

# Effects of environment during growth on the sensitivity of leaf conductance to changes in humidity

JAMES A. BUNCE

USDA-ARS, Climate Stress Laboratory, Beltsville Agricultural Research Center, 10300 Baltimore Ave., Beltsville, MD 20705–2350, USA

## Abstract

Soybeans (*Glycine max*) and grain amaranth (*Amaranthus hypochondriacus*) were grown at a range of temperatures, carbon dioxide concentrations and light conditions in controlled environment chambers, and the response of leaf conductance to water vapour to changes in humidity was then measured under a standard set of conditions. The sensitivity of conductance was analysed in terms of (i) the absolute sensitivity of conductance to changes in leaf to air water vapour pressure difference (LAVPD), (ii) the sensitivity of conductance relative to the absolute value of conductance, and (iii) the slope of the relationship between conductance and an index incorporating assimilation rate, carbon dioxide concentration and relative humidity. The sensitivity of conductance varied substantially with growth conditions for all three analyses in both species. The growth temperature of 25 °C increased the sensitivity of conductance by all three measures compared with growth at 20 or 30 °C in amaranth, with little difference between 25 and 30 °C in soybean. Growth at elevated carbon dioxide decreased sensitivity in amaranth by all three measures, and decreased the absolute but not the relative sensitivity in soybean. Growth at reduced photon flux density and growth at high stand density reduced sensitivity in amaranth by all three measures. In soybean, growth at high stand density reduced sensitivity by all three measures, but growth at low photon flux density increased the relative sensitivity. The sensitivity of leaf conductance to changes in humidity varied by a factor of two or more with growth environment by all measures of sensitivity in both the C3 and the C4 species.

*Keywords:* carbon dioxide concentration, humidity, light, stomatal conductance, temperature, vapour pressure difference

*Received 13 February 1997; revised version accepted 3 June 1997*

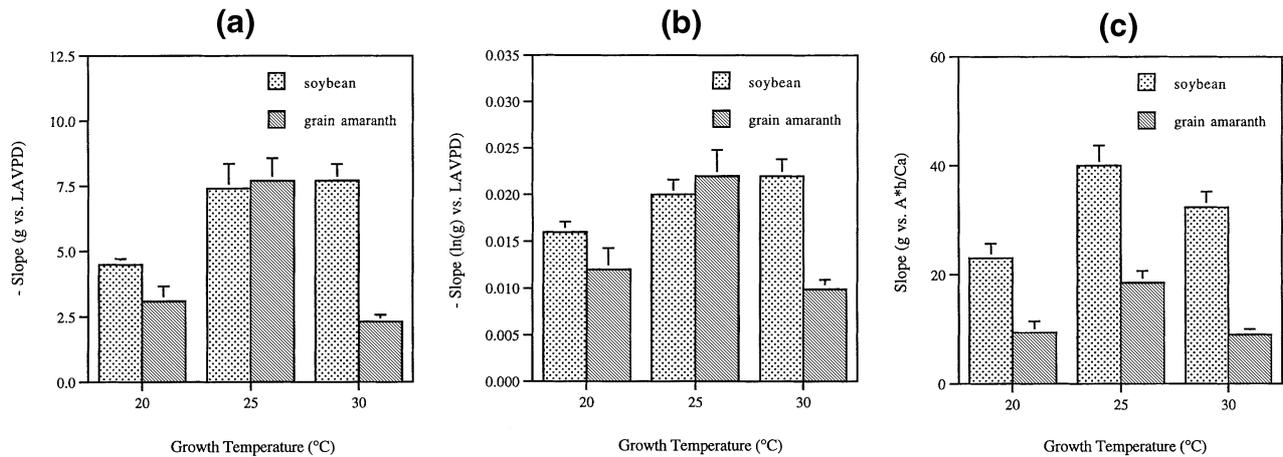
## Introduction

Leaf to air water vapour pressure difference (LAVPD) is one of the most important environmental variables influencing stomatal conductance. Because leaf conductance to water vapour affects the exchange of energy between vegetation and the atmosphere, stomatal responses to changes in LAVPD have an impact on meteorology and climate. In fact, climate models have suggested that the sensitivity of stomatal conductance to change in LAVPD may have a significant impact on temperature and precipitation (Sellers *et al.* 1996). The issue is of considerable current interest because increasing concentrations of carbon dioxide in the atmosphere may reduce stomatal conductance and alter the sensitivity of conductance to LAVPD (Bunce 1993; Hollinger 1987). The

