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Conservative Decrease in Water Potential in Existing Leaves during New Leaf Expansion in Temperate and Tropical Evergreen *Quercus* Species

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• *Background and Aims* This study aimed at clarifying how the water potential gradient ($\Delta\Psi$) is maintained in the shoots of evergreen trees with expanding leaves, whose leaf water potentials at the turgor loss point (Ψ_{tlp}) are generally high.

• *Materials* The water relations were examined in current-year expanding (CEX) and 1-year-old (OLD) leaves on the same shoots in temperate (Osaka, Japan) and tropical (Bogor, Indonesia) areas. A temperate evergreen species, *Quercus glauca* growing in both sites, was compared with a temperate deciduous species, *Q. serrata*, in Osaka, and two tropical evergreen species, *Q. gemelliflora* and *Q. subsericea*, in Bogor.

• *Key Results* (1) In Osaka, the midday leaf water potential (Ψ_{midday}) was slightly higher in OLD (-0.5 MPa) than in CEX leaves (-0.6 MPa), whereas Ψ_{tlp} was significantly lower in OLD (-2.9 MPa) than in CEX leaves (-1.0 MPa). In Bogor, Ψ_{midday} was also higher in OLD leaves (-1.0 MPa) despite the low Ψ_{tlp} (-1.9 MPa), although stomatal conductance was not always low in OLD leaves. In the branch bearing CEX and OLD leaves, most of the hydraulic resistance (86 %) exists in the current-year branch, leading to differences in water supply between CEX and OLD leaves. The removal of buds just before breaking did not affect the high Ψ_{midday} in OLD leaves after 1 month. Ψ_{midday} in OLD leaves thus appears to be independent of that in CEX leaves.

• *Conclusions* The moderate decrease in Ψ_{midday} in OLD leaves would contribute to maintenance of $\Delta\Psi$ in the shoots during leaf expansion.

Key words: Cohesion–tension theory, hydraulic resistance, leaf expansion, leaf water potential, pressure–volume curve, *Quercus*, turgor maintenance, water potential gradient.

INTRODUCTION

The leaf water potential at the point where leaf cells incipiently lose their turgor (i.e. the turgor loss point, Ψ_{tlp}) is high (less negative) in expanding leaves. Ψ_{tlp} of leaves early in their development was approx. -1 MPa in *Acer saccharum* and *Populus* spp. (Tyree *et al.*, 1978). Ψ_{tlp} gradually decreases with leaf maturation, and Ψ_{tlp} of mature leaves ranges from -1.5 to -3 MPa in many broad-leaved tree species (Hinckley *et al.*, 1978; Roberts *et al.*, 1980; Parker *et al.*, 1982; Saito *et al.*, 2003). The high Ψ_{tlp} of expanding leaves potentially causes problems in water flow in the pathway which continues from the soil, through the plant, and into the surrounding atmosphere (the soil–plant–atmosphere continuum).

Water flow in the soil–plant–atmosphere continuum has been explained by the cohesion–tension (C–T) theory (Dixon and Joly, 1894). The C–T theory proposes that when water is transpired from a leaf, a negative gradient of hydrostatic pressure develops in the continuous column of water inside the xylem conduits. This is because hydrogen bonds hold water molecules together and there are resistances to water flow in the xylem conduits that extend from the roots to the leaves. The gradient of water potential thus formed causes water to flow in the tree.

The C–T theory has been widely accepted since the introduction of the pressure-chamber technique, which has been used successfully to measure the negative pressure that develops in the xylem of leaves and branches (Scholander *et al.*, 1965). According to the C–T theory, water potential of the leaves at the end of the water transport pathway must be lower than those in other parts of the plant. In other words, the low leaf water potential (Ψ_{leaf}) ensures stable water flow through the plant.

However, cells of expanding leaves cannot maintain turgor if the leaf water potential at midday (Ψ_{midday}) decreases to the level that is usually experienced by mature leaves. This is the result of the higher Ψ_{tlp} in expanding leaves than that in mature leaves. Ψ_{midday} usually does not decrease below Ψ_{tlp} (Tyree and Jarvis, 1982; Saito *et al.*, 2003; Saito and Terashima, 2004). Indeed, turgor loss inhibits physiological activities in plant cells (Hayashi and Takagi, 2003; Heidecker *et al.*, 2003). Ψ_{leaf} of expanding leaves will remain high and this indicates that large water potential gradients do not develop in the plant during this period. Thus, water flow within the soil–plant–atmosphere continuum may be unstable when new leaves are expanding.

The shoots of evergreen tree species are particularly interesting because new leaves, which are usually located at the tips of shoots, expand in the presence of mature

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leaves on the same shoots. This situation does not occur in common deciduous trees which have only a single flush of leaves per season (Kikuzawa, 1982). In the shoots of evergreen trees, water from the roots must pass through branches that bear mature leaves to reach the expanding leaves. If Ψ_{leaf} in the mature leaves decreased below the Ψ_{tlp} of the expanding leaves, cell turgor could potentially be lost in the expanding leaves because water would move preferentially along the steeper pressure gradient into the mature leaves. Thus, it was hypothesized that the water potential gradient ($\Delta\Psi$) within the shoot is maintained despite the high Ψ_{tlp} of expanding leaves. The Ψ_{leaf} of 1-year-old (OLD) leaves would play an important role in the water flow in the plants.

The aim of the present study was to clarify whether $\Delta\Psi$ was maintained in the shoots of evergreen trees while new leaves are expanding. We conducted three experiments in a temperate climate (Osaka, Japan) and a tropical climate (Bogor, Indonesia) using *Quercus* species. Firstly, changes in Ψ_{leaf} and Ψ_{tlp} in current-year and OLD leaves was followed throughout leaf expansion in Osaka (expt 1). Secondly, the relationship between Ψ_{leaf} in current-year expanding (CEX) leaves and in OLD leaves was examined again in Bogor, because the Ψ_{leaf} of OLD leaves could be lower due to the drier atmosphere there (expt 2). Thirdly, buds were removed just before breaking and the effect on Ψ_{leaf} of OLD leaves was examined in Osaka to test whether the expanding current-year shoot controls Ψ_{leaf} of OLD leaves (expt 3). These experiments clarified the role of OLD leaves in turgor maintenance in expanding leaves.

MATERIALS AND METHODS

Experiment 1: leaf development in Osaka

The study site in Osaka was in a secondary forest on the Toyonaka Campus of Osaka University (central Honshu Island, Japan; 34 °N, 135 °E, 55 m a.s.l.). In 2002, the mean annual temperature was 16·6 °C, with the highest mean monthly temperature (28·8 °C) in July and the lowest (6 °C) in January. The annual precipitation was 819 mm (Toyonaka Meteorological Observatory). During the period of measurement in 2002, the mean monthly temperatures were 16·1, 19·5 and 23·4 °C in April, May and June, respectively. Monthly precipitation totalled 61, 114 and 103 mm in the corresponding months.

