Modeling of stomatal conductance for estimating ozone uptake of *Fagus crenata* under experimentally enhanced free-air ozone exposure

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Abstract

We examined a performance of the multiplicative stomatal conductance model to estimate of stomatal ozone uptake for *Fagus crenata*. Parameterization of the model was carried out by in-situ measurements in a free-air ozone exposure experiment. The model performed fairly well under ambient conditions, with low ozone concentration. However, the model overestimated stomatal conductance under enhanced ozone condition due to ozone-induced stomatal closure. A revised model that included a parameter representing ozone-induced stomatal closure showed better estimation of ozone uptake. Neglecting ozone-induced stomatal closure induced a 20% overestimation of the stomatal uptake of ozone. The ozone-induced stomatal closure was closely related to stomatal ozone uptake, rather than AOT40 (accumulated concentrations of ozone exceeding 40 nmol mol^{-1}). Our results suggest that ozone-induced stomatal closure should be implemented to stomatal conductance model for estimating ozone uptake for *F. crenata* and the implementation will contribute to adequate risk assements of ozone impacts on *F. crenata* forests in Japan.

Key words: *Fagus crenata*; ozone uptake; stomatal conductance modeling; Japan; ozone

1. INTRODUCTION

Tropospheric ozone (O_3) concentrations are increasing in East Asia as a result of rapid increases in emissions of the main O_3 precursors, such as nitrogen oxides and volatile organic compounds (Naja and Akimoto, 2004). It is expected that ground surface O_3 concentrations will increase especially in East Asian countries (Ohara et al., 2007; Yamaji et al., 2008). Ozone is an important phytotoxic air pollutant that causes damage to plants (e.g., NIES, 1980, 1984; Serengil et al., 2011). Many experimental studies using open-top chambers have determined the sensitivity to O_3 of various Japanese forest tree species since the 1990s (Yamaguchi et al., 2011). Kohno et al. (2005) suggested that 8–15 μmol $mol^{-1}\cdot h$ AOT40 (accumulated exposure over a threshold of 40 nmol mol^{-1}) from April to September was a critical level for sensitive forest species grown in Japan, i.e., able to induce a 10% growth reduction. In Europe, the critical level for O_3 is considered to be 5 μmol $mol^{-1}\cdot h$ AOT40 for forest trees (Mills et al., 2010) or 9μmol $mol^{-1}\cdot h$ AOT40 for all type of vegetation (Directive 2008/50/EC). However, application of AOT40 as an index to assess O_3 impacts may be inappropriate at the regional scale (Kobayashi, 1999; Matyssek and Innes, 1999). Stomatal uptake or flux of O_3 is more closely related to the O_3 impacts (Omasa et al., 2002; Paoletti and Manning, 2007; Karlsson et al., 2007). In Europe, critical levels for O_3 risk based on stomatal O_3 uptake have been proposed for the potato, tomato, wheat and several tree species (Mills et al., 2010). The stomatal flux-based approach is also expected to provide better assessments of O_3 damage to plants in East Asia (Watanabe and Yamaguchi, 2011). However, there are only a few reports on stomatal O_3 uptake modeling for

Japanese tree species (Hoshika et al., 2011a, b).

Fagus crenata is the representative tree species, deciduous and broadleaved, in the cool-temperate forests of Japan (Koike et al., 1998). Previous studies have been indicated this species is highly sensitive to O_3 (Kohno et al., 2005; Takeda and Aihara, 2007). Accurate parameterization of stomatal conductance model is essential to develop a stomatal flux-based approach for assessments of O_3 impact on *F. crenata* forest in Japan.

Ozone is known to induce stomatal closure, and may therefore limit stomatal O_3 uptake (e.g., Wittig et al., 2007). However, current models for estimating stomatal O_3 uptake of forest trees do not take account of the effects of O_3 on stomatal conductance (Hoshika et al., 2011a,b). We should therefore also validate the model with field measurements under elevated O_3 conditions, to evaluate O_3 uptake by *F. creanta*.

Our main objective was to test a performance of the multiplicative stomatal conductance model to estimate stomatal O_3 uptake for *F. crenata* and to investigate whether model should include the O_3 effects on stomatal conductance as an additional parameter. Parameterization of the model was carried out by measurements under a free-air O_3 exposure experiment.