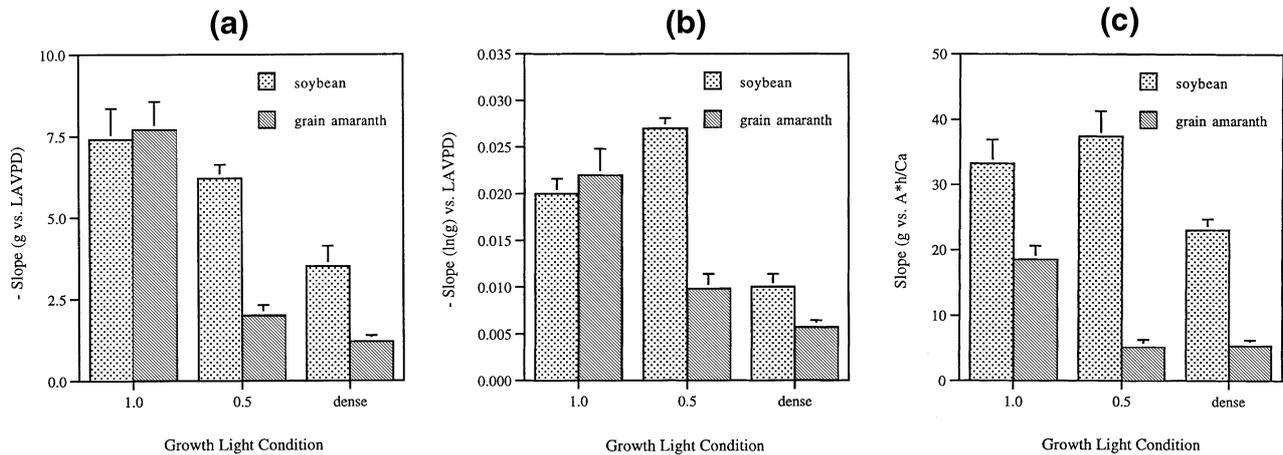
accuracy of climate models may therefore depend on correct predictions of responses of stomatal conductance to LAVPD, and knowledge of how such responses may change with increasing atmospheric carbon dioxide and temperature.

While there is considerable information on how the sensitivity of leaf conductance to change in LAVPD varies with other environmental factors at the time of measurement, there is relatively little information on how sensitivity may vary with environmental conditions during leaf development. The purpose of this work was to examine responses of leaf conductance to changes in LAVPD measured under standard conditions for plants grown at a range of temperatures, carbon dioxide concentrations and light conditions, to determine how much stomatal sensitivity to LAVPD may vary with growth

Correspondence: fax +1/310-504-6626, e-mail jabunce@aol.com



**Fig. 1** Slopes of responses of leaf conductance to changes in humidity for soybean and grain amaranth plants grown at a range of temperatures. (a) conductance vs. leaf to air water vapour pressure difference (LAVPD); (b)  $\ln$  (conductance) vs. LAVPD; (c) conductance vs.  $A^*h/Ca$ .  $A$  is net carbon dioxide exchange rate, and  $h$  is relative humidity. Units for conductance are  $\text{mmol m}^{-2} \text{s}^{-1}$ , for LAVPD are  $\text{mPa Pa}^{-1}$ , for  $A$  are  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , and for  $Ca$  are  $\text{cm}^3 \text{m}^{-3}$ . Error bars represent standard errors of the mean for  $n = 4-7$ .



