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Research paper

Coping with low light under high atmospheric dryness: shade acclimation in a Mediterranean conifer (*Abies pinsapo* Boiss.)

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Plant species living in the understory increase carbon (C) allocation toward leaf production for maximizing light capture at the expense of roots and stems, with negative consequences for the whole-plant hydraulic conductance. Moreover, under some conditions, the high atmospheric evaporative demand occurring in Mediterranean areas may be not well buffered by the canopy, which might be the case for relict conifer *Abies pinsapo* Boiss. growing in the forest understory. We hypothesized that acclimation to combined understory shade and high atmospheric dryness can be achieved through the adjustment of water losses to cope with the restriction in water transport. The results reveal high structural plasticity in *A. pinsapo* that allows light harvesting of this species to maximize light capture in the forest understory, and maintain a positive C balance under low light conditions. However, growth in the understory resulted in reduced leaf-specific conductivity, up to approximately four to five times, implying decreased plant capacity to supply water to the leaves. In order to cope with the high atmospheric evaporative demand in the understory, there is an adjustment of the stomatal conductance to the hydraulic conductivity by means of a reduction in the stomatal density in understory individuals, which is due to the almost complete lack of stomata in the adaxial side of the needles. To the extent of our knowledge, such a drastic phenotypic response found in a conifer when growing under shaded conditions had not been previously reported.

Keywords: carbon allocation, forest understory, leaf-specific conductivity, stomatal conductance, vapor pressure deficit.

Introduction

Understory saplings contribute significantly to the open gap regeneration in natural forests (Walters and Reich 1999, Renninger et al. 2007, Schoonmaker et al. 2010), and therefore, the strategies of trees improving their survival under the environmental conditions imposed by the forest canopy are of ecological and silvicultural importance. These strategies have long been associated with changes in the morphology of leaves and shoots (Carpenter and Smith 1981, Givnish 1988, Abrams and Kubiske 1990, Niinemets and Sack 2006), especially with

the increase in the allocation of carbon (C) toward leaf production in order to maximize light capture at the expense of roots and stems (Givnish 1988, Landhäuser and Lieffers 2001, Percy 2007).

One possible consequence of a limited C allocation to the stem may be the reduction of its diameter (Niinemets et al. 2002, 2004a) with a negative influence on the ratio between sapwood area and leaf area, i.e., the so-called Huber value (HV), which finally implies a decrease in the water transport ability to the leaves (Cochard 1992, Protz et al. 2000, Gotsch

et al. 2010). In fact, shading has been associated with a reduction of the whole-plant hydraulic conductance (Schoonmaker et al. 2010), mainly due to decreased leaf-specific conductivity (LSC) (Schultz and Matthews 1993, Shumway et al. 1993, Aasamaa et al. 2002, Brodrigg et al. 2005, Schoonmaker et al. 2010, Peltoniemi et al. 2012). Such reduction in LSC can be counterbalanced by reducing leaf transpiration, in order to achieve a balance between the water flow to the leaves and the water flow from the leaves to the atmosphere (Martínez-Vilalta et al. 2009), then diminishing the risk of drought-induced cavitation due to an excessive water tension in the xylem (Tyree and Sperry 1989).

Conifer shoots are assumed to be well coupled with the atmosphere, with a stomatal conductance much smaller than its boundary layer conductance (Martin et al. 1999). A strong coupling condition with the atmosphere implies that vapor pressure deficit (VPD) is the main factor controlling transpiration (Daudet et al. 1999, Pereira 2004). Assuming this, stomatal conductance constitutes the dominant controller of water loss in conifers (Martin et al. 1999). Both stomatal size and density modify the maximum stomatal conductance (Franks and Beerling 2009), and changes in stomatal density are most frequently associated with life in the shade (Givnish 1988, Youngblood and Ferguson 2003).

Besides light availability, evaporative demand has long been considered to be lower for plants growing in the understory (Niinemets and Valladares 2004, Bladon et al. 2006, Niinemets and Anten 2009, Schoonmaker et al. 2010), due to the effect of the forest canopy on the solar radiation regime and air mixing in the understory (Geiger et al. 2009). As a consequence, the daily fluctuations of air temperature (T) and relative humidity (RH) are dampened when compared with those in the open areas (von Arx et al. 2012), although this consideration cannot be absolutely generalized. Thus, von Arx et al. (2013) stated that this smoothing effect of the forest canopy on T and RH, the two main variables determining the VPD, depends on the overall ambient weather conditions, the structure of the forest canopy and the physiographic situation of the stand. For instance, the variation in mean day T and RH between the understory and a nearby open area for a broadleaved forest canopy at ~600 m altitude during summer is ~2 °C and 5%, respectively, while for a pine forest canopy with similar conditions, the variation is <0.5 °C and ~1.5% (von Arx et al. 2012). Furthermore, von Arx et al. (2013) determined that the smoothing in VPD between open-field and understory conditions was lower with sparse canopies (leaf area index below four), finding even no smoothing effect on VPD with dry soils below these canopies. Under these conditions, the difference in evaporative demand between understory and open field could be functionally negligible, leaving the light availability as the main—or even the single—differential factor between both situations. Therefore, it could happen that the high atmospheric evaporative demand occurring in Mediterranean

areas (Peguero-Pina et al. 2011) is not well buffered by the canopy, which would imply that understory saplings also have to cope with similar evaporative conditions to those in the open field. Furthermore, due to competition for water by overstory individuals, drought can be even more severe in the understory of Mediterranean forests than in open habitats (Valladares and Pearcy 2002, Niinemets 2010a). This might be the case for pinsapo fir (*Abies pinsapo* Boiss.), a Mediterranean fir that may cope with these conditions in order to survive in the understory.

Abies pinsapo is a relict species that occurs in some restricted areas of the Mediterranean mountain ranges in Spain and Morocco (Latorre and Artero 2012). This species is included within the group of 'Mediterranean firs', which refers to a group of *Abies* Mill. species that occupy disconnected areas around the Mediterranean Basin (Aussenac 2002). *Abies pinsapo* has to cope with high VPD values during a moderately dry summer (Fernández-Cancio et al. 2007, Peguero-Pina et al. 2011). Living under this stressful environment requires special morphological or physiological adaptations (Vilagrosa et al. 2003, Latorre and Artero 2012). Peguero-Pina et al. (2011) compared open-field specimens of *A. pinsapo* and *A. alba* Mill., another fir species naturally occurring in the humid montane or subalpine altitudinal belts of mountain ranges in Europe, where the climatic conditions include the lack of summer drought and a very low VPD during the whole vegetative period (Peguero-Pina et al. 2007). In terms of hydraulic traits, Peguero-Pina et al. (2011) found a much higher LSC in the branches of *A. pinsapo*, which was interpreted as an adaptation to the high VPD during the vegetative period resulting in higher water transport ability to the transpiring needles.

Assuming that (i) living in the forest understory modifies the hydraulic architecture of trees toward a lower LSC due to an increase of C allocation to leaves, (ii) the VPD beneath the forest canopy may not be different from that in open fields and (iii) a high LSC may be critical for coping with dry atmospheres, we hypothesize that the functioning of *A. pinsapo* individuals in the understory may be quite different from those in the open field. The aim of this study is to characterize the phenotypic response of *A. pinsapo* individuals to low light regime when growing beneath a Mediterranean forest canopy, in terms of foliage and shoot architecture, hydraulic architecture and water balance. We hypothesize that adjustment to co-occurring limited light and water availabilities leads to profound changes in both plant light and water-harvesting efficiencies.

Materials and methods

Study site and climatic conditions

The study was carried out in the natural regeneration of a population of *A. pinsapo* planted in 1913 on a NE-facing slope of the southern 'Sistema Ibérico' range (Orcajo, Spain; 41°05'N, 01°30'W; 1150 m above sea level). This site is characterized

by the existence of a summer aridity period typical of the Mediterranean-type climates, as occurs in its natural distribution area (see meteorological data in [Peguero-Pina et al. 2011](#)). The study site shows high VPD values during summer, while convective rains are relatively frequent during the hottest period of the year. The forest is characterized by an uneven-aged, mixed stand co-dominated by *A. pinsapo*, *Pinus pinaster* Ait. and *Pinus sylvestris* L. No symptoms of forest decline were observed, indicating a healthy status of all tree specimens in the study site. The naturally regenerating stand has an irregular distribution of saplings in the understory of up to six stems per square meter and isolated specimens in the open field. The canopy has a dominant tree height reaching up to 15 m, while the height of singular 100-year-old *A. pinsapo* trees exceeds 20 m. Measurements were made in ~22-year-old understory individuals (4 m height, 20 cm breast height diameter; denoted as UI) and in ~17-year-old open-field individuals (2.5 m height, 13 cm breast height diameter; denoted as OI).