2. MATERIALS AND METHODS

2.1 Experimental site

The study was carried out at Sapporo Experimental Forest, Hokkaido University, in northern Japan (43°04' N, 141°20' E, 15 m a.s.l., annual mean temperature: 13.5 ˚C, total precipitation: 1254 mm in 2011). Ten saplings of ten-year-old Siebold beech (*F. crenata*) were exposed to ambient air, and another ten saplings were exposed to an experimentally enhanced O_3 regime during daytime, using a free-air O_3 exposure system similar to that developed at the Technical University of Munich (TUM) in Germany (Nunn et al., 2002; Werner and Fabian, 2002). Each plot is 5.5 m × 7.2 m × 5.5 m in size (height quoted last). The distance between the O_3 and ambient plot was about 20 m. There is a small stand composed of maple (*Acer mono*), elm (*Ulmus davidiana*), ash (*Fraxinus mandschrica* var. *japonica*) and birch (*Betula platyphylla* var. *japonica*). Soil type was brown forest soil. Ozone generated from oxygen is diluted with ambient air in a pressurized tank and infused into the canopies through a system of 48 teflon tubes hanging down from a fixed grid above the trees. The target O_3 concentration was 60 nmol mol^{-1} during daylight hours. The mean O_3 concentrations observed during daytime were 25.7 ± 11.4 nmol mol^{-1} under ambient condition and 56.7 ± 10.5 nmol mol^{-1} under enhanced O_3 condition. The enhanced O_3 regime began on August 6th, 2011. Ozone concentrations were recorded continuously at canopy height by an O_3 monitor (Mod. 202, 2B Technologies, Boulder CO, USA). At the start of O_3 fumigation, the mean tree height was 3.3 ± 0.4 m and the mean stem diameter at breast height was 26.7 ± 5.9 mm. Meteorological data (photosynthetic photon flux density, air temperature, relative humidity and wind speed) were continuously recorded throughout the experimental period, until leaf-fall. Soil moisture was measured in the root layer (20 cm depth) using dielectric sensors (10HS soil moisture sensor; Decagon Devices, Pullman WA, USA). Average soil moisture was 28.8 ± 4.8% during the experimental period. Values were almost equal to the field capacity of the study site

(32%).

2.2 Parameterization of the stomatal conductance model

Diurnal courses of the stomatal conductance were measured using a steady-state diffusion porometer (Model LI-1600, Li-Cor instruments, Lincoln, NE, USA), from the end of July to the end of October 2011. All measurements were conducted on fully expanded sun leaves at the top of the canopy of 5 to 6 trees in each ambient and enhanced O_3 treatment. Pooled data from all 767 measurements were used to estimate the parameters of the stomatal conductance model.

Our stomatal conductance model was based on the multiplicative algorithm described by Jarvis (1976) and modified by Emberson et al. (2000), as follows:

$$g_{sw} = g_{max} \cdot f_{phen} \cdot f_{light} \cdot \max\{f_{min}, (f_{temp} \cdot f_{VPD} \cdot f_{SWP})\} \quad (1)$$

where g_{max} is the maximum stomatal conductance (mmol H_2O m^{-2} Projected Leaf Area (PLA) s^{-1}). The other functions are limiting factors of g_{max} and are scaled from 0 to 1. Here, f_{min} is the minimum stomatal conductance, f_{phen} is the variation in stomatal conductance with leaf age, and f_{light}, f_{temp}, f_{VPD}, and f_{SWP} depend respectively on the photosynthetically relevant photon flux density at the leaf surface (PPFD, μmol photons m^{-2} s^{-1}), the temperature (T, °C), the vapor pressure deficit (VPD, kPa), and the volumetric soil water potential (MPa).

The response of g_{sw} to phenology (f_{phen}) during the experiments is described as follows:

for DOY ≤ (EGS-f_{phen_a}),

$f_{phen} = 1$;

for (EGS-f_{phen_a}) < DOY ≤ EGS,

$$f_{phen} = (1 - f_{phen\_b}) \cdot ((EGS - DOY)/f_{phen\_a}) + f_{phen\_b}, \quad (2)$$

where DOY is the day of the year. Here EGS is the date denoting the end of the growing season, chosen as Nov. 7th when all leaves have fallen. The parameter f_{phen_a} represents the number of days during the decline of f_{phen} to the minimum value. The parameters f_{phen_b} denote the maximum fraction of g_{max} at EGS.

The response of g_{sw} to PPFD, i.e., f_{light}, is specified as:

$$f_{light} = 1 - \exp(a \cdot PPFD) \quad (3)$$

where a is a species-specific parameter defining the shape of the exponential relationship.