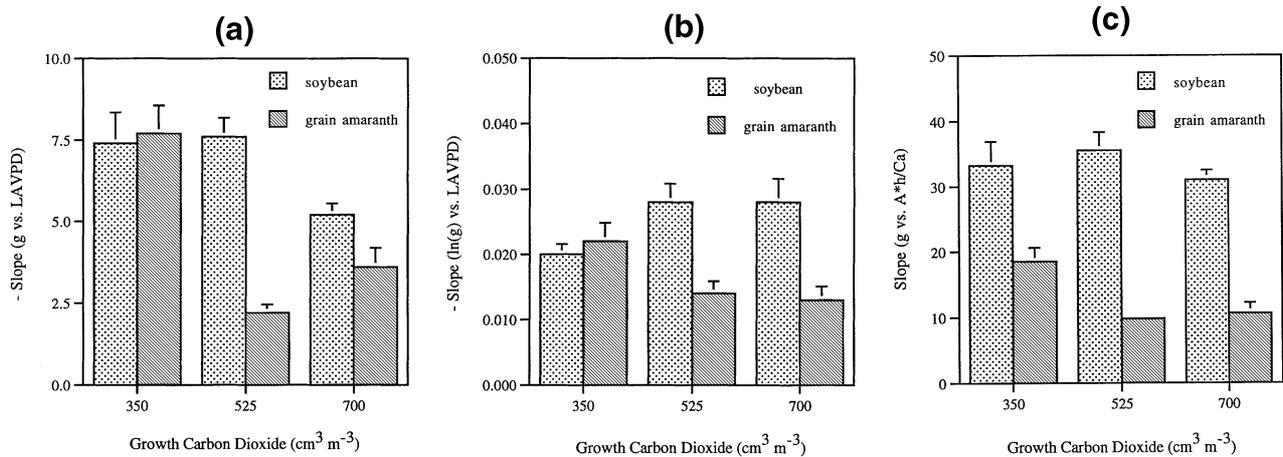
**Fig. 2** Slopes of responses of leaf conductance to changes in humidity for soybean and grain amaranth plants grown in different light environments. '1.0' indicates a PPFD of  $1.0 \text{ mmol m}^{-2} \text{s}^{-1}$ , '0.5' indicates at PPFD of  $0.5 \text{ mmol m}^{-2} \text{s}^{-1}$ , and 'dense' indicates a PPFD of  $1.0 \text{ mmol m}^{-2} \text{s}^{-1}$  and a density of 123 plants  $\text{m}^{-2}$ . (a) conductance vs. leaf to air water vapour pressure difference (LAVPD); (b)  $\ln$  (conductance) vs. LAVPD; (c) conductance vs.  $A^*h/Ca$ .  $A$  is net carbon dioxide exchange rate, and  $h$  is relative humidity. Units for conductance are  $\text{mmol m}^{-2} \text{s}^{-1}$ , for LAVPD are  $\text{mPa Pa}^{-1}$ , for  $A$  are  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , and for  $Ca$  are  $\text{cm}^3 \text{m}^{-3}$ . Error bars represent standard errors of the mean for  $n = 4-7$ .

conditions. We also investigated whether two different relativization schemes could factor out any variation in stomatal sensitivity to LAVPD induced by changes in environment during growth.

## Materials and methods

Soybean, *Glycine max* (L.) Merr. cv. Clark, and grain amaranth, *Amaranthus hypochondriacus* L. were grown in controlled environment chambers. All plants were grown in pots filled with vermiculite and flushed daily with a complete nutrient solution. The lights were on for 14 h per day in all experiments. In the temperature and carbon dioxide treatment comparisons, plants were grown one

per pot in 15 cm diameter pots, and the photosynthetic photon flux density was  $1.0 \text{ mmol m}^{-2} \text{s}^{-1}$ . Comparisons were made among plants grown at 20, 25 and 30 °C at a carbon dioxide concentration of  $350 \pm 30 \text{ cm}^3 \text{m}^{-3}$ , and among plants grown at 350, 525 and  $700 \text{ cm}^3 \text{m}^{-3}$  carbon dioxide concentrations at 25 °C. Plants were also grown at 25 °C,  $350 \text{ cm}^3 \text{m}^{-3}$  carbon dioxide concentration and either a low photon flux of  $0.5 \text{ mmol m}^{-2} \text{s}^{-1}$ , or a high photon flux ( $1.0 \text{ mmol m}^{-2} \text{s}^{-1}$ ). At the higher photon flux plants were also grown at a stand density of 123 plants  $\text{m}^{-2}$  by growing 9 plants per 30 cm diameter pot. For all growth conditions, the dew point temperature was  $18 \pm 2$  °C, thus air saturation deficit for water vapour increased with growth temperature. Gas exchange was



