

RESEARCH PAPER



Seasonal change in response of stomatal conductance to vapor pressure deficit and three phytohormones in three tree species

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ABSTRACT

Stomatal behavior under global climate change is a central topic of plant ecophysiological research. Vapor pressure deficit (VPD) and phytohormones can affect stomata of leaves which can affect gas exchange characteristics of plant. The role of VPD in regulating leaf gas exchange of three tree species was investigated in Jinan, China. Experiments were performed in June, August, and October. Levels of three phytohormones (GA₃, IAA, ABA) in the leaves of the three trees were determined by high-performance liquid chromatography in three seasons. The responses of stomatal conductance (g_s) to an increasing VPD in the leaves of the three trees had peak curves under different seasons, which differed from the prevailing response pattern of g_s to VPD in most literature. The peak curve could be fitted with a Log-Normal Model ($R^2 = 0.838-0.995$). The VPD/RH values of the corresponding maximum of g_s (g_{s-max} -VPD/RH) could be calculated by fitted models. The g_{s-max} -RH could be affected by environmental conditions, because of positive correlation between g_{s-max} -RH and the mean monthly temperature in 2010 ($R^2 > 0.81$). Two typical stomatal models (the Leuning model and the optimal stomatal behavior model) were used to estimate g_s values, but they poorly predicted g_s in the three trees. The concentration of ABA was positively correlated to sensitivity in response of stomatal conductance to VPD in the leaves of the tree species during the different seasons.

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1. Introduction

Stomata are the major pathway for acquiring carbon and limiting water loss between vegetation and the atmosphere. Stomatal behavior under global climate change is a central topic of plant ecophysiological research because it affects plant growth, vegetation distribution and ecosystem function. Global climate change significantly affects plant productivity and their water transport and use patterns, which would be reflected in the water use efficiency (WUE) of individual plants, communities, and ecosystems, and ultimately, in the vegetation distribution pattern, species composition, and ecosystem structure. Therefore, it is important to understand stomatal movement because it is a factor in changing the planet and in modeling the climate and weather of the current and future Earth.¹⁻⁴ To study the WUE of plants would be helpful in understanding and forecasting the responses of terrestrial vegetation to global climate change and to the adoption of adaptive strategies. In addition to the environmental factors which affect the stomatal regulation, including light, temperature, relative humidity, etc., phytohormones also participate in the stomatal regulation, including ABA, auxin, cytokinin, ethylene, gibberellin, etc.⁵⁻⁷

The prevailing pattern regarding the relationship between g_s and atmospheric water content is that increasing vapor pressure deficit (VPD) leads to reduction of g_s .⁸⁻¹¹ Stomatal conductance is closely linked to atmospheric water content.¹² The Ball-

Woodrow-Berry (BWB) model employs a linear relationship between the stomatal conductance (g_s) and relative humidity (RH).⁸ However, the models based on vapor pressure deficit generally perform better than those based on relative humidity.¹³ VPD is a more appropriate variable in describing stomatal responses to air humidity;¹⁴ therefore, relative humidity is replaced by vapor pressure deficit in the stomatal response models after Leuning's model publication.¹⁵ The main stomatal models until now could be divided into two types. One type is the empirical model which is widely used because of readily estimated parameters from measured data and simple implementation at different scales. The typical empirical models were developed by Jarvis,¹⁴ Ball et al.,⁸ Leuning¹⁵ et al. The other type of stomatal model is based on the theory of optimal stomatal behavior.¹⁶ Although abundant research has been carried out about stomatal response and many models have been established, the physiological mechanism controlling the response of stomata to VPD (or RH) is inadequately understood.^{8,13-15}

Some of the present stomatal conductance models have shown poor predictions compared with measured data. For example, evaluation of g_s by Jarvis' model was better than the BWB model with *Aneurolepidium chinense*,¹⁷ but both stomata models poorly predicted values of g_s under high RH conditions.¹³ Liu et al.¹⁸ suggested that the Leuning model may not be appropriate for measured data analysis and ecosystem simulation applications in arid and semiarid zones by comparing the predicted with measured data of three major species in a semi-arid site. In addition,

many studies have shown different patterns of the response of g_s to VPD. *Vitis Pseudoreticulata* indicates a maximal g_s at RH 70% with increasing RH, which showed a stress to stomatal regulation at high RH.¹⁹ Three distinct phases were used to describe the pattern of g_s to VPD response in *Pseudotsuga menziesii*¹² with g_s first increasing then decreasing when VPD increased. The leaves of *Pseudotsuga menziesii* showed a maximal g_s at intermediate VPD. Soni et al.²⁰ also have reported similar relationships between stomatal conductance and VPD in *Selaginella bryopteris*. High RH reduces the efficacy of transpiration.²¹⁻²³ All of the above-mentioned reports concerning the response of g_s to VPD show a different response pattern of g_s to VPD from the prevailing opinion. However, little scholars pay attention to explore or analyze the environmental or plant characteristics and the possible causes of this phenomenon.

The response of stomata to environmental and physiological factors is complex. Phytohormones are important factors that affect stomatal regulation. Increased levels of abscisic acid (ABA) while a plant is under stress promote stomatal closure and/or inhibit stomata opening in order to avoid excessive water loss.²⁴⁻²⁹ The response of g_s to VPD is correlated to the level of ABA.³⁰ The other phytohormones, including indole-3-acetic acid (IAA) and Gibberellins (GAs), are known to antagonize the effects of ABA on stomatal behavior.^{31,32} High ratios of GA_3/ABA and IAA/ABA have been shown to maintain stomata open in order to keep growing under high temperature and drought conditions.³³ Larger ratios of IAA/ABA could lead to better growth of plants,³⁴ and also GA_3/ABA affected the growth rate of plants.^{35,36}

It is important and urgent to study the stomatal behavior, and understand the characteristics of stomatal and physiological factors' response to global climate change. Therefore, in this study, the response patterns of stomatal conductance to VPD in three temperate trees under different seasons were characterized. The characteristics of these response patterns are discussed. We also investigated whether the phytohormones in the leaves of the three trees had an influence on the relationship between g_s and VPD. In addition, the two stomatal models (Leuning model and the optimal stomatal model) were simulated and we compared the predicted and measured stomatal conductance values in the leaves of the three trees in different seasons.