The parameter for of air temperature (T, ˚C) is expressed as:

$$f_{temp} = \left(\frac{T - T_{min}}{T_{opt} - T_{min}}\right)\left\{\left(\frac{T_{max} - T}{T_{max} - T_{opt}}\right)^{\left(\frac{T_{max} - T_{opt}}{T_{opt} - T_{min}}\right)}\right\} \quad (4)$$

where T_{opt}, T_{min}, and T_{max} respectively denote the optimum, minimum, and maximum temperature (˚C) for stomatal conductance.

The response of g_{sw} to the vapor pressure deficit (VPD, kPa) is given by:

$$f_{VPD} = \frac{(1 - f_{min}) \cdot (VPD_{min} - VPD)}{VPD_{min} - VPD_{max}} + f_{min} \qquad (5)$$

where VPD_{min} and VPD_{max} denote the threshold of VPD (kPa) for attaining minimum and full stomatal opening, respectively. If VPD exceeds VPD_{min} then f_{VPD} is set to f_{min}. If VPD is lower than VPD_{max} then f_{VPD} is 1.

Terms describing modification of g_{sw} by the soil moisture (i.e., f_{SWP}) were not used in this study. As mentioned above, the soil moisture was nearly equal to the field capacity throughout the experimental period, and no reductions in stomatal conductance due to soil water content were recorded (data not shown).

Parameter estimation was carried out using a boundary line analysis. First, the g_{sw} data were divided into classes with the following step-wise increases for each variable: 200 μmol photons m^{-2} s^{-1} for PPFD (when PPFD values were less than 200 μmol photons m^{-2} s^{-1}, PPFD classes at 50 μmol photons m^{-2} s^{-1} steps were adopted), 2 °C for T and 0.2 kPa for VPD. A function was fitted against each model variable based on 98th percentile values per each class of environmental factors. The g_{max} value corresponds to a maximum value recorded throughout the experiment. We took f_{min} as the 5th percentile values during the daytime period of conductance.

2.3 f_{O3} function

In the present study, lower g_{sw} was found in the enhanced O_3 treatment than in controls. A further parameter was tested in the model:

$$g_{sw} = g_{max} \cdot f_{phen} \cdot f_{O3} \cdot f_{light} \cdot \max\{f_{min}, (f_{temp} \cdot f_{VPD} \cdot f_{SWP})\} \quad (6)$$

where the new parameter f_{O3} represents a response of g_{sw} to O_3.

The value of f_{O3} was estimated by a linear regression analysis between O_3 indices and the ratio of daily g_{max} to the control value. AOT40 and cumulative O_3 uptake were tested as O_3 indices. Daily g_{max} was identified as the daily maximum record of g_{sw} of the measured trees when PPFD exceeded 500 μmol photons m^{-2} s^{-1} and VPD was less than 1.5 kPa. When applying the model in control conditions, we assumed O_3 had no effect on g_{sw}, i.e., $f_{O3} = 1$.

2.4 Estimation of AOT40 and stomatal ozone uptake

AOT40 was estimated using the hourly mean O_3 concentrations during daylight hours (6:00 to 17:00 CET):

$$AOT40 = \sum \max([O_3] - 40, 0) \quad (7)$$

where $[O_3]$ is the hourly mean O_3 concentration (nmol mol^{-1}).

Stomatal O_3 uptake (F_{st}; nmol O_3 m^{-2} s^{-1}) was calculated as:

$$F_{st} = [O_3] \cdot g_{sO3} \cdot \frac{r_c}{r_b + r_c} \quad (8)$$

where r_b is the leaf boundary layer resistance (mol^{-1} m^2 s), and g_{sO3} is the stomatal conductance for O_3 (g_{sO3} =g_{sw}/1.65; mmol O_3 m^{-2} PLA s^{-1}); the factor 1.65 accounts for the difference in diffusivity of water in air and in ozone. Here, r_c is the leaf surface resistance (=

$1/(g_{sO3}+g_{ext})$; s m^{-1}), and g_{ext} is the external leaf or cuticular conductance (s m^{-1}), chosen as 0.0004 m s^{-1} (Mills et al., 2010).