Air temperature (T , °C) and relative humidity (RH, %) were measured both in the open field and in the understory using three Hobo Pro temp/RH data loggers (Onset Computer, Bourne, MA, USA) located at 1.30 m above the soil surface in both sites. Incident solar radiation was measured both in the open field and in the understory with three Hobo Pendant temp/light (Onset Computer) located at 1.30 m above the soil in both sites. The measurements were recorded every 30 min during the growing season (April to September) in years 2010, 2011 and 2012. Vapor pressure deficit (kPa) was calculated for both open and understory conditions from mean values of T and RH according to [Rundel and Jarrell \(1989\)](#). The mean daily quantum flux density (Q_{int} , mol m⁻² day⁻¹) was calculated for both understory and open conditions from the mean values of incident solar radiation ([Cescatti and Zorer 2003](#)).

The study of canopy gaps in the forest understory was performed using hemispherical photographs, obtained with a digital camera with a lens adapter, providing a focal length equivalent to 8 mm in a 35 mm format ([Esteso-Martínez et al. 2010](#)). A total of 39 photographs were analyzed with the Gap Light Analyzer software version 2.0 (GLA V2.0, [Frazer et al. 1999](#)). Using this software, we calculated canopy openness (non-weighted proportion of canopy gaps) and sunfleck distribution. Moreover, the potential number of sunflecks per day during the growing season (from April to September) was calculated.

Shoot and needle morphology and anatomy

Morphological and anatomical parameters were measured on 10 current-year shoots in both the open field and the understory. Shoot length (cm) and diameter before and after bark removal (mm) were measured with a micrometer. Pith diameter was identified by staining the xylem with phloroglucinol-HCl and measured using a digital caliper. The conductive xylem area (m²) was calculated as the difference between the stem

and the pith area. Needle and stem dry mass was estimated after drying the plant material in a ventilated oven at 60 °C for 48 h. Total shoot leaf area was determined using Ballotini balls by measuring the mass increase after covering the needles with an adhesive and subsequently with a monolayer of small glass beads (0.08–0.11 mm in diameter) ([Thompson and Leyton 1971](#)). Shoot and leaf projected area were calculated with Image-J software (<http://rsb.info.nih.gov/nih-image/>) using orthogonal pictures of the whole shoot with needles attached in front of a white screen and of all the needles detached and laid flat on a horizontal surface. The ratio between leaf projected area and shoot dry mass (leaf area ratio, LAR), the ratio between the shoot projected area and total shoot leaf area and the ratio between basal shoot area and total shoot leaf area (HV) were subsequently calculated. The leaf mass per area (LMA, g cm⁻²) was calculated as the ratio between the dry mass of the needles and the total shoot leaf area.

The middle segments of three-year-old shoots were transversely sectioned with a sliding microtome (Microm HM 350 S, Thermo Scientific, Walldorf, Germany). Sections with a thickness of 20 µm were stained with safranin, dehydrated by means of ethanol series, and permanently mounted on slides with Eukitt mounting medium. The cross-sections were viewed with a Leica DM LB microscope (Leica Microsystems, Wetzlar, Germany) and photographed with a digital camera (Moticam 2300, Motic, Hong Kong, China). The resulting micrographs were analyzed using Image-J software (<http://rsb.info.nih.gov/nih-image/>), to measure the diameter of 25 tracheids of the earlywood per year (1, 2 and 3 years old) and shoot (10 shoots per condition), yielding 750 tracheids per condition.

Needle length, width and thickness were measured with a digital caliper on 100 fresh needles (5 needles × 4 current-year shoots × 5 trees) per light condition. Thickness and width were measured in the middle of the needle. The stomatal density (number mm⁻²) was also measured on both the adaxial and abaxial sides of another set of 50 needles (10 needles of the current-year shoot × 5 trees) per light condition. This was made by counting the number of stomata in a 1-mm large central part of the needle in micrographs obtained with scanning electron microscopy (VP-SEM S-3400N, Hitachi, Tokyo, Japan) using a low vacuum range of 6–270 Pa. The total number of stomata per shoot was estimated as the product of the stomatal density and the total shoot leaf area. The stomatal size (µm²) was calculated for both the adaxial and abaxial sides of the needles by measuring the dimensions of the stomatal pore on a maximum of 10 stomata per needle.

Xylem vulnerability to cavitation

The Cavitron technique ([Cochard 2002](#), [Cochard et al. 2005](#), [2010](#)) was used to study xylem vulnerability to cavitation of *A. pinsapo* grown in the open field and in the understory. The percentage loss of conductivity (PLC) was measured on 10

healthy branches from 10 different mature well-established trees at each light condition. Straight south-exposed branches (>0.35 m in length, <0.01 m in diameter) were collected, placed in plastic bags and transported to the laboratory. In the laboratory, the side shoots and all the needles were removed, and the main shoots were wrapped in wet paper, put in a plastic bag and stored at 5 °C to prevent dehydration. The technique consists of spinning xylem segments (0.275 m in length) centered on a dedicated rotor to expose them to large negative pressures and then measure the effect of the negative pressure on their conductivity (K). Both sample ends were inserted in transparent vials containing ultrapure water with 10 mM KCl and 1 mM CaCl₂ filtered to 0.2 μm. The solution in the vials was degassed at the start of the measurement but remained in contact with the atmosphere during centrifugation. The water level in the vial, where the basal segment end was inserted, was adjusted to 1.5 cm before each K determination. The water level in the other vial was constant and set to 1 cm. The difference in water levels creates a pressure gradient, and hence a water flow through the sample, which allows K to be estimated (Cochard 2002, Cochard et al. 2005). The maximum sample conductivity (K_{\max}) was measured at low speed and high pressure (−0.1 or −0.25 MPa). Dividing K_{\max} by sample basal wood area yielded sample-specific conductivity. Xylem pressure was then lowered stepwise by increasing the rotational velocity, and K was determined again. The sample PLC was computed as:

$$\text{PLC} = 100 \times (1 - K/K_{\max}) \quad (1)$$

Following Pammenter and Van der Willigen (1998), a sigmoid function was fitted to each curve:

$$\text{PLC} = 100 / (1 + e^{(s(P-P_{50})/25)}) \quad (2)$$

where P_{50} is the pressure causing 50% loss in hydraulic conductivity and s is the slope of the curve at this point. The water potential (−MPa) at which 12% (P_{12}) loss of conductivity occurs was calculated as follows:

$$P_{12} = P_{50} + 50 / s \quad (3)$$

Stem hydraulic conductivity

The hydraulic conductivity (K_h , kg m s^{−1} MPa^{−1}) of stem segments obtained from 10 south-exposed branches (0.5–1 m long) was determined in *A. pinsapo* trees growing in the open field and in the understory. One branch per tree was collected and three stem segments per branch (1, 2 and 3 years old, 2–5 cm long and >1 mm in diameter) were prepared as described in Mayr et al. (2002). The measurement pressure was set to 4 kPa. The flow rate was determined with a PC-connected balance (Sartorius BP221S, 0.1 mg precision, Sartorius AG,

Göttingen, Germany) by recording the change in weight every 10 s and fitting linear regressions over 200 s intervals. The conductivity measurements were carried out with distilled, filtered (0.22 μm) and degassed water containing 0.005% (volume/volume) Micropur (Katadyn Products, Wallisellen, Switzerland) to prevent microbial growth (Mayr et al. 2006). The specific hydraulic conductivity (K_s , kg m^{−1} s^{−1} MPa^{−1}) was calculated by dividing K_h by the conductive xylem area, and the LSC (kg m^{−1} s^{−1} MPa^{−1}) was calculated by dividing K_h by the total leaf area supported by the measured segment.

Water potential, gas exchange and δ¹³C measurements

Shoot water potential (MPa) in OI and UI was measured at pre-dawn and at midday with the Scholander pressure chamber during the midsummer of 2012. During the same period, net CO₂ assimilation (A_N), stomatal conductance (g_s) and transpiration (E) were recorded at 10 h (solar time) in fully developed shoots of OI and UI with a portable gas exchange system (CIRAS-2, PP-Systems, Amesbury, MA, USA) fitted with an automatic conifer cuvette (PLC-C, PP-Systems). Photosynthesis was stabilized at a cuvette CO₂ concentration (C_a) of 400 μmol mol^{−1}, at photosynthetic photon flux densities (PPFD) incident on the needle surface of 77 μmol m^{−2} s^{−1} for trees grown in the understory and at 1381 μmol m^{−2} s^{−1} for trees in the open field and at ambient temperature and relative humidity. After the steady-state rates were observed in these conditions, PPFD was suddenly increased to ~1400 μmol m^{−2} s^{−1} for trees grown in the understory to simulate the effect of sunfleck on the same shoots previously measured. After 10 min, when photosynthesis was stabilized, g_s , A_N and E were again recorded. The intrinsic water-use efficiency (WUE) was calculated as the ratio between A_N and g_s .

