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短 報

Seasonal variations in the vessel size of temperate, diffuse-porous species and its relationship to leaf water potential

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温帯の散孔材樹種における道管サイズの季節変化と水分条件との関係

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Vessel lumen area and leaf water potential (ψ_{leaf}) at predawn and midday were measured periodically to reveal seasonal variations in the vessel sizes of diffuse-porous species and to determine the factors that cause this variation. Two deciduous species (*Cercidiphyllum japonicum* and *Liriodendron tulipifera*) and one evergreen species (*Quercus glauca*) were studied. In most of the samples, mean vessel lumen area (MVLA) initially increased then eventually decreased after a period, but this period varied among species. In *C. japonicum*, the decrease in MVLA coincided with a decrease in predawn ψ_{leaf} . In contrast, MVLA variations in most samples of *L. tulipifera* and *Q. glauca* did not show such a relationship. However, one of the *L. tulipifera* samples had an earlier decrease in MVLA that coincided with a lower predawn ψ_{leaf} . There was no clear relationship between the variation in vessel size and midday ψ_{leaf} , because midday ψ_{leaf} showed daily variation depending on vapor pressure deficit, which had less seasonality. Thus, we concluded that there are species-specific annual patterns in vessel size variation that are likely determined by internal factors. Low water availability is one of the external factors inducing the formation of narrower vessels.

Key words: Annual ring, diffuse-porous wood, tree water relation, vessel size

散孔材における道管サイズの季節変化、およびその変化を引き起こす要因を明らかにするため、道管形成の観察と夜明け前と日中の葉の水ポテンシャル (ψ_{leaf}) の測定を定期的に行った。試料には落葉樹のカツラとユリノキ、常緑樹のアラカシを用いた。全ての樹種で道管サイズは一年の成長の始めに増加し、ある時期から減少していた。減少が始まる時期は樹種によって異なっており、カツラでは夜明け前の ψ_{leaf} の低下と対応が見られた。一方でユリノキとアラカシでは、道管サイズと夜明け前の ψ_{leaf} の対応は見られなかった。ただし夜明け前の ψ_{leaf} が他よりも低下していた個体では道管サイズの減少が早く始まっていたため、乾燥ストレスの影響が示唆された。日中の ψ_{leaf} は日変動が大きく、道管サイズとの明確な関連性は見られなかった。したがって、年輪内の道管サイズの変化は主に内的要因によって決まっており、乾燥ストレスは外部要因として道管サイズを減少させると考えられた。

キーワード：年輪、散孔材、水分生理、道管サイズ

1. Introduction

Diffuse-porous wood shows a less distinct variation in vessel diameter than ring-porous wood does. While ring-porous trees produce obviously large-diameter vessels in their initial period of annual growth, diffuse-porous trees produce vessels of similar size throughout a ring. However, on close examination, a variation in vessel diameter characterized by a decrease in the terminal period of annual growth can be found in diffuse-porous woods in temperate regions (Sass and Eckstein 1995,

Schume et al. 2004). We also found annual cyclicity in vessel diameter, even in seasonal tropical climates (Ohashi et al. 2009a, 2009b) and humid tropical climates with less seasonality (Ohashi et al. 2011). These observations indicate that diffuse-porous nature is primarily regulated by internal factors, but it can also be influenced by external factors.

Since most tropical trees lack distinct annual rings, the variation of vessel diameter is a useful indicator for detecting annual rings. However, factors that trigger this variation are not fully understood because research

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focusing on the variation of vessel diameter in a diffuse-porous wood is scarce. Understanding such factors is important for the validation and improvement of annual ring detection methods for tropical trees. Additionally, revealing the factors and timing that contribute to vessel variation is helpful in understanding the phenology and water relations of trees because vessels transport water and vessel diameters determine potential hydraulic conductivity.