Leaf boundary layer resistance (r_b) was calculated from the wind speed, u (m s^{-1}), and the cross-wind leaf dimension, L_d (0.05 m for *F. crenata*, obtained as the mean value of 30 leaves × 6 trees in each O_3 treatment) (Mills et al., 2010):

$$r_b = 1.3 \cdot 150 \cdot (L_d/u)^{0.5} \tag{9}$$

where the factor 1.3 accounts for differences in diffusivity between heat and O_3.

2.5 Data analysis

Simple regression analysis was used to test the relationship between measured and estimated g_{sw}, and the relationship between O_3 indices and the ratio of daily g_{ma} to control. The analysis was performed using SPSS software (SPSS, Chicago, USA).

3. Results

3.1 Parameterization of the stomatal conductance model

We set the g_{max} value as 474 mmol H_2O m^{-2} PLA s^{-1} (Table 1). The value of f_{min} was chosen as 0.11 (fraction). The limiting functions of g_{sw} (i.e. f_{light}, f_{temp}, f_{VPD} and f_{phen}) were defined by boundary line analysis (Table 1; Fig. 1). The response of g_{sw} to PPFD followed a typical light-response curve, with a light saturation point above 500 µmol m^{-2} s^{-1} (Fig. 1A). The response of g_{sw} to air temperature indicated an optimal temperature of 26 ˚C for stomatal opening (Fig.

1B). More than 1.1 kPa of VPD induced stomatal closure (Fig. 1C). Stomatal conductance decreased to 40% of g_{max} at 3.0 kPa of VPD. The value of f_{phen} was 1.0 until mid-September, but decreased in October.

Stomatal conductance under elevated O_3 conditions was lower than under ambient conditions after 2-weeks of O_3 exposure (Fig. 1D). The ratio of daily g_{max} in enhanced O_3 condition to that in ambient condition decreased linearly with increasing both cumulative O_3 uptake and AOT40 (Fig. 2). However, the value of determination coefficient was higher when we applied cumulative O_3 uptake as an index (R^2=0.67 and 0.44 for cumulative O_3 uptake and AOT40, respectively).

3.2 Test of f_{O3} function

The model estimation of g_{sw} showed good agreement with the measurement values in ambient conditions (R^2=0.77) (Fig. 3). In the enhanced O_3 treatment, however, the model without f_{O3} overestimated g_{sw} (Fig. 4A). When f_{O3} was included, the performance of the model under elevated O_3 conditions was better (Fig. 4B).

Fig. 5 shows the hourly mean F_{st} and the cumulative O_3 uptake estimated by the model with and without f_{O3} in the enhanced O_3 treatment. Almost constant values of maximum hourly mean F_{st} were provided by the model without f_{O3} (upper edge of plots for hourly mean F_{st}, 10 to 15 nmol O_3 m^{-2} s^{-1} from August to September). In contrast, when f_{O3} was included in the model, a decrease in maximum hourly mean F_{st} was discernible after mid-August. As a result, lower F_{st} in the model with f_{O3} than that without f_{O3} was recorded to the end of October. The cumulative O_3 uptake estimated by the model with f_{O3}

was 20% lower than that by the model without f_{O3} in the enhanced O_3 treatment.

4. Discussion

The stomatal conductance model without f_{O3} overestimated g_{sw} under elevated O_3 condition (Fig. 4A), although it showed good performance under ambient condition with low O_3 concentration (Fig. 3). When f_{O3} was included in the model, the fit of estimated g_{sw} with the measured g_{sw} became better (Fig. 4B). An ozone-induced reduction in g_{sw} was observed after 2-weeks of O_3 exposure (Fig. 1D). The decrease in daily maximum g_{sw} was directly related to increases in cumulative O_3 uptake (Fig. 2A). As a result, the model without f_{O3} may overestimate cumulative O_3 uptake by up to 20% under elevated O_3 conditions (Fig. 5). In general, O_3 induces stomatal closure (Wittig et al., 2007). Weber et al. (1993) reported a linear decrease in g_{sw} with increases in cumulative O_3 uptake by the ponderosa pine (*Pinus ponderosa*). In previous modeling studies with crops, a development of f_{O3} was successfully achieved for potato (Pleijel et al., 2002), rice (Oue et al., 2008) and wheat (Grüters et al., 1995; Danielsson et al., 2003; Oue et al., 2009) since elevated O_3 treatment cause a reduction in g_{sw} before a limitation of f_{phen} due to natural senescence. Our result is a first achievement for a forest tree species under free-air conditions.