2. Materials and methods

2.1. Sites description

The study site is located in Jinan (36°35'36"-36°40'04" N, 116°54'29"-117°02'01" E), Shandong Province, China. Jinan is located in a typical warm-temperate humid/semi-humid climate which is characterized by a long summer with mean annual precipitation of 688.9 mm. The regional dominant vegetation belongs to needle-broadleaved mixed forest. The climate diagram is shown in Figure 1. The climatic data were collected from the China Meteorological Administration (<http://www.cma.gov.cn/>), and show average values from 1951 to 2010.³⁷

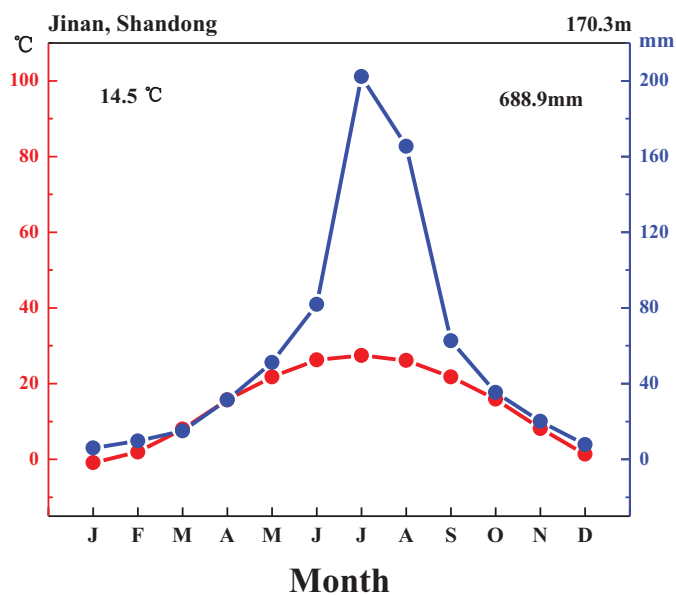


Figure 1. Climate diagram for Jinan, Shandong, China.

2.2. Plant material

Fraxinus chinensis Roxb., *Populus alba* L. var. *pyramidalis* Bge. and *Populus tomentosa* Carr. are widely distributed in China. *Fraxinus chinensis* Roxb. grow rapidly and are distributed widely in both north and south of China. *Populus alba* L. var. *pyramidalis* Bge. has the natural distribution area in the north of China with drought and salinity tolerance. *Populus tomentosa* Carr. is distributed in the central and northern parts of China and its optimum tree specie for shelter forest. All three tree species are also excellent gree tree species. Therefore, they were chosen to measure the response characteristics of stomatal conductance to VPD in different seasons as the typical tree species.

P. alba var. and *P. tomentosa* were 1-year-old seedlings. Cuttings (20–25 cm) of the same clone were collected from Urumqi (Xinjiang Uygur Autonomous Region) for *P. alba* var., and from Jinan (Shandong Province) for *P. tomentosa*. The cuttings were planted in plastic pots outside at the end of March 2010. The plants were well watered every day and supplemented with Hoagland nutrient solution once a week. The *F. chinensis* were adult trees.

Measurements were made on the leaves of the plants. The regimes of the experimental plants and the leaf chamber temperature (T_b) during measurements are shown in Table 1.³⁷

2.3. Gas exchange measurements

Measurements of leaf stomatal response to air humidity in the three trees were carried out on sunny days in June (early summer), August (midsummer) and October (late autumn), 2010. The 3–7 individuals for each species of the three trees were selected to measure leaf stomatal conductance response, with fully developed and sun-exposed leaves in adult trees and the seventh/eighth leaves from the top in seedlings, between 8:00 and 12:00 am (to avoid midday depression in

Table 1. Features of the experimental plants and leaf chamber temperature (T_b) during measurements.

Plant species	Month	T_b (°C)	Chest diameter of trees (mm)	Height of trees (m)
<i>F. chinensis</i>	June	30	150-200	7-8
	August	30		
	October	20		
<i>P. alba</i> var.	June	31	Seedling	0.5–1.3
	August	31		
	October	25		
<i>P. tomentosa</i>	June	32	Seedling	0.5–1.5
	August	31		
	October	26		

photosynthesis) in Jinan,^{38–40} using a portable photosynthesis system LI-COR LI-6400 equipped with a red/blue LED source (LI-COR Inc., Lincoln, NE, USA). Photosynthetic photon flux density (PPFD) at 8:00 am (PPFD >200 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$) could exceed the light compensation point for most tree species in Jinan.^{41–44} Prior to leaf gas exchange measurements, cuvette PPFD was adjusted to 1500 $\mu\text{mol}/(\text{m}^2\cdot\text{s})$ as light saturation and constant leaf chamber temperature (Table 1, the third column) with ambient CO_2 content in order to determine the stomatal conductance response to vapor pressure deficit. The vapor pressure deficit was controlled with the LI-COR 6400 humidity control function.