Quercus glauca, an evergreen, and *Q. serrata*, a deciduous broad-leaved tree species (both Fagaceae), were used. These species are common in the warm temperate forests of Japan. Healthy mature trees of *Q. glauca* (10·8 m tall) and of *Q. serrata* (16·8 m tall) were used. Several shoots at the middle of the canopy in these trees were used for leaf sampling. The photosynthetic photon flux density (PPFD) relative to that in the open site for these shoots was approx. 25 %.

Phenological events and measurements are summarized in Table 1. In 2002, changes in leaf area were monitored by measuring width and length of leaves every second or third day after bud break. The bud break occurred around 4 April for *Q. glauca* and 6 April for *Q. serrata* (Saito

et al., 2006). The leaf area attained 99 % of the final size on 24 April in *Q. glauca* and on 26 April in *Q. serrata*. These dates were designated as the dates of full leaf expansion (FLE) for these species. Leaf ages in the text preceded by a − or + sign denote the number of days before or after FLE, respectively.

Pre-dawn and midday leaf water potential. Changes in leaf water potential (Ψ_{leaf}) were followed from 15 April to 19 July throughout leaf development using a pressure chamber (Soilmoisture Equipment, Santa Barbara, CA, USA). Ψ_{leaf} was measured before dawn (Ψ_{predawn}) and at midday (Ψ_{midday}, 1200–1400 h). For *Q. glauca*, the measurements were conducted separately on current-year and OLD leaves. For each set of measurements, at least six leaves from three trees were used for each species. One of the three trees was the tree used for the measurements of leaf area expansion and pressure–volume curves, and others were growing adjacent to the tree.

Pressure–volume measurements. Shoots about 300 mm in length were cut from the trees, and the bases of the shoots were cut again under water. Each cut end of the shoots was submerged in water in a flask. The shoot was covered with a plastic bag and kept in a dark room. The next day, water drops were carefully removed from each sample leaf. Immediately after cutting, Ψ_{leaf} was measured with the pressure chamber. Leaf weight was measured before and after the measurement of Ψ_{leaf}, and mean values were recorded. Then, decreases in Ψ_{leaf} and leaf

TABLE 1. *Phenological events and measurements*

Date	Month	Year	Events and measurments
Leaf development in Osaka			
4	April	2002	Bud break in *Q. glauca*
6			Bud break in *Q. serrata*
15 April to 19 July			Ψ_{midday}, Ψ_{predawn}, P–V curve, leaf area
24	April		Full leaf expansion in *Q. glauca*
26			Full leaf expansion in *Q. serrata*
Leaf development in Bogor			
6–31	October	2003	P–V curve
14			Installing a weather station
19			Ψ_{predawn}, Ψ_{midday}, g_{s} in CEX and OLD
27			Ψ_{midday}, g_{s} in CEX and OLD
28			Ψ_{predawn}, Ψ_{midday}, g_{s} in CEX and OLD
4 October to 3 November		2004	P–V curve, hydraulic resistance
7	October		Installing a weather station
22			Ψ_{predawn}, Ψ_{midday}, g_{s} in CMT and OLD
25			Ψ_{midday}, g_{s} in CMT and OLD
Debudding experiment in Osaka			
6	April	2005	Ψ_{predawn}, Ψ_{midday}, g_{s}
7			Removing buds from treatment shoots
8–9			P–V curve
10			Bud break in control shoots
18			Leaf area
28–29			Ψ_{midday}, g_{s}
30			Full leaf expansion
30 April to 1 May			P–V curve

weight were followed as the leaf dried on the laboratory bench. Data were plotted with the aid of a calculation program working on spreadsheet software and a pressure–volume (P–V) curve was constructed (Scholander *et al.*, 1965; Maruyama and Morikawa, 1983; Turner, 1988; Saito *et al.*, 2006). For each leaf, more than 12 data points (in a few cases, seven points) were obtained in an initial curve, and four to six points were obtained in the liner part. From the P–V curves, the leaf water potential when leaf cells incipiently lose their turgor (Ψ_{tlp}; MPa) and the ratio of leaf dry weight to leaf fresh weight at water saturation (DW/SW) were obtained.

Experiment 2: leaf development in Bogor

The study site was in the Bogor Botanical Garden (6 °S, 106 °E, 235–250 m a.s.l.), about 60 km south of Jakarta (western Java, Indonesia). The seasonal change in temperature is small, but precipitation varies widely. The mean annual temperature in 2002 was 27·4 °C, with the highest mean monthly temperature (28·5 °C) in October and the lowest (25·7 °C) in February (Center for Soil and Agro-Climate in Bogor). The rainy season usually lasts October to April, and the short dry period from June to August, although the monthly precipitation varies widely from year to year (Hatta *et al.*, 2005). The highest monthly precipitation (779 mm) was recorded in January and the lowest (134 mm) was in August in 2002· The annual precipitation was as much as 4551 mm in 2002, so that Bogor city is called 'Kota Hujan' (rainy city). In October 2002, the mean temperature was 28·5 °C and the precipitation was 199·8 mm. The midday vapour-pressure deficit (VPD) on sunny days was usually >0·04 Pa Pa^{-1}.

On 14 October in 2003 and 7 October 2004, a custom-built weather station was installed at the nursery of Treub Laboratory, which is located about 0·75 km from the sample trees. PPFD, air temperature and relative humidity were recorded at 10-min intervals on a data logger (Thermic, 2300A, Eto-denki, Tokyo, Japan) until the end of October.

Two saplings of *Q. glauca* (8 m tall), a temperate species native to Japan, were used for the experiment in Bogor. The saplings had been planted >5 years ago and were growing healthily. Also, one sapling of *Q. gemelliflora* (8 m tall) and one mature tree of *Q. subsericea* (30 m tall) were used. Both are tropical species from west Malesia. All the trees were growing adjacently with about 20 m in distance with other neighbouring trees in an open location, and were not irrigated or pruned by gardeners. New leaves expanded, over a range of dates, on the shoots of both *Q. gemelliflora* and *Q. glauca*. In contrast, new leaves expanded simultaneously in mid-October in most shoots of *Q. subsericea*.

A phenological study conducted in the garden at Bogor (Hatta *et al.*, 2005) reported that leaf longevity was >14 months in *Q. gemelliflora*, from 13 to 18 months in *Q. glauca*, and 10 months or >24 months in *Q. subsericea*. Thus, the actual age of the mature old leaves could not be identified. Accordingly, the leaves that had expanded one-cycle before the latest expansion were called 'one-cycle-old leaves'.