**Fig. 3** Slopes of responses of leaf conductance to changes in humidity for soybean and grain amaranth plants grown at a range of carbon dioxide concentrations. (a) conductance vs. leaf to air water vapour pressure difference (LAVPD); (b)  $\ln$  (conductance) vs. LAVPD; (c) conductance vs.  $A^*h/Ca$ .  $A$  is net carbon dioxide exchange rate, and  $h$  is relative humidity. Units for conductance are  $\text{mmol m}^{-2} \text{s}^{-1}$ , for LAVPD are  $\text{mPa Pa}^{-1}$ , for  $A$  are  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , and  $Ca$  are  $\text{cm}^3 \text{m}^{-3}$ . Error bars represent standard errors of the mean for  $n = 4-7$ .

**Table 1**  $r^2$  values of regression equations relating leaf conductance to water vapour pressure deficit for soybean and grain amaranth plants grown in different environments. Regressions related leaf conductance ( $g$ ) or the natural log of conductance to the vapour pressure deficit ( $vpd$ ) or to the product of assimilation rate ( $A$ ) and relative humidity ( $h$ ) divided by the external carbon dioxide concentration ( $Ca$ ). Temperature ( $T$ ) is in  $^{\circ}\text{C}$ , carbon dioxide concentration ( $\text{CO}_2$ ) is in  $\text{cm}^3 \text{m}^{-3}$ , and photosynthetic photon flux density (PPFD) is in  $\text{mmol m}^{-2} \text{s}^{-1}$ . 'Dense' indicates plants grown at  $1.0 \text{ mmol m}^{-2} \text{s}^{-1}$  PPFD at a density of  $123 \text{ plants m}^{-2}$ . All regressions were significant at  $P = 0.05$

Environment			$r^2$ of regression					
			$g$ vs. $vpd$		$\ln(g)$ vs. $vpd$		$g$ vs. $A^*h/Ca$	
$T$	$\text{CO}_2$	PPFD	soybean	amaranth	soybean	amaranth	soybean	amaranth
25	350	1.0	0.30	0.73	0.30	0.73	0.55	0.80
25	525	1.0	0.65	0.40	0.66	0.40	0.63	0.54
25	700	1.0	0.55	0.68	0.70	0.69	0.70	0.80
30	350	1.0	0.54	0.32	0.52	0.33	0.69	0.47
20	350	1.0	0.48	0.81	0.48	0.82	0.66	0.83
25	350	0.5	0.61	0.47	0.58	0.19	0.76	0.26
25	350	dense	0.27	0.17	0.27	0.16	0.31	0.27

measured on the fourth mainstem leaf from the bottom in grain amaranth and on the fourth mainstem trifoliolate leaf from the bottom in soybean. For the high stand density treatment, gas exchange measurements were made on interior plants. Measurements were made within a few days after these leaves had reached full area expansion. These leaves had the highest conductances of any leaves on the plants at that time.

During leaf gas exchange measurements, plants were in a controlled environment chamber at the growth conditions. Leaf gas exchange was measured using an open gas exchange system enclosing whole leaves or leaflets, with control of light, temperature, carbon dioxide concentration and humidity (Bunce 1993). All measurements were made at a leaf temperature of  $30 \pm 0.5$   $^{\circ}\text{C}$ , a photosynthetic photon flux density of  $1.8 \text{ mmol m}^{-2} \text{s}^{-1}$ ,

an external carbon dioxide concentration of  $350 \pm 10 \text{ cm}^3 \text{m}^{-3}$ , and an air speed of about  $2 \text{ m s}^{-1}$ . Leaf conductance to water vapour, including stomatal, cuticular and boundary layer conductances was calculated assuming water vapour saturation of internal air at the measured leaf temperature. LAVPD was referenced to air outside the boundary layer. Leaves were initially equilibrated at a LAVPD of  $15-20 \text{ mPa Pa}^{-1}$ . The LAVPD was then increased in 3-5 gradual steps to a maximum of  $32-36 \text{ mPa Pa}^{-1}$ . Following each change in LAVPD, rates of water vapour and carbon dioxide exchange were recorded after rates were stable (within system error) for about 10 min. At least four leaves were measured for each growth treatment.