Ozone-induced reduction in g_{sw} limited stomatal O_3 uptake, and may therefore minimize further negative effects of O_3 on plants. This O_3-induced reduction in g_{sw} was closely related to cumulative O_3 uptake rather than AOT40, which is an exposure-based index (Fig. 2). A previous study suggested that spatial maps of stomatal O_3 uptake are strongly correlated with maps of AOT40 for cool-temperate forest trees such as *F. crenata* in Japan, because humid and cool climates promote stomatal opening (Hoshika et al., 2011a; b). The present study found that stomatal O_3 uptake for *F. crenata* may be limited under elevated O_3 exposure, because of O_3-induced stomatal closure. Ozone-induced stomatal closure may therefore decouple stomatal O_3 uptake from high O_3 levels.

Present study did not include a function of soil moisture in stomatal conductance model. As soil moisture was almost equal to field capacity throughout the experiment, no reductions in stomatal conductance due to soil water deficit were recorded (data not shown). In Japan, annual precipitation is generally high, ranging from 1100 mm to 2300 mm. Sirisampan et al. (2003) reported that soil water content had no effect on stomatal conductance in six tree species in central Japan. However, Hoshika et al. (2012) demonstrated including the soil moisture function improved the model performance for Japanese zelkova in the summer of 2010 when low precipitation was observed. The function of soil moisture in stomatal conductance model may be significant in estimating stomatal O_3 uptake of *F. crenata* for short period drought during the growing season.

Previous studies have highlighted the importance of g_{max} in determining the uptake of O_3 by stomata (e.g., Tuovinen et al., 2007). Wieser et al. (2000) suggested that g_{max} depends on the region. Koike (2008) reported that properties of leaf gas exchange traits for *F. crenata* were strongly related to the regions in Japan. Accumultion of g_{max} information in different regions may provide a better understanding of the relation between g_{sw} and environmental conditions, including pollution.

Based on the results of present study, we conclude our hypothesis was supported. When O_3-induced stomatal closure was not taken into account in a stomatal conductance model, the model overestimated g_{sw} under conditions of enhanced O_3. Our results presented here contribute a development of stomatal O_3 uptake modeling for *F. crenata* especially under elevated O_3 condition. There are only a few reports of stomatal conductance modeling to develop the stomatal flux-based approach in East Asia such as Japan (rice: Oue et al., 2008; wheat: Oue et al., 2009; several tree species: Hoshika et al., 2011a, b, 2012). Further validations of the model parameters should be implemented and will contribute to adequate risk assessments of O_3 impacts on cool-temperate forest trees in Japan.

Acknowledgements

This work was partly supported by the Environment Research and Technology Development Fund (B-1105) of the Ministry of the Environment, Japan and for a Grant-in-aid from the Japanese Society for Promotion of Science (Type B 23380078)..

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Figure captions

Fig.1 Parameterization of the stomatal conductance model for *Fagus crenata* (A: f_{light} ; B: f_{temp}; C: f_{VPD}; D f_{phen}). The parameters f_{light}, f_{temp}, f_{VPD} and f_{phen} respectively depend on the photosynthetic photon flux density at the leaf surface (PPFD, μmol photons m^{-2} s^{-1}) for photosynthesis, the temperature (T, °C), the vapor pressure deficit (VPD, kPa) and phenology.

