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# Stomatal Response to Humidity in Common Bean (*Phaseolus vulgaris*): Implications for Maximum Transpiration Rate, Water-use Efficiency and Productivity

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*Abstract*

Twelve common bean (*Phaseolus vulgaris* L.) cultivars were grown under greenhouse conditions to study the response of net photosynthesis ($A$) and transpiration ($E$) to variation in the leaf-to-air humidity gradient ($\nu$). Large differences were observed between cultivars in maximum rates of $A$ and $E$. The variation in $A$ correlated with both leaf nitrogen content and specific leaf area. Thin leaves had higher nitrogen contents per unit dry weight, but thick leaves had higher nitrogen content per unit surface area. Photosynthetic nitrogen-use efficiency did not correlate with nitrogen content on either a mass or a surface area basis. Very little variation was found between cultivars in the sensitivity of total leaf conductance ($g$) to increasing $\nu$, when sensitivity was defined as the slope of $\ln(g)$ versus $\nu$ ($\partial \ln(g)/\partial \nu$). No significant correlation existed between $\partial \ln(g)/\partial \nu$ and variation in maximum conductance values. Much steeper slopes (greater sensitivity) were found in the response of stomatal conductance alone ($g_s$) to the leaf–leaf surface humidity gradient ($\nu_s$). The sensitivity of stomatal response correlated positively with variation in maximum conductance among cultivars, and, since stomatal conductance was in series with a fixed boundary layer conductance, this positive correlation made possible the uniform sensitivity of $g_{(\text{total})}$ with respect to $\nu_{(\text{leaf-air})}$ despite the wide variation in $g_{\max}$. All cultivars reached their maximum $E$ at very similar $\nu$ values, and all showed a relatively constant $E$ over a wide range of high $\nu$. The implications of this relative homeostasis in $E$ are discussed in the context of possible hydraulic limitations on $E$. Considerable recent interest has focussed on the use of carbon isotope discrimination ($\Delta$) as a useful screening character in crop breeding programmes, and $\Delta$ has been found to correlate positively with yield in *P. vulgaris*. We found that $\Delta$, measured on bulk leaf tissue, positively correlated with both $A_{\max}$ and $g_{\max}$ between bean cultivars, but did not correlate with instantaneous measures of intercellular $CO_2$ ($c_i$) when $\nu$ was held constant across all measurements. This apparent discrepancy may be due, at least in part, to variation in leaf temperatures among cultivars under normal growing conditions. Leaf-energy-budget simulations indicated that the observed range of maximum leaf conductance at low $\nu$ would generate up to 3·0°C variation in leaf temperatures under field conditions of low to moderate windspeed. Given the strong stomatal response to $\nu$, this variation in leaf temperature could cause variation in carbon isotope discrimination, which reflects long-term $c_i$ values. Such a mechanism of producing variation in $c_i$ would not be apparent in $c_i$ measurements taken under cuvette conditions where leaf temperature was held constant.

## Introduction

The nature of the direct response of stomata to humidity is a topic of considerable current debate (Grantz 1990) and much functional significance (Cowan and Farquhar 1977; Farquhar 1978). Since transpiration ($E$) is equal to the product of leaf conductance ($g$) and $\nu$, the water vapour pressure gradient between leaf and atmosphere divided by total atmospheric pressure, it is often hard to distinguish with certainty between stomatal closure due to large $\nu$ (direct humidity response) and stomatal closure due to high $E$ (a feedback response). Furthermore, there is debate concerning what measure of humidity is most likely

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to be sensed by plants (Aphalo and Jarvis 1991), and at what point in the diffusion pathway such measures are likely to be made (Bunce 1985). Despite these mechanistic uncertainties, variation in the response to humidity clearly has potentially important functional ramifications for plant productivity and water-use efficiency (Cowan and Farquhar 1977). It also plays a key role in determining maximum $E$, and therefore is important in balancing water loss rates from the canopy with the hydraulic conductance of the liquid-phase transport between root and shoot (Tyree and Sperry 1989; Jones and Sutherland 1991).

Common bean (*Phaseolus vulgaris* L.) cultivars are known to differ in water-use efficiency, and in the mean ratio of stomatal conductance to photosynthetic capacity during growth (Ehleringer 1990; Ehleringer *et al* 1991). These differences could potentially be explained by differential sensitivities of leaf conductance to humidity. We attempted to describe the nature of the response of stomatal conductance to changes in humidity, how those responses differed between cultivars, and how they affected intercellular $CO_2$ concentrations and photosynthetic performance. The stomatal response was analysed to determine whether (1) stomata closed uniformly across the leaf surface, and the degree to which lack of homogeneity affected photosynthesis, (2) different stomatal sensitivities to humidity existed among the bean cultivars, and (3) stomatal closure was able to set a stable maximum transpiration rate in the face of highly variable humidity gradients and wind speeds. Since $\nu$ is determined in part by leaf temperature and since leaf temperature is determined in part by leaf conductance and transpiration rate, maximum stomatal conductances will interact with stomatal sensitivity to humidity in determining diurnal patterns of stomatal opening. These interactions were explored in computer simulations after quantitative descriptions of the stomatal responses to humidity had been acquired.

