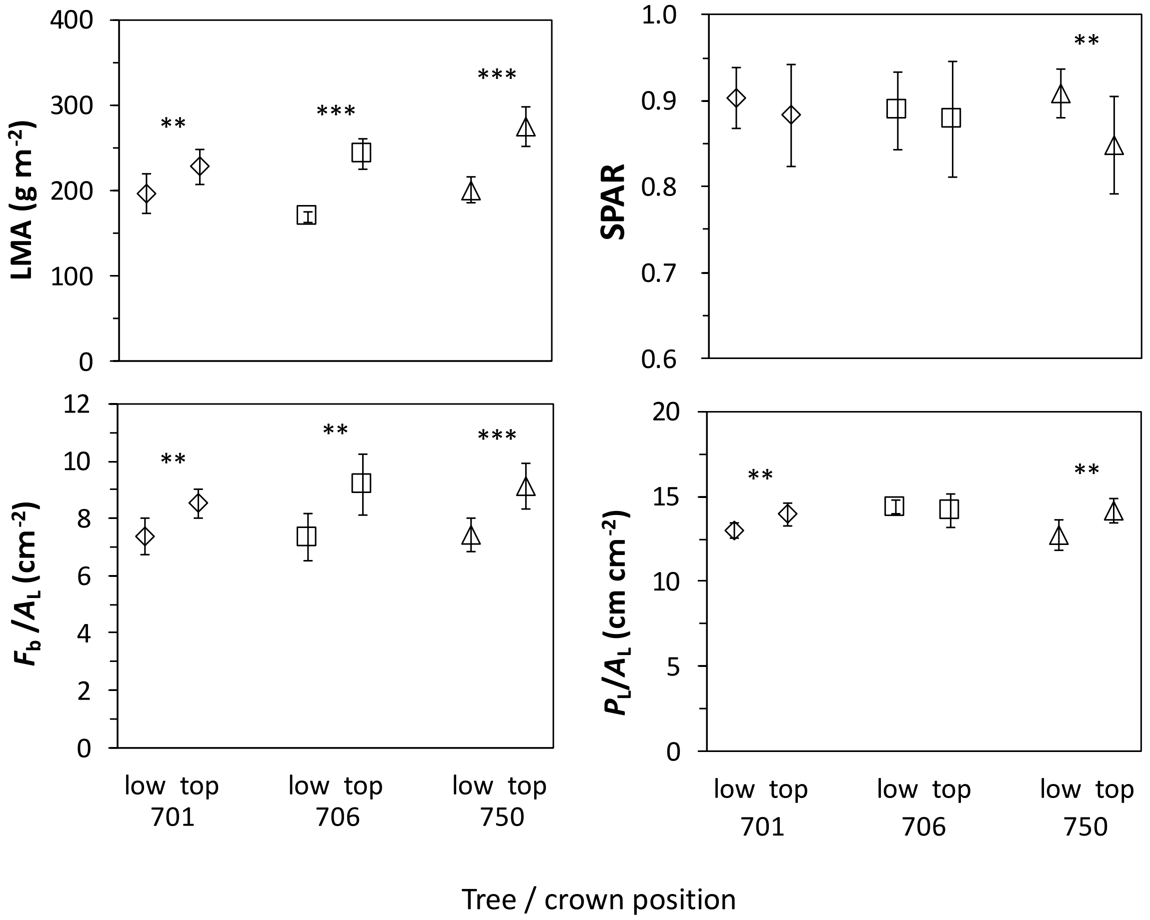

LMA (g m-2)
400
300
200
100
0
**
***
***
SPAR
1.0
0.9
0.8
0.7
0.6
**
Fb /AL (cm-2)
12
10
8
6
4
2
0
**
**
***
PL/AL (cm cm-2)
20
15
10
5
0
**
**
low top
701
low top
706
low top
750
low top
701
low top
706
low top
750
Tree / crown position