Leaf water relations. In 2003, diurnal changes in Ψ_{leaf} and stomatal conductance (g_s) were monitored from 0500 to 1600 h on 19 and 28 October and from 1100 to 1300 h on 27 October (Table 1) at intervals of approx. 1 h. These values were obtained from the CEX leaves which were at around FLE and the OLD leaves on the same shoot, simultaneously. In 2004, measurements were conducted from 0500 to 1600 h on 22 October and from 1100 to 1400 h on 25 October. Ψ_{leaf} and g_s were obtained from the current-year mature (CMT) leaves and OLD leaves on the same shoot, simultaneously. $\Psi_{predawn}$ was measured between 0500 and 0600 h.

Stomatal conductance (g_s) was measured using a porometer (AP4; Delta-T Devices, Cambridge, UK). Then, each of the leaves was severed, placed in a humidified plastic bag, and brought to the Treub Laboratory within 30 min by motorbike. Ψ_{leaf} was measured with the pressure chamber inside the laboratory. At each set of the measurements, four to five leaves were measured. After sorting Ψ_{leaf} values of diurnal change, daily minimum leaf water potential (Ψ_{midday}) was obtained by averaging the six lowest points from the minimum value. Similarly, midday g_s was calculated. The leaves used for the calculation of Ψ_{midday} were not always identical to those used for the midday g_s. Two erroneous points of Ψ_{leaf} in OLD leaves below −2 MPa which were obtained on 28 October 2003 were omitted, because the other six points measured at the same time were all greater than −1·29 MPa. P–V measurement was conducted in the Treub Laboratory. Shoots were sampled in the early morning and were re-hydrated for >2 h. P–V curves of CEX and CMT leaves were measured on the sampling day and that of OLD leaves on the next day.

Hydraulic resistance in the shoots. To describe the difference in water status for the leaves in different positions, the hydraulic resistances of the component parts of a whole one-cycle-old shoot were examined. The values were not standardized on the length and cross-sectional area of the xylem in the branch.

The whole 1-cycle-old shoots, each of which contained only one current-year shoot, were selected (approx. 350 mm in total length; see Fig. 1). At around 0800 h, the shoots were cut at the bud-scale scars at the base of one-cycle-old shoots, and immediately placed in a humidified plastic bag. In the Treub Laboratory, the shoots were re-cut at 10 mm from the cut end under water, and covered with a plastic bag for at least 30 min for absorbing water.

The shoot was supported horizontally by a clamp, and exposed to the air of the laboratory at around 22 °C, with a VPD of 0·013 Pa Pa^{-1} and a PPFD of 6 μmol m^{-2} s^{-1}. A plastic tube which was 5 mm in diameter and 1500 mm in length was filled with distilled water. One end of the tube was connected tightly to the distal end of the shoot. Bubbles in the tube were carefully removed. Then, the other end was submerged in distilled water in a plastic bag placed inside the pressure chamber.

A pressure of 0·1 MPa was applied to the water column in the tube using the pressure chamber. Water leaked neither
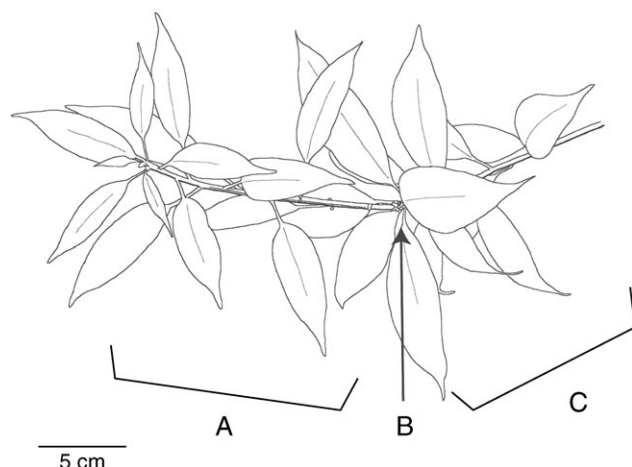
FIG. 1. Morphology of a typical shoot of *Quercus subsericea* in Bogor. (A) A current-year shoot with current-year expanding (CEX) leaves; (B) the junction with bud-scale scars between the current-year and one-cycle-old shoots; (C) a one-cycle-old shoot with one-cycle-old (OLD) leaves. 'one-cycle-old' leaves and shoots denote the mature old leaves and shoots which had expanded one-cycle before the latest expansion when measurements were conducted.

from the connection between the tube and the shoot, nor from any injuries on the shoot. After about 10 min, the top part of the current-year shoot bearing two or three leaves (about 15 mm in length) was cut from the shoot. Water exuded from the cut end was collected using pink tissue paper held in small plastic tubes (20 mm long). The pink colour made it easier to watch wetting of the paper. The wet tissue paper and tube were weighed at 5-min intervals for 45 min and the amount of water exuded (W, mg) was obtained. The total hydraulic conductance of the shoot (G_t; mg s^{-1} MPa^{-1}) was calculated as follows:

$$G_t = W/TP \qquad (1)$$

where T is time (s) and P is the pressure applied (MPa). In practice, W was plotted against T, and the slope of the regression line was designated as G_t. The total hydraulic resistance (R_t) was calculated as the reciprocal of G_t. No apparent reduction in G_t was detected during the observation for 45 min. The effect of xylem embolisms was minimized by sampling the shoots early in the morning and by applying sufficient hydrostatic pressure to the shoot.

After this measurement, a pressure of 0·01 MPa was applied to the shoot using a water column 1 m in height created using a plastic bottle (1·5 L) and the same type of tubing. After connection of the tube to the shoot, the current-year shoot was cut at about 10 mm above the bud-scale scars. The hydraulic conductance of the remaining branch was measured in the same manner to obtain the sum of the hydraulic resistance in the junction and that in the one-cycle-old shoot ($R_j + R_o$). The resistance of the current-year shoot (R_c) was calculated for each shoot as follows:

$$R_c = R_t - (R_j + R_o) \qquad (2)$$

Then, the junction was cut just below the bud-scale scars. R_o was measured and R_j was calculated. These procedures were repeated using more than five shoots for each species.