**List of Variables Used in the Text**

| *Variable* | *Definition* | *Units* |
|---|---|---|
| $A$ | net photosynthesis | $\mu$mol $m^{-2}$ $s^{-1}$<br>nmol $g^{-1}$ $s^{-1}$<br>nmol $(mgN)^{-1}$ $s^{-1}$ |
| $E$ | transpiration | mmol $m^{-2}$ $s^{-1}$ |
| $g$ | leaf conductance to gas-phase diffusion | mol $m^{-2}$ $s^{-1}$<br>nmol $g^{-1}$ $s^{-1}$ |
| $g$ subscripts: | 'none' total conductance to water vapour<br>c total conductance to $CO_2$<br>s stomatal<br>b boundary layer<br>l upper leaf surface<br>u lower leaf surface | |
| $A_{max}$; $g_{max}$ | indicates measurement conditions of light saturation, $\nu = 15$ mbar $bar^{-1}$, leaf temperature 30°C, ambient $CO_2$ 350 $\mu$L $L^{-1}$ | |
| $E_{max}$ | same as above, except $\nu$ varies from 15–35. This was sometimes a true maximum, sometimes merely the values of $E$ at $\nu = 35$ mbar $bar^{-1}$ | |
| $c$ | $CO_2$ concentration<br>subscripts i and a = leaf intercellular airspaces and ambient respectively | $\mu$L $L^{-1}$ |
| $\nu$ | leaf to bulk air vapour diffusion gradient | mbar $bar^{-1}$ |
| $\nu_s$ | leaf to leaf surface vapour diffusion gradient | mbar $bar^{-1}$ |
| $\Delta$ | carbon isotope discrimination between plant and atmosphere | ‰ |

| *Variable* | *Definition* | *Units* |
|---|---|---|
| $\delta$ | difference in carbon isotope ratio between sample and PDB standard | ‰ |
| $\Psi$ | leaf water potentital | MPa |

Two notations are used in regards to linear responses of diffusional conductance to changes in the vapour pressure gradient. Throughout the Results section, partial derivative notation is used to emphasise the dependency of the reported linearity on controlled cuvette conditions. In the Discussion and Appendix, simpler notation is substituted to enhance legibility of algebraic expressions. The correspondence of these two notations is as follows:

| | | | | |
|---|---|---|---|---|
| slopes: | $\frac{\partial g}{\partial v} = m$ | $\frac{\partial g_s}{\partial v_s} = m_s$ | $\frac{\partial \ln(g)}{\partial v} = m^*$ | $\frac{\partial \ln(g_s)}{\partial v_s} = m_s^*$ |
| $y$-intercepts: | $B$ | $B_s$ | $B^*$ | $B_s^*$ |

## Materials and Methods

Individuals representing 12 common bean (*Phaseolus vulgaris*) cultivars were grown during fall and winter in the greenhouse at the University of Utah. These cultivars were A54, San Christobal G5201, Victor, G4523, San Juan Select, Viva, Othello, CZ 18183, CO33142, CO22625, and UNS 117. Plants received supplementary lighting (a mixed bank of alternating Na-vapour and Metal Halide high intensity discharge lamps) so that maximum irradiance was 1·6 mmol $m^{-1}$ $s^{-1}$ at noon, and total irradiance was 38 mol $m^{-2}$ $day^{-1}$. Day/night temperatures were 30/20°C, and the leaf-to-air vapour pressure gradient divided by total atmospheric pressure gradient ($v$) was 30 and 38 mbar $bar^{-1}$ for the daytime mean and midday maximum, respectively. Several rotating fans, strong enough to cause leaf fluttering, stirred the air around the plants at all times during growth.

PVC pots (1 m tall and 0·15 m diameter) provided a total soil volume of 15 L. The soil mix was perlite : clay-loam : vermiculite : bark compost : silica sand : peat, in the ratios of 6:2:2:2:1:1. The set of 12 cultivars were all grown and measured on two separate occasions. The main purpose of the replication was to verify the repeatability of the results, but extra steps were taken with the second set of seedlings to ensure that no nutritional deficiencies were present. Plants were watered once every three days during both experiments, but received slightly different fertilisation regimes. During the first measurement cycle (treatment 1) plants received a commercial fertiliser, Peters 20:20:20 (N:P:K), dissolved in the water-supply at a total concentration of 150 ppm/nutrient. During the second set of plantings (treatment 2), plants were watered with de-ionised water every third day, and immediately following received 0·5 strength Hoagland's solution. This second treatment thus received a similar dosage of N:P:K as the first treatment, but was further supplemented with all micronutrients. Gas exchange measurements within each treatment required 6–8 weeks to complete, and cultivar plantings were staggered so that all plants were 6 weeks old at the time of measurement.