Carbon isotope discrimination (δ¹³C), as a long-term indicator of WUE (Bacelar et al. 2012), was obtained for trees grown in the open field and in the understory by analyzing fully developed needles collected from five different current-year shoots. Samples were analyzed by an elemental analyzer coupled to an isotope ratio mass spectrometer (EA-IRMS, Delta V Advantage, Thermo Fisher Scientific, Inc., MA, USA) and expressed relative to Vienna PDB.

Statistical analysis

All data are expressed as means ± standard error. Student's *t*-tests were used to compare the parameters for trees grown in the open field and in the understory. All statistical analyses were carried out using SAS version 8.0 (SAS, Cary, NC, USA).

Results

Differences in climatic conditions in the open field and in the understory

In the open field, the mean monthly values of RH ranged from 30 to 70% and those for air temperature from 8 to 27 °C,

resulting in a range of mean VPD from 0.3 to 2.6 kPa, reaching values of up to 5 kPa during summer (Figure 1). In the understory, mean RH was 1.9% higher, whereas mean T and VPD were 0.67 °C and 0.01 kPa lower than those measured in the open field, respectively. Despite these variations, most of the differences in the mean monthly values of diurnal RH, T and VPD between the open field and the understory were not statistically significant ($P > 0.05$). On the other hand, Q_{int} was consistently, on average ~ 4.7 times, lower in the understory than in the open field ($P < 0.05$). In the understory, the canopy openness was $\sim 20\%$, with an average potential number of 25 sunflecks per day at the point where the photograph was taken. Eighty percent of the total number of sunflecks were shorter than 10 min, of which 50% were shorter than 2 min. In contrast, only 5% of the total number of sunflecks were longer than 30 min.

Effects of light conditions on shoot and needle structure

Values of current-year shoot length and shoot projected area were not statistically different at $P < 0.05$ among OI and UI (Table 1; see also Figure 2). In contrast, shoot dry mass, shoot diameter, leaf projected area, total shoot leaf area and LMA

were lower in the understory than in the open field (Table 1). The low values of leaf projected area and total shoot leaf area in UI can be explained by the lower number of needles of the understory shoots (Table 1). In consequence, UI shoots had a higher shoot projected area/total shoot leaf area ratio, a higher LAR and a lower HV. Needles were longer, wider and thinner in UI than in OI (Table 1, Figures 2 and 3). Needles of UI also showed a lower stomatal density and whole number of stomata per shoot (Table 1), lacking them for the most part on the adaxial side of the needle surface (Figures 2 and 3). In spite of this, the stomatal size did not show statistically significant differences between UI and OI, both in the adaxial and abaxial sides of the needles (Table 1).

The mean values of the diameter of tracheids in the stem xylem were $16.78 \pm 0.10 \mu\text{m}$ for individuals growing in the open field and were $13.73 \pm 0.11 \mu\text{m}$ for individuals growing in the understory (Table 1). This difference was statistically different at $P < 0.05$.

Differences in hydraulic parameters

K_h was approximately three times lower for trees grown in the understory. K_s was also significantly lower in UI, although the

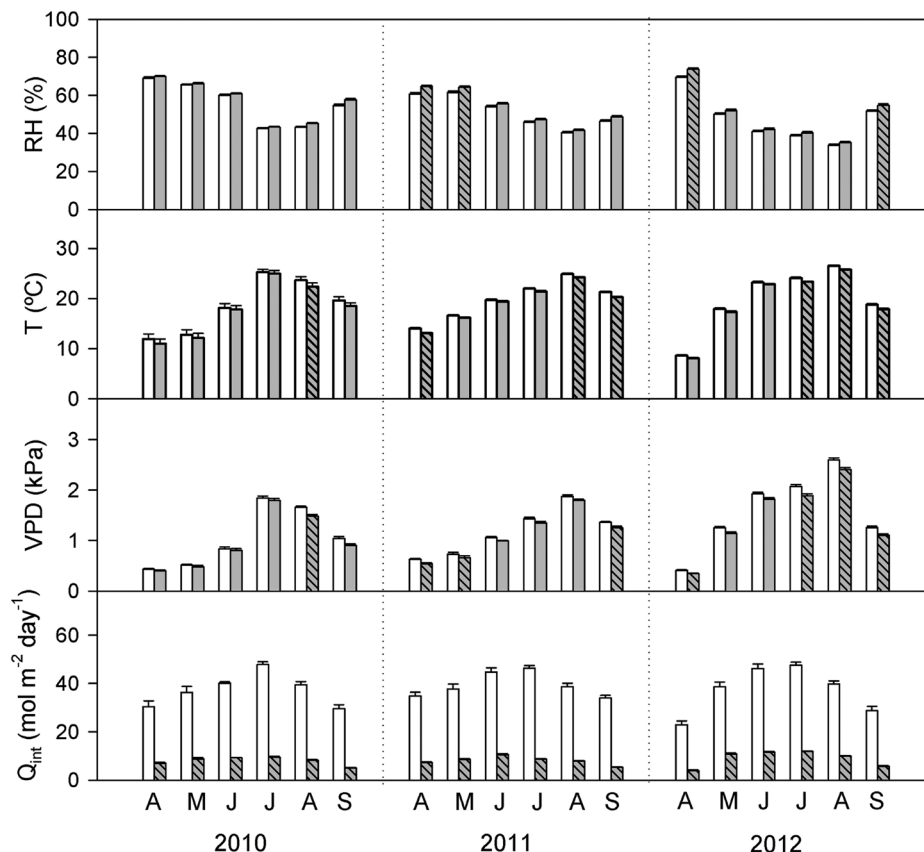


Figure 1. Mean diurnal (from dawn to sunset) air temperature (T , °C), relative humidity (RH, %), vapor pressure deficit (VPD) and mean daily quantum flux density (Q_{int} , mol m⁻² day⁻¹) in the open field (white bars) and in the understory (gray bars) of the studied site during three consecutive growing seasons (from April to September 2010, 2011 and 2012). Striped bars indicate significant statistical differences between open-field and understory values. Values are mean \pm SE.

Table 1. Shoot and needle morphological and anatomical parameters for *A. pinsapo* grown in the open field (OI) and in the understory (UI). LAR, leaf area ratio; HV, Huber value; LMA, leaf mass area. Data are mean \pm SE and different letters indicate significant differences between OI and UI at $P < 0.05$.

	OI	UI
Shoot length (cm)	3.8 \pm 0.5a	3.5 \pm 0.4a
Shoot dry mass (g)	0.76 \pm 0.08a	0.25 \pm 0.04b
Number of needles	118 \pm 4a	63 \pm 4b
Shoot diameter (mm)		
With bark	3.8 \pm 0.4a	2.1 \pm 0.3b
Without bark	1.1 \pm 0.2a	0.6 \pm 0.1b
Shoot projected area (cm ²)	7.6 \pm 0.5a	7.5 \pm 1.1a
Leaf projected area (cm ²)	17.4 \pm 0.9a	10.5 \pm 1.0b
Total shoot leaf area (cm ²)	62.3 \pm 5.3a	27.4 \pm 2.5b
Shoot projected area/total shoot leaf area	0.13 \pm 0.01a	0.27 \pm 0.02b
LAR (cm ² g ⁻¹)	22.70 \pm 1.59a	42.35 \pm 1.67b
HV	1.5 $\times 10^{-4}$ \pm 0.3 $\times 10^{-4}$ a	1.0 $\times 10^{-4}$ \pm 0.2 $\times 10^{-4}$ b
Tracheids diameter (μ m)	16.78 \pm 0.10a	13.73 \pm 0.11b
Needle length (mm)	9.1 \pm 0.1a	13.1 \pm 0.2b
Needle thickness (mm)	1.0 \pm 0.0a	0.5 \pm 0.0b
Needle width (mm)	1.9 \pm 0.0a	1.5 \pm 0.0b
LMA (g cm ⁻²)	0.010 \pm 0.001a	0.007 \pm 0.001b
Stomata density (no. mm ⁻²)		
Adaxial side	58.5 \pm 3.5a	1.1 \pm 0.5b
Abaxial side	78.3 \pm 2.2a	51.5 \pm 5.2b
Stomata size (μ m ²)		
Adaxial side	1001 \pm 50a	979 \pm 40a
Abaxial side	968 \pm 40a	1064 \pm 55a
Number of stomata per shoot	238,032a	55,230b