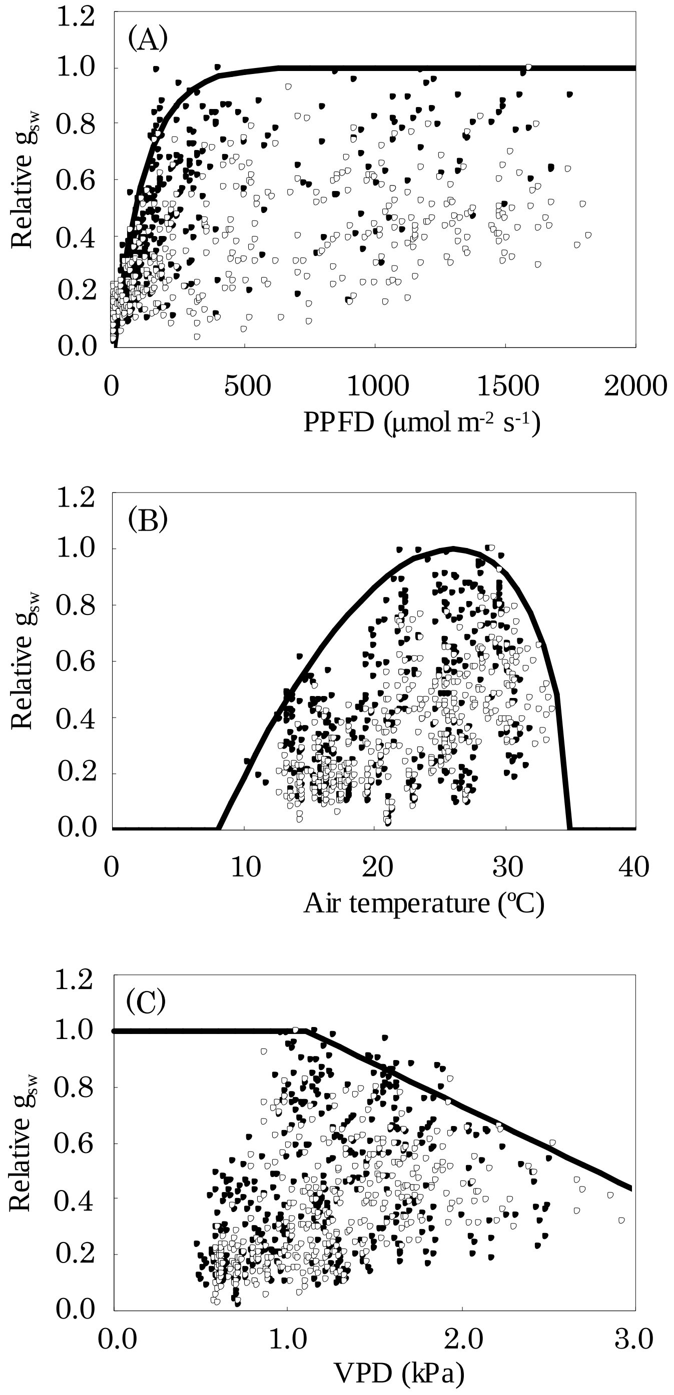
Fig.2 Ratio of daily g_{max} for enhanced O_3 and ambient conditions, as a function of O_3 indices (A: cumulative O_3 uptake; B: AOT40). The daily g_{max} was determined as the daily maximum record of g_{sw} of the trees analyzed when PPFD>500μmol photons m^{-2} s^{-1} and VPD<1.5 kPa.

Fig.3 Relations between measured and estimated g_{sw} under ambient conditions.

Fig.4 Relations between measured and estimated g_{sw} using the model without f_{O3} (A) and with f_{O3} (B) under enhanced O_3 levels.

Fig.5 Stomatal O_3 uptake for *Fagus crenata* in an enhanced O_3 treatment using the proposed model with or without f_{O3}. Grey and black circles show the hourly mean F_{st} (nmol O_3 m^{-2} s^{-1}) estimated by the model with and without f_{O3}, respectively. Grey and black lines are cumulative O_3 uptake (mmol O_3 m^{-2}) estimated by the model with and without f_{O3}.

Fig.1


(A)
Relative gsw
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
500
1000
1500
2000
PPFD (μmol m-2 s-1)
(B)
Relative gsw
0
10
20
30
40
Air temperature (ºC)
(C)
Relative gsw
0.0
1.0
2.0
3.0
VPD (kPa)

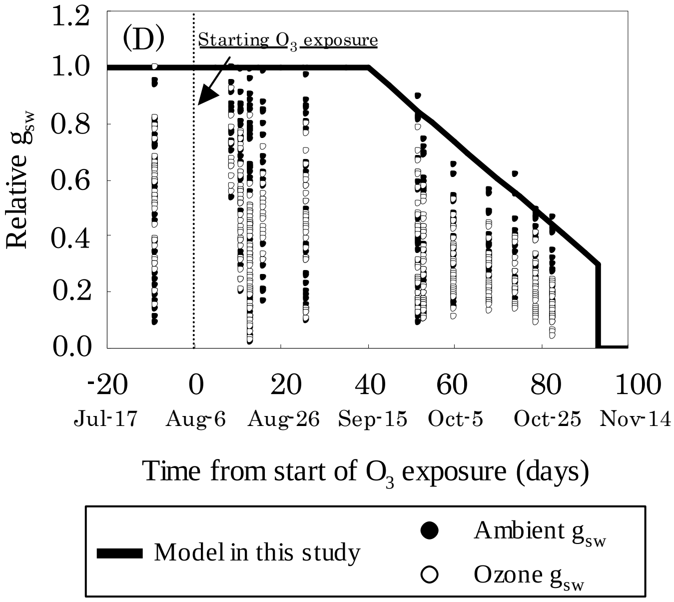

(D)
Starting O3 exposure
Relative gsw
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-20
0
20
40
60
80
100
Jul-17
Aug-6
Aug-26
Sep-15
Oct-5
Oct-25
Nov-14
Time from start of O3 exposure (days)
Model in this study
Ambient gsw
Ozone gsw