Water status is one of the factors that affect vessel diameter. Since turgor pressure is a driving force for cell enlargement (Ray et al. 1972) and the subsequent process is extension with water uptake, less water availability would result in the formation of smaller sized vessels. Doley and Leyton (1968) found that even a slight reduction of water potential in *Fraxinus* had a depressing effect on cell division and expansion. In addition, water-control experiments have shown that low water availability causes a reduction in vessel diameter and a simultaneous increase in vessel density (Schume et al. 2004, Searson et al. 2004, Stevenson and Mauseth 2004, Arend and Fromm 2007, Fichot et al. 2009). Since these changes increase conductive safety (Mauseth and Stevenson 2004), it is reasonable to consider these changes as reactions that decrease embolism risk. Thus, tree water status is related to turgor maintenance and conductive safety, both of which affect vessel diameter.

Another factor affecting vessel characteristics is the status of phytohormones such as indole-3-acetic acid (IAA), which induces vessel differentiation (Digby and Wareing 1966, Zakrzewski 1983). Since IAA is mainly produced by young leaves (Hess and Sachs 1972), flushing and maturing of leaves causes seasonal variations in IAA concentration and results in the variation of vessel diameter. However, studies have shown contrasting results on the effect of IAA on vessel formation. While some studies show that higher IAA concentrations induce the production of wider vessels (Digby and Wareing 1966, Doley and Leyton 1968), others show that higher IAA concentrations induce the production of narrower vessels (Aloni and Zimmermann 1983, Tuominen et al. 1997). In addition, Zakrzewski (1991) found that there is an optimal IAA concentration for inducing the production of wide vessels, and concentrations higher or lower than the optimal concentration result in narrower vessel diameters. Thus,

it seems difficult to formulate a hypothesis on the effect of phytohormones on seasonal variation of vessel diameter, but leaf phenology and shoot elongation could be factors affecting the seasonal variation of vessel diameters through phytohormone production.

In this study, we aimed to investigate the factors that cause periodic variations of vessel size in diffuse-porous species. Although our final goal is to reveal the contributing factors in tropical areas, temperate trees were selected in the case study because they experience distinct seasons and have clear annual rings. Since Sass and Eckstein (1995) pointed out the influence of rainfall amount on the variation of vessel lumen size, we focused especially on tree water status variation. Sass and Eckstein (1995) have also indicated that phytohormone concentration and leaf and shoot elongation influence vessel size, and hence, we also considered these factors. In this study, our objectives were to (1) investigate seasonal variation of vessel size in diffuse-porous woods, (2) examine whether species-specific variation is observed, and (3) investigate how tree water status affects vessel size.

2. Materials and methods

2.1. Research site and sample selection

Research was conducted at the Yoshida North Campus of Kyoto University (Kyoto, Japan, 35°01'N, 135°47'E). The site has a temperate humid climate with high precipitation, more than 100 mm per month from March to October and more than 200 mm per month in June, July, and September. The weather in 2009, when the research was carried out, was characterized by very high precipitation (more than 300 mm) in July and low precipitation (around 50 mm) in September (Fig. 1).

Cercidiphyllum japonicum (CJ), *Liriodendron tulipifera* (LT), and *Quercus glauca* (QG) planted for ornamental purposes were chosen for this study. *C. japonicum* and *Q. glauca* are indigenous species widely distributed in natural forests of Japan, whereas *L. tulipifera* is an introduced species from North America. *C. japonicum* and *L. tulipifera* are deciduous trees and *Q. glauca* is an evergreen tree. Vessel porosity of all species is diffuse-porous, and vessel arrangement of *Q. glauca*, in particular, is usually described as radial-porous (Fig. 2). All species have distinct annual rings, which are

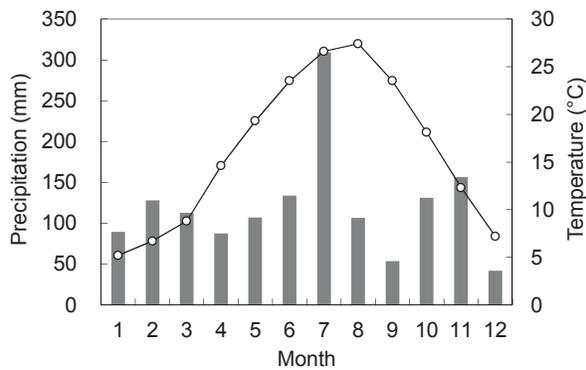


Fig. 1 Mean monthly precipitation (bar) and mean monthly temperature (line) in Kyoto city in 2009. Data were provided by Japan Meteorological Agency (<http://www.jma.go.jp>).