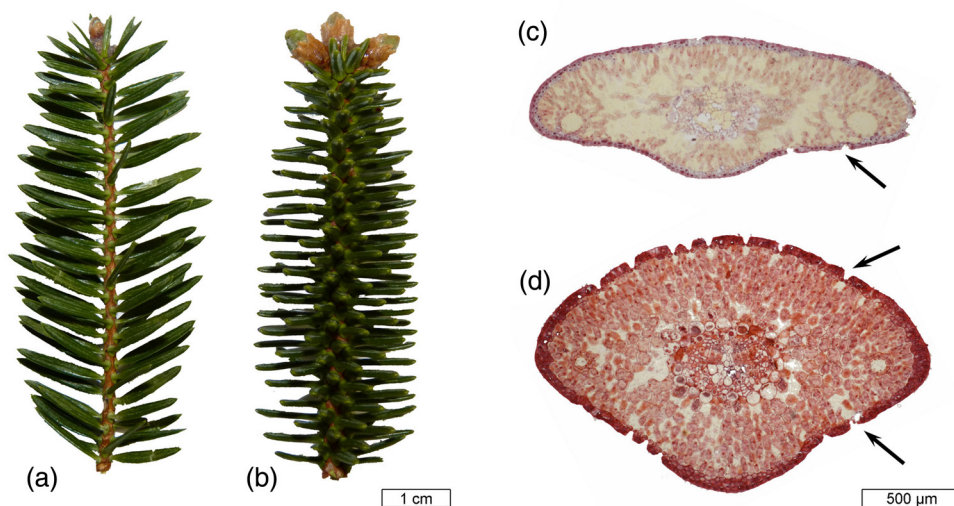


Figure 2. Representative shoot silhouette images and needle cross-sections for understory (a, c) and open-field (b, d) grown *A. pinsapo*. Arrows indicate the stomata.

differences between both conditions were smaller than those found for K_h (Table 2). This fact can be explained by the lower values of conductive xylem area found in trees grown in the understory (data not shown), which partially compensated the lower K_h values here measured. Leaf-specific conductivity was also significantly lower for the UI (Table 2), which indicates a lower capacity to supply water to the leaves.

In relation to the resistance to drought-induced cavitation, the values of P_{50} were not statistically different (ca -3.75 MPa for OI and UI, Figure 4). Despite this, P_{12} was statistically significantly lower for OI than for UI (-2.85 and -2.25 MPa respectively, Figure 4). The lowest water potential values registered during summer (Ψ_{min}) were -2.14 ± 0.15 and -1.73 ± 0.12 MPa for OI and UI, respectively (Figure 4).

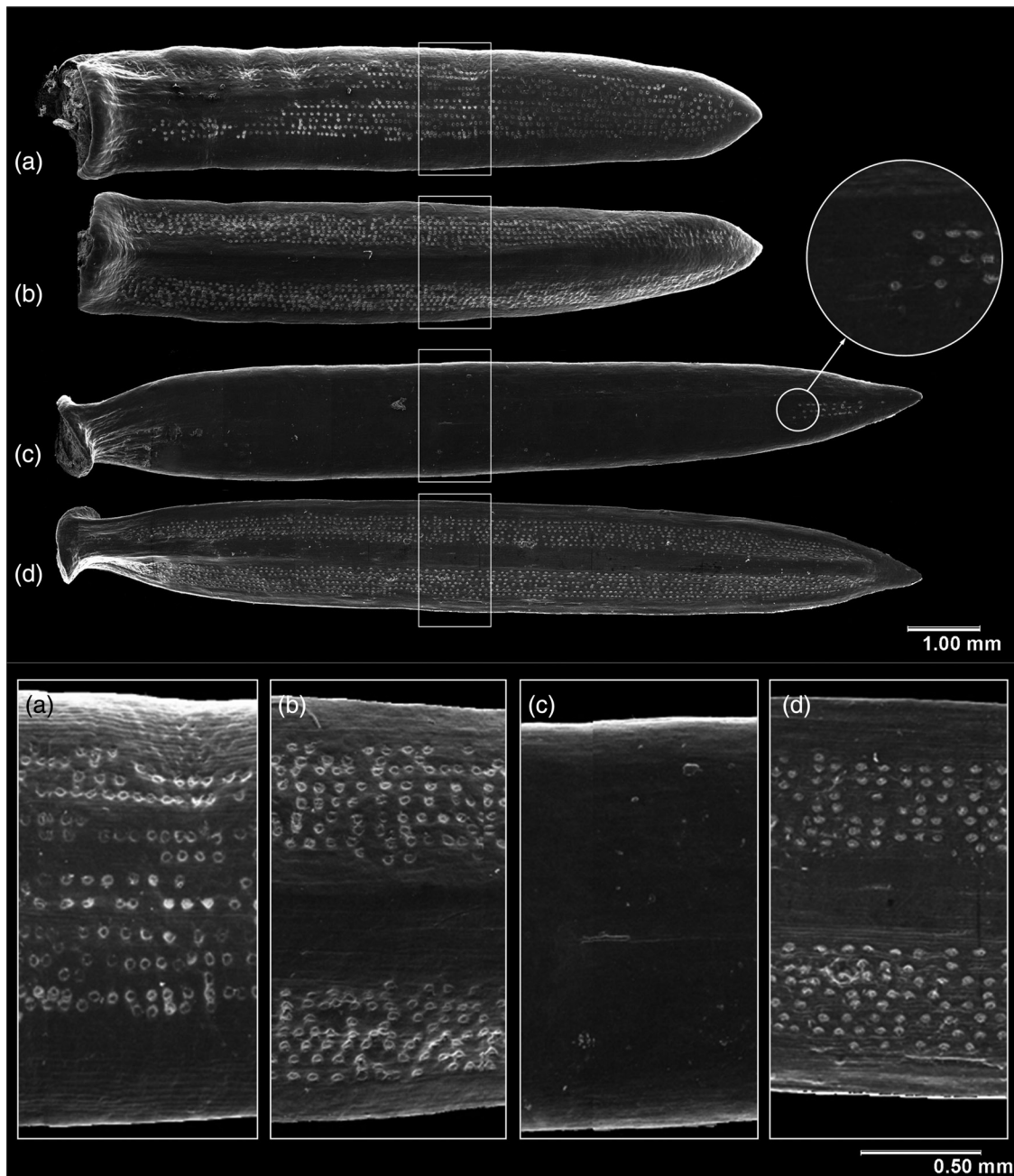


Figure 3. Scanning electron microscopy images of needles of *A. pinsapo* grown in the open field (a, adaxial; b, abaxial) and in the understory (c, adaxial; d, abaxial). Note the absence of stomata for the most part on the adaxial side of the understory needle.

Although Ψ_{\min} was significantly lower for OI, the difference between Ψ at midday and at predawn ($\Delta\Psi$) was almost equal for both conditions (0.54 ± 0.10 and 0.62 ± 0.12 MPa for UI and OI, respectively).

Shading effects of photosynthetic traits

The values of g_s , A_N and E measured through the summer were much lower in needles of UI than in OI ($P < 0.05$, Table 3). The decrease in A_N in the understory, when compared with the open field, was much higher than that

registered for g_s . Thus, the instantaneous WUE was much lower in trees grown in the understory (Table 3). This was further confirmed by a statistically significant difference ($P < 0.05$) in $\delta^{13}\text{C}$ between both kinds of trees, with a lower value in UI needles (Table 3), which can be interpreted as an indicator of a lower intrinsic WUE during the vegetative period in these specimens. When PPFD was suddenly increased to $\sim 1400 \mu\text{mol m}^{-2} \text{s}^{-1}$ in UI, the values of g_s , A_N and E only slightly increased and this increase was not statistically significant (Table 3).

Table 2. Mean (\pm SE) hydraulic conductivity (K_h , $\text{kg m s}^{-1} \text{MPa}^{-1}$), specific hydraulic conductivity (K_s , $\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$) and LSC ($\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$) in 1-, 2- and 3-year-old stem segments for *A. pinsapo* grown in the open field (OI) and in the understory (UI). Different letters indicate significant differences between OI and UI at $P < 0.05$.