Fig.2

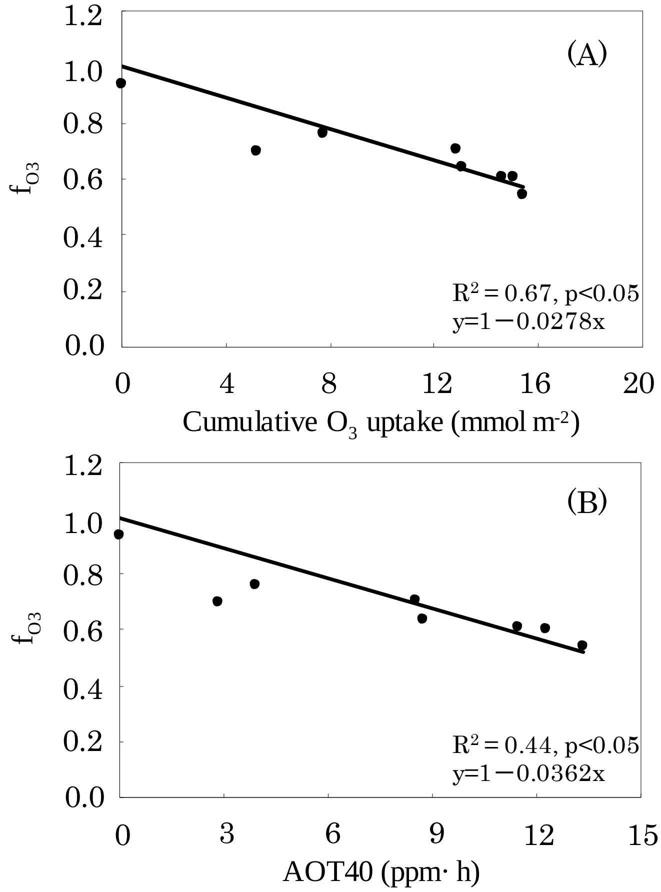

1.2
1.0
0.8
0.6
0.4
0.2
0.0
f O3
(A)
R2 = 0.67, p<0.05
y=1－0.0278x
0
4
8
12
16
20
Cumulative O3 uptake (mmol m-2)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
f O3
(B)
R2 = 0.44, p<0.05
y=1－0.0362x
0
3
6
9
12
15
AOT40 (ppm· h)

Fig.3

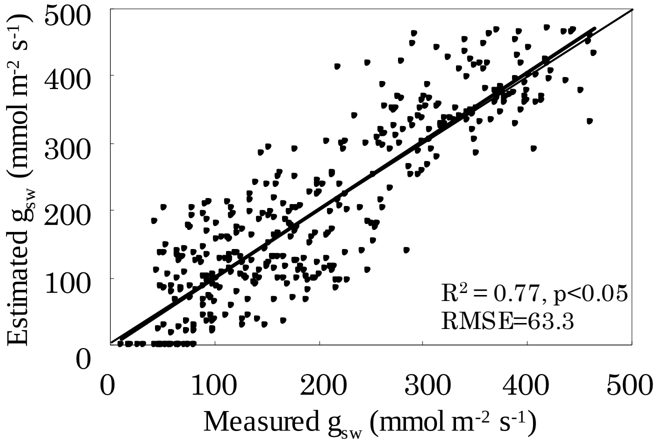

500
400
300
200
100
0
0
100
200
300
400
500
Estimated g_{sw} (mmol m^{-2} s^{-1})
Measured g_{sw} (mmol m^{-2} s^{-1})
R^2 = 0.77, p<0.05
RMSE=63.3