Gas-exchange measurements were conducted using an open steady-state system described in Ehleringer (1983). The system had been modified to include Tylan flow controllers for mixing gases and the equilibrium dewpoint temperature of the air was measured using dew-point mirrors (model 911, Dew All). Measurements were performed at the University of Utah at an elevation 1400 m, total atmospheric pressure of 860 mbar. Leaf boundary-layer conductance in the cuvette was measured with wet filter paper backed with Teflon on one side, and was 1·15 and 1·35 mol $m^{-2}$ $s^{-1}$ on the upper and lower surfaces, respectively. 'Upper' and 'lower' refer to orientation within the cuvette, and differences in boundary layer conductance were related to air circulation patterns with the cuvette fan located on the chamber floor beneath the leaf. During measurements, leaves were always oriented with the adaxial surface facing up in the cuvette. Calculation of net photosynthesis ($A$), transpiration ($E$), and intercellular $CO_2$ ($c_i$) followed von Caemmerer and Farquhar (1981), but calculation of leaf conductance was modified to include a two-sided leaf model with upper and lower conductances in parallel. The calculation of stomatal conductance ($g_{stomatal}$) from total and boundary layer conductances followed the derivation of Leuning and Sands (1989), but was modified to include separate upper and lower boundary layer conductances. The ratio of upper to lower stomatal conductance was assumed to be 0·25 at all times, which was consistent with both upper and lower surface measurements made with

a single-surface porometer (Ehleringer 1990). Sensitivity analysis calculations indicated that moderate departures from this assumption (upper and lower conductance ratios of 0·1–0·5) would have caused only slight errors, and that this approach was more accurate than assuming fully hypostomatous or amphistomatous distributions (stomatal ratios of 0 and 1·0, respectively). Total conductance to $CO_2$ ($g_c$) was calculated from measured conductance to water vapour as:

$$g_c = \frac{g_{s.u}\, g_{b.u}}{1{\cdot}6g_{b.u} + 1{\cdot}37g_{s.u}} + \frac{g_{s.l}\, g_{b.l}}{1{\cdot}6g_{b.l} + 1{\cdot}37g_{s.l}}, \quad (1)$$

where subscripts s and b refer to stomatal and boundary layer conductances respectively, u and l refer to the upper and lower leaf surfaces, and 1·6 and 1·37 are the ratios of diffusivities of $H_2O$ to $CO_2$ in the stomatal pores and leaf boundary layer, respectively.

Gas exchange data were collected on the fourth or fifth leaf of the main stem just after full leaf expansion. One leaf was measured on each of three plants for each cultivar within each treatment. A leaf was placed in the gas-exchange cuvette and allowed to acclimate for 1 h prior to measurement. During all data collection, leaf temperature was maintained near the photosynthetic temperature optimum at 30°C, and irradiance was saturating for photosynthesis at 1·8 mmol $m^{-2}$ $s^{-1}$. Initially, at a $\nu$ of 10 mbar $bar^{-1}$, ambient $CO_2$ was varied to generate an abbreviated $A$–$c_i$ response over the physiologically relevant range of 200–280 $\mu$L $L^{-1}$. After this, $c_a$ was maintained at a constant 350±1 $\mu$L $L^{-1}$ while $\nu$ was increased in units of approximately 5 mbar $bar^{-1}$ up to a maximum of 35–40 mbar $bar^{-1}$. At least 30 min equilibration time was allowed between collection of each point following changes in $\nu$. All reported values of $A$, $E$, $g$, and $c_i$ represent fully stable readings under steady-state conditions of at least 10 min. Responses tended to be slower at high $\nu$ values, and up to 60 min were sometimes needed to achieve fully stable values.

Simulated values of $A$ and $c_i$, which appear at several points in the discussion, are based on observed $g$ and a previously determined $A$–$c_i$ relationship. These simulated values were obtained by simultaneously solving equations describing the diffusional supply of $CO_2$ to the leaf mesophyll and the functional dependence of $A$ on $c_i$ during carboxylation (Farquhar and Sharkey 1982). Predicted values were:

$$A = \frac{c_a\left(g_c - \frac{E}{2}\right) + y\left(\frac{g_c}{s} + \frac{E}{2s}\right)}{\left(1 + \frac{g_c}{s} + \frac{E}{2s}\right)} \quad \text{and} \quad c_i = \frac{A - y}{s}, \quad (2, 3)$$

where $g_c$ is total leaf conductance to $CO_2$, and $s$ and $y$ are the slope and $y$-intercept, respectively, of linear approximations of the $A$ versus $c_i$ response curves previously measured empirically over the immediate region of interest.