	Shoot age (years)	OI	UI
K_h ($\text{kg m s}^{-1} \text{MPa}^{-1}$)	1	$0.6 \times 10^{-6} \pm 1.8 \times 10^{-7}\text{a}$	$0.2 \times 10^{-6} \pm 0.5 \times 10^{-7}\text{a}$
	2	$1.6 \times 10^{-6} \pm 3.2 \times 10^{-7}\text{a}$	$0.5 \times 10^{-6} \pm 1.1 \times 10^{-7}\text{b}$
	3	$3.6 \times 10^{-6} \pm 9.1 \times 10^{-7}\text{a}$	$1.2 \times 10^{-6} \pm 3.5 \times 10^{-7}\text{b}$
K_s ($\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$)	1	$4.3 \times 10^{-1} \pm 5.2 \times 10^{-2}\text{a}$	$2.8 \times 10^{-1} \pm 3.1 \times 10^{-2}\text{b}$
	2	$4.0 \times 10^{-1} \pm 2.3 \times 10^{-2}\text{a}$	$2.5 \times 10^{-1} \pm 3.1 \times 10^{-2}\text{b}$
	3	$4.7 \times 10^{-1} \pm 4.9 \times 10^{-2}\text{a}$	$2.9 \times 10^{-1} \pm 3.5 \times 10^{-2}\text{b}$
LSC ($\text{kg m}^{-1} \text{s}^{-1} \text{MPa}^{-1}$)	1	$5.3 \times 10^{-5} \pm 10.7 \times 10^{-6}\text{a}$	$1.4 \times 10^{-5} \pm 2.4 \times 10^{-6}\text{b}$
	2	$8.3 \times 10^{-5} \pm 12.8 \times 10^{-6}\text{a}$	$1.6 \times 10^{-5} \pm 3.4 \times 10^{-6}\text{b}$
	3	$14.0 \times 10^{-5} \pm 27.7 \times 10^{-6}\text{a}$	$2.6 \times 10^{-5} \pm 5.9 \times 10^{-6}\text{b}$

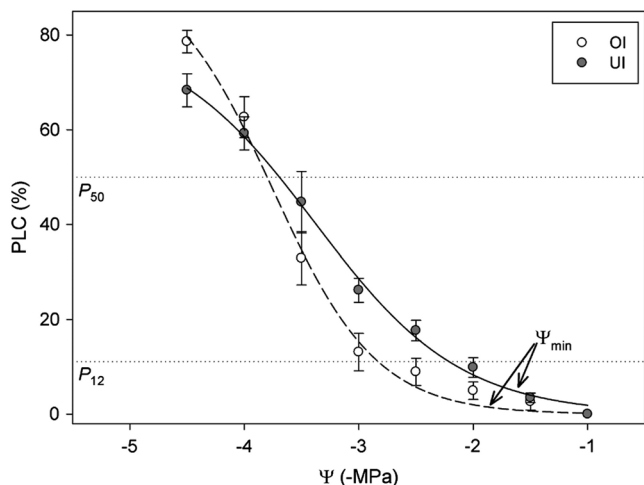


Figure 4. Relationships between the percentage loss of conductivity (PLC) and shoot water potential (Ψ) for the open-field (white, OI) and understory (gray, UI) *A. pinsapo* individuals. The 50% (P_{50}) and 12% (P_{12}) losses of conductivity are indicated by dotted lines. Values of minimum shoot water potential during midsummer (Ψ_{\min}) are indicated by arrows. Values are expressed as mean \pm SE.

Discussion

Abies pinsapo shows a great phenotypic response to the change in the light environment when living in the forest understory, as seen from both morphological and physiological parameters. A reduction in Q_{int} of approximately five times when compared with the value registered in the open field implies a drastic limitation to the photosynthetic capacity in the understory. To cope with such environmental constraint, UI increase approximately two times their dry mass allocation to leaf tissue (LAR), which has been also reported in several other conifer species (Niinemets 2010b). The increment in LAR is mainly due to a higher reduction in shoot dry mass more than to a reduction in leaf projected area (Table 1). Second, by reducing their total shoot leaf area, UI also increase by two times the ratio of shoot projected area to total shoot leaf area. Third, by reducing the total to projected needle area ratio and leaf thickness (Table 1), the needles increase the exposure of leaf area for given total

Table 3. Mean (\pm SE) net CO_2 assimilation (A_N), stomatal conductance (g_s), transpiration (E), instantaneous WUE and C isotope discrimination ($\delta^{13}\text{C}$) for *A. pinsapo* grown in the open field (OI) and in the understory (UI). Means with different letters are significantly different between OI and UI at $P < 0.05$.

	OI	UI	UI (Sunfleck)
g_s ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$)	$79 \pm 8\text{a}$	$30 \pm 6\text{b}$	$36 \pm 7\text{b}$
A_N ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$)	$11.0 \pm 1.0\text{a}$	$1.3 \pm 0.4\text{b}$	$3.3 \pm 1.4\text{b}$
E ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$)	$1.60 \pm 0.34\text{a}$	$0.74 \pm 0.13\text{b}$	$0.93 \pm 0.17\text{b}$
WUE	$142 \pm 15\text{a}$	$46 \pm 15\text{b}$	–
($\mu\text{mol CO}_2 \text{mol}^{-1} \text{H}_2\text{O}$)			
$\delta^{13}\text{C}$ (‰)	$-26.99 \pm 0.01\text{a}$	$-28.51 \pm 0.12\text{b}$	–

leaf area, essentially becoming flatter resembling leaves in broad-leaved species (Figure 2). With these modifications, UI increase their efficiency for using low light levels by the avoidance of self-shading (Niinemets and Kull 1995a, 1995b, Mori and Takeda 2004). And finally, UI reduce the needle mass per area, contributing to increase in light capture area with a lower investment in needle mass (Stenberg 1996). Although UI have a much lower A_N than OI, these morphological modifications can contribute to maintaining a positive C balance in UI shoots even under a low light regime (Sprugel et al. 1996, Mori and Takeda 2004, Renninger et al. 2007).

By increasing the C allocation to needles at the expense of the stem, UI branches show a reduced diameter when compared with OI (Table 1), which causes a drastic reduction in K_h in conifer branches (Cochard 1992, Cruziat et al. 2002). An increase in K_s can partially improve the ability for water supply to transpiring needles, even when the ratio between the basal area of the branch and the total shoot leaf area—or HV—decreases as a consequence of a main C allocation to leaves. However, this does not seem to be the strategy of UI, with K_s being ~ 1.5 times lower than the value measured in OI due to the formation of narrower tracheids. Other studies have also found similar effects of shade on K_s in conifer species. Protz et al. (2000) reported a reduction in K_s associated with narrower tracheids in individuals of *Pinus contorta* Dougl. ex Loud.

growing in a closed canopy. More recently, Renninger et al. (2007) showed that suppressed specimens of *Pseudotsuga menziesii* (Mirb.) Dougl. and *Tsuga heterophylla* (Raf.) Sarg. had also lower K_s values than released trees, which were partially explained by the formation of narrower and shorter tracheids. On the contrary, Schoonmaker et al. (2010) did not observe a decline in K_s in four conifer species living in the forest understory, which suggests that this parameter does not show a single response as a result of understory shading.

The decrease in K_h showed by UI stems, as a combination of both the reduction in stem diameter and K_s , is not accompanied by a proportional reduction in total shoot leaf area, and therefore it implies a decrease in LSC of approximately four times in 1-year shoots and approximately five times in 2- and 3-year shoots, as compared with OI specimens (Table 2). A similar result was reported by Schoonmaker et al. (2010), which found an overall LSC decrease in the understory environment in four conifer species. This drastic decrease in LSC of UI branches seriously compromises the water transport efficiency to the transpiring needles, especially under high VPD values. Several studies have demonstrated a reduction in the amount of leaf area in relation to the conducting sapwood area with increasing evaporative demand in several conifers (Callaway 1992, Mencuccini and Grace 1995, DeLucia et al. 2000, Mencuccini and Bonosi 2001). However, in terms of C gain, a lower allocation to photosynthetic tissue seems to be incompatible with the low light availability in the understory. Therefore, the lower LSC in UI could only be compatible with low VPD values, which are often associated with the understory environment (Schoonmaker et al. 2010). However, our meteorological data indicate that VPD values in the understory were similar to those in the open field (Figure 1), probably due to the canopy structure and topographic position of the stand that allow a mixing of the air between the understory and the open field, as has been previously reported (von Arx et al. 2013). In fact, the values registered, with maximum ~ 5 kPa, are quite close to those registered in a semi-arid Mediterranean-type climate (Martínez-Cob et al. 2008). Therefore, the hydraulic architecture of UI branches, with a reduced K_s and a reduced allocation to conducting tissue, is in contradiction with the long-term changes in functional traits reported for conifer species under high VPD (Poyatos et al. 2007). Although a certain contribution of radiation in the transpiration could be considered under a non-perfect coupling condition with the atmosphere (Pereira 2004), the geometry of *A. pinsapo*, which is very similar to that described by Martin et al. (1999) for *Abies amabilis* Dougl. ex Forbes, should suggest a strong coupling with the atmosphere. Under this premise, it can be assumed that VPD would be the main contributing factor to water loss by transpiration in *A. pinsapo*.