Fig.4

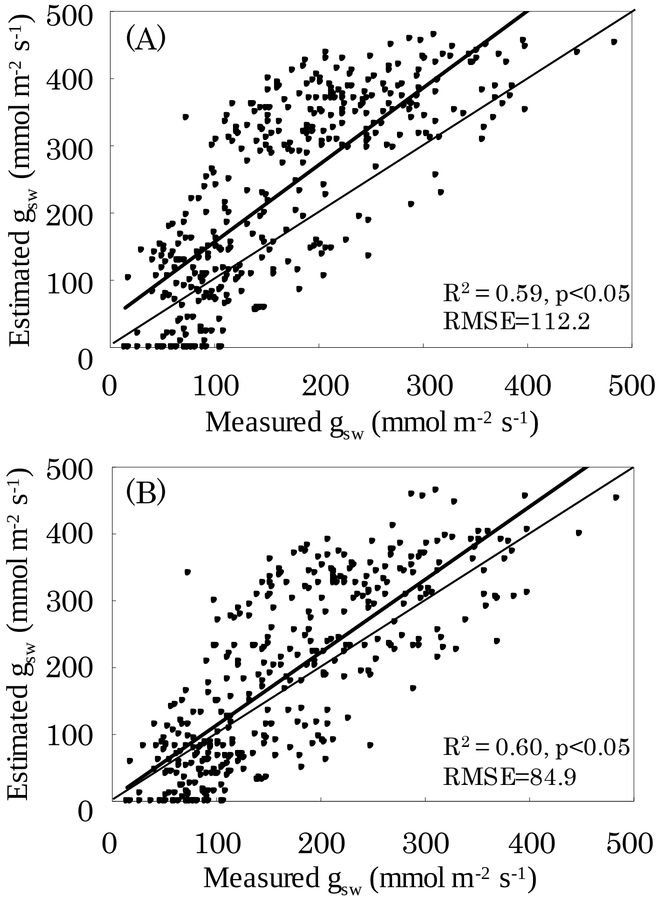
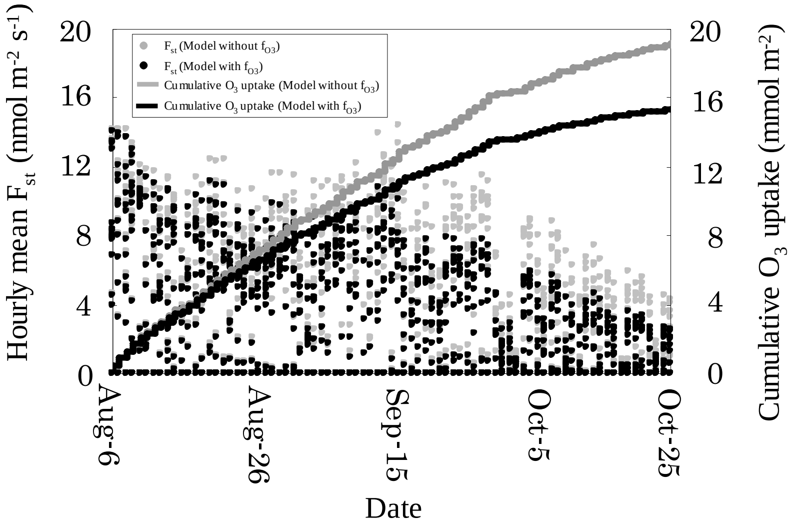

(A)
Estimated g_{sw} (mmol m^{-2} s^{-1})
Measured g_{sw} (mmol m^{-2} s^{-1})
R^2 = 0.59, p<0.05
RMSE=112.2
(B)
Estimated g_{sw} (mmol m^{-2} s^{-1})
Measured g_{sw} (mmol m^{-2} s^{-1})
R^2 = 0.60, p<0.05
RMSE=84.9

Fig.5


Hourly mean F_st (nmol m-2 s-1)
20
16
12
8
4
0
F_st (Model without f_O3)
F_st (Model with f_O3)
Cumulative O3 uptake (Model without f_O3)
Cumulative O3 uptake (Model with f_O3)
Cumulative O3 uptake (mmol m-2)
Aug-6
Aug-26
Sep-15
Oct-5
Oct-25
Date

Tables

Table 1 Summary of model parameters in stomatal conductance for *Fagus crenata*

Parameter			This study
g_{max} (mmol H_2O m^{-2} PLA s^{-1})			474
f_{min}		(fraction)	0.11
f_{phen}	f_{phen_a}	(days)	53
	f_{phen_b}	(fraction)	0.3
f_{light}	a	(constant)	-0.0086
f_{temp}	T_{opt}	(°C)	26
	T_{min}	(°C)	8
	T_{max}	(°C)	35
f_{VPD}	VPD_{max}	(kPa)	1.1
	VPD_{min}	(kPa)	4.1