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# Photosynthetic and stomatal responses of two tropical and two temperate trees to atmospheric humidity

S.-Y. PARK* and A. FURUKAWA**

*Environmental Biology Division, National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba 305, Japan**
*Faculty of Science, Nara Women's University, Kitauoya-Nishimachi, Nara 630-8506, Japan***

## Abstract

The effects of leaf to air vapour pressure differences (ΔW) on net photosynthetic rate ($P_N$) and stomatal conductance ($g_s$) were examined in the leaves of two tropical rain forest trees, *Eugenia grandis* and *Pongamia pinnata*, and two temperate evergreen trees, *Viburnum awabuki* and *Daphniphyllum macropodum*. A single leaf was set inside a small chamber and ΔW was varied from 7 to 24 mmol $mol^{-1}$ at 25 and 500 μmol $m^{-2}$ $s^{-1}$ of photon flux density. $P_N$ and $g_s$ of the two tropical rain forest trees decreased with increasing ΔW, while the two temperate evergreen trees were not highly responsive to ΔW. *P. pinnata* was more sensitive to ΔW in its stomatal response, and had a higher stomatal density and higher stomatal index than did the two temperate trees and another tropical tree. Significant reductions in $g_s$ and intercellular $CO_2$ concentrations in the two tropical trees at high ΔW suggest that the decline of $P_N$ was due to the decrease in $g_s$. The responses of $P_N$ and $g_s$ indicated that the tropical trees were more sensitive to ΔW than were the temperate ones.

*Additional key words*: *Daphniphyllum macropodum*; *Eugenia grandis*; intercellular $CO_2$ concentration; *Pongamia pinnata*; stomatal density; transpiration rate; *Viburnum awabuki*.

## Introduction

Understanding of the strategies of plants grown under stressed environmental conditions may be beneficial in managing regeneration of seedlings cultivated in disturbed areas and assist in providing optimum growth conditions (David *et al.* 1996, Marsden *et al.* 1996). Responses of photosynthesis and stomata to changes in water vapour

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**Present address*: Department of Agronomy, College of Agriculture, KyungPook National University, Sankyuk-Dong 1370, Bukgu, Taegu, 702-701 South Korea. Fax: +82-53-958-6880, e-mail: shinypark@hotmail.com.
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pressure differences between the atmosphere and leaf air spaces ($\Delta W$) in both $C_3$ and $C_4$ species have been reported, though the sensitivity to $\Delta W$ varies between species (Schulze 1986, Grantz 1990, Loreto and Sharkey 1990, Mott 1990, Aphalo and Jarvis 1991, Berg *et al.* 1997). Experiments on humidity responses of woody species in tropical rain forests are scarce. Since plants in that area are considered to be growing under high humidity, their growth is not expected to be restricted by low humidity. However, the relative humidity often falls to as low as 50 % on sunny days. Thus, tree species grown in humid tropical areas may have their photosynthetic carbon gain suppressed by comparatively low relative humidity. From these aspects, we examined the effects of $\Delta W$ on $P_N$ and $g_s$ of tropical rain forest trees.

## Materials and methods

**Plants**: Two tropical trees, *Eugenia grandis* Wight (*Myrtaceae*) and *Pongamia pinnata* (L.) Pierre (*Leguminosae*), and two temperate trees, *Daphniphyllum macropodum* Mig. (*Euphorbiaceae*) and *Viburnum awabuki* K. Koch (*Caprifoliaceae*), were used. Seeds of *E. grandis* and *P. pinnata* collected from Malaysia were germinated on vermiculite and transplanted into pots. *V. awabuki* was propagated from cuttings and *D. macropodum* was purchased from the local nursery. Trees were transferred to plastic pots (11 cm diameter, 15 cm deep) containing vermiculite, perlite, peat moss, and fine gravel (2 : 2 : 1 : 1, v/v). Each pot contained 5 g of *Magamp-K* and 15 g of magnesia lime. Trees were grown at 25 °C and 70 % relative humidity in a phytotron greenhouse. The natural photoperiod was extended to 15 h using fluorescent tubes. Plants were watered twice a day to maintain high soil moisture.

**Gas exchange** was measured in a controlled environment room (2.7×4.0×2.8 m high) at 25 °C, 70 % relative humidity, and 500 μmol $m^{-2}$ $s^{-1}$ of photon flux density (PFD) at plant height. The radiation source was 400 W phosphorous metal halide lamps (*BOC, Toshiba*, Tokyo, Japan). Gas exchange was measured using a portable photosynthesis system (*LI-6400, LiCor*, Lincoln, NE, USA). A leaf chamber (40×30 ×30 cm high), covered with a transparent *Teflon* film, was set inside a controlled environment room containing a sensor head of a *LI-6400* portable photosynthesis system was installed inside this chamber. Measurements of intercellular $CO_2$ ($C_i$), $g_s$, $P_N$, and transpiration ($E$) were calculated following Caemmerer and Farquhar (1981).