An enhanced resistance to embolism might be another way to cope with this situation (Maherali and DeLucia 2000) through a plastic response of the xylem physiology to different

levels of aridity during the conifer growth (Corcuera et al. 2012). In fact, Schoonmaker et al. (2010) reported a higher P_{50} in four conifers when growing in the understory with a lower value of atmospheric evaporative demand. However, our data indicate that UI and OI of *A. pinsapo* did not show statistically significant differences in P_{50} values (Figure 4), which suggests a similar resistance to drought between both sets of trees. In conifers, a strong linear relationship between P_{50} and the lethal water potential associated with recovery failure has been reported (Brodribb and Cochard 2009), while angiosperms die closer to P_{88} (Choat 2013, Urli et al. 2013). In spite of the similarity in P_{50} , the comparison of the first section of the vulnerability curves indicates that UI are even more vulnerable to drought-induced cavitation at water potential values higher than P_{50} , with P_{12} being significantly higher in UI (Figure 4). It should be noted that the risk of embolism in the field could be considered small for both conditions because Ψ_{\min} were in both cases quite far from P_{12} , which is considered an estimation of the xylem water potential at which embolism begins (López et al. 2013). Therefore, both UI and OI show a similar and high safety margin, as judged by the difference between Ψ_{\min} and P_{50} (Choat 2013). In consequence, it can be suggested that the strategy to cope with shade conditions in this particular ecological situation, i.e., low light but high VPD, consists in keeping Ψ_{\min} within the safety range, more than being able to resist a higher tension in the xylem.

In a steady state, the water uptake—as the product of LSC and the water potential gradient through the system ($\Delta\Psi$)—must be balanced by the water losses to the atmosphere by transpiration (Martínez-Vilalta et al. 2009), which in conifers can be approximated to the product of g_s and VPD due to their high coupling with the atmosphere (Martin et al. 1999):

$$LSC\Delta\Psi = VPDg_s \quad (4)$$

In this study, VPD values were found to be nearly equal between OI and UI, whereas LSC in OI reached approximately four times the values in UI. In spite of this, the difference between Ψ at midday and at predawn ($\Delta\Psi$) did not differ significantly between OI and UI in midsummer. Therefore, according to Eq. (4), a similar value in $\Delta\Psi$ between the two sets of trees under such conditions can only be achieved through a drastic reduction in g_s in UI. Our results confirmed this, because g_s during midsummer did not exceed $30 \text{ mmol m}^{-2} \text{ s}^{-1}$ in UI, whereas it reached $79 \text{ mmol m}^{-2} \text{ s}^{-1}$ in OI (Table 3).

The differences found in g_s in both sets of specimens may be explained by the existence of anatomical differences, both in terms of stomatal size and density (Franks and Beerling 2009). In our case, no statistically significant differences were found in the stomatal size between OI and UI, but large differences were found in the stomatal density and the whole number of stomata per shoot (Table 1). The decrease in the number of

stomata in the needle and, consequently in the whole shoot, is due to the almost complete lack of stomata in the adaxial side of the needles in UI (Figure 3). It should be noted that the few stomata that can be found on the adaxial side of the UI needles are located at the apex. Furthermore, some of them seem to be aborted or immature (Figure 5) because they had failed to complete their development, which may indicate that not all the initial stomata become fully developed in the mature needles of UI (Croxdale 2000). In this sense, Casson and Hetherington (2010) showed that light quantity is a factor that positively influences the differentiation of epidermal cells to stomatal cells, which might explain the differential morphology of the adaxial epidermis of OI and UI needles found in this study. To the extent of our knowledge, such a drastic phenotypic response found in a conifer when growing under shaded conditions had not been previously reported.

The whole number of stomata per shoot in OI was approximately four times higher than in UI, which coincides with the ratio of LSC values between both sets of specimens. Consequently, a long-term adjustment between the water transport ability and the maximum transpiration rate for a given VPD is achieved for both OI and UI of *A. pinsapo*. This trade-off between LSC and the maximum value of g_s has been previously reported in *Pinus palustris* Mill. (Addington et al. 2004). The extreme reduction in maximum g_s may be interpreted as an efficient way for avoiding an excessive risk of xylem cavitation under high VPD with a low LSC, even when the stomata

are fully open. Moreover, this adjustment in the maximum g_s to LSC favors maximum stomatal opening for both UI and OI to maximize C gain without a risk of cavitation. Besides the existence of a stomatal limitation to net CO₂ assimilation, the drastic reduction of the relationship between C gain and water loss in UI—both in terms of WUE and $\delta^{13}\text{C}$ (Table 3)—indicates that the photosynthetic activity is more strongly limited by the low light availability in the understory than it is by reductions of g_s in accordance with past observations (Le Roux et al. 2001, Niinemets et al. 2004b). A prolonged dark period can result in slowed induction of photosynthesis in the dark (Tinoco-Ojanguren and Pearcy 1992, 1993), especially when combined with limited water availability (Aasamaa et al. 2002). Low rate of induction development could explain why A_N of UI only moderately improved when PPFD was temporarily increased due to the occurrence of a sunfleck (Table 3).

Conclusions

The great phenotypic plasticity of *A. pinsapo* allows this species to develop shoots for maximizing light capture in the forest understory, by increasing its dry mass allocation to leaf tissue and by reducing mutual needle shading and needle mass per area. These modifications enable shoots of understory individuals to maintain a positive C balance and, therefore, the trees can grow under environmental conditions where the photosynthetic activity is severely limited by the low light availability.

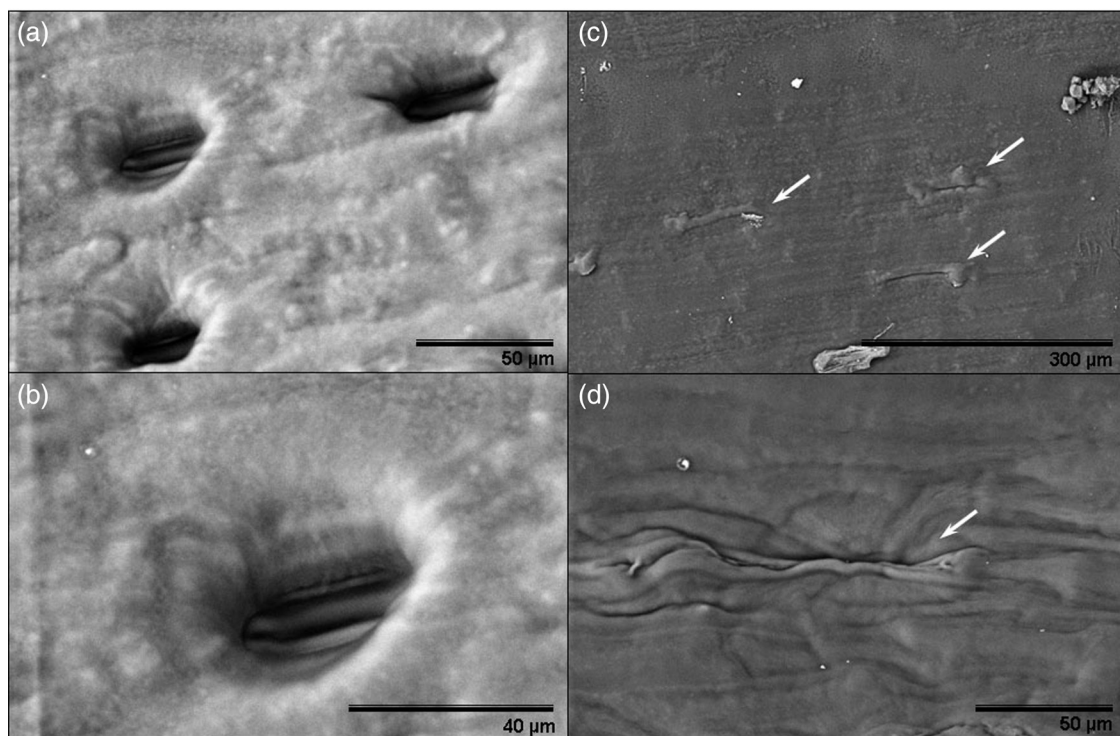


Figure 5. Scanning electron microscopy micrographs of fully developed (a, b) and aborted (c, d) stomata in needles of *A. pinsapo* grown in the understory.