2.4. Stomatal response models

Two typical stomatal models were used to compare the estimated g_s and measured g_s data. One is the Leuning model¹⁵ as one of the empirical models; the other is the optimal stomatal model which was modified by Medlyn et al.^{45,46}

The Leuning model reads

$$g_s = g_0 + a \frac{A_n}{(C_s - \Gamma)(1 + D/D_0)} \quad (1)$$

where g_s is the stomatal conductance; A_n is the net photosynthetic rate; D and C_s are vapor pressure deficit and the CO_2 concentration at the leaf surface, respectively; g_0 is the conductance as $A_n \rightarrow 0$ when leaf irradiance $\rightarrow 0$; and D_0 and a are empirical coefficients.

The optimal stomatal model reads

$$g_s \approx g_0 + 1.6 \left(1 + \frac{g_l}{\sqrt{D}} \right) \frac{A_n}{C_s} \quad (2)$$

where g_s is the stomatal conductance; A_n is the net photosynthetic rate; D and C_s are vapor pressure deficit and the CO_2 concentration at the leaf surface, respectively; g_0 is the conductance as $A_n \rightarrow 0$ when leaf irradiance $\rightarrow 0$; and g_l and a are empirical coefficients.

2.5. Phytohormone measurements

Measurement of the leaf phytohormones in this study was done by high-performance liquid chromatography (HPLC). Plant leaf samples were collected and powdered with liquid nitrogen, and extracted with cold methyl alcohol at 4 °C. After centrifugation (8000 rpm, 4 °C, 10 min) and complete removal of the methyl alcohol with a rotating evaporator, the dried samples were

dissolved in phosphate buffer solution (pH 8.0) and chloroform. Further clean-up was achieved by using chloroform and cross-linked polyvinylpyrrolidone (PVPP) to remove pigment and phenols, respectively. Final clean-up of the extract was done by extracting with ethyl acetate at pH 2.8 (adjusted with formic acid). Finally, the extracts were centrifuged, evaporated and then the dried samples were stored in 1.5 ml of mobile phase solution (methanol/ H_2O , 40/60, 0.5% acetic acid) until HPLC analysis was performed. The concrete step about extraction and purification of the leaves followed the procedure described by Fu et al.⁴⁷ Detection limits and recoveries of standards of GA_3 , IAA, and ABA are shown in Table 2.³⁷

Phytohormones were monitored on a diode array detector (SPD-M10Avp) with detection wavelength at λ 254 nm using HPLC (Shimadzu Lc-10ADvp, Japan). The samples were separated on a C18 reversed-phase column using the above mobile phase solution, the column temperature was to keep at 38 °C. All reagents were HPLC-grade. The GA_3 , IAA and ABA standards were purchased from Sigma (St. Louis, USA).

2.6. Statistical analysis

Significant differences among multiple means in the concentration of phytohormones were tested using a least significant difference test (SPSS v. 16.0). The response curves were fitted using non-linear regression method by SigmaPlot (v. 10.0). The differences between our measured data and simulated data based on the two models were, respectively, tested with F-test (SPSS) and Root Mean Square Error (RMSE, σ).

The sensitivity of g_s to VPD in the published reports was described by $dg_s/d(\text{VPD})$.^{48,49} The change trend of g_s to VPD was assumed to decrease monotonously in those researches. However, the result in this paper showed that the change trend of g_s to VPD was one top line in three tree species in different seasons. So the test of the sensitivity of g_s to VPD in the three tree species in the three different seasons was carried out using Root Mean Square Error of stomatal conductance in a certain range of VPD (Equation 3). RMSE could be a more appropriate way to reflect the sensitivity of g_s to VPD.

RMSE shows the variation from the average.⁴⁸ A high σ indicates that the data points tend to be very far from the mean.

$$\sigma = \sqrt{\frac{\sum_{i=1}^n (g_s - g_s^*)^2}{n}} \quad (3)$$

where g_s is the measured value; g_s^* is the value of $y = x$ in expressing the g_s model prediction, and g_s^* is the average value of measured data in expressing the sensitivity of g_s to VPD; n is the number of the measured samples.

Table 2. Detection limits and recoveries of standards of GA_3 , IAA, and ABA.

Phytohormones	Quantitative detection limit (ng/L)	Recovery (100%)
GA_3	8.43	95.2 \pm 7.9
IAA	3.77×10^{-2}	96.3 \pm 8.2
ABA	12.5	95.9 \pm 3.9

3. Results

The experiment was conducted in June (early summer), August (midsummer) and October (late autumn), 2010 in the central campus of Shandong University in Jinan. The net photosynthetic rate (A_n), transpiration rate (E) and g_s were determined with a gradient of increasing VPD at constant PFD and T_b applied to the measured leaf in the cuvette (Figure 2). Figure 2 shows the responses of g_s , A_n , E and WUE to VPD in the leaves of *F. chinensis*, *P. alba* var. and *P. tomentosa* in June, August, and October in Jinan.³⁷ Table 3 shows the concentrations of ABA, GA₃, IAA and the ratios of GA₃/ABA and IAA/ABA in different seasons.³⁷

3.1. The response of gas exchange to VPD

Figure 2 shows the variations of stomatal conductance, photosynthetic rates, transpiration rates, and water use efficiency in the three trees along a gradient of vapor pressure deficit. There is a particular stomatal response pattern to VPD in our results, which shows that g_s is low under low VPD, and g_s

increases with increasing VPD following by a peak at intermediate VPD, and then g_s declines steadily with further increases in VPD. There is a maximal g_s (g_{s-max}) with increasing VPD. This stomatal response pattern to high VPD shows a similar trend with most stomatal response patterns to VPD at high VPD in the literature,⁹⁻¹¹ which also show that g_s decreased with further increasing VPD. Photosynthetic rates show a smoother change with increasing VPD in the three trees, but a significant increase in E with increasing VPD.

A_n , E and g_s of *F. chinensis* were highest in August; A_n , E , and g_s of *P. alba* var. were highest in October.