In referring to the rate at which $g$ declines in response to increasing $\nu$, the phrase 'stomatal sensitivity' is often employed. Our usage of this term differs in an important way from the most common one in that we use it to refer to the proportional rate of change ($\partial \ln(g)/\partial \nu$), not the absolute rate of change ($\partial g/\partial \nu$). We consider this to be a more useful definition, because the effect of stomatal closure on parameters such as $c_i$ and $A/E$ depends not on the absolute value of $g$, but on the ratio of $A/g$. Thus, leaves with very different photosynthetic capacities are necessarily expected to differ in $\partial g/\partial \nu$, simply because $g_{max}$ must scale with $A_{max}$, but $\partial \ln(g)/\partial \nu$ describes the effect of stomatal closure in a manner independent of maximum values.

Total leaf nitrogen was measured on the same leaves used for the gas-exchange measurements using a CHN elemental analyser (model 2400, Perkin Elmer).

Carbon isotope discrimination ($\Delta$) was determined separately on leaf tissues. Leaves were dried and ground to pass through a 40 mesh screen. A 2–3 mg subsample of the tissue was flash combusted, the $CO_2$ produced was purified cyrogenically, and the $\delta^{13}C$ value of the $CO_2$ was measured on the isotope ratio mass spectrometer (delta S, Finnigan MAT). The instrument error, defined as twice the standard deviation, associated with each observation was ±0·01‰, and the error between repeated analyses of the same ground tissue was ±0·11‰ (1 SD). The $\Delta$ values were calculated from carbon isotope ratios (against the PDB standard) using a value for $\delta_{air}$ of −8‰ (Farquhar *et al.* 1989).

Carbon isotope discrimination ($\Delta$) in $C_3$ plants is related to photosynthetic gas exchange; because $\Delta$ is in part determined by $c_i/c_a$, the ratio of $CO_2$ concentrations in the leaf intercellular spaces to that in the atmosphere (Farquhar *et al.* 1989). This ratio, $c_i/c_a$, differs between plants because of

variation in stomatal opening, affecting the supply rate of $CO_2$, and because of variation in the chloroplast demand for $CO_2$. Of the models linking $C_3$ photosynthesis and $^{13}C/^{12}C$ composition, the one developed by Farquhar *et al.* (1982) has been the most extensively tested. In its simplest form, their expression for discrimination in leaves of $C_3$ plants is

$$\Delta = a + (b-a)\frac{c_i}{c_a}, \tag{4}$$

where $a$ is the fractionation occurring due to diffusion of air (4·4‰), and $b$ is the net fractionation caused by carboxylation (mainly discrimination by RuBP carboxylase, approximately 27‰). Since transpiration ($E$) and net carbon assimilation ($A$) share a common diffusion pathway, the stomata, instantaneous water-use efficiency can be determined without an estimate of $g$,

$$\frac{A}{E} = \frac{c_a\left(1-\frac{c_i}{c_a}\right)}{1{\cdot}6v}, \tag{5}$$

and the relationship between $\Delta$ and $A/E$ is apparent; both are a function of $c_i/c_a$.

Carbon isotope ratio showed a strong correlation between greenhouse and field grown material for these particular cultivars (Fig. 1). This suggested that the relative limitations imposed on photosynthesis by diffusional (stomatal) and biochemical events were similar in the two diverse environments. This pattern was consistent with previous datasets showing consistency of cultivar rankings in $\Delta$ (or $\delta^{13}C$) between years, sites, and developmental stages (Ehleringer *et al.* 1990, 1991; White *et al.* 1990).

In evaluating genetic variation in gas-exchange parameters, the $\Delta$ values used were those from field-grown observations because the air isotopic composition was more constant, and variation in plant carbon isotopic composition under these conditions should more accurately reflect biological behaviour.

Leaf water potentials were measured with a Scholander pressure chamber. Plants were well watered in both sets of experiments, and no differences were detected in water potential between cultivars. Mean leaf water potentials averaged −0·28 and −0·85 MPa for predawn and midday values, respectively.