Experiment 3: removal of the current-year shoot

On the Toyonaka Campus in Osaka, one mature *Q. glauca* tree was selected just before bud break in March 2005· The tree was growing adjacent to the trees used in the experiment in 2002· Ten healthy shoots were randomly selected from a large branch (70 mm in diameter) and divided into the control shoots and the 'treatment' shoots to be debudded. $\Psi_{predawn}$ was measured between 0530 and 600 h on 6 April (Table 1) using ten OLD leaves on the shoots adjacent to the sample shoots. Then, Ψ_{midday} and g_s were measured from 1000 to 1300 h using five OLD leaves on the control and treatment shoots, respectively. Ψ_{leaf} was measured with the pressure chamber and g_s was measured with a steady-state porometer (Li-1600, LiCor, Lincoln, NE, USA). On 7 April, all buds on the treatment shoots were removed with a razor blade. On 8 and 9 April, Ψ_{tlp} values were obtained from the P–V curves using eight leaves from four shoots similar to the sample shoots.

The date of FLE of the control shoots in 2005 was 30 April. The FLE was estimated from leaf area measured on 18 April 2005 with the detailed time course of expansion in 2002· On 28 and 29 April, Ψ_{midday} and g_s were measured between 1000 and 1200 h using ten OLD leaves each on the control and debudded shoots. On 30 April and 1 May, these shoots were sampled to measure P–V curves for OLD leaves. Ψ_{tlp} and DW/SW were obtained.

The weather station was placed in the open place beside the sample tree during the measurements. PPFD, air temperature and relative humidity were recorded at 10-min intervals using the data logger. The g_s data were recalculated using the flow rate of dry air provided by the porometer and the ambient air temperature and relative humidity from the weather station as described in Saito *et al.* (2003).

Statistical analysis

All statistical tests were conducted according to the methods of Sokal and Rohlf (1995). Regression lines were calculated using the least-squares method. Significant differences between means were detected using the *t*-test.

RESULTS

Experiment 1: leaf development in Osaka

Figure 2A shows representative P–V curves for *Q. glauca* leaves in Osaka. As leaves aged, the turgor loss point (Ψ_{tlp}) became more negative, changing from about −1·0 MPa on −8 day to a value of about −2·5 MPa on +45 day. The relative water content when turgor loss occurs decreased as leaves aged, though this tendency did not apply to OLD leaves. Similar changes were observed in P–V curves of *Q. glauca* in Bogor (Fig. 2B), but the
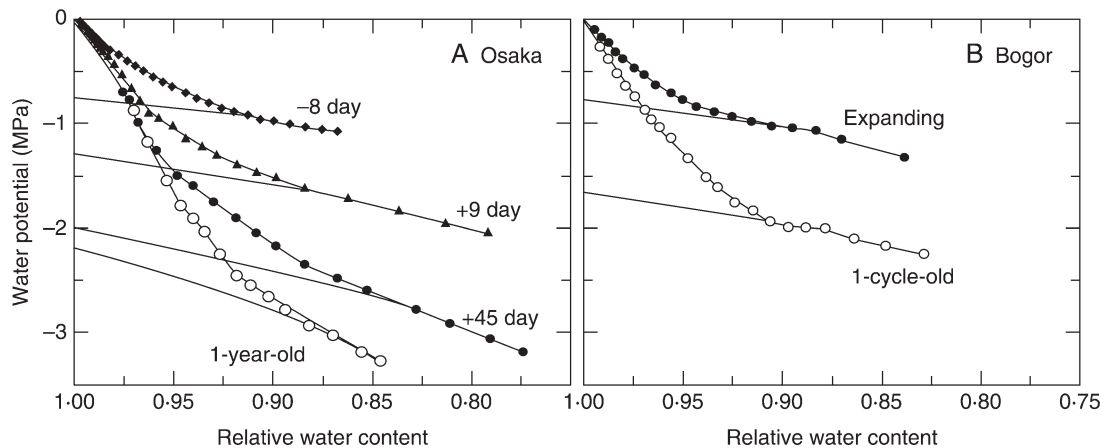
FIG. 2. Representative pressure–volume curves for *Q. glauca* leaves measured in (A) Osaka and (B) Bogor. Data points represent the measured leaf water potential and the curves extrapolated denote the estimated osmotic potential. Leaf age in (A) represents the number of days before (−) and after (+) full leaf expansion.

turgor of OLD leaves was lost when < 10 % water was lost from the leaves and Ψ_{leaf} was −2 MPa.

The measurement of Ψ_{leaf} was conducted on mostly sunny days. The predawn leaf water potential ($\Psi_{predawn}$) was around −0·1 MPa in both species throughout the experiment (Fig. 3A and B). The stable and high value for $\Psi_{predawn}$ indicates that the sample leaves did not experience long-term water stress during leaf development.

Ψ_{midday} of the current-year leaves before FLE stayed constant at approx. −0·6 MPa in both species. The abrupt increase in Ψ_{midday} in +3 day and +6 day in both species resulted from cloudy weather. Subsequently, Ψ_{midday} of current-year leaves in *Q. glauca* decreased gradually to a value of −1·5 MPa on +27 day and Ψ_{midday} of *Q. serrata* decreased to approx. −2·2 MPa on +39 day. In *Q. glauca*, Ψ_{midday} of OLD leaves was approx. −0·5 MPa before FLE and decreased gradually, in parallel with that of the current-
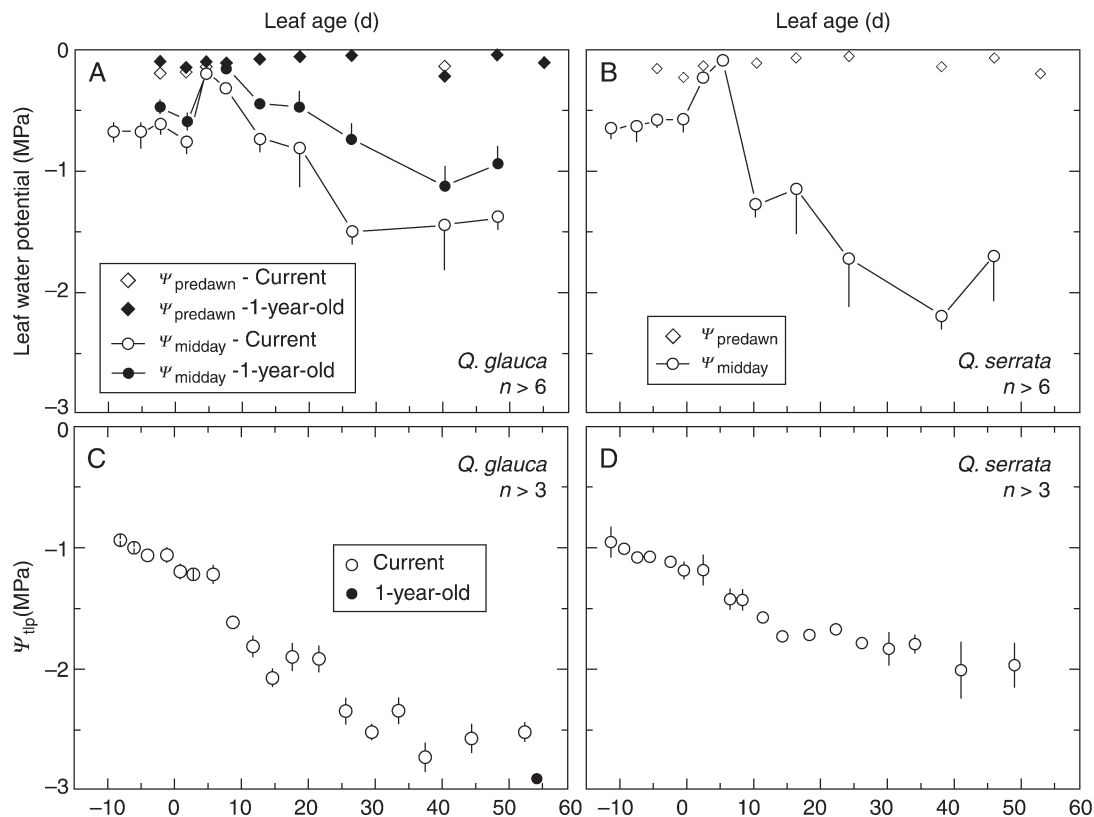