3.2. The response of WUE to VPD

Water use efficiency (WUE) reflects the relationship between photosynthetic production and water consumption.⁵⁰ The instantaneous WUE is calculated as the ratio of A_n to E which could be affected by light, temperature, relative humidity, etc. The three trees show the same pattern of WUE to VPD, that is WUE decreased gradually with

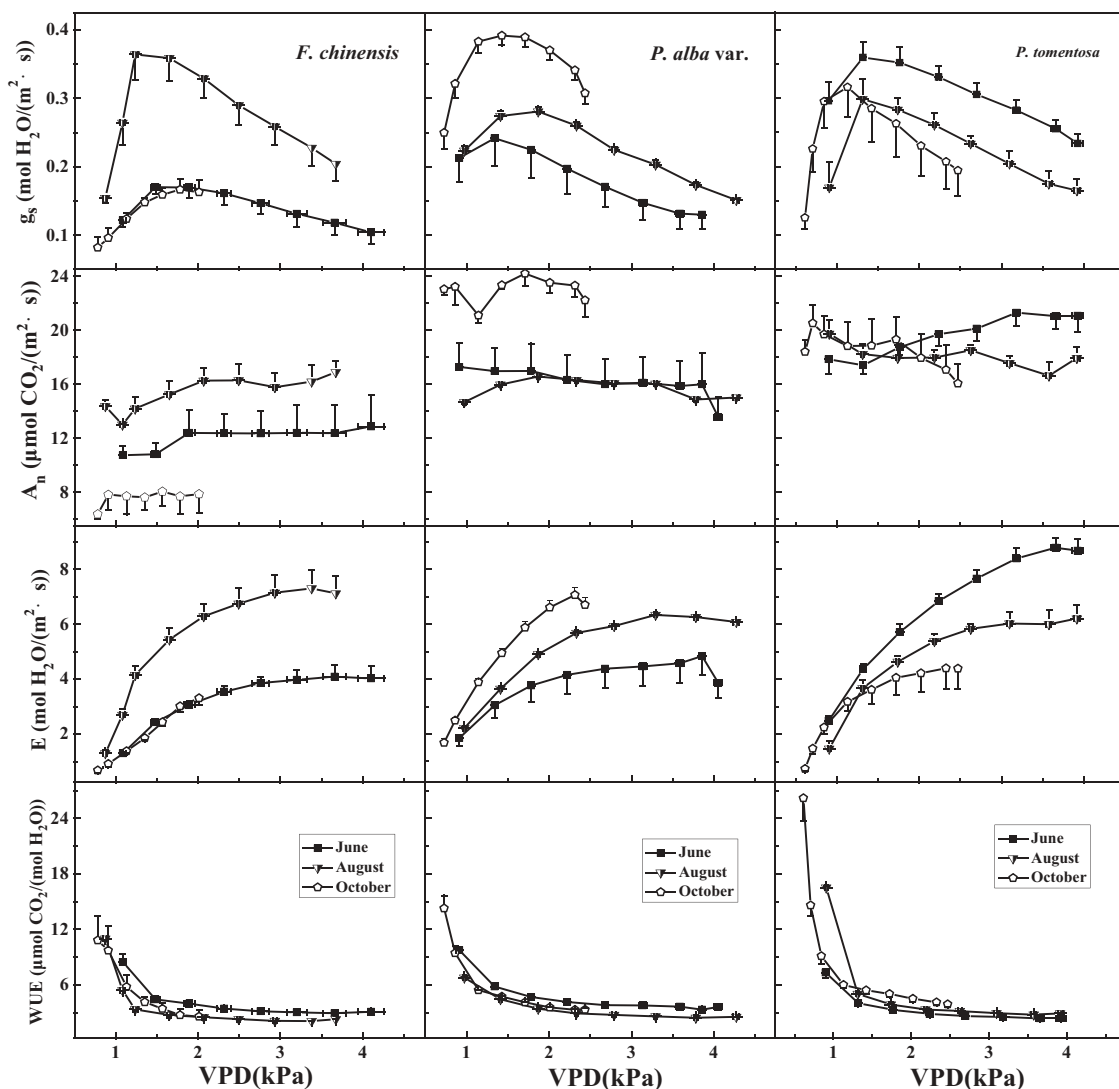


Figure 2. The responses of stomatal conductance (g_s), net photosynthesis rate (A_n), transpiration rate (E) and water use efficiency (WUE) to vapor pressure deficit (VPD) in *F. chinensis*, *P. alba* var. and *P. tomentosa* in Jinan, China. Error bars represent standard errors of g_s , A_n and E with 3–7 replicates measurements.

Table 3. The concentration of phytohormone (GA₃, IAA, ABA) and the ratios of GA₃/ABA and IAA/ABA in three tree species in Jinan.

Plant species	Month	GA ₃ (ng/(g FW))	IAA (ng/(g FW))	ABA (μg/(g FW))	GA ₃ /ABA (10 ⁻³)	IAA/ABA (10 ⁻³)
<i>F. chinensis</i>	June	311.15 ± 14.75 ^a	10.74 ± 0.85 ^a	5.50 ± 1.05 ^a	56.57	1.84
	August	60.50 ± 2.07 ^{b,c}	1.94 ± 0.29 ^{b,c}	13.63 ± 0.94 ^b	4.44	0.14
	October	12.80 ± 0.49 ^b	2.85 ± 0.32 ^c	3.21 ± 0.72 ^c	3.99	0.89
<i>P. alba</i> var.	June	934.18 ± 31.75 ^d	5.21 ± 0.41 ^d	4.13 ± 1.28 ^{a,c}	226.19	1.26
	August	230.31 ± 18.65 ^{a,c}	1.79 ± 0.37 ^{b,c}	18.20 ± 1.74 ^d	12.66	0.10
	October	842.50 ± 28.50 ^d	1.37 ± 1.06 ^b	14.06 ± 1.76 ^b	59.92	0.10
<i>P. tomentosa</i>	June	324.22 ± 7.55 ^a	4.15 ± 1.05 ^d	13.47 ± 0.60 ^b	24.07	0.31
	August	315.33 ± 8.07 ^a	4.87 ± 0.27 ^d	16.24 ± 0.83 ^d	19.41	0.30
	October	414.71 ± 10.48 ^a	2.25 ± 0.33 ^{b,c}	22.99 ± 1.64 ^e	18.04	0.10

Note: The same letter in the same column represents no significant difference (Duncan test, $P < 0.05$, $n = 3-5$).

increasing VPD. Under low VPD, WUE decreased sharply due to the steep increase in E (Figure 2). However, WUE decreased slowly and smoothly with further increased in VPD. Variations in WUE along a gradient of increasing VPD are shown in Figure 2.

3.3. Models test

Figures 3 and 4 show a comparison of the measured data with the simulated stomatal conductance provided by the two models.³⁷ There are obvious differences between the measured data and the predicted data.

3.4. Phytohormones in the leaves of the three trees

GA₃, IAA and ABA concentration in the leaves of the three tree species in Jinan are shown in Table 3.³⁷ The measurement results showed that the ABA concentrations in the leaves of the three trees in June were lower than in August. The highest GA₃/ABA and IAA/ABA in the leaves were found in all three trees in June.

The concentration of GA₃ in the leaves decreased with season in *F. chinensis*, especially GA₃ in late autumn (Table 3). The concentration of IAA and ABA changed more moderately than GA₃ in the three trees in the different seasons. GA₃ concentration in the leaves of *F. chinensis* decreased sharply,

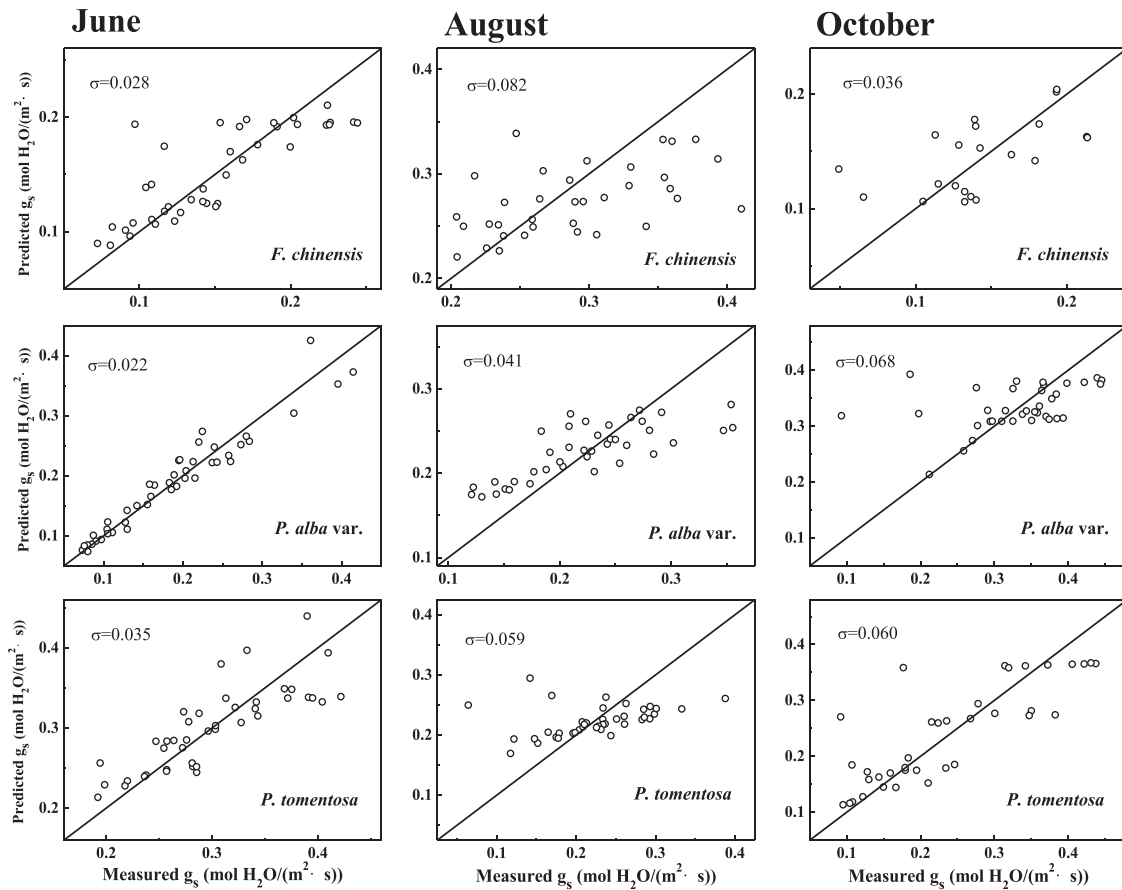


Figure 3. Comparison of stomatal conductance predicted by the Leuning model and measured data for the three tree species in Jinan, China. The diagonal line is the 1:1 relationship between predicted data and measured data. The σ value is the difference between the 1:1 line and measured data (Root Mean Square Error, RMSE).

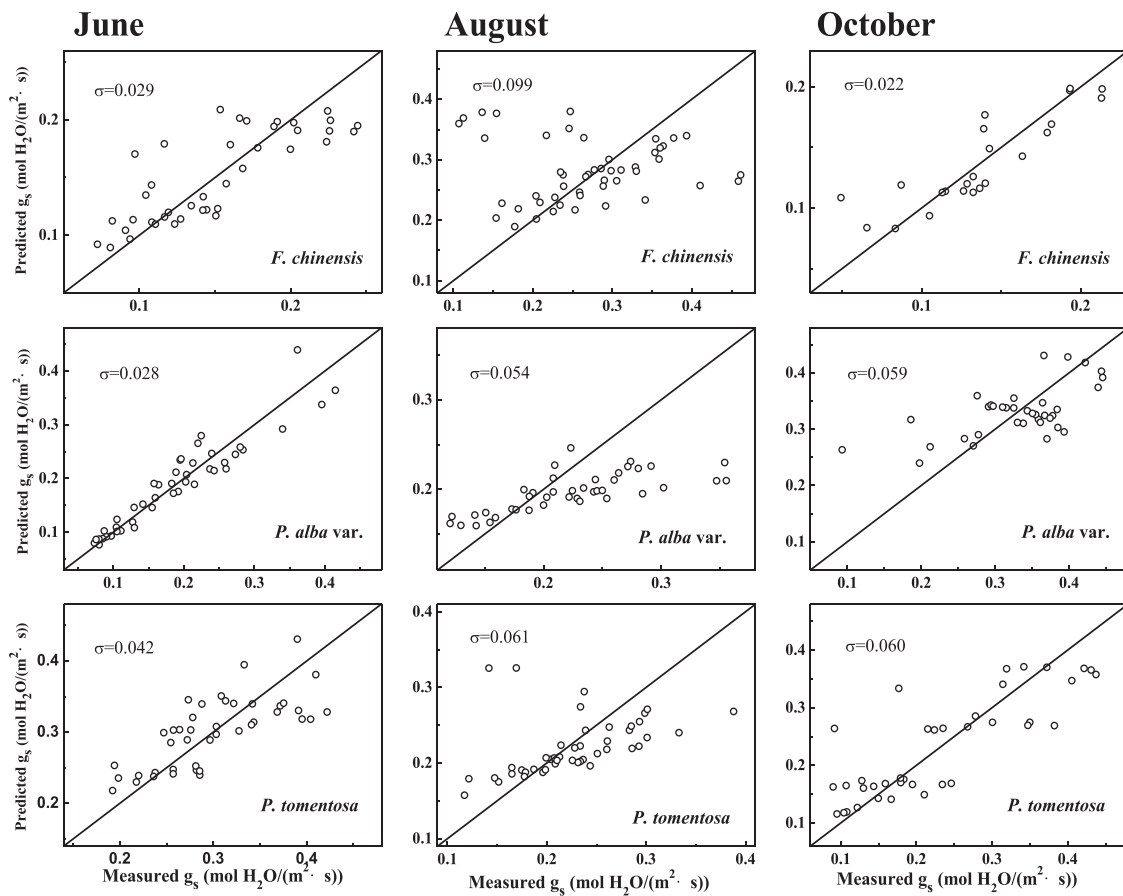


Figure 4. Comparison of stomatal conductance predicted by the optimal stomatal model and measured data for the three tree species in Jinan, China. The diagonal line is the 1:1 relationship between predicted data and measured data. The σ value is the difference between the 1:1 line and measured data (Root Mean Square Error, RMSE).

whereas it increased in the leaves of *P. alba* var. and *P. tomentosa* in October compared with that in August.

4. Discussion

4.1. Stomatal response to vapor pressure deficit

Our results showed that the stomatal conductance increased with the increasing VPD under low VPD conditions, and reached a peak value under a certain VPD condition; then, the stomatal conductance gradually decreased following a further increasing VPD. This changing pattern of g_s to VPD was of a feature of peak curve. Many researchers have shown that the stomatal aperture decreases at high humidity

in many tree species.^{51–54} The stomatal conductance showed a maximal value (g_{s-max}) in a certain range of VPD along with increasing VPD in the three trees, which was similar to the published results by Woodruff et al. in Douglas-fir.¹² The response pattern of g_s to VPD in this study did not agree with the prevailing stomatal response pattern to VPD.^{8,9,11} A Log-Normal Model was selected to fit the measured data using a nonlinear regression method (SigmaPlot, V. 10.0). Then, we tested the fitting models, and the results showed high correlation coefficients and goodness of fit ($g_s = a \cdot \exp(-0.5(\ln(D/c)/b)^2)$, $D = VPD$, $R^2 = 0.845–0.996$). The g_{s-max} and VPD/RH corresponding to g_{s-max} (g_{s-max} -VPD/RH) were calculated based on this model (Table 4).³⁷ Environmental factors significantly affect the leaves' performance (like

Table 4. Nonlinear simulation formula of g_s to VPD and g_{s-max} corresponding to VPD and RH.

Plant species	Month	Nonlinear simulation formula		VPD (kPa, $g_s = g_{s-max}$)	RH (%, $g_s = g_{s-max}$)	Mean monthly VPD in 2010 (kPa)	Mean monthly temperature in 2010 (°C)
		$g_s = a \cdot \exp(-0.5(\ln(D/c)/b)^2)$, $D = VPD$	Correlation coefficient (R^2)				
<i>F. chinensis</i>	June	$g_s = 0.17 \exp(-0.5(\ln(D/1.91)/0.75)^2)$	0.962	1.91	60.14	1.43	25.8
	August	$g_s = 0.36 \exp(-0.5(\ln(D/1.77)/0.63)^2)$	0.845	1.77	57.88	0.61	25.1
	October	$g_s = 0.17 \exp(-0.5(\ln(D/1.96)/0.76)^2)$	0.994	1.96	12.31	0.78	15.3
<i>P. alba</i> var.	June	$g_s = 0.24 \exp(-0.5(\ln(D/1.34)/0.89)^2)$	0.979	1.34	69.98	1.43	25.8
	August	$g_s = 0.28 \exp(-0.5(\ln(D/1.68)/0.83)^2)$	0.995	1.68	64.18	0.61	25.1
	October	$g_s = 0.4 \exp(-0.5(\ln(D/1.46)/0.75)^2)$	0.973	1.46	49.21	0.78	15.3
<i>P. tomentosa</i>	June	$g_s = 0.36 \exp(-0.5(\ln(D/1.6)/0.99)^2)$	0.968	1.60	64.11	1.43	25.8
	August	$g_s = 0.29 \exp(-0.5(\ln(D/1.74)/0.71)^2)$	0.901	1.74	60.45	0.61	25.1
	October	$g_s = 0.31 \exp(-0.5(\ln(D/1.25)/0.64)^2)$	0.838	1.25	56.59	0.78	15.3

stomatal length, width, and area).⁵⁵⁻⁶¹ Arve et al. found that the regulation of stomatal movement and the response of g_s to environmental factors mainly depended on the developing period of the leaves,^{56,58} which was consistent with our results. Therefore, the g_{s-max} -VPD of leaves from each tree species showed little variation among the different seasons during the experimental period.

VPD was calculated from RH and temperature.⁶² Mean monthly temperature and RH were different in the three seasons. Therefore, the g_{s-max} -RH of the leaves among the seasons was different. The g_{s-max} -RH is about 60–70% in June at about 25.8°C, 57–65% in August at about 25.1°C, and 12–57% in October at about 15.3°C. The trends of g_{s-max} -RH in the three trees in the different seasons and the mean monthly temperature (°C) in different seasons in 2010 were the same ($R^2 > 0.81$) (Table 4, the sixth and eighth columns), which shows that g_{s-max} -RH is related to the climatic conditions. Table 4 shows the fitting formulas, the related correlation coefficients of fitting model, the g_{s-max} -VPD/RH and the mean monthly VPD/RH in 2010.

Therefore, the present data show that an optimal g_s occurs at optimal VPD. When VPD is higher or lower than the optimal VPD, g_s decreases. The optimal VPD could be affected by the environmental conditions. The pattern in response of g_s to VPD could be described with a Log-Normal Model. All three tree species reached the maximum stomatal conductance in a certain range of VPD in the different seasons.

4.2. Comparison of two stomatal models predicting g_s with measured g_s

Models of stomatal conductance play an important scientific role in summarizing commonly observed trends in stomatal behavior, and advancing our understanding of ecosystem responses to global change. They are widely used for predicting climate impacts on biospheric carbon cycles, hydrological cycles, and nutrient cycles. The prevailing pattern regarding the relationship between g_s and atmospheric water content is that increasing VPD leads to the reduction of g_s .⁸⁻¹¹ Two types of stomatal conductance models are widely used to simulate the gas exchange of leaves.^{13,17,18} Two typical stomatal models (the Leuning model and the optimal stomatal model) were used to compare the predicted g_s and measured g_s data.^{14,45,46} The data of g_s under increasing VPD are simulated by the Leuning model and the optimal stomatal model. Both models performed poorly in predicting g_s in the three trees (Figures 3 and 4) in different seasons in Jinan. There are obvious differences ($P < .05$, F-test) in the measured data and the predicted data of the three tree species in August and *P. alba* var. in October with those two models. The difference between the 1:1 line and the measured data (σ , RMSE) was bigger in the three tree species in August and *P. alba* var. in October with those two models than the others (Figures 3 and 4). These results show that the present data on the response of stomatal conductance to VPD cannot be explained well with either of the two stomatal models.

4.3. Patterns of WUE in three tree species

The net photosynthesis rate did not show a significant variation with increasing VPD even at high VPD in the three tree

species in Jinan. Assimilation continued undiminished in spite of the declined g_s at low and high VPD, which demonstrated that A_n was not sensitive to VPD. However, net photosynthetic rate (A_n) in the three trees varied strongly among the different species. In addition to the plant species, environment factors (like temperature and precipitation) could lead to the different assimilation performances by the trees.²⁰ Transpiration rates (E) in the three trees showed a steady increase, and increased steeply at the lower range of VPD. These findings were similar to those of Soni et al. in *S. bryopteris*.²⁰

However, A_n of *F. chinensis* in October decreased sharply (Figure 2, the second row), and the same result was found in g_{s-max} -RH (12.31%) (Table 4, the fourth row). IAA, CTK (cytokinin) and GAs could inhibit leaf senescence, GA₃ could promote the growth of plant stem and leaf, and the other phytohormones like ABA and ETH (ethylene) could promote leaf senescence.⁶³⁻⁶⁷ Growing periods in trees and the environmental conditions could affect the level of phytohormones in the leaves.⁴⁷ GA₃ concentration in the leaves of *F. chinensis* decreased sharply, which was associated with the senescence in plant leaves.^{32,66,68} Leaf function was limited by senescence of the leaves of *F. chinensis*, and A_n of *F. chinensis* reduced significantly in October. Therefore, the optimal RH of *F. chinensis* was just 12.3% in October which showed the poor adaptation of the leaf stomata to RH in autumn.

The different response patterns of A_n and E to VPD lead to a sharp reduction in WUE at low VPD, and then a smooth decrease in WUE was observed. The last row of Figure 2 demonstrates that the WUE was significantly higher at lower ranges of VPD than at higher ranges of VPD for all three species of trees.

4.4. Sensitivity of g_s to VPD variation and the concentration of phytohormones

Phytohormones are of vital importance for regulating the stomatal aperture, which is crucial for minimizing water loss and maximizing CO₂ exchange. In particular, ABA plays a major role in regulating stomatal function in adaptation to various environmental stresses, such as drought, cold, air pollutants, etc.²⁴⁻²⁷ Inadequate stomatal closure at high vapor pressure deficit may be mediated by abscisic acid.³⁰ In addition, other phytohormones also contribute to stomatal aperture regulation, such as auxin, cytokinin, ethylene and salicylic acid.^{31,33,34} Gibberellins and IAA are major phytohormones involved in various aspects of plant growth and development, and they are also known to antagonize the effects of ABA on stomatal movement.^{31,32}

Stomata of different species vary in their sensitivity in response of g_s to VPD. The different levels of ABA observed in the different seasons, affect the sensitivity in response of g_s to VPD. The response of g_s to VPD was very sensitive to the level of ABA because ABA could lead to an obvious inhibition of g_s in leaves.⁶⁹ Sensitivity of g_s to VPD along the gradient of increasing VPD was assessed by Root Mean Square Error (RMSE, σ) of g_s data selected the same range of VPD for different trees, respectively. RMSE is a frequently used measure of the differences among several values observed, which

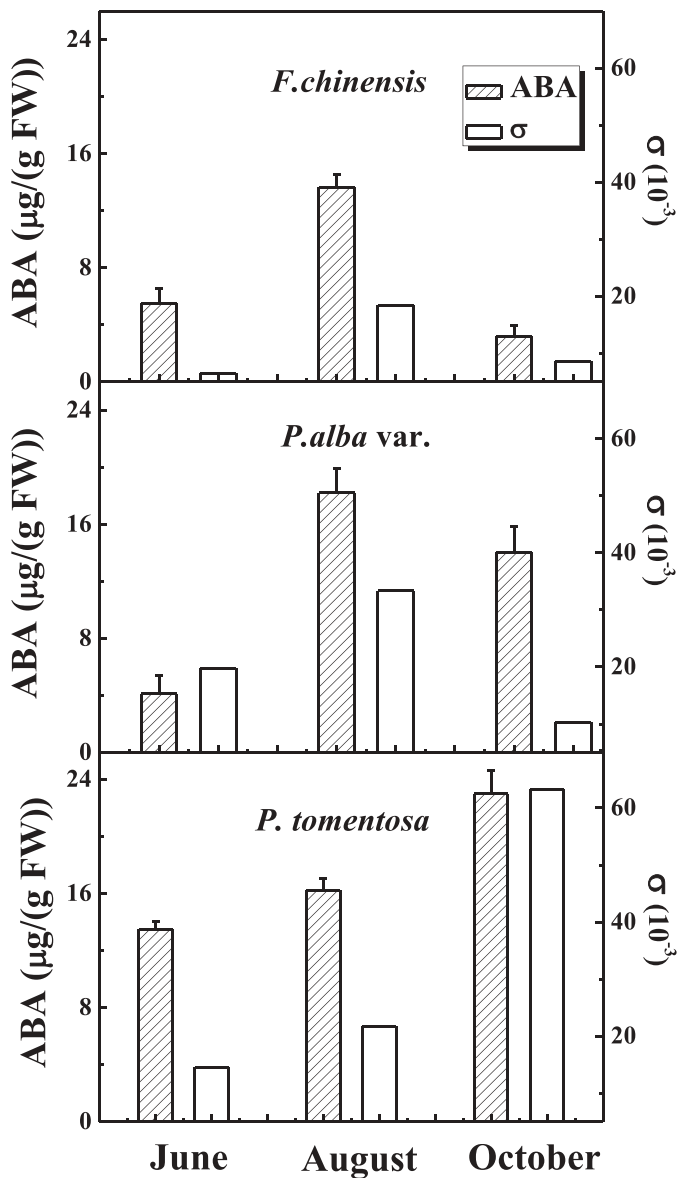


Figure 5. The concentration of abscisic acid (ABA) and the sensitivity of stomatal conductance (g_s) to vapor pressure deficit (VPD) in the three trees in different seasons in Jinan. The sensitivity of g_s to VPD is described by Root Mean Square Error (RMSE, σ , $n = 9$).

represents the sample standard deviation of the differences. The larger σ as a sensitivity parameter related to the higher sensitivity of g_s to VPD. Figure 5 demonstrates that the value of σ correlated positively with the concentration of ABA in the leaves of the three trees.³⁷ Therefore, the higher sensitivity of response of g_s to VPD was related to the higher concentration of ABA in the leaves of the three trees.

5. Conclusion

We can safely infer from this study that the response of g_s to a gradient of increasing VPD can be fitted very well with a Log Normal Model ($R^2 = 0.845\text{--}0.996$). Therefore, it can be considered that there is a $g_{s\text{-max}}$ in response of g_s to VPD in all measured plants which illustrated that there is an optimal range of vapor pressure deficit in the trees. The $g_{s\text{-max}}\text{-VPD}$ of leaves

from each tree species showed little variation among the different seasons during the experimental period but a positive relationship between $g_{s\text{-max}}\text{-RH}$ and the mean monthly temperature in 2010 ($R^2 > 0.81$). Two typical stomatal models (the Leuning model and the optimal stomatal model) were used to estimate g_s values, and we found a large difference between the predicted data and experimental data in the three tree species in different seasons. The concentration of ABA was positively correlated to sensitivity in response of stomatal conductance to VPD in the leaves of the three tree species in different seasons in Jinan.

However, this study mainly focused on deciduous broad-leaved tree species and did not have enough energy and time to discuss other kinds of plants, such as herbs, conifers and so on. Therefore, the response characteristics of other kinds of plants need to be further studied and validated. At the same time, the meaning of parameters in nonlinear simulation formula of g_s to VPD needs further study and discussion.

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Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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