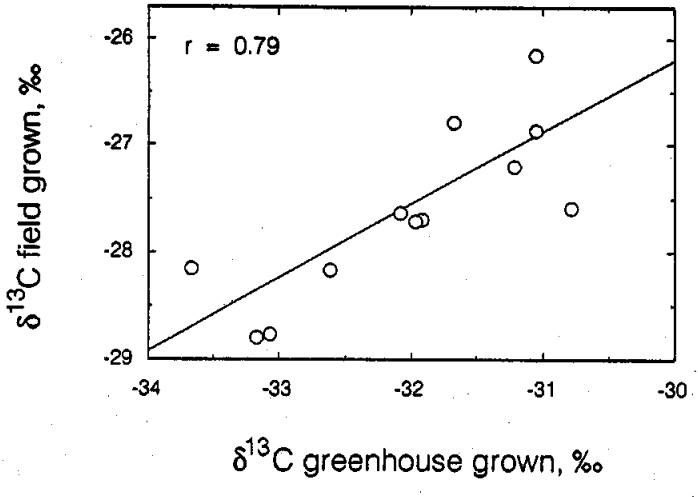


**Fig. 1.** A comparison of $\delta^{13}C$ from the greenhouse grown gas-exchange leaves and leaves of the same cultivars grown at Cortez, Colorado, under field conditions. Greenhouse values represent three bulked leaves of each cultivar, and Cortez values represent 10 bulked leaves. The line is the least squares best fit: $\delta^{13}C$, field grown = $-5{\cdot}87 + 0{\cdot}687\ \delta^{13}C$, greenhouse ($r = 0{\cdot}79$; $P = 0{\cdot}002$).

Field observations of maximum leaf conductance were collected on 21 non-overlapping common bean cultivars growing at Cortez, Colorado. The $g_{max}$ estimates were made by repeatedly sampling leaf conductances during high light periods between 0900 and 1200 hours over a 4-day period (21–25 July 1989). Leaf conductances were measured with a Li-cor 1600 null balance porometer so that independent estimates were obtained of the stomatal conductance on the adaxial and abaxial surfaces.

## Results

Substantial differences were observed in maximum leaf gas-exchange rates between cultivars (Table 1), with a greater than 1·5 fold variation between mean cultivar values for both $A$ at a $c_i$ value of 250 $\mu$L L$^{-1}$ and $g$ at a $\nu$ of 15 mbar bar$^{-1}$, respectively. Both $A$ and $g$ were somewhat higher when plants were fertilised with Hoagland's solution during growth, but there were no significant cultivar by fertiliser treatment interactions.

Consequently, gas exchange data were lumped within cultivars for further analyses of relationships between gas-exchange parameters.

The typical response of $g$ to increasing $\nu$ was a continuous reduction in total leaf conductance over the entire measurement range (Fig. 2*B*). Although a linear fit to the data for each leaf always produced a high $r^2$ ($r^2 > 0{\cdot}95$), natural log transformation of $g$ made the response to $\nu$ more fully linear, increasing the $r^2$ value (mean $r^2 = 0{\cdot}99$) and eliminating an otherwise consistent pattern in the residuals. The tight fit of ln-transformed data to a linear model indicated that, for each leaf, the percentage rate of change in $g$ per unit change in $\nu$ was constant throughout the measurement range, and $g$ approached its minimum value at high $\nu$ in an asymptotic fashion.

Total leaf conductance is the result of two conductances in series: stomatal and leaf boundary layer. The leaf boundary layer conductance is a function of both leaf size and wind speed, and, in the cuvette, was approximately 1·3 mol m$^{-2}$ s$^{-1}$ (per side). Total leaf conductance ($g$) is thus related to stomatal (subscript s) and boundary layer (subscript b) conductance as

$$g = \frac{g_s g_b}{(g_s + g_b)}. \tag{6}$$

For the amphistomatous bean leaves, this equation was first applied to each side of the leaf separately, and the results added together to obtain $g_{total}$. Stomatal conductance was therefore necessarily higher than total conductance at all times (Fig. 2*B*), and $g_s$ differed from $g$ more at low $\nu$, when $g_s$ was high, than at high $\nu$. This was because at high $\nu$, $g_s$ approached the magnitude of $g_b$, and total conductance was co-limited by these two portions of the total diffusion path, but, when $g_s$ declined at high $\nu$, $g_s$ became much smaller than $g_b$ and $g$ was approximately equal to $g_s$. Due to these relationships with $g_b$, $g_s$ was noticeably more curvilinear than $g$, but the ln-transformation of $g_s$ also produced a linear relationship to $\nu$ (mean $r^2 = 0{\cdot}99$). $g_s$ was related to the vapour pressure gradient between the inside of the leaf and the leaf surface inside the boundary layer ($\nu_s$). Bunce (1987) concluded that this relationship was the most robust mechanistically, since only the stomatal conductance is under direct plant control and only the humidity inside the boundary layer can be directly sensed by the leaf epidermis.