FIG. 3. Predawn ($\Psi_{predawn}$) and midday (Ψ_{midday}) leaf water potentials in (A) evergreen *Q. glauca* and (B) deciduous *Q. serrata*, and leaf water potential at the turgor loss point (Ψ_{tlp}) in (C) *Q. glauca* and (D) *Q. serrata*. Measurements were conducted in Osaka. Values are means ± s.d.

TABLE 2. *Leaf mass per area (LMA) and leaf area in the three* Quercus *species*

	LMA (g m^{-2})		Leaf area (cm^2)	
	Expanding (CEX)	One-cycle-old (OLD)	Expanding (CEX)	One-cycle-old (OLD)
Q. gemelliflora	48·6 ± 3·3	96·7 ± 13·5**	35·3 ± 10·6	30·7 ± 9·2 n.s.
Q. glauca	50·3 ± 12·0	92·3 ± 9·8**	35·7 ± 10·0	40·4 ± 9·4 n.s.
Q. subsericea	35·4 ± 7·2	79·1 ± 6·1**	27·2 ± 8·9	32·5 ± 10·8 n.s.

Measurements were conducted in Bogor using 16 leaves from six leaves in 2003 and ten leaves in 2004. (mean ± s.d.)
** and n.s. denote $P < 0.01$ and not significant for statistical significances in the differences between leaf ages for a species.

year leaves. Ψ_{midday} of OLD leaves was always greater (less negative) than that of the current-year leaves, by approx. 0·15 MPa before FLE and by >0·30 MPa afterwards.

The leaf water potential at the turgor loss point (Ψ_{tlp}) decreased gradually as the leaves matured in both species (Fig. 3C and D). Before FLE, Ψ_{tlp} was approx. −1·0 MPa in both species, but decreased slowly. After FLE, Ψ_{tlp} in *Q. glauca* decreased to a minimum value of approx. −2·5 MPa by +40 day. The corresponding value in *Q. serrata* was approx. −1·8 MPa by +40 day. In *Q. glauca*, Ψ_{tlp} in OLD leaves was −2·91 MPa. Ψ_{midday} of current-year leaves appeared to parallel the gradual decrease in Ψ_{tlp} of these leaves. In addition, a lower PPFD on 1-year-old shoots was reported for solitary grown *Q. glauca* trees (Miyazawa *et al.*, 2004), but the shoots of *Q. glauca* were arranged sparsely in the sample trees in this study. Thus, PPFD relative to that in the open site would be around 25 % in both CEX and OLD leaves.

Experiment 2: three Quercus species in Bogor

Leaf area and leaf mass per area. The actual age of the current-year leaves could not be identified in Bogor due to the limited study period. Instead, LMA and DW/SW were used as an indicator of leaf age. The LMA of CEX leaves ranged from 35 to 50 g m^{-2} and was approximately half that of OLD leaves in the three species (Table 2). Also, DW/SW obtained from P–V curves was approximately

50 % and 80 % of the values for OLD leaves in CEX and CMT leaves, respectively. Based on the detailed time courses for changes in LMA and DW/SW with leaf age in Osaka in 2002 (Saito *et al.*, 2006), CEX leaves were around FLE and CMT leaves were more than +20 days. The leaf area ranged from 27 to 40 cm^2 and neither of the differences between CEX and OLD leaves was significant in the three species (Table 2).

Micrometeorology. All field measurements were conducted on sunny days. During the day (1000 to 1400 h) on 19, 27 and 28 October 2003, PPFD, air temperature and VPD was generally more than 1000 μmol m^{-2} s^{-1}, 30 °C and 0·03 Pa Pa^{-1}, respectively. On 22 and 25 October 2004, those values were more than 1000 μmol m^{-2} s^{-1}, 32 °C and 0·035 Pa Pa^{-1}, respectively. Thus, the measurements would not have been biased by the difference between 2003 and 2004·

Leaf water relations. In all the species, $\Psi_{predawn}$ was approx. −0·2 MPa irrespective of the leaf ages (data not shown). Ψ_{midday} of CEX leaves was high (more than −1·0 MPa; Fig. 4A) as was observed in Osaka (Fig. 3A). Ψ_{midday} in OLD leaves was also generally high (more than −1·1 MPa) and was similar to that in CEX leaves on the same shoot. For example, Ψ_{midday} values were −1·02 and −1·14 MPa, respectively, in CEX and OLD leaves on the same shoot of *Q. gemelliflora*. Although the value of CEX leaves was slightly higher than that of OLD leaves,
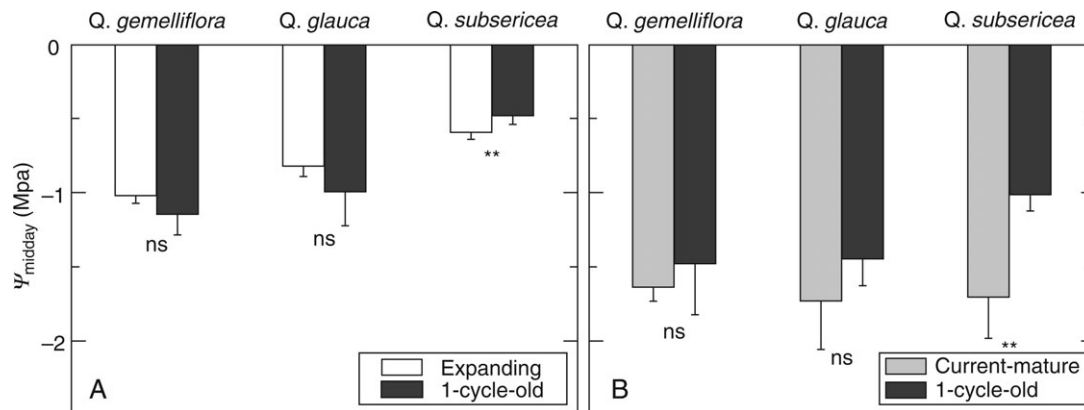