As a consequence, individuals of *A. pinsapo* growing in the understory reduce their hydraulic conductance decreasing its capacity to supply water to the leaves. In order to cope with the high atmospheric VPD during the growing season in the understory, there is an adjustment of the maximum stomatal conductance to the hydraulic conductivity by means of a reduction in the stomatal density.

Limited hydraulic conductance has also major implications for plant response to possible rapid increases in light availability due to gap formation in the understory. Past research has demonstrated that conifers, in general, have a limited capacity to cope with such rapid changes (Tucker and Emmingham 1977, Tucker et al. 1987), partly reflecting limited hydraulic conductance (Niinemets 2012). Clearly more work is needed to gain insight into the overall capacity of trees to acclimate to combined stress conditions that are expected to be particularly severe after light conditions rapidly increase exposing trees with shade-adapted foliar and stem characteristics to high light.

Conflict of interest

None declared.

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References

- Aasamaa K, Söber A, Hartung W, Niinemets Ü (2002) Rate of stomatal opening, shoot hydraulic conductance and photosynthesis characteristics in relation to leaf abscisic acid concentration in six temperate deciduous trees. *Tree Physiol* 22:267–276.
- Abrams MD, Kubiske ME (1990) Leaf structural characteristics of 31 hardwood and conifer tree species in Central Wisconsin: influence of light regime and shade-tolerance rank. *For Ecol Manag* 31:245–253.
- Addington RN, Mitchell RJ, Oren R, Donovan LA (2004) Stomatal sensitivity to vapor pressure deficit and its relationship to hydraulic conductance in *Pinus palustris*. *Tree Physiol* 24:561–569.
- Aussenac G (2002) Ecology and ecophysiology of circum-Mediterranean firs in the context of climate change. *Ann For Sci* 59:823–832.
- Bacelar ELVA, Moutinho-Pereira JM, Gonçalves BMC, Brito CVQ, Gomes-Laranjo J, Ferreira HMF, Correia CM (2012) Water use strategies of plants under drought conditions. In: Aroca R (ed) *Plant responses to drought stress*. Springer, Berlin, pp 145–170.
- Bladon KD, Silins U, Landhäusser SM, Liefvers VJ (2006) Differential transpiration by three boreal tree species in response to increased evaporative demand after variable retention harvesting. *Agric For Meteorol* 138:104–119.
- Brodribb T, Cochard H (2009) Hydraulic failure defines the recovery and point of death in water-stressed conifers. *Plant Physiol* 149:575–584.
- Brodribb TJ, Holbrook NM, Zwieniecki MA, Palma B (2005) Leaf hydraulic capacity in ferns, conifers and angiosperms: impacts on photosynthetic maxima. *New Phytol* 165:839–846.
- Callaway RM (1992) Morphological and physiological responses of three California oak species to shade. *Int J Plant Sci* 153:434–441.
- Carpenter SB, Smith ND (1981) A comparative study of leaf thickness among southern Appalachian hardwoods. *Can J Bot* 59:1393–1396.
- Casson SA, Hetherington AM (2010) Environmental regulation of stomatal development. *Curr Opin Plant Biol* 13:90–95.
- Cescatti A, Zorer R (2003) Structural acclimation and radiation regime of silver fir (*Abies alba* Mill.) shoots along a light gradient. *Plant Cell Environ* 26:429–442.
- Choat B (2013) Predicting thresholds of drought-induced mortality in woody plant species. *Tree Physiol* 33:669–671.
- Cochard H (1992) Vulnerability of several conifers to air embolism. *Tree Physiol* 11:73–83.
- Cochard H (2002) A technique for measuring xylem hydraulic conductance under high negative pressures. *Plant Cell Environ* 25:815–819.
- Cochard H, Damour G, Bodet C, Tharwat I, Poirier M, Améglio T (2005) Evaluation of a new centrifuge technique for rapid generation of xylem vulnerability curves. *Physiol Plant* 124:410–418.
- Cochard H, Herbette S, Barigah T, Badel E, Ennajeh M, Vilagrosa A (2010) Does sample length influence the shape of xylem embolism vulnerability curves? A test with the Cavitron spinning technique. *Plant Cell Environ* 33:1543–1552.
- Corcuera L, Gil-Pelegrin E, Notivol E (2012) Differences in hydraulic architecture between mesic and xeric *Pinus pinaster* populations at the seedling stage. *Tree Physiol* 32:1442–1457.
- Croxdale J (2000) Stomatal patterning in angiosperm. *Am J Bot* 87:1069–1080.
- Cruziat P, Cochard H, Améglio T (2002) Hydraulic architecture of trees: main concepts and results. *Ann For Sci* 59:723–752.
- Daudet FA, Le Roux X, Sinoquet H, Adam B (1999) Wind speed and leaf boundary layer conductance variation within tree crown. Consequences on leaf-to-atmosphere coupling and tree functions. *Agric For Meteorol* 97:171–185.
- DeLucia EH, Maherali H, Carey EV (2000) Climate-driven changes in biomass allocation in pines. *Glob Change Biol* 6:587–593.
- Esteso-Martínez J, Peguero-Pina JJ, Valladares F, Morales F, Gil-Pelegrin E (2010) Self-shading in cork oak seedlings: functional implications in heterogeneous light environments. *Acta Oecol* 36:423–430.
- Fernández-Cancio A, Navarro-Cerillo R, Fernández-Fernández R, Gil-Hernández P, Manrique-Menéndez E, Calzado-Martínez C (2007) Climate classification of *Abies pinsapo* Boiss. forests in southern Spain. *Inv Agric Sist Rec For* 16:222–229.
- Franks PJ, Beerling DJ (2009) Maximum leaf conductance driven by CO₂ effects on stomatal size and density over geologic time. *Proc Natl Acad Sci USA* 106:10343–10347.
- Frazer GW, Canhan CD, Lertzman KP (1999) Gap Light Analyzer (GLA), Version 2.0: Imaging software to extract canopy structure and gap light transmission indices from true-color fisheye photographs, users manual and program documentation. Simon Fraser University, Burnaby, British Columbia and the Institute of Ecosystem Studies, Millbrook, New York.
- Geiger R, Aron RH, Todhunter P (2009) *The climate near the ground*. Rowman and Littlefield Publishers, Inc., Lanham, MD.
- Givnish TJ (1988) Adaptation to sun and shade: a whole-plant perspective. *Aust J Plant Physiol* 15:63–92.
- Gotsch SG, Geiger EL, Franco AC, Goldstein G, Meinzer FC, Hoffmann WA (2010) Allocation to leaf area and sapwood area affects water relations of co-occurring savanna and forest trees. *Oecologia* 163:291–301.
- Landhäusser SM, Liefvers VJ (2001) Photosynthesis and carbon allocation of six boreal tree species grown in understory and open conditions. *Tree Physiol* 21:243–250.
- Latorre AVP, Artero BC (2012) Phenomorphology and ecomorphological traits in *Abies pinsapo*. A comparison to other Mediterranean species. *Phytocoenologia* 42:15–27.