Sensitivity of leaf conductance to change in humidity was compared among growth conditions using three

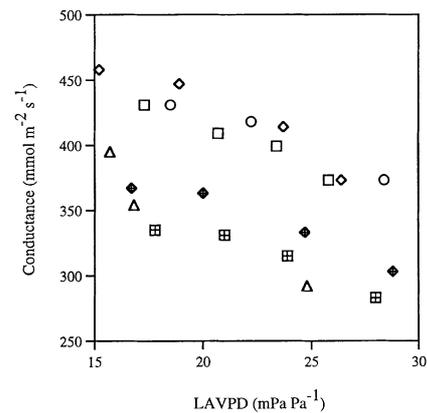
**Table 2** Mean values of leaf conductance to water vapour for soybean and grain amaranth plants grown in different environments at a leaf to air water vapour pressure difference (LAVPD) of 20 mPa Pa<sup>-1</sup>. Values were obtained from the regressions of conductance on LAVPD which included all measurements for a given environmental treatment. 'Dense' indicates plants grown at 1.0 mmol m<sup>-2</sup> s<sup>-1</sup> PPFD at a density of 123 plants m<sup>-2</sup>

Environment			Conductance (mmol m <sup>-2</sup> s <sup>-1</sup> )	
T (°C)	CO <sub>2</sub> (cm <sup>3</sup> m <sup>-3</sup> )	PPFD (mmol m <sup>-2</sup> s <sup>-1</sup> )	Soybean	Amaranth
25	350	1.0	380	340
25	525	1.0	300	240
25	700	1.0	310	280
30	350	1.0	400	240
20	350	1.0	320	270
25	350	0.5	280	220
25	350	dense	310	230

methods. Linear regressions of the absolute value of conductance on LAVPD were determined separately for each leaf. Mean slopes for the different growth conditions were then compared using analysis of variance. Regressions of the natural log of conductance on LAVPD were treated similarly, and were used to compare the sensitivity of conductance relative to the absolute value of conductance. The slope of the natural log of conductance directly indicates relative sensitivity since (cf. Comstock & Ehleringer 1993):

$$\frac{d(g)/g}{d(\text{LAVPD})} = \frac{d(\ln(g))}{d(\text{LAVPD})}$$

Although the first of these methods assumes a linear response of conductance to LAVPD, and the second a curvilinear response, the correlation coefficients were not consistently higher for one type of regression than for the other (see later). The third method was to determine separately for each leaf regressions of conductance on the product of assimilation rate, relative humidity and the reciprocal of the external carbon dioxide concentration (i.e. the Ball-Berry model; Collatz *et al.* 1991). While Leuning (1995) proposed a modification of the Ball-Berry formulation, the modified formulation gave lower  $r^2$  values with the present data, so was not used. Mean slopes for the different growth conditions were then compared using analysis of variance. The mean of the slopes of the individual leaves is not exactly equivalent to the slope of the regression of the combined data for all leaves of a given treatment, but the patterns of the response of stomatal sensitivity to the growth environment obtained from these two methods of summarizing the data were very similar.



**Fig. 4** Leaf conductance to water vapour at 3–4-values of leaf to air water vapour pressure difference (LAVPD) for each of six soybean leaves from different plants. Plants were grown at 25 °C, 350 cm<sup>3</sup> m<sup>-3</sup> carbon dioxide concentration and at photosynthetic photon flux density of 1.0 mmol m<sup>-2</sup> s<sup>-1</sup>. Each type of symbol represents a different leaf.