FIG. 4. Midday leaf water potential (Ψ_{midday}) in the three *Quercus* species in Bogor. Data were collected from (A) expanding leaves and one-cycle-old leaves on the same shoot, and (B) current-year mature leaves and one-cycle-old leaves on the same shoot. Values are means ± s.d. (n=6). ** and ns denote $P < 0.01$ and not significant for statistical significances in the difference between leaf ages for a species.
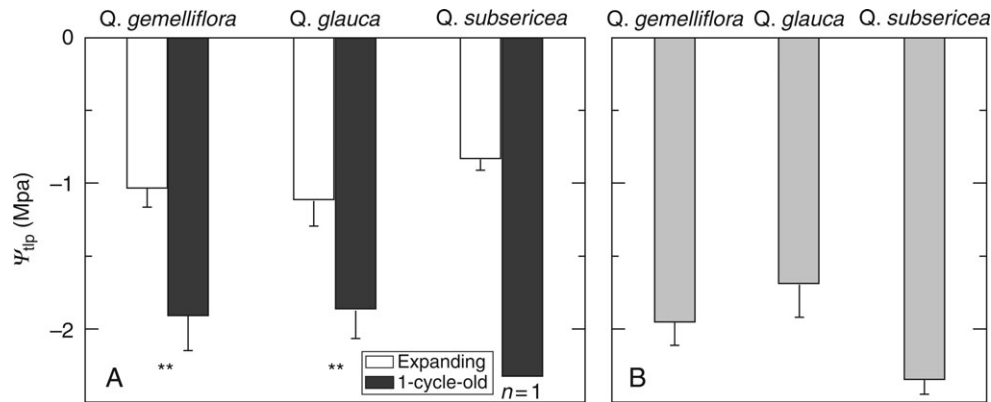
FIG. 5. Leaf water potential at the turgor loss point (Ψ_{tlp}) in the three *Quercus* species in Bogor. Data was collected from (A) expanding leaves and the one-cycle-old leaves on the same shoot, and (B) current-year mature leaves. Values are means $\pm$ s.d. ($n > 5$). ** and ns denote $P < 0.01$ and not significant for statistical significances in the difference between leaf ages for a species.

the inverse water flow from CEX toward OLD leaves would not be significant. On the shoot with CMT leaves, Ψ_{midday} of CMT leaves decreased to less than -1.5 MPa in the three species (Fig. 4B). Ψ_{midday} of OLD leaves also decreased to the level of CMT leaves. For example, the Ψ_{midday} values were -1.64 and -1.49 MPa in CMT and OLD leaves on the same shoot in *Q. gemelliflora*. A significant difference in Ψ_{midday} between leaf ages was observed only in *Q. subsericea* in Fig. 4A and B.

The leaf water potential at the turgor loss point (Ψ_{tlp}) was significantly lower in OLD leaves than in CEX leaves on the same shoot in all the three species (Fig. 5A). For example, Ψ_{tlp} were -1.05 and -1.92 MPa, respectively, in CEX and OLD leaves of *Q. gemelliflora*. After maturation of the leaves, Ψ_{tlp} of CMT leaves (Fig. 5B) was similar to the Ψ_{tlp} values of OLD leaves measured with CEX leaves. Ψ_{tlp} of CMT leaves (Fig. 5B) was lower than Ψ_{midday} of CMT leaves in *Q. gemelliflora* and *Q. subsericea* (Fig. 4B), although slightly higher in *Q. glauca* (-1.69 MPa and -1.73 MPa, respectively). In addition, Ψ_{tlp} of OLD leaves in *Q. glauca* in Bogor (-1.86 MPa; Fig. 5A) was much higher than that in Osaka (-2.91 MPa; Fig. 3C).

Stomatal conductance. In the expanding shoots, g_s in CEX leaves was similar in the three species and was around 100 mmol m^{-2} s^{-1} (Table 3). But, g_s in OLD leaves varied and was highest in *Q. gemelliflora*, followed by *Q. glauca* and *Q. subsericea*. In the mature shoots, g_s in

CMT leaves was 3–7 times higher than CEX leaves and was highest in *Q. gemelliflora* followed by *Q. glauca* and *Q. subsericea*. Also, g_s values in CMT leaves were twice that in OLD leaves on the same shoot. g_s of OLD leaves in mature shoots was lower than that in OLD leaves in expanding shoots.

PPFD on the sample leaves varied from 22 to 50 µmol m^{-2} s^{-1} in 2003, and from 68 to 397 µmol m^{-2} s^{-1} in 2004, with large standard deviations due to sunflecks. Between the years, the effects of different PPFD on g_s would not be large. Among the species, PPFD values on *Q. subsericea* leaves were below 68 µmol m^{-2} s^{-1} and tended to be lower than those of other two species. Between the leaves with different ages, a clear difference in PPFD was not found in any of the three species. Thus, the values of g_s would reflect the capacity of the leaves.

g_s of CMT leaves of *Q. glauca* in Bogor was approx. seven times the value measured in Osaka (Miyazawa *et al.*, 2004). The much higher precipitation in October in Bogor (200 mm) than Osaka (50 mm) may have ensured an abundant water supply to the leaves. In addition, it may be worth mentioning that different instruments were used in these two studies.

Hydraulic resistance in the shoots. The resistances in the respective parts of the shoot (Table 4) are expressed as proportions to R_t because of large differences in R_t (from 0.56 to 5.42 MPa s mg^{-1}). R_c was >86 % of R_t when new leaves

TABLE 3. *Midday stomatal conductance (g_s, mmol m^{-2} s^{-1}) in the three* Quercus *species in Bogor*

	Expanding shoot		Mature shoot	
	Expanding leaves (CEX)	One-cycle-old leaves (OLD)	Current-year mature leaves (CMT)	One-cycle-old leaves (OLD)
Q. gemelliflora	165.5 ± 66.0	482.5 ± 89.3**	556.7 ± 98.5	309.5 ± 34.5**
Q. glauca	71.0 ± 15.9	276.5 ± 61.3**	471.7 ± 31.4	221.5 ± 34.9**
Q. subsericea	135.5 ± 62.5	115.8 ± 28.3 n.s.	340.8 ± 64.6	107.0 ± 9.7**

Data were collected from expanding leaves (CEX) and one-cycle-old (OLD) leaves on the same shoots, or from current-year mature leaves (CMT) and OLD on the same shoots.