As $\nu$ increased, stomatal closure was sufficiently great to counteract fully the increased diffusion gradient. In many cases, the transpiration rate reached a maximum value, usually between a $\nu$ of 25 and 30 mbar bar$^{-1}$, after which $E$ actually declined slightly. In all cases, variation in $E$ between $\nu$ of 25 and 35 mbar bar$^{-1}$ was very slight. At the highest values of $\nu$, bean leaves were prone to exhibit oscillations in stomatal conductance which increased in intensity as $\nu$ increased. This behaviour was strongly influenced by growth conditions, and was less marked for plants grown under breezy conditions and high light. Only in cases where the oscillations damped out, and steady-state values were eventually achieved, were leaves included for analysis. While approximately 50% of the leaves showed

**Table 1. The importance of nutrient regime and cultivar differences between dry-beans (*Phaseolus vulgaris*) in determining variation in gas-exchange parameters**
Realised net photosynthetic rate ($A$), intercellular $CO_2$ concentration ($c_i$), leaf conductance to water vapour ($g$), maximum transpiration rate ($E_{max}$), and biochemical capacity for photosynthesis (values of $A$ observed at experimentally controlled values of $c_i$), and the response of $g$ to short-term variation in the leaf-to-air humidity gradient ($\nu$). 12 cultivars were grown under two slightly different fertiliser regimes (Treatments 1 and 2) and, for each cultivar within each treatment, the response of leaf gas-exchange to decreasing $\nu$ was measured on 1 leaf from each of 3 different plants (in two cases 4 leaves were sampled). This gave a total sample size of 74 response curves for the entire analysis. Measures of photosynthetic capacity ($A$ at $c_i = 250$) were obtained by varying the ambient $CO_2$ concentration ($c_a$) in order to vary $c_i$, and all other data were collected while varying $\nu$ from 15 to 35 mbar bar$^{-1}$ while holding $c_a$ constant at 350 $\mu$L L$^{-1}$. In all analyses below, the degrees of freedom for the mean squares are 1, 11, 11, and 50 for Treatments, Cultivars, Treatment × Cultivar and the error terms, respectively. Significance levels of each factor in the two-way ANOVAS and the interaction terms are indicated following each mean square as $^*P \leqslant 0{\cdot}05$, $^{**}P \leqslant 0{\cdot}01$ and $^{***}P \leqslant 0{\cdot}001$. NS = not significant

| Parameter | Mean squares and significance levels | | | | Teatment means and range of cultivar means | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Treatment 1 | | | Treatment 2 | | |
| | Treatment | Cultivar | Treatment × Cultivar | Error | Mean | Min. | Max. | Mean | Min. | Max. |
| $A$, when $\nu = 10$ mbar bar$^{-1}$ and $c_i = 250$ $\mu$L L$^{-1}$: | | | | | | | | | | |
| $A$, $\mu$mol m$^{-2}$ s$^{-1}$ | 173·5*** | 50·7*** | 17·5NS | 9·5 | 27·0 | 20·1 | 35·3 | 31·4 | 24·0 | 36·9 |
| $A$, when $\nu = 15$ mbar bar$^{-1}$ and $c_a = 350$ $\mu$L L$^{-1}$: | | | | | | | | | | |
| $A$, $\mu$mol m$^{-2}$ s$^{-1}$ | 526·9*** | 67·5*** | 15·2NS | 9·5 | 28·2 | 22·4 | 35·6 | 33·6 | 25·7 | 39·1 |
| $A$, nmol g$^{-1}$ | $122{\cdot}8 \times 10^3$*** | $28{\cdot}7 \times 10^3$*** | $12{\cdot}4 \times 10^3$NS | $7{\cdot}8 \times 10^3$ | 544·4 | 348·9 | 680·4 | 633·7 | 494·4 | 759·9 |
| $g$, when $c_a = 350$ $\mu$L L$^{-1}$: | | | | | | | | | | |
| $g$, mol m$^{-2}$ s$^{-1}$, when $\nu = 15$ mbar bar$^{-1}$ | 0·059*** | 0·026*** | 0·004NS | 0·003 | 0·555 | 0·425 | 0·651 | 0·674 | 0·499 | 0·768 |
| $g$–$\nu$, slope ($\nu = 15$–35 mbar bar$^{-1}$) | $111{\cdot}0 \times 10^{-6}$*** | $17{\cdot}6 \times 10^{-6}$*** | $6{\cdot}4 \times 10^{-6}$NS | $3{\cdot}6 \times 10^{-6}$ | −0·012 | −0·014 | −0·009 | −0·014 | −0·017 | −0·010 |
| $g$, % decline ($\nu = 15$–35 bar bar$^{-1}$) | 7·3NS | 39·0* | 17·2NS | 19·0 | 44·3 | 39·2 | 49·8 | 43·6 | 37·9 | 47·1 |
| $c_i$ when $c_a = 350$ $\mu$L L$^{-1}$ and $\nu = 15$ mbar bar$^{-1}$: | | | | | | | | | | |
| $c_i$, $\mu$L L$^{-1}$ | 57·8NS | 171·0** | 175·0*** | 51·3 | 263·0 | 251·0 | 279·0 | 265·0 | 250·0 | 273·0 |
| $E_{max}$ (over range $\nu = 15$–35 mbar bar$^{-1}$) | 122·0*** | 11·3*** | 1·89NS | 1·78 | 7·46 | 5·61 | 8·80 | 8·68 | 6·28 | 10·5 |
| Specific Leaf Area, cm$^2$ g$^{-1}$ | 350·1NS | 4385·1*** | 794·0* | 382·0 | 195·5 | 238·0 | 146·0 | 190·7 | 254·3 | 158·7 |