## Results

For all individual leaves of both species and for all growth conditions, increasing LAVPD decreased leaf conductance. Variation in the slope of the response among individual leaves within a treatment was fairly small for all three types of regressions, as indicated by standard errors usually less than 20% of the mean (Figs 1, 2 and 3). Regressions developed using all data for each treatment had  $r^2$  values ranging from 0.17 to 0.83 (Table 1). The regressions using leaf conductance or the natural log of conductance had very similar  $r^2$  values (Table 1). The low  $r^2$  values for the combined data were usually attributable to variation among leaves in the maximum conductance, rather than variation in the slope of the response. This is illustrated by the data for soybeans grown at 25 °C, 350 cm<sup>3</sup> m<sup>-3</sup> carbon dioxide concentration and high PPFD, which had one of the lowest overall  $r^2$  values (Fig. 4). The regressions incorporating photosynthetic rates usually had somewhat higher  $r^2$  values, indicating that normalizing for differences in photosynthetic capacity removed some variation among leaves (Table 1). Leaf conductances at a standard LAVPD of 20 mPa Pa<sup>-1</sup> (calculated from the linear regressions) varied by a factor of about 1.5 among growth environments in both species, with lower values for the elevated carbon dioxide and lower light environments (Table 2).

Growth conditions affected the sensitivity of leaf conductance to changes in humidity by all three measures of sensitivity in both species, as indicated by significant treatment effects at  $P = 0.05$ , using analysis of variance. It is not clear that any of the three methods of assessing the sensitivity of conductance greatly reduced the amount of variation with growth condition (Figs 1, 2 and 3). The

growth temperature of 25 °C resulted in greater sensitivity of conductance than either 20 or 30 °C in amaranth by all three measures of sensitivity (Fig. 1). The response of soybean was similar except that the difference between the 25 and 30 °C growth conditions was small (Fig. 1). Growth at reduced photon flux density and growth at high stand density both reduced sensitivity in amaranth by all three measures (Fig. 2). In soybean, growth at high stand density reduced sensitivity by all three measures, but growth at low photon flux density increased the relative sensitivity (Fig. 2). Growth at elevated carbon dioxide decreased sensitivity in amaranth by all three measures, with the largest change between 350 and 525 cm<sup>3</sup> m<sup>-3</sup> (Fig. 3). Growth at elevated carbon dioxide decreased the absolute sensitivity in soybean, but increased the relative sensitivity (Fig. 3).

## Discussion

In a study of variation in stomatal sensitivity to LAVPD among cultivars of bean, Comstock & Ehleringer (1993) found that the substantial variation in absolute sensitivity was correlated with maximum conductance, such that the relative sensitivity was quite uniform across cultivars. A similar result was obtained by Morison & Gifford (1983) in analysing the effects of short-term changes in carbon dioxide concentration on stomatal sensitivity to LAVPD. The same relativization procedure used by Comstock & Ehleringer (1993), i.e. use of the slope of the natural log of conductance vs. LAVPD to indicate sensitivity of conductance to LAVPD relative to the absolute value of conductance, did not substantially reduce the environmentally induced variation in sensitivity in the present study. With few exceptions, the same patterns of response to growth environment were apparent in both the absolute and relative sensitivities.

The Ball–Berry model (Collatz *et al.* 1991) is appealing in that it links stomatal response with assimilation rate and carbon dioxide concentration and uses relative humidity rather than LAVPD, potentially removing variation caused by differences in photosynthetic capacity, and photon flux density and temperature during the measurement (cf. Leuning 1995). However, it was not notably successful in removing variation caused by the different growth conditions in this study. Others have also found that the Ball–Berry model slope was not constant for a given species. For example, the slope of the fit to the Ball–Berry model varied by a factor of 1.4 from November to February in a eucalypt species (Leuning 1990), and by more than a factor of 4 in a California grassland over the course of a year (Valentini *et al.* 1995). Our data indicate that variation in growth temperature alone caused about a two-fold variation in the Ball–Berry slope.

The plants in these experiments were grown in controlled environment chambers, and thus the data should not be directly extrapolated to plants grown in the field. However, Wilson & Bunce (1997) found that the sensitivity of stomatal conductance to LAVPD was substantially greater in soybean plants grown in the field in Beltsville, MD, than in plants grown in controlled environment chambers. This, and the literature indicating seasonal variation in stomatal sensitivity to LAVPD in the field (Leuning 1990; Valentini *et al.* 1995) suggests that the variation in sensitivity with growth environment is likely to occur under more natural conditions.