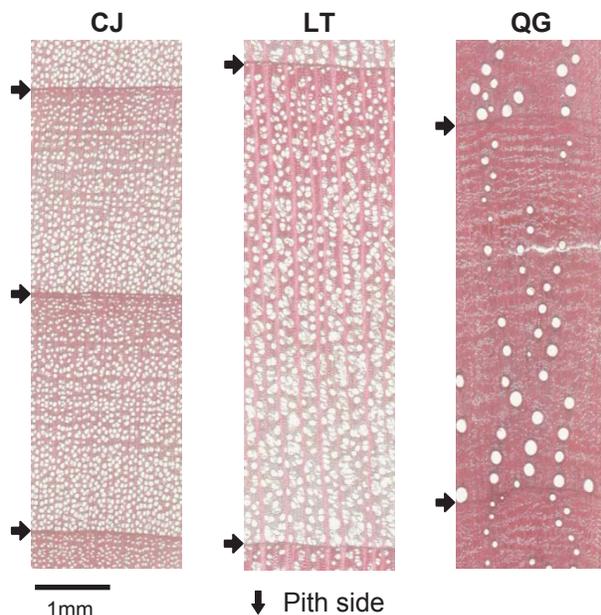


Fig. 2 Cross section from each species. Each arrow points to a distinct annual ring boundary. CJ: *Cercidiphyllum japonicum*; LT: *Liriodendron tulipifera*; QG: *Quercus glauca*.

demarcated by bands of flattened wood fibers in the terminal part of a ring for *C. japonicum* and *Q. glauca* and by bands of axial parenchyma for *L. tulipifera*. Formation of reaction wood, which affects vessel size and number, was examined by checking the G-layer of wood fibers under a microscope. Reaction wood was not observed in this study.

Three trees for each species were selected for this study. Core samples were collected from breast height with an increment borer (5 mm in diameter) about every 4 weeks from late May to late September of 2009 to estimate periodical growth during each sampling period.

Sampling positions were selected to be vertically or horizontally 2–3 cm apart from the first sampling site. In the last sampling, the cores that were used for vessel analysis were collected from approximately 5 cm below the fifth sampling site to reduce the influence of wound reactions caused by the previous samplings.

2.2. Vessel analysis

Cross sections of each sample were cut using a sliding microtome and stained with safranin. The sections were scanned at 4000 dpi resolution to digitize the image by using POLASCAN 4000 (Polaroid Corp., Waltham, MA, USA). Images of vessels that were broken during sectioning were restored, and color of the whole image was binarized using Adobe Photoshop 5.5 (Adobe Systems, San Jose, CA, USA). The lumen area and the distance between the cambium and the centroid of each vessel were measured using Image J (National Institutes of Health, Bethesda, MD, USA).

Mean vessel lumen area (MVLA) and vessel frequency (VF) were calculated at 0.2-mm intervals along the radius to examine radial variation. Tangential length of the analysis area was determined on the basis of the mean vessel frequency of each species as follows: 0.4 mm for *C. japonicum* (225 mm^{-2}), 1.0 mm for *L. tulipifera* (76 mm^{-2}), and 2.5–3.0 mm for *Q. glauca* (5 mm^{-2}). The vessels around the edge of the analysis area were excluded if their centroids were outside of the area. Weighted moving average (WMA) was applied to reduce the noise and smooth out radial variations. The number of points (filter length) used for the smoothing was different among samples because the optimum number depends on the annual growth width. In our previous paper (Ohashi et al. 2011), we have suggested that the optimum filter length for the detection of annual variation is approximately 50% of the annual growth width. The filter length, however, seemed to be a little too long for detecting the variations in one growing season. We, therefore, tested the filter length of 25% of annual growth width.

2.3. Estimation of tree water status

Leaf water potential (ψ_{leaf}) of each tree was measured using a pressure chamber (PC-40; Daiki Rika Kogyo Co., Saitama, Japan) every week from May 27 to September 30, 2009. Predawn ψ_{leaf} was measured within 1 h of dawn to estimate the water availability of the trees, and midday

ψ_{leaf} was measured between 1:00 PM and 2:00 PM to estimate the intensity of drought stress during the daytime.

Since there was a strong negative correlation between the midday ψ_{leaf} and midday vapor pressure deficit (VPD) of atmosphere in all species ($r = -0.849$ to -0.720 , $P < 0.001$), the midday ψ_{leaf} on all days during the research period was estimated from VPD and smoothed with a 15-point WMA to emphasize the seasonal variation.

3. Results and discussion

3.1. *Cercidiphyllum japonicum*

MVLA of all samples showed distinct seasonal variation, whereas VF did not show such a pattern (Fig. 3). MVLA initially increased, but eventually decreased in late May. The rapid decrease in MVLA was most pronounced from late May to late June, with a simultaneous increase in VF. Since predawn ψ_{leaf} of *C. japonicum* in that period was lower than that of the other 2 species, it seems that low water availability prompted the decrease in MVLA and the increase in VF. These simultaneous variations in MVLA and VF are similar to the reaction to reduce embolism risk, as indicated by Stevenson and Mauseth (2004). Moreover, after June, MVLA continued to decrease as well as the predawn ψ_{leaf} . Estimated midday ψ_{leaf} had a tendency to be low in late June and mid-August; similar tendency and values of ψ_{leaf} were also found in *L. tulipifera* and *Q. glauca* (Figs. 4 and 5), because their midday ψ_{leaf} was estimated from VPD. Moreover, in these periods, MVLA decreased and VF increased. Thus, MVLA in *C. japonicum* is considered to be linked with water availability and drought stress at midday.

3.2. *Liriodendron tulipifera*

MVLA in *L. tulipifera* sample 1 (LT1) increased initially then decreased in early July and continued to decrease until the end of the study period (Fig. 4). Since predawn ψ_{leaf} in LT1 was high for nearly the entire growth period, the variation of MVLA in LT1 was not caused by water availability. However, we cannot exclude the possibility that midday drought stress in late June triggered the MVLA decrease. Although the variation of MVLA in *L. tulipifera* sample 2 (LT2) was similar to that in LT1, water availability seemed to affect vessel formation in LT2. The

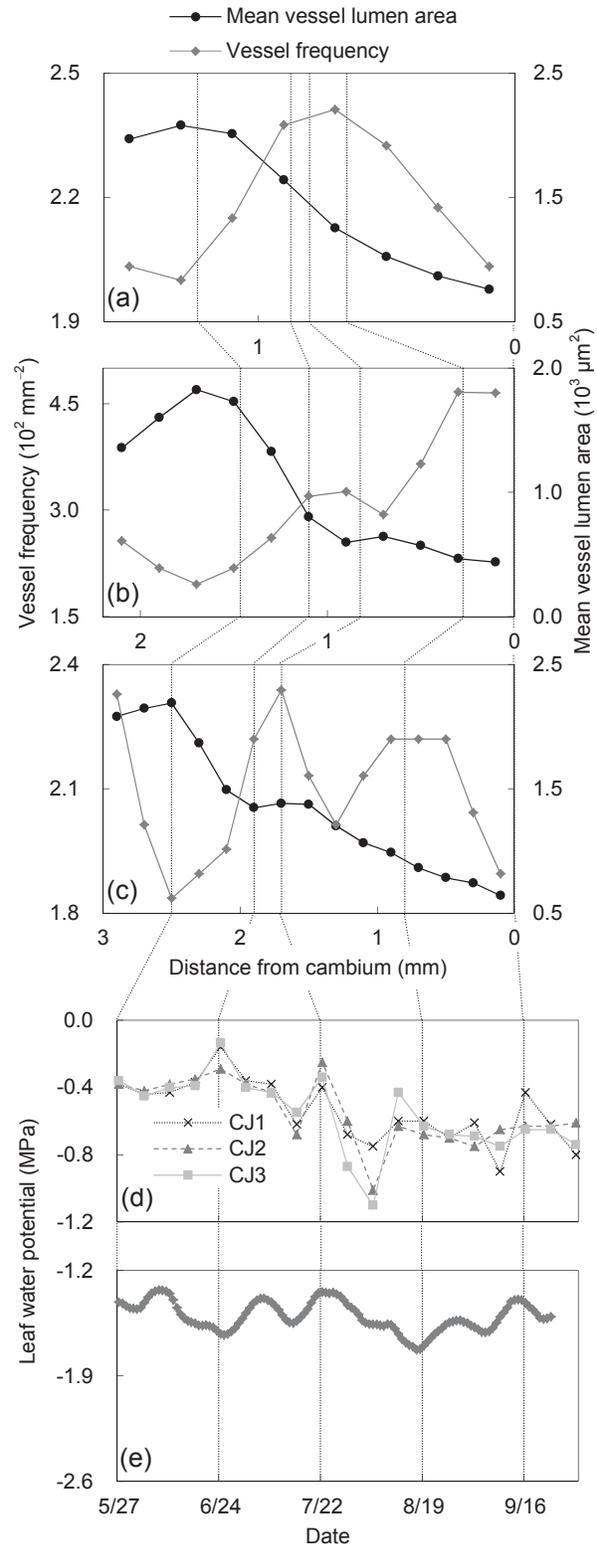


Fig. 3 Variation in mean vessel lumen area (MVLA), vessel frequency (VF) and leaf water potential in *Cercidiphyllum japonicum* (CJ). (a) MVLA and VF smoothed by 3-point weighted moving average (WMA) in CJ1, (b) CJ2, and (c) CJ3; (d) predawn leaf water potential of each sample; (e) estimated midday leaf water potential. The dotted lines in (a), (b), and (c) are estimated growth width between each sampling.

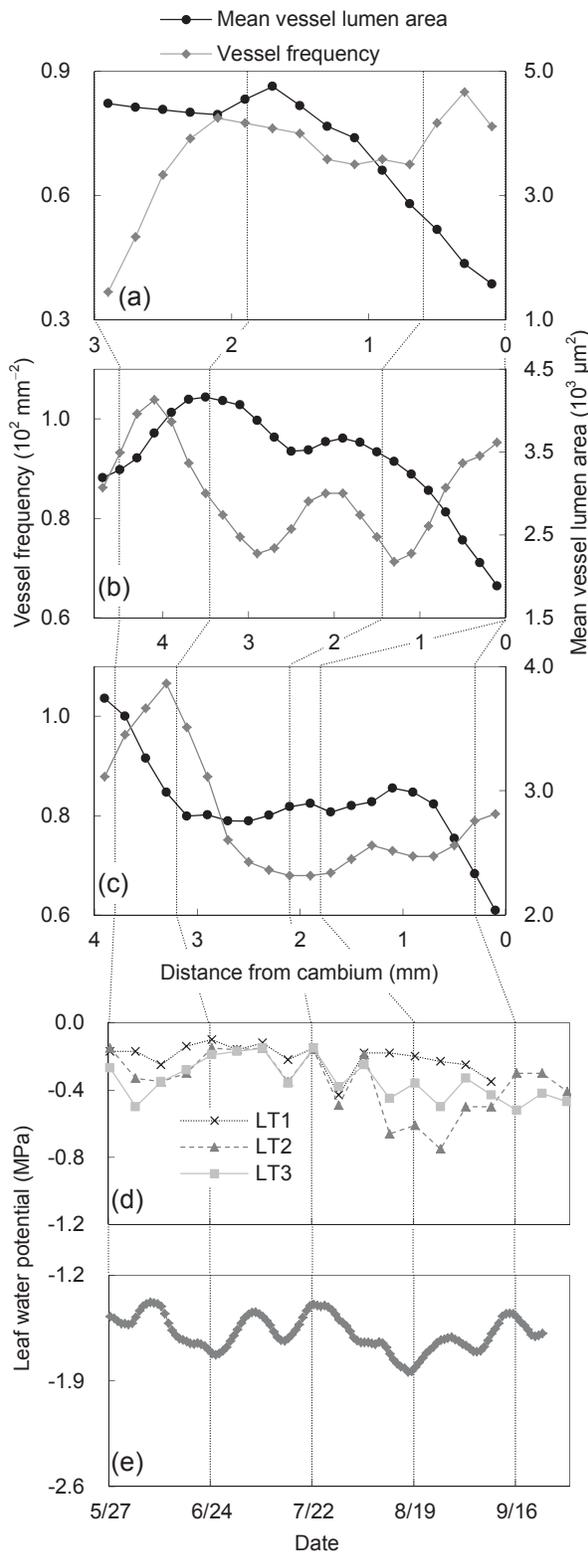


Fig. 4 Variations in mean vessel lumen area (MVLA), vessel frequency (VF) and leaf water potential in *Liriodendron tulipifera* (LT). (a) MVLA and VF smoothed by 3-point weighted moving average (WMA) in LT1, (b) smoothed by 5-point WMA in LT2 and (c) LT3; (d) predawn leaf water potential of each sample; (e) estimated midday leaf water potential. The dotted lines in (a), (b), and (c) are estimated growth width between each sampling. Predawn leaf water potential data of LT1 is lacking on and after September 16.

decrease in MVLA was slower in July, when predawn ψ_{leaf} recovered, and the decrease in MVLA was rapid in August, when predawn ψ_{leaf} decreased; these variations are attributable to changes in water availability. The decrease in MVLA started earlier (in June) in *L. tulipifera* sample 3 (LT3) than in LT1 and LT2, and predawn ψ_{leaf} was lower in LT3 than in LT1 and LT2. Consequently, there is a possibility that low water availability triggered the decrease in MVLA. The decrease in MVLA in LT3 was slower in July; this also implies the effect of water availability; however, MVLA did not decrease when predawn ψ_{leaf} became low again in August. VF also did not show a common pattern in *L. tulipifera*; only the increases in the initial period were common but they would not be influenced by water availability. Therefore, overall trends of MVLA in *L. tulipifera* seem to be formed independently of water status, but the trends could be modified by water availability.

3.3. *Quercus glauca*

MVLA of all samples showed seasonal variation, decreasing toward the end of the ring, but it was more variable in *Q. glauca* than in the other 2 species (Fig. 5). Since predawn ψ_{leaf} was almost always high until late August and midday ψ_{leaf} also did not correlate with the decrease in MVLA or the increase in VF, the variations of both MVLA and VF were not affected by water status. Since *Q. glauca* is reported to be tolerant to dry conditions (Yoshikawa et al. 1996), vessel formation was unlikely to be affected by the soil moisture conditions in the observation year. The variable pattern of MVLA may be caused by statistical errors. The low vessel frequency (range, 1 to 12 mm⁻²) and small sample size can produce statistical noise.

3.4. Factors affecting vessel size

We observed a general pattern of vessel size; it first increases then decreases after a period, except in the case of LT3. Sass and Eckstein (1995) have suggested that vessel formation at the beginning of cambial activity is mainly controlled by internal factors; therefore, the initial increase should not be affected by water availability. The timing of the decrease and the variation in shape pattern were similar within each species, except LT, but were different among species. This species-specific variation means that the seasonal variation of vessel size is

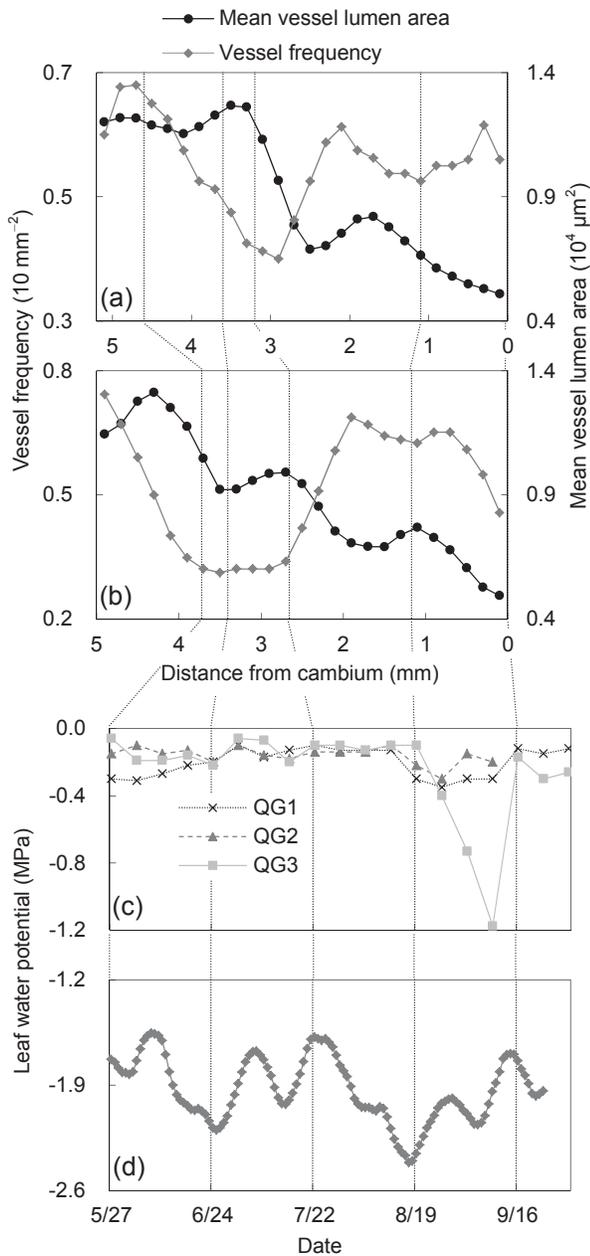


Fig. 5 Variation in mean vessel lumen area (MVLA), vessel frequency (VF) and leaf water potential in *Quercus glauca* (QG). (a) MVLA and VF smoothed by 7-point weighted moving average (WMA) in QG1 and (b) QG3; (c) predawn leaf water potential of each sample; (d) estimated midday leaf water potential. The dotted lines in (a) and (b) are estimated growth width between each sampling. The vessel analysis of QG2 was not done because of the very narrow ring width, and predawn leaf water potential data of QG2 is lacking on and after September 16.

genetically determined. Differences in shoot elongation patterns among species would promote species specificity because flushing and maturing of leaves causes changes in phytohormone concentrations (Hess and Sachs 1972). Leaf emergence in the 3 species is as follows. *C.*

japonicum flushes 2 leaves on each spur, then continues adding leaves on the elongating shoots (heterophyllous subtype of the intermediate type described by Kikuzawa (1983)). *L. tulipifera* flushes leaves simultaneously and continues adding small leaves with elongating shoots (flush and succeeding subtype of the intermediate type described by Kikuzawa (1983)). *Q. glauca* flushes all the leaves at once (flush type described by Kikuzawa (1983)). The 2 former species continue adding leaves even in August, i.e., production of phytohormones is extended for some time. This observation seems consistent with the monotonous decrease in vessel size of *Q. glauca*, unlike in the other 2 species. The relationship between leafing phenology and vessel formation is one of the topics to be investigated in future studies. Low water availability would prompt vessel sizes to become smaller and vessel numbers to become larger, as seen in *C. japonicum*. However, intensity of the effect depends on sensitivity to environmental changes and plasticity of vessel formation, which also vary among species. In terms of sensitivity to water availability, *C. japonicum* was most sensitive, *Q. glauca* was least sensitive, and *L. tulipifera* had intermediate sensitivity. This observation is consistent with the riparian nature of *C. japonicum* (Kubo et al. 2000) and drought tolerant characteristics of *Q. glauca* (Yoshikawa et al. 1996). Our results imply that tree water status is one of the factors that cause seasonal variations in vessel size. We, therefore, conclude that each tree species has a species-specific seasonal variation pattern of vessel size, and the pattern is modified by environmental factors such as water availability.

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