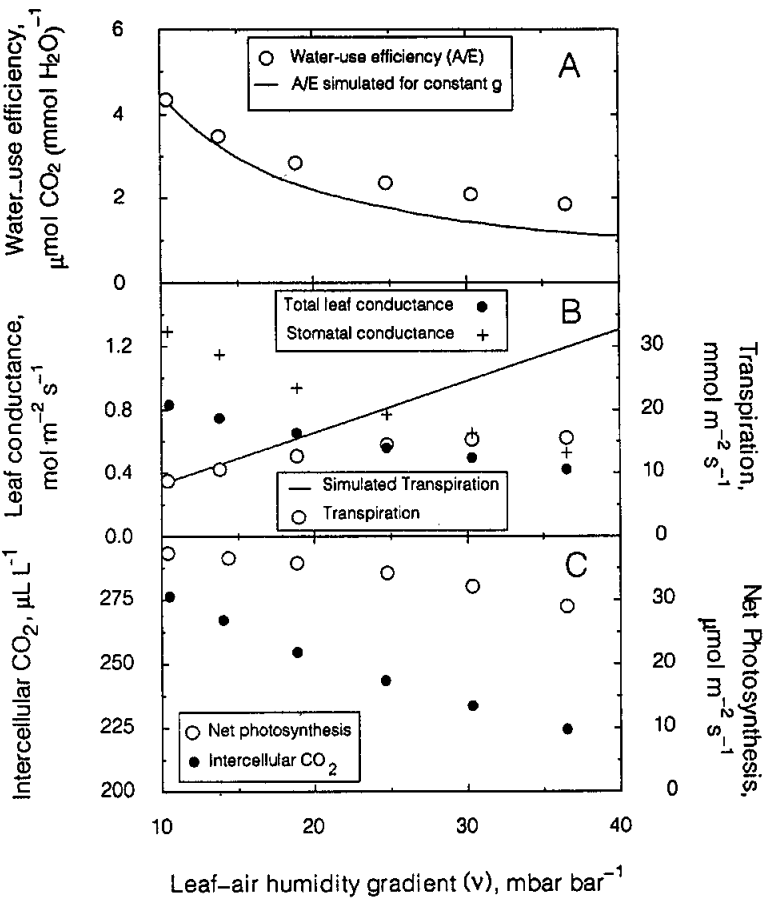


**Fig. 2.** A typical response of leaf gas exchange to an increasing leaf-air humidity gradient ($v$). (*A*) The response of water-use efficiency to $v$: the open circles refer to actual data points; the line represents a simulation of what water-use efficiency would have been if there had been no stomatal response to increasing $v$. (*B*) The response of leaf conductance ($g$, closed circles) and transpiration ($E$, open circles) to increasing $v$: the line represents a simulation of how $E$ would have increased with $v$ under cuvette conditions (constant leaf temperature) if $g$ had remained unchanged at its maximum value. (*C*) The decline in intercellular $CO_2$ ($c_i$, closed circles) caused by stomatal closure, and the associated decrease in net photosynthesis ($A$, open circles).

some tendency towards limited oscillations beginning at $v \sim 30$ mbar bar$^{-1}$, only 7 out of 151 leaves had to be excluded from further analysis.

As $g$ declined with increasing $v$, intercellular $CO_2$ also declined substantially, and this caused a moderate decline in net photosynthesis (Fig. 2*C*). In contrast to $E$, which changed significantly at low $v$, but was eventually stabilised by decreasing $g$ at high $v$, $A$ showed an accelerating decline with increasing $v$. This was caused by several factors, the most important being that decreasing $c_i$ values became progressively more limiting to photosynthesis.