There has been little systematic investigation of how sensitivity of stomatal conductance to LAVPD varies with growth conditions. Similar to the results presented here for soybean, high light and cool temperatures during growth reduced the relative sensitivity of conductance to LAVPD in several C3 species (Bunce 1981). These are also conditions which increased photosynthetic capacity. Hollinger (1987), and Bunce (1993) found lower relative sensitivity of conductance to LAVPD in several species grown and measured at elevated CO<sub>2</sub>. However, Chen *et al.* (1994) found the Ball–Berry slope to be higher in plants grown at elevated CO<sub>2</sub> in big bluestem. Wilson & Bunce (1996) found that the effect of growth at elevated CO<sub>2</sub> on the relative stomatal sensitivity to LAVPD depended on the assay temperature in soybean.

While much more work would be required to clearly define general patterns of effects of growth conditions on the sensitivity of leaf conductance to changes in LAVPD, the present data at least indicate that substantial variation in sensitivity occurs under controlled conditions. Those incorporating stomatal responses to LAVPD into predictions of leaf conductance should be aware that large errors could result from assuming a constant sensitivity for a given species. Neither of the relativization schemes examined eliminated changes in sensitivity with growth environment. Sensitivity of conductance to LAVPD varied by a factor of at least two with growth conditions in these experiments, for all three indices of sensitivity.

## References

- Bunce JA (1981) Comparative responses of leaf conductance to humidity in single attached leaves. *Journal of Experimental Botany*, **32**, 629–634.
- Bunce JA (1993) Effects of doubled atmospheric carbon dioxide concentration on the responses of assimilation and conductance to humidity. *Plant, Cell and Environment*, **16**, 189–197.
- Chen D-X, Coughenour MB, Knapp AK, Owensby CE (1994) Mathematical simulation of C<sub>4</sub> grass photosynthesis in ambient and elevated CO<sub>2</sub>. *Ecological Modelling*, **73**, 63–80.

- Collatz GJ, Grivet C, Ball JT, Berry JA (1991) Physiological and environmental regulation of stomatal conductance, photosynthesis and transpiration: a model that includes a laminar boundary layer. *Agricultural and Forest Meteorology*, **54**, 107–136.
- Comstock J, Ehleringer JE (1993) Stomatal response to humidity in common bean (*Phaseolus vulgaris*): implications for maximum transpiration rate, water-use efficiency and productivity. *Australian Journal of Plant Physiology*, **20**, 669–691.
- Hollinger DT (1987) Gas exchange and dry matter allocation responses to elevation of atmospheric CO<sub>2</sub> concentration in seedlings of three species. *Tree Physiology*, **3**, 193–202.
- Leuning R (1990) Modelling stomatal behavior and photosynthesis of *Eucalyptus grandis*. *Australian Journal of Plant Physiology*, **17**, 159–175.
- Leuning R (1995) A critical appraisal of a combined stomatal-photosynthesis model for C<sub>3</sub> plants. *Plant, Cell and Environment*, **18**, 339–355.
- Morison JIL, Gifford RM (1983) Stomatal sensitivity to carbon dioxide and humidity. A comparison of two C<sub>3</sub> and two C<sub>4</sub> grass species. *Plant Physiology*, **71**, 789–796.
- Sellers PJ, Bounoua L, Collatz GJ, Randall DA, Dazlich DA, Los SO, Berry JA, Fung I, Tucker CJ, Field CB, Jensen TG (1996) Comparison of radiative and physiological effects of doubled atmospheric CO<sub>2</sub> on climate. *Science*, **271**, 1402–1406.
- Valentini R, Gamon JA, Field CB (1995) Ecosystem gas exchange in a California grassland: seasonal patterns and implication for scaling. *Ecology*, **76**, 1940–1952.
- Wilson KB, Bunce JA (1997) Effects of carbon dioxide concentration on the interactive effects of temperature and water vapor on stomatal conductance in soybeans. *Plant, Cell and Environment*, **20**, 230–238.