Figure5

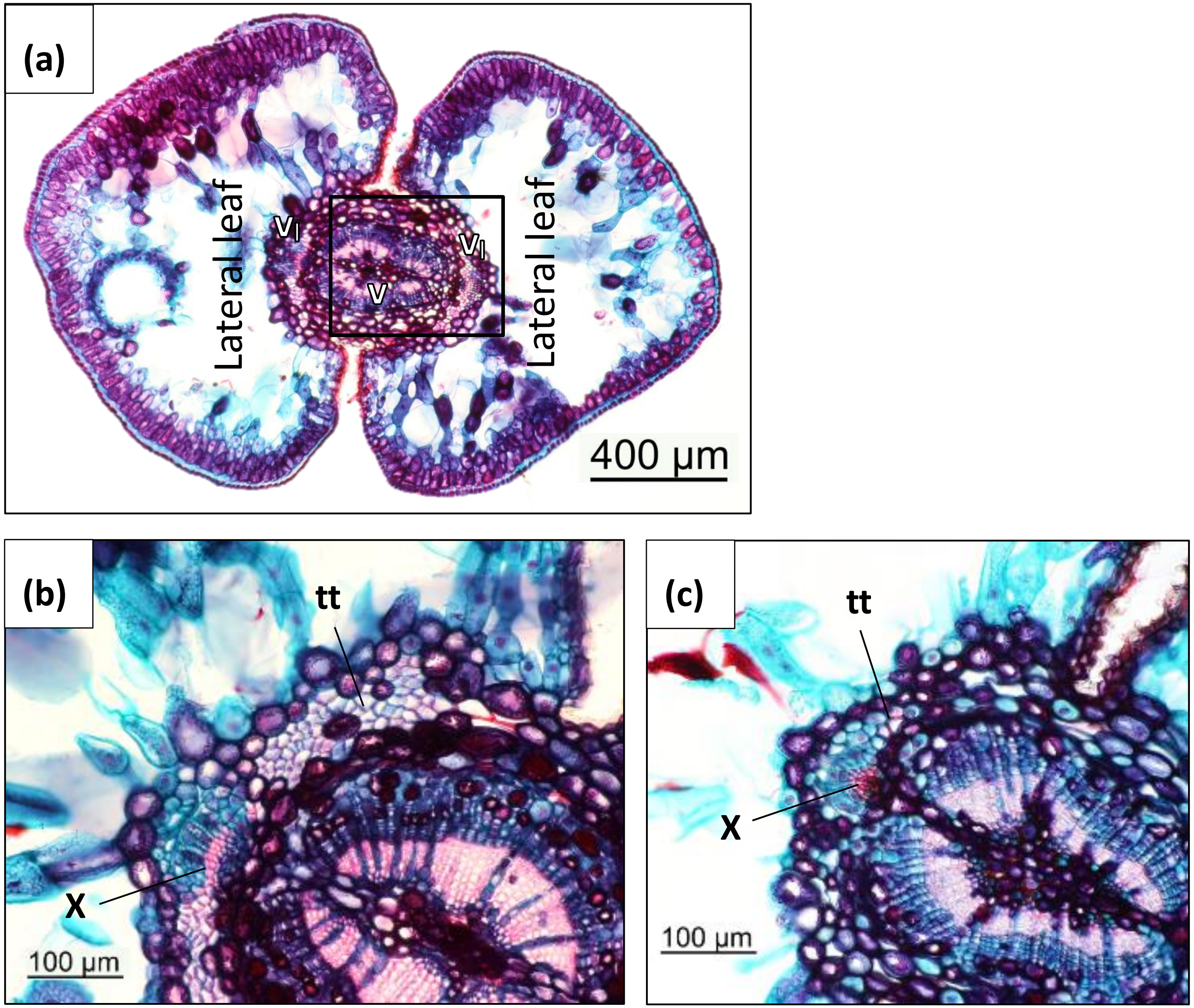
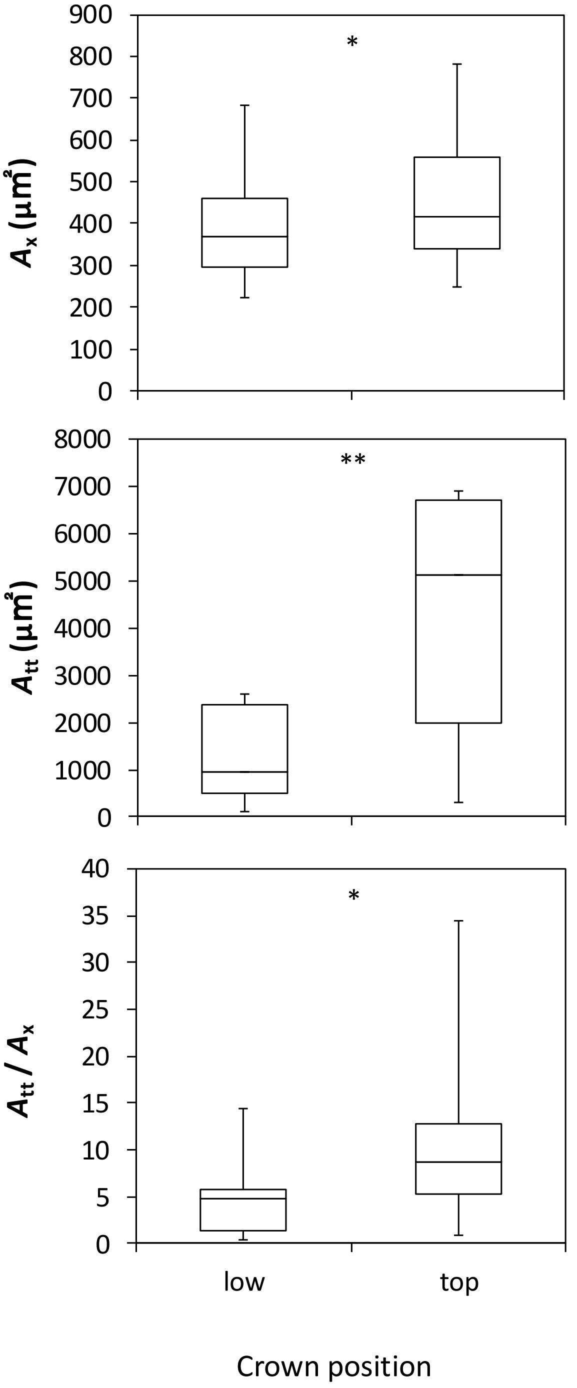

(a)
Lateral leaf
v$_l$
v
v$_l$
Lateral leaf
400 µm
(b)
tt
x
100 µm
(c)
tt
x
100 µm

Figure 6



900
800
700
600
500
400
300
200
100
0
A_x (µm²)
*
8000
7000
6000
5000
4000
3000
2000
1000
0
A_{tt} (µm²)
**
40
35
30
25
20
15
10
5
0
A_{tt} / A_x
*
low
top
Crown position