Values were mean $\pm$ standard deviations of six largest points in the data of diurnal measurements.

** and n.s. denote $P < 0.01$ and not significant for statistical significance in the difference between leaf ages.

TABLE 4. *Hydraulic resistances of the parts of shoots*

		Relative hydraulic resistance		
	n	Current-year branch (R_c)	Junction (R_j)	One-cycle-old branch (R_o)
Q. gemelliflora	6	0.868 ± 0.077	0.098 ± 0.050	0.034 ± 0.029
Q. glauca	7	0.859 ± 0.086	0.106 ± 0.061	0.034 ± 0.033
Q. subsericea	5	0.953 ± 0.040	0.026 ± 0.018	0.021 ± 0.024

The one-cycle-old branches with one current-year branch were examined for the three *Quercus* species in Bogor.

Values are relative to the total hydraulic resistance of the whole shoot (mean $\pm$ standard deviation).

The parts of the shoots are current-year branch, the junction between current and one-cycle-old branch, and the one-cycle-old branch.

were at FLE. R_j was $< 10\%$ and R_o was only about 3 % of R_t in the three species.

Experiment 3: removal of the current-year shoot

Ψ_{midday} in OLD leaves did not change much from bud break to FLE in both the control and debudded shoots (from around -0.8 MPa to around -1.0 MPa; Fig. 6A). Also, Ψ_{tlp} in OLD leaves did not change much during the corresponding period in both shoots (from -2.64 MPa to around -2.4 MPa; Fig. 6B). The difference between the control and debudded shoots was not significant in both Ψ_{midday} and Ψ_{tlp} ($P > 0.05$). There were large differences between Ψ_{midday} and Ψ_{tlp} in OLD leaves both before and after FLE, so that the daily minimum turgor of leaf cells was about 1.5 MPa.

g_s of OLD leaves was around 6.8 mmol m^{-2} s^{-1} in both shoots before bud break (Fig. 6C). At FLE, g_s increased in the control shoot (8.0 mmol m^{-2} s^{-1}), but decreased in the debudded shoot (5.7 mmol m^{-2} s^{-1}), so that the difference in g_s was significant between the shoots ($P < 0.01$). In addition, $\Psi_{predawn}$ was -0.14 ± 0.07 (average $\pm$ standard deviation) before bud break, suggesting that the plants were not experiencing serious water stress. Ψ_{midday} values at FLE in 2005 were slightly lower than in 2002 (Fig. 3A).

All measurements were conducted on sunny days. Both before bud break and at FLE in April 2005, air temperature and VPD during the day (1000–1400 h) were generally above 20 °C and 0.02 Pa Pa^{-1}, respectively. PPFD at FLE was 1300 μmol m^{-2} s^{-1} and higher by 300 μmol m^{-2} s^{-1} than that before bud break. This may partly explain the larger g_s after FLE in the control shoots. The higher g_s in control shoots than debudded shoots at FLE indicates that the development of current-year shoots enhances hydraulic conductance of 1-year-old shoots. Probably, basipetal transport of IAA (indole-3-acetic acid) from expanding leaves and shoots promoted cambial activity of the 1-year old stem (Funada *et al.*, 2001).

DISCUSSION

The results support the hypothesis that the gradient in water potential in the shoot is maintained even when new leaves are expanding. Despite the low Ψ_{tlp} below -1.8 MPa in OLD leaves (Figs 3C and 5A), Ψ_{midday} was usually greater than -1 MPa in these leaves (Figs 3A and 4A). This value was higher than Ψ_{midday} and Ψ_{tlp} in CEX leaves. Thus, the moderate decrease in Ψ_{midday} in OLD leaves played a central role in maintaining the water potential gradient both in temperate and tropical areas.

Leaf development in Osaka

From the data measured in Osaka, changes in cell turgor in CEX leaves were examined. The minimum level of turgor at midday can be estimated using the Ψ_{midday} value and the P–V curves (Pavlik, 1984; Saito *et al.*, 2003). From the results, a minimum turgor pressure of approx. 0.3 MPa was maintained before FLE in both species. After FLE, a minimum turgor of approx. 0.6 MPa was consistently maintained in *Q. glauca*. On the other hand, zero turgor probably occurred in *Q. serrata* on +25 and +39 days due to decreases in Ψ_{midday} (Fig. 3B and D). This would result from the optimistic stomatal
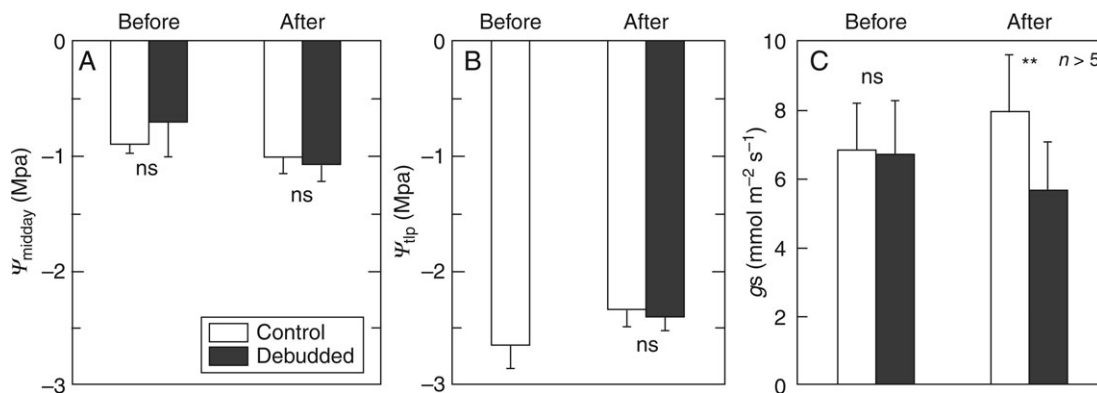

FIG. 6. (A) Leaf water potential at midday (Ψ_{midday}), (B) leaf water potential at the turgor loss point (Ψ_{tlp}), and (C) stomatal conductance at midday (g_s) in 1-year-old leaves of *Q. glauca*. Debudded shoots had the buds that would generate the current-year shoots removed before bud break. Measurements were conducted before bud break (Before) and after full leaf expansion (After) on the control shoots in Osaka. Values are means $\pm$ s.d. ($n>5$). ** and ns denote $P < 0.01$ and not significant for statistical significances in the difference between leaf ages for a species.

opening in deciduous species in comparison to evergreen species (Sobrado, 1986). However, $\Psi_{predawn}$ remained sufficiently high even on these days (Fig. 3B). Thus, cell turgor was almost consistently maintained in CEX leaves in both species.