Several recent studies have indicated that stomata sometimes close in a patchy rather than a uniform manner under stressful conditions (Terashima *et al.* 1988; Downton *et al.* 1988). It has been suggested that if $g$ were to approach 0 in some leaf patches, while retaining a high or at least moderate value in other patches, the ratio of $A/g$ (and hence $c_i$) measured by bulk gas-exchange would reflect in the patches in which $g \gg 0$. Under such a patchy regime, $A$ values would appear to fall well below the expected $A$–$c_i$ response curve deter-

mined when $g$ was uniform throughout the leaf. One way to evaluate the potential importance of non-uniform stomatal closure in gas-exchange data is by comparing $A$–$c_i$ relationships obtained at an initial, low $\nu$ value (generated by varying ambient $CO_2$, $c_a$), with the same plot for data obtained under constant $c_a$, but with progressively higher $\nu$ causing stomatal closure (Fig. 3). In this dataset such comparisons indicated that most of the change in $A$ could be accounted for using the response of $g$ to increasing $\nu$ and assuming a fixed $A$–$c_i$ response (simulated values, Fig. 3). This suggests that stomatal closure may have been largely uniform. Cheeseman (1991), however, has pointed out that substantial variation in stomatal conductance may occur between patches without noticeably changing $A$–$c_i$ relationships if $g$ does not equal 0 in any of the patches. Slightly higher-than-expected $A$ was occasionally observed as $\nu$ increased from 10 to 20 mbar $bar^{-1}$. A larger deviation, towards lower than expected $A$, was frequently observed at $\nu \geqslant 30$ mbar $bar^{-1}$; the magnitude of the deviation appeared to be associated with the tendency towards large oscillations in $g$. Such a deviation was consistent with the interpretation that non-uniform stomatal closure did occur at very high values of $\nu$, but was not quantitatively important over the range of $\nu$ measured. It should be noted that these very high $\nu$ values represent the most extreme environmental conditions that the plants experienced in the greenhouse. The apparent trajectory of the $A$–$c_i$ response at $\nu = 35$ mbar $bar^{-1}$, however, suggests that, with further increases in $\nu$, a strong pattern of non-uniform stomatal closure might have developed. On average, $A$ at $\nu = 35$ mbar $bar^{-1}$ was 5% lower than the $A$ value predicted based on the initial $A$–$c_i$ response (Fig. 3, vertical projection between squares and curve described by circles), and 3% lower than the value predicted from a simulation assuming the same total $g_c$, but perfectly

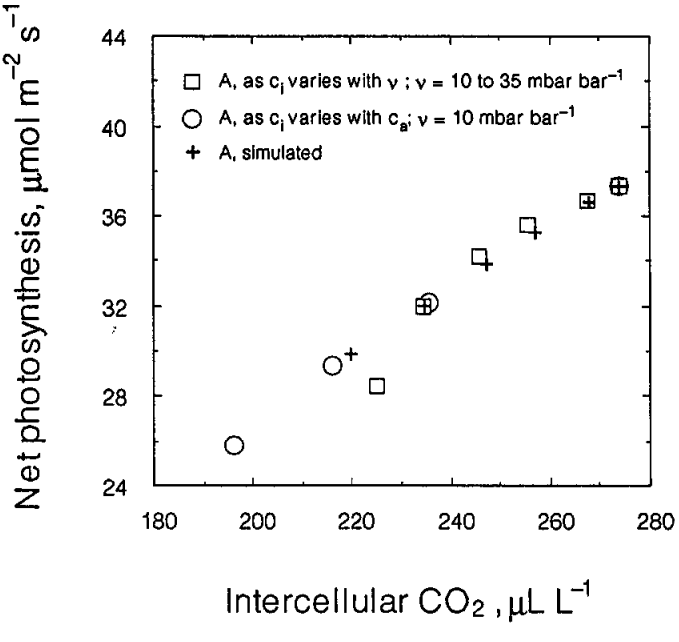


**Fig. 3.** A comparison of the relationship between net photosynthesis ($A$) and intercellular $CO_2$ ($c_i$) when the data were collected on the same leaf by: (1) varying ambient $CO_2$ ($c_a$) while the leaf-air humidity gradient ($\nu$) was held constant at 10 mbar $bar^{-1}$ (circles), or (2) when $c_a$ was constant at 350 µL $L^{-1}$ and $\nu$ was varied from 10 to 35 mbar $bar^{-1}$ causing stomatal closure (squares). Simulated data (+) indicate how stomatal closure during the response to increasing $\nu$ would have affected $A$ and $c_i$ if the $A$–$c_i$ relationship measured at $\nu = 10$ mbar $bar^{-1}$ had remained unchanged throughout the $\nu$ response.

uniform stomatal closure (Fig. 3, +). Modelled predictions of $A$ based on uniform stomatal closure accounted for 88% of the observed decrease in $A$ as $v$ increased from 10 to 35 mbar $bar^{-