- Le Roux X, Bariac T, Sinoquet H, Genty B, Piel C, Mariotti A, Girardin C, Richard P (2001) Spatial distribution of leaf water-use efficiency and carbon isotope discrimination within an isolated tree crown. *Plant Cell Environ* 24:1021–1032.
- López R, López de Heredia U, Collada C, Cano FJ, Emerson BC, Cochard H, Gil L (2013) Vulnerability to cavitation, hydraulic efficiency, growth and survival in an insular pine (*Pinus canariensis*). *Ann Bot* 111:1167–1179.
- Maherali H, DeLucia EH (2000) Xylem conductivity and vulnerability to cavitation of ponderosa pine growing in contrasting climates. *Tree Physiol* 20:859–867.
- Martin TA, Hinckley TM, Meinzer FC, Sprugel DG (1999) Boundary layer conductance, leaf temperature and transpiration of *Abies amabilis* branches. *Tree Physiol* 19:435–443.
- Martínez-Cob A, Playán E, Zapata N, Cavero J, Medina ET, Puig M (2008) Contribution of evapotranspiration reduction during sprinkler irrigation to application efficiency. *J Irrig Drain Eng* 134:745–756.
- Martínez-Vilalta J, Cochard H, Mencuccini M et al. (2009) Hydraulic adjustment of Scots pine across Europe. *New Phytol* 184:353–364.
- Mayr S, Wolfschwenger M, Bauer H (2002) Winter-drought induced embolism in Norway spruce (*Picea abies*) at the Alpine timberline. *Physiol Plant* 115:74–80.
- Mayr S, Wieser G, Bauer H (2006) Xylem temperatures during winter in conifers at the alpine timberline. *Agric For Meteorol* 137:81–88.
- Mencuccini M, Bonosi L (2001) Leaf/sapwood area ratios in Scots pine show acclimation across Europe. *Can J For Res* 31:442–456.
- Mencuccini M, Grace J (1995) Climate influences the leaf area/sapwood area ratio in Scots pine. *Tree Physiol* 15:1–10.
- Mori A, Takeda H (2004) Functional relationships between crown morphology and within-crown characteristics of understory saplings of three codominant conifers in a subalpine forest in central Japan. *Tree Physiol* 24:661–670.
- Niinemets Ü (2010a) Responses of forest trees to single and multiple environmental stresses from seedlings to mature plants: past stress history, stress interactions, tolerance and acclimation. *Forest Ecol Manag* 260:1623–1639.
- Niinemets Ü (2010b) A review of light interception in plant stands from leaf to canopy in different plant functional types and in species with varying shade tolerance. *Ecol Res* 25:693–714.
- Niinemets Ü (2012) Commentary. Optimization of foliage photosynthetic capacity in tree canopies: towards identifying missing constraints. *Tree Physiol* 32:505–509.
- Niinemets Ü, Anten NPR (2009) Packing the photosynthesis machinery: from leaf to canopy. In: Laik A, Nedbal L, Govindjee (eds) *Photosynthesis in silico: understanding complexity from molecules to ecosystems*. Springer, Berlin, pp 363–399.
- Niinemets Ü, Kull O (1995a) Effects of light availability and tree size on the architecture of assimilative surface in the canopy of *Picea abies*: variation in needle morphology. *Tree Physiol* 15:307–315.
- Niinemets Ü, Kull O (1995b) Effects of light availability and tree size on the architecture of assimilative surface in the canopy of *Picea abies*: variation in shoot structure. *Tree Physiol* 15:791–798.
- Niinemets Ü, Sack L (2006) Structural determinants of leaf light-harvesting capacity and photosynthetic potentials. In: Esser K, Lüttge UE, Beyschlag W, Murata J (eds) *Progress in botany*. Springer, Berlin, pp 385–419.
- Niinemets Ü, Valladares F (2004) Photosynthetic acclimation to simultaneous and interacting environmental stresses along natural light gradients: optimality and constraints. *Plant Biol* 6:254–268.
- Niinemets Ü, Ellsworth DS, Lukjanova A, Tobias M (2002) Dependence of needle architecture and chemical composition on canopy light availability in three North American *Pinus* species with contrasting needle length. *Tree Physiol* 22:747–761.
- Niinemets Ü, Cescatti A, Christian R (2004a) Constraints on light interception efficiency due to shoot architecture in broad-leaved *Nothofagus* species. *Tree Physiol* 24:617–630.
- Niinemets Ü, Sonninen E, Tobias M (2004b) Canopy gradients in leaf intercellular CO₂ mole fractions revisited: interactions between leaf irradiance and water stress need consideration. *Plant Cell Environ* 27:569–583.
- Pammenter NW, Van der Willigen C (1998) A mathematical and statistical analysis of the curves illustrating vulnerability of xylem to cavitation. *Tree Physiol* 18:589–593.
- Pearcy RW (2007) Responses of plants to heterogeneous light environments. In: Pugnaire FI, Valladares F (eds) *Functional plant ecology*. CRC Press, Boca Raton, FL, USA, pp 213–246.
- Peguero-Pina JJ, Camarero JJ, Abadía A, Martín E, González-Cascón R, Morales F, Gil-Pelegrín E (2007) Physiological performance of silver-fir (*Abies alba* Mill.) populations under contrasting climates near the south-western distribution limit of the species. *Flora* 202:226–236.
- Peguero-Pina JJ, Sancho-Knapik D, Cochard H, Barredo G, Villarroya D, Gil-Pelegrín E (2011) Hydraulic traits are associated with the distribution range of two closely related Mediterranean firs, *Abies alba* Mill. and *Abies pinsapo* Boiss. *Tree Physiol* 31:1067–1075.
- Peltoniemi M, Duursma R, Medlyn B (2012) Co-optimal distribution of leaf nitrogen and hydraulic conductance in plant canopies. *Tree Physiol* 32:510–519.
- Pereira AR (2004) The Priestley-Taylor parameter and the decoupling factor for estimating reference evapotranspiration. *Agric For Meteorol* 125:305–313.
- Poyatos R, Martínez-Vilalta J, Čermák J et al. (2007) Plasticity in hydraulic architecture of Scots pine across Eurasia. *Oecologia* 153:245–249.
- Protz CG, Silins U, Lieffers VJ (2000) Reduction in branch sapwood hydraulic permeability as a factor limiting survival of lower branches of lodgepole pine. *Can J For Res* 30:1088–1095.
- Renninger HJ, Meinzer FC, Gartner BL (2007) Hydraulic architecture and photosynthetic capacity as constraints on release from suppression in Douglas-fir and western hemlock. *Tree Physiol* 27:33–42.
- Rundel PW, Jarrell WM (1989) Water in the environment. In: Pearcy RW, Ehleringer J, Mooney HA, Rundel PW (eds) *Plant physiological ecology: field methods and instrumentation*. Chapman and Hall, London, pp 29–56.
- Schoonmaker AL, Hacke UG, Landhäusser SM, Lieffers VJ, Tyree MT (2010) Hydraulic acclimation to shading in boreal conifers of varying shade tolerance. *Plant Cell Environ* 33:382–393.
- Schultz HR, Matthews MA (1993) Xylem development and hydraulic conductance in sun and shade shoots of grapevine (*Vitis vinifera* L.): evidence that low light uncouples water transport capacity from leaf area. *Planta* 190:393–406.
- Shumway DL, Steiner KC, Kolb TE (1993) Variation in seedling hydraulic architecture as a function of species and environment. *Tree Physiol* 12:41–54.
- Sprugel DG, Brooks JR, Hinckley TM (1996) Effects of light on shoot geometry and needle morphology in *Abies amabilis*. *Tree Physiol* 16:91–98.
- Stenberg P (1996) Simulations of the effects of shoot structure and orientation on vertical gradients in intercepted light by conifer canopies. *Tree Physiol* 16:99–108.
- Thompson FB, Leyton L (1971) Method for measuring leaf surface area of complex shoots. *Nature* 229:572.
- Tinoco-Ojanguren C, Pearcy RW (1992) Dynamic stomatal behavior and its role in carbon gain during lightflecks of a gap phase and an understory *Piper* species acclimated to high and low light. *Oecologia* 92:222–228.
- Tinoco-Ojanguren C, Pearcy RW (1993) Stomatal dynamics and its importance to carbon gain in two rainforest *Piper* species. II.

- Stomatal versus biochemical limitations during photosynthetic induction. *Oecologia* 94:395–402.
- Tucker GF, Emmingham WH (1977) Morphological changes in leaves of residual western hemlock after clear and shelterwood cutting. *For Sci* 23:195–203.
- Tucker GF, Hinckley TM, Leverenz JW, Jiang SM (1987) Adjustments of foliar morphology in the acclimation of understory Pacific silver fir following clearcutting. *For Ecol Manag* 21:249–268.
- Tyree MT, Sperry JS (1989) Vulnerability of xylem to cavitation and embolism. *Annu Rev Plant Phys* 40:19–38.
- Urli M, Porté AJ, Cochard H, Guengant Y, Burrett R, Delzon S (2013) Xylem embolism threshold for catastrophic hydraulic failure in angiosperm trees. *Tree Physiol* 33:672–683.
- Valladares F, Pearcy RW (2002) Drought can be more critical in the shade than in the sun: a field study of carbon gain and photo-inhibition in a Californian shrub during a dry El Niño year. *Plant Cell Environ* 25:749–759.
- Vilagrosa A, Bellot J, Vallejo VR, Gil-Pelegrín E (2003) Cavitation, stomatal conductance, and leaf dieback in seedlings of two co-occurring Mediterranean shrubs during an intense drought. *J Exp Bot* 54:2015–2024.
- von Arx G, Dobbertin M, Rebetez M (2012) Spatio-temporal effects of forest canopy on understory microclimate in a long-term experiment in Switzerland. *Agric For Meteorol* 166–167:144–155.
- von Arx G, Pannatier EG, Thimonier A, Rebetez M (2013) Microclimate in forests with varying leaf area index and soil moisture: potential implications for seedling establishment in a changing climate. *J Ecol* 101:1201–1213.
- Walters MB, Reich PB (1999) Low-light carbon balance and shade tolerance in the seedlings of woody plants: do winter deciduous and broad-leaved evergreen species differ? *New Phytol* 143:143–154.
- Youngblood A, Ferguson DE (2003) Changes in needle morphology of shade-tolerant seedlings after partial overstory canopy removal. *Can J For Res* 33:1315–1322.