The high Ψ_{midday} of CEX leaves (about -0.7 MPa) could potentially disturb the water potential gradient in the shoots of *Q. glauca*. But in reality, Ψ_{midday} in OLD leaves (about -0.5 MPa) was slightly higher than that in CEX leaves (Fig. 3A). Thus, the water potential gradient would be maintained in the shoot. Moreover, Ψ_{tlp} in OLD leaves (-2.9 MPa) was considerably lower than Ψ_{midday} (Fig. 3C). Consequently, the daily minimum turgor in OLD leaves was as much as 1.7 MPa. This suggests that the high Ψ_{midday} in OLD leaves is not for maintaining the critical level of turgor of the leaves, which is around 0.5 MPa in the various plants (discussed in Saito and Terashima, 2004). Alternatively, the conservative decrease in Ψ_{leaf} in OLD leaves ensures a water potential gradient within the shoot during leaf expansion and consequently maintains turgor in CEX leaves.

Expanding leaves and one-cycle-old leaves in Bogor

The moderate decrease in Ψ_{leaf} in OLD leaves would play a more critical role in expansion of new leaves in Bogor. The present results show that Ψ_{midday} in OLD leaves was again high (more than -1.2 MPa) and comparable to that in CEX leaves (Fig. 4A). In contrast, Ψ_{tlp} of OLD leaves was below -1.8 MPa (Fig. 5A). Thus, the daily minimum turgor of OLD leaves was approx. 0.5 MPa in *Q. gemelliflora*, 0.8 MPa in *Q. glauca*, and would be 1.3 MPa in *Q. subsericea*. This high Ψ_{midday} in OLD leaves was maintained in spite of high g_s during leaf expansion (Table 3). Values of g_s in OLD leaves were comparable to those of deciduous species in temperate areas (Saito *et al.*, 2003). After maturation of the current-year leaves, Ψ_{midday} of OLD leaves also decreased following the decrease in Ψ_{midday} of CMT leaves (Fig. 4B), as was observed in Osaka (Fig. 3A).

Stomatal conductance (g_s) of CEX leaves was generally smaller than that of OLD leaves (Table 3), but Ψ_{midday} values of CEX leaves were similar to those of OLD leaves (Fig. 4A). These results indicate that there is a large resistance to water flow between OLD leaves and CEX leaves. In fact, >85 % of the total resistance of the branch existed in the current-year branch in all three species (Table 4). Ikeda and Suzaki (1984) reported that the hydraulic conductivity of current-year shoots was small relative to other parts of trees of *Lithocarpus edulis* Makino (Fagaceae) as well. These results suggest that the large hydraulic resistance of current-year shoots and their small g_s contribute to maintaining stable water conditions in the CEX leaves.

The high Ψ_{midday} in OLD leaves suggests that the stem on the pathway of water flow to CEX leaves should have higher water potential than CEX leaves. Indeed, shoots were re-hydrated before leaf or flower buds began to expand during a severe dry season in savanna in Costa Rica (Reich and Borchert, 1984; Borchert, 1994). In contrast, the inverse gradient of water potential is observed between developing flowers or fruits and subsidiary leaves growing on the same shoot (Trolinder *et al.*, 1993; Nobel *et al.*, 1994; Chapotin *et al.*, 2003). Even backflows of water from fruit to stem via the xylem were observed (Lang and Thorpe, 1989; Lang, 1990). With the inverse gradient, water would be transported by the phloem along with photosynthates (Nobel *et al.*, 1994). A similar situation is assumed at the start of the leaf expansion. However, this study dealt with the leaves at a later stage of expansion.

Removal of current-year shoots

As the current-year leaves matured, Ψ_{midday} of OLD leaves decreased following the decreasing Ψ_{midday} of CMT leaves (Figs 3A and 4B). This result suggests that CEX leaves control Ψ_{leaf} of OLD leaves by sending a hormonal signal such as ABA to OLD leaves. The ABA could cause stomatal closure (Davies *et al.*, 1994), resulting in low g_s and high Ψ_{midday} in OLD leaves. However, the Ψ_{midday} of OLD leaves was not significantly affected by the removal of buds (Fig. 6A). In addition, g_s of OLD leaves in the debudded shoots was not high, but significantly lower than in the control shoots at FLE (Fig. 6C). This rejects the assumption that Ψ_{midday} of OLD leaves is controlled by a signal from the expanding current-year shoot. The hydration of OLD leaves by root pressure at the whole-tree level is not likely, because leaf expansion did not always occur simultaneously in all the shoots in Bogor. It is likely that the charging of water occurs in each shoot that will produce CEX leaves, but the mechanism is not clear at present.

This apparently autonomous behaviour of water relations in OLD leaves contrasts with the previous reports that g_s was tightly regulated by hydraulic signals propagated along the water transport pathway within an individual. For example, when the covering of a large part of the crown was removed suddenly, the g_s of other foliage decreased within 1 min in some conifer trees (Whitehead *et al.*, 1996; Pepin *et al.*, 2002).

Turgor maintenance in leaf cells is usually examined in individual leaves. In contrast, this study showed for the first time that turgor of CEX leaves is maintained by physiological adjustment at the shoot level. In this process, adjusting $\Delta\Psi t$ is indispensable for ensuring an adequate water supply to the shoot tip. In further examinations into $\Delta\Psi$, the water in the stem that could behave as a buffer for water use in CEX and OLD leaves should be considered. If the buffering effect is large, water transport to CEX leaves will occur even when the Ψ_{leaf} of OLD leaves is lower than that in CEX leaves. Thus, it is not necessary for the Ψ_{leaf} of OLD leaves to be high for water flow to CEX leaves. However, in the shoots of *Quercus* in this study, Ψ_{leaf} of OLD leaves was higher or similar to that of CEX leaves. Thus, water buffer is not required for water supply to CEX leaves. Also, it is possible that CEX and OLD leaves use a different xylem conduit in the branch. Farther studies including a mathematical modelling are required.

CONCLUSIONS

Ψ_{midday} of OLD leaves did not decrease below Ψ_{tlp} of CEX leaves despite the much lower Ψ_{tlp} in OLD leaves in both temperate and tropical areas. This small decrease in Ψ_{midday} in OLD leaves did not result from stomatal closure and was not affected by removal of the current-year shoot. The results suggest that the conservative decrease in Ψ_{midday} in OLD leaves contributes to the maintenance of a water potential gradient in the shoots of evergreen trees.

ACKNOWLEDGEMENTS

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