Ambient air was passed through soda lime to remove all $CO_2$ and then humidified by passing through distilled water. Dehumidifying the air, using two thermostatic glass condenser columns set to a known dew point, controlled the partial pressure of water vapour in the air entering the leaf chamber. The temperature of the condenser was maintained by a constant temperature water bath (*RTE-111, Neslab Instrument*, Newington, USA). The desired $CO_2$ concentration of the air was adjusted with a mass-flow controller (model *5877, Ueshima Brooks Co.*, Tokyo, Japan) by injecting 10 % $CO_2$ supplied from a cylinder. The leaf to be tested was inserted entirely into this small chamber, and the chamber temperature was kept at 25 °C using a circulating water bath (*RTE-4B, Neslab Instrument*, Newington, USA).

The potted plants were brought into the controlled environment room a day before the measurement and watered well. All experiments were conducted on current year leaves to ensure comparable physiological conditions. Before decreasing the vapour pressure inside the leaf chamber, the leaf was kept at 350 μmol($CO_2$) $mol^{-1}$, vapour pressure difference of 0.6 kPa, 25 °C, and 500 μmol $m^{-2}$ $s^{-1}$ of PFD to attain a steady rate of gas exchange.

**Measurements of stomatal and epidermal cell density**: Impressions were made on the abaxial epidermis in the four species using a cellulose acetate film, and stomata and epidermal cell densities were determined under a light microscope. The stomatal index (SI) was estimated by:

$$SI = SD / (SD + ED)$$

where SD and ED are stomatal and epidermal cell densities, respectively (Meidner and Mansfield 1968).

## Results

Photosynthetic responses to ΔW were much more significant in the tropical trees than in the temperate ones (Fig. 1). With increasing ΔW, $P_N$ of the two tropical trees, *E. grandis* and *P. pinnata*, decreased by 49 and 54 %, respectively. In contrast, the two temperate evergreen trees, *V. awabuki* and *D. macropodum*, showed only slight decreases in $P_N$. The interspecific differences in response of $g_s$ to ΔW were similar to those of $P_N$ (Fig. 1). The relative decreases in $g_s$ of *E. grandis* and *P. pinnata* were 63 and 73 %, respectively, *i.e.*, greater than the decreases in $P_N$. $C_i$ also decreased with increasing ΔW in the two tropical trees, but no remarkable decreases could be detected in the temperate trees (Fig. 1).

The values of $E$ were not different between the two temperate tree species or between the two tested tropical trees (Fig. 1). However, with increasing ΔW, an increase in $E$ was followed by a decrease in the two temperate trees, but no such changes were detected in the two tropical trees.

Differences in stomatal indices were found between the four tree species (Table 1). However, the interspecific differences in stomatal densities and stomatal indices did not reflect the interspecific differences in the responses of $P_N$ or $g_s$ to ΔW.

## Discussion

Stomata close in response to an increase in ΔW (Schulze and Hall 1982). We demonstrated that also $g_s$ decreased in the two tropical trees tested. However, in these trees, the $g_s$ was fairly constant over the range of ΔW tested. ΔW may affect stomatal apertures through two mechanisms: (*a*) a negative feedback of $E$ on the leaf water potential of the water conducting system (Raschke and Kuehl 1969); this

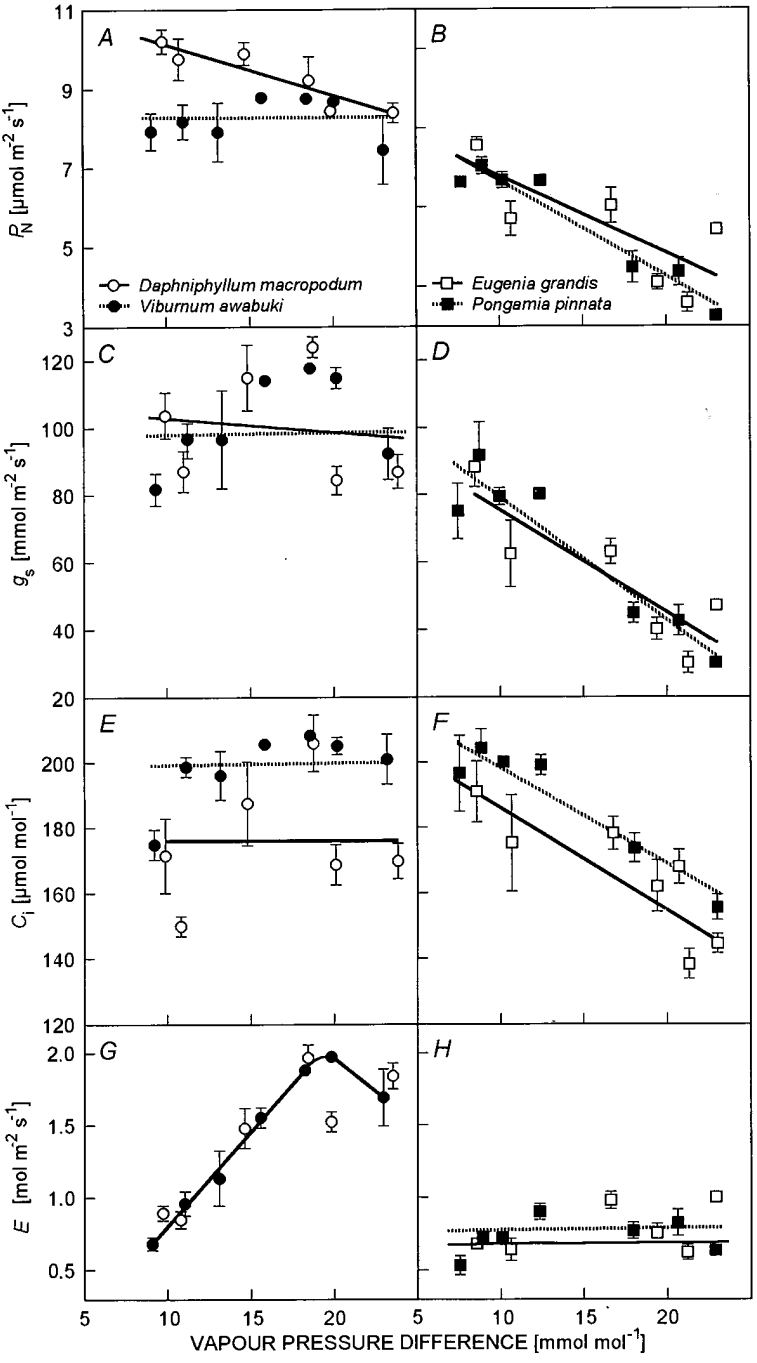


Fig. 1. The responses of net photosynthetic rate ($P_N$), stomatal conductance ($g_s$), intercellular $CO_2$ concentration ($C_i$), and transpiration rate ($E$) of *E. grandis*, *P. pinnata*, *V. awabuki*, and *D. macropodum* to different ΔW. Vertical bars indicate ± standard error ($n$ = 4).

hypothesis is derived from the observation that stomatal closure is caused by a decrease in leaf water potential as a result of an increase in water loss from a leaf (Meidner and Mansfield 1968). (*b*) The "feed forward" response (Farquhar 1978). Schulze *et al.* (1972) found that stomatal closure was induced before the decline of leaf water potential and speculated from this result that stomata respond directly to atmospheric humidity. However, our results are not easily explained using either of these hypotheses. The two tested temperate trees showed increases in $E$ with fairly constant $g_s$, while $E$ in the two tropical trees was not enhanced but $g_s$ was reduced. Thus, it is difficult to explain that the increased $\Delta W$ enhanced water loss from leaves and induced stomatal closure.

Table 1. Stomatal and epidermal cell densities [$mm^{-2}$] and stomatal indices of the two tropical (*E. grandis* and *P. pinnata*) and the two temperate (*V. awabuki* and *D. macropodum*) trees.

| Species | Stomatal density | Cell density | Stomatal index |
|---|---|---|---|
| *E. grandis* | 398 ± 52 | 962 ± 43 | 0.29 ± 0.03 |
| *P. pinnata* | 219 ± 44 | 1280 ± 96 | 0.15 ± 0.03 |
| *V. awabuki* | 169 ± 21 | 722 ± 44 | 0.19 ± 0.02 |
| *D. macropodum* | 277 ± 37 | 794 ± 80 | 0.26 ± 0.03 |

Bunce (1996) reported that low $CO_2$ concentrations eliminated the stomatal response to $\Delta W$, but the effect of $\Delta W$ became apparent at the ambient levels of $CO_2$. He also showed that increased $\Delta W$ enhanced $E$ at low $CO_2$ concentrations, but the response of $E$ was not clear at higher $CO_2$ concentrations. This shows that $C_i$ should influence the stomatal response to $\Delta W$. $C_i$ in the two tropical trees was reduced at high $\Delta W$, but there was no clear difference in the temperate trees. Besides this hypothesis, there is a possibility that the two tropical trees have lower amounts of water available for transpiration in leaves or have higher resistance to water transport from roots to leaves than do the two temperate trees.

We determined the stomatal and epidermal cell frequencies since they may modify the rate of gas exchange (Poole *et al.* 1996). Furthermore, Nátr and Pazourek (1986) documented that stomatal density is a useful indicator for rapid initial classification of plants into groups related to their probable growth potentials. However, we found that stomatal frequencies or stomatal indices did not reflect the $P_N$ or sensitivity to $\Delta W$.

It may be conceptually simple that in humid tropical areas water is not a limiting factor for growth and survivorship of tree seedlings. However, on a clear day in the tropical rainforest of Malaysia, $\Delta W$ often exceeds 30 mmol $mol^{-1}$ (values not shown). Under such conditions, leaves of tropical tree seedlings may experience a high water demand and the efficiency of $CO_2$ fixation may decline. Thus, the ambient humidity surrounding the leaf is an important environmental factor determining the survivorship of tropical tree seedlings in tropical areas. From this aspect, we consider that water management to keep $\Delta W$ low will enhance the

survivorship of seedlings in tropical areas, especially in disturbed open areas where temperature and radiation are high and $\Delta W$ increases.

## References

Aphalo, P.J., Jarvis, P.G.: Do stomata respond to relative humidity? - Plant Cell Environ. **14**: 127-132, 1991.

Berg, D., Maier, K., Otteken, D., Terjung, F.: Picosecond fluorescence decay studies on water-stressed pea leaves: energy transfer and quenching processes in photosystem 2. - Photosynthetica **34**: 97-106, 1997.

Bunce, J.A.: Does transpiration control stomatal responses to water vapour pressure deficit? - Plant Cell Environ. **19**: 131-135, 1996.

Caemmerer, S. von, Farquhar, G.D.: Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. - Planta **153**: 376-387, 1981.

David, C.P., Proctor, J.T.A., Tsujita, M.J.: Whole-plant net $CO_2$ exchange of raspberry as influenced by air and root-zone temperature, $CO_2$ concentration, irradiation, and humidity. - J. amer. Soc. hort. Sci. **121**: 838-845, 1996.

Farquhar, G.D.: Feedforward responses of stomata to humidity. - Aust. J. Plant Physiol. **5**: 787-800, 1978.

Grantz, D.A.: Plant response to atmospheric humidity. - Plant Cell Environ. **13**: 667-679, 1990.

Loreto, F., Sharkey, T.D.: Low humidity can cause uneven photosynthesis in olive (*Olea europea* L.) leaves. - Tree Physiol. **6**: 409-415, 1990.

Marsden, B.J., Lieffers, V.J., Zwiazek, J.J.: The effect of humidity on photosynthesis and water relations of white spruce seedlings during the early establishment phase. - Can. J. Forest Res. **26**: 1015-1021, 1996.

Meidner, H., Mansfield, T.A.: Physiology of Stomata. - McGraw-Hill, London 1968.

Mott, K.A.: Sensing of atmospheric $CO_2$ by plants. - Plant Cell Environ. **13**: 731-737, 1990.

Nátr, L., Pazourek, J.: Genotypic differences and heterogeneity in the stomatal frequency of barley leaves. - Photosynthetica **20**: 363-370, 1986.

Poole, I., Weyers, J.D.B., Lawson, T., Raven, J.A.: Variations in stomatal density and index: implications for palaeoclimatic reconstructions. - Plant Cell Environ. **19**: 705-712, 1996.

Raschke, K., Kuehl, U.: Stomatal response to changes in atmospheric humidity and water supply. Experiments with leaf sections of *Zea mays* in $CO_2$-free air. - Planta **87**: 36-48, 1969.

Schulze, E.-D.: Carbon dioxide and water vapor exchange in response to drought in the atmosphere and in the soil. - Annu. Rev. Plant Physiol. **37**: 247-274, 1986.

Schulze, E.-D., Hall, A.E.: Stomatal responses, water loss and $CO_2$ assimilation rates of plants in contrasting environments. - In: Lange, O.L., Nobel, P.S., Osmond, C.B., Ziegler, H. (ed.): Physiological Plant Ecology II. Pp. 181-230. Springer-Verlag, Berlin - Heidelberg - New York 1982.

Schulze, E.-D., Lange, O.L., Buschbom, U., Kappen, L., Evenari, M.: Stomatal responses to changes in humidity in plants growing in the desert. - Planta **108**: 259-270, 1972.