Evaluating the effects of stomata development and senescence on the seasonal variation in stomatal conductance

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Abstract:

We examined the influence of phenological changes in stomata on the seasonal variation of stomatal conductance using a Jarvis-type conductance model that included functions representing the active stomatal density and chlorophyll concentration of leaves. We studied the leaves of three 12-year-old oak trees (Quercus serrata). Stomatal conductance was measured under controlled ambient conditions (i.e., photosynthetic photon flux density, leaf temperature, and specific humidity deficit) in a chamber. Our analyses showed that stomatal conductance cannot be explained by environmental variables alone. Stomatal conductance decreased with increasing stomatal density, where the number of stomata included guard mother cells (GMC), in spring. On the other hand, time series of stomatal conductance showed a correlation with the increases in active stomatal density. Chlorophyll concentration was a good index of the low conductance in autumn, and the active stomata density was a good index of the leaf-unfolding period. These results imply that phenological progress of stomata must be included in land surface models for the accurate prediction of seasonal variations in water, energy, and CO₂ cycles.

KEYWORDS active stomata; phenological change of stomata; seasonal variation; stomatal conductance

INTRODUCTION

The exchange of CO₂ and H₂O between trees and the atmosphere occurs through the stomata of leaves. Therefore, to quantify CO₂, water and heat cycles in vegetated areas, it is important to understand the responses of stomata to environmental variables. Seasonal variations in stomatal conductance cannot be expressed by variations in environmental factors (e.g., photosynthetic photon flux density, temperature, specific humidity deficit and soil water content) alone, especially in spring and autumn (e.g., Kosugi et al., 1995; Sirisampan et al., 2003). Matsumoto et al. (2005) examined the dependence of stomatal conductance on chlorophyll concentration and meteorological variables using a Jarvis-type stomatal conductance model. They reported that decreases in stomatal conductance were strongly correlated with chlorophyll concentration and suggested that other limiting factors may affect conductance during the leaf-unfolding season. Kosugi et al. (2006) used an improved Ball-type stomatal conductance model to analyse the effects of leaf physiology on seasonal fluctuations in gas exchange in a warm-temperate evergreen broadleaf forest in Japan and found that physiological restraints could explain CO₂ uptake on the canopy scale during leaf expansion.

Stomata grow in the leaf-unfolding season and senesce in autumn, with related phenological and physiological changes, and the development and differentiation of stomata. The effects of these changes on stomatal conductance have been investigated extensively (e.g., Zeiger et al., 1987; Willmer and Fricker, 1996). However, there have been a few studies on the influence of phenological changes in stomata on stomatal conductance through parameters of stomatal conductance models. Uddling and Pleijel (2006) used three types of model to evaluate the phenological development of spring wheat, and represented the phenological effects using the parameter thermal time.

In this paper, we focused on stomatal shape and number to identify the factors limiting stomatal conductance, especially in spring and autumn, and performed quantitative evaluation of the influences of these factors on stomatal conductance of oak (Quercus serrata) trees using a stomatal conductance model.

METHODS

Site description

The study was conducted at the Nagoya University experimental farm in Togo, Aichi Prefecture, Japan (35°6'33''N, 137°4'58''E). Three 12-year-old oak (Quercus serrata) trees were planted on 3 April 2003 and fully isolated. The leaves of each tree did not make contact with those of the other trees.

Measured variables

We conducted observations on 19 days from April to December 2005, on 1 to 4 days in each month except August. Using a portable open-air gas analyser (LI-6400; LI-COR, Lincoln, NE, USA) to measure stomatal conductance, we carried out examinations using intact leaf samples under natural soil water conditions. Atmospheric conditions in the chamber (photosynthetic photon flux density [PPFD] 750–800 μmol m⁻² s⁻¹, leaf temperature 17–28°C, and specific humidity deficit < 8 g kg⁻¹) were controlled to maintain optimal conditions for stomatal openness. It was confirmed using the same trees that the stomatal conductance showed almost maximal values under the conditions described above (Matsumoto et al., 2005). We define stomatal conductance obtained under these conditions as gₛopt. The CO₂ concentration in the chamber was not regulated and had an average value of 368.3 ppm with standard deviation of ±17.4 ppm for the measurement period. We found no seasonal trends in CO₂ concentration. Accordingly, the CO₂ concentration probably did not significantly affect the seasonal variation of gₛopt.

We sampled one leaf from each tree on each observa-

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tornation day. Leaves were cryopreserved after the observations on each day and leaf chlorophyll concentrations were later examined by extracting the total chlorophyll (chlorophylls $a$ and $b$) using dimethyl sulfoxide. We measured the light absorbance of the extract using a spectrophotometer (UV-3510; Hitachi, Tokyo, Japan) and calculated the total chlorophyll concentration from the absorbance (Barnes et al., 1992). In addition, we used a chlorophyll meter (SPAD-502; Minolta, Tokyo, Japan) to obtain the “SPAD value” (dimensionless), which is a good indicator of leaf chlorophyll concentration (Hoshino, 1996).

We also measured the phenological changes in stomata using the method of Hirose et al. (1992). One drop of instant adhesive was placed onto a glass coverslip, and a leaf sample was then placed over the adhesive. When the adhesive had dried, we carefully removed the leaf from the coverslip. Next, we observed and photographed the coverslip under a microscope. Then, we cut squares measuring 100 mm x 100 mm, trying to avoid leaf veins as much as possible, and measured the stomatal density in these samples. Two samples were obtained from each tree on each observation day for stomatal density measurements.

The volumetric soil water content ($\theta$ %) was measured using a dielectric aqua meter (ECH2O EC-20; Decagon Devices, Pullman, WA, USA) buried to a depth of 20 cm. From 9 May to 6 June, $\theta$ was measured manually only on days during which the LI-6400 was in operation. From 5 July to the end of the observation season, $\theta$ was measured automatically using a data logger (Em5; Decagon Devices).

**Stomatal conductance model**

For quantitative evaluation of the influence of phenological changes in stomata on stomatal conductance, we used a Jarvis-type stomatal conductance model (Jarvis, 1976). Jarvis (1976) described the relationship between $g_s$ and several variables using an empirical model in which maximum stomatal conductance ($g_{s\ max}$) is reduced by functions of variables associated with stomatal conductance. We used only $\theta$ as an environmental variable in the Jarvis-type model. It was not necessary to consider other meteorological variables (i.e., PPFD, leaf temperature, and specific humidity deficit) because they were controlled at optimal levels during the conductance measurement as noted above. To examine the effects of leaf aging on $g_{s\ opt}$, we used the model shown in Eq. (1), to which the functions of $C$ and $S$ were added as indicators of seasonal change:

\[
g_{s\ opt} = g_{s\ max} \cdot f_1(\theta) \cdot f_2(C) \cdot f_3(S),
\]

where $g_{s\ max}$ is the maximum stomatal conductance (mol H2O m$^{-2}$ s$^{-1}$), and $f_1(\theta), f_2(C),$ and $f_3(S)$ are functions of the volumetric soil water content ($\theta$ %), chlorophyll concentration ($C$, mg dm$^{-2}$) and active stomatal density ($S$, number mm$^{-2}$; defined later), respectively. Each function is independent of the other variables and varies from 0 to approximately 1. The chlorophyll concentration is used as an index of leaf physiological properties (Matsumoto et al., 2005). Each function operates as a reduction factor for $g_{s\ max}$ and is written as follows:

\[
f_1(\theta) = 1 - \exp[k_s(\theta_{\ min} - \theta)],
\]

\[
f_2(C) = \frac{C}{C_{\ max} + k_s},
\]

\[
f_3(S) = \frac{1}{1 + \left((S_{\ max} - S)/S_{\ min} - S_{\ opt}\right)^k_s},
\]

where $\theta_{\ min}$ is the minimum volumetric soil water content, $C_{\ max}$ is the value of $C$ at $f_2(C) = 1$, $S_{\ max}$ is the maximum value of $S$, $S_{\ min}$ is the value of $S$ at $f_3(S) = 0.5$, and $k_s, k_1,$ and $k_2$ are constants connected to the curvature of each response curve. Equations (2) and (3) are from a previous study (Matsumoto et al., 2005), and Equation (4) was created for this study.

The parameter values were estimated by a nonlinear least-squares technique using the equation solver in Microsoft Excel to minimise the root mean square error (RMSE) between the measured and predicted values of $g_{s\ opt}$. To estimate values expressing actual phenomena, we imposed the following constraints: $g_{s\ max}$ cannot be smaller than the maximum $g_{s\ opt}$ observed, and each fitted line represented by Eqs. (2)–(4) must agree roughly with the upper boundaries of groupings in the plots of each variable and $g_{s\ opt}$. Table I lists the parameter values obtained according to the above procedure, and Table II presents the model precision.

As we had no data for $\theta$ on 26 October and 9 November, we estimated $\theta$ on these dates by the following procedure (estimates shown by the open symbols in Figure 2). The lowest value of $\theta$ was 16.4% on 15 November. The total precipitation from 26 October to 15 November was 1.2 mm. During this period, $\theta$ showed a progressive decrease due to the small amount of rain. The period from 15 November to 5 December, during which a complete set of $\theta$ values were obtained, also had little rain. Based on the rate of decrease in $\theta$ from 15 November to 5 December, we then assumed a constant rate of decrease in $\theta$ from 26 October to 5 December. This method is simple, but the estimated $\theta$ values cannot be considered accurate. However, the value of $f_1$ was 0.73 at $\theta = 16.4\%$. The values of $f_1$ for the estimated $\theta$ on 26 October and 9 November were more than 0.73 because soil moisture levels on these days were higher than 16.4%. Thus, the estimation had little influence on our analysis.

**RESULTS AND DISCUSSION**

**Seasonal variation in each variable**

The value of $g_{s\ opt}$ began to increase at the beginning of June, peaked in the middle of September, and then decreased rapidly in late autumn (Figure 1a). The chlorophyll concentration increased gradually in spring and dropped quickly in late autumn (Figures 1b and 1c). As the rapid drop in chlorophyll concentration in autumn agreed well with that in $g_{s\ opt}$, chlorophyll concentration correlated with $g_{s\ opt}$ in autumn. Many previous works have reported the disappearance of chlorophyll during

| Sample tree no. | RMSE (mol H2O m$^{-2}$ s$^{-1}$) |
|----------------|----------------------------------|
| Sample tree no. 1 | 0.0293 |
| Sample tree no. 2 | 0.0288 |
| Sample tree no. 3 | 0.0397 |
| Average           | 0.0321 |

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Table I. Fitted parameter values of the model.

| Parameter       | Value |
|-----------------|-------|
| $g_{s\ max}$    | 0.23  |
| $C_{max}$       | 3.60  |
| $\theta_{\ min}$ | 6.56  |
| $S_{\ max}$ (number 10$^4$ mm$^{-1}$) | 9.72 |
| $S_{\ min}$ (number 10$^4$ mm$^{-1}$) | 30.28 |
| $k_1$           | 0.90  |
| $k_2$           | 0.14  |
| $k_3$           | 9.45  |
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senescence (e.g., Thomas et al., 1991; Rosenthal and Camm, 1997), and our results agree with these investigations. However, while \( g_{\text{opt}} \) increased, chlorophyll concentration was almost constant from spring to summer. In addition, while \( g_{\text{opt}} \) was higher in September and October than in May and the beginning of July, the seasonal variation in \( g_{\text{opt}} \) roughly followed that of \( S \) in the spring.

The soil was remarkably dry, with \( \theta < 10\% \) from late May to the beginning of June and from the end of July to the beginning of September (Figure 2). The temporary decrease in \( g_{\text{opt}} \) during June seemed to be strongly affected by the extremely low \( \theta \). The drying of the soil under these conditions affected \( g_{\text{opt}} \). However, we cannot discuss this effect in detail because we could not measure \( g_{\text{opt}} \) in August.

Relationship between estimated and observed \( g_{\text{opt}} \)

Two methods were used to estimate \( g_{\text{opt}} \): one included the function of \( S \) shown in Eq. (4) (open squares in Figure 4), while the other did not (shaded triangles in Figure 4). The parameter values shown in Table I were used for both simulations. The seasonal variation in the estimated \( g_{\text{opt}} \) that included \( S \) matched the observed \( g_{\text{opt}} \) better than the estimated \( g_{\text{opt}} \) without \( S \), especially in spring (Figure 4).

Evaluation of active stomata and stomatal conductance

To quantitatively examine the effects of each variable on the variability in \( g_{\text{opt}} \), we calculated the difference in RMSE between the estimated and observed \( g_{\text{opt}} \), using the following method. We defined the contribution index \( \alpha \) to the variability in \( g_{\text{opt}} \) as follows:

\[
\alpha = \beta - \omega, \tag{5}
\]

where \( \beta \) is the RMSE of the model in which a function for a certain variable was excluded, and \( \omega \) is the RMSE obtained from the model containing all functions in Eqs. (2)–(4). The parameter values shown in Table I were used to obtain \( \alpha \) for the three environmental variables. A larger \( \alpha \) means that the variable excluded to calculate \( \beta \) has a more significant influence on \( g_{\text{opt}} \). We divided

Figure 1. Seasonal variations in \( g_{\text{opt}} \) (a), chlorophyll concentration (b), SPAD value (c), stomatal density (d) and active stomatal density (e) from April to December 2005. Error bars indicate maximum and minimum values.

Figure 2. Seasonal variation in soil water content and precipitation. Open symbols indicate estimated values.

Figure 3. Morphology of stomata on 27 April (a), 27 April (b), 17 June (c), and 2 December (d) in 2005: (a) shows a guard mother cell (GMC) and (b) shows an immature stomata.

Thus, we defined mature stomata (Figure 3c), in which the GMC turned to guard cells, as active stomata capable of effective water vapour exchange. The active stomatal density (\( S \), number mm\(^{-2}\)) increased rapidly in spring and decreased gradually in autumn (Figure 1e). The seasonal variation in \( g_{\text{opt}} \) roughly followed that of \( S \) in the spring.

Figure 3. Seasonal variations in \( g_{\text{opt}} \) (a), chlorophyll concentration (b), SPAD value (c), stomatal density (d) and active stomatal density (e) from April to December 2005. Error bars indicate maximum and minimum values.
the sampling period into five subperiods to calculate seasonal variation of $\alpha$ quantitatively (Table III). These subperiods were divided by taking the seasonal variations of $S$, $C$, and $G_{\text{act}}$ into account. Table III summarises the properties of the subperiods.

Figure 5 shows the contribution index ($\alpha$) of each function in each subperiod (I–V; see Table III for subperiod definitions). The influence of both the density of active stomata and the chlorophyll concentration was as large as the influence of meteorological factors. Thus, not only environmental factors, but also changes in leaf properties (e.g., active stomatal density and chlorophyll concentration) of a plant should be evaluated in the leaf-unfolding and leaf-senescent seasons.

CONCLUDING REMARKS

In spring, stomatal conductance is strongly dependent on the phenological changes of stomata, as indicated by the density of active stomata. In contrast, in autumn, stomatal conductance was correlated with physiological properties, as indicated by chlorophyll concentration. The influence of both the density of active stomata and the chlorophyll concentration on stomatal fluctuation was as large as the influence of meteorological factors. Thus, not only environmental factors, but also changes in leaf properties (e.g., active stomatal density and chlorophyll concentration) of a plant should be evaluated in the leaf-unfolding and leaf-senescent seasons.

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REFERENCES

Barnes JD, Balaguer L, Manrique R, Elvira S, Davison AW. 1992. A reappraisal of the use of DMSO for the extraction and determination of chlorophyll $a$ and $b$ in lichens and higher plants. Environmental and Experimental Botany 32: 85–100.

Hirose T, Izuta T, Miyake H, Totsuka T. 1992. A stomatal impression method using a fast-sticking adhesive. Japanese Journal of Crop Science 61: 159–160 (in Japanese).

Kosugi Y, Kobashi S, Shibata S. 1995. Modelling stomatal conductance in leaves of several temperate evergreen broadleaf trees. Journal of the Japanese Society of Revegetation Technology 20: 158–167 (in Japanese).

Kosugi Y, Takanashi S, Matsuo N, Tanaka K, Tanaka H. 2006. Impact of leaf physiology on gas exchange in a Japanese evergreen broad-leaved forest. Agricultural and forest meteorology 139: 182–199.

Matsumoto K, Ohta T, Tanaka T. 2005. Dependence of stomatal conductance on leaf chlorophyll concentration and meteorological variables. Agricultural and forest meteorology 132: 44–57.

Rosenthal SI, Camm EL. 1997. Photosynthetic decline and pigment loss during autumn foliar senescence in western larch (Larix occidentalis). Tree Physiology 17(12): 767–775.

Sirisampan S, Hiyama T, Hashimoto T, Fukushima Y. 2003. Diurnal and seasonal variations of stomatal conductance in a secondary temperate forest. Journal of the Japan Society of Hydrology and Water Resources 16(2): 113–130 (in Japanese with English summary).

Thomas C, Davis SD, Tallman G. 1991. Response of stomata of senescing and non-senescent leaves of Nicotiana glauca to change in intercellular concentrations of leaf carbon dioxide. Plant, Cell and Environment 14(9): 971–978. DOI: 10.1111/j.1365-3040.1991.tb00867.x.

Uddling J, Pleijel H. 2006. Changes in stomatal conductance and net photosynthesis during phenological development in spring wheat: implication for gas exchange modelling. International Journal of Biometeorology 51(1): 37–48.

Willmer C, Fricker M. 1996. Stomata. Chapman and Hall, London, UK. 375 pp.

Zeiger E, Farquhar GD, Cowan IR (eds). 1987. Stomatal function. Stanford University Press. Stanford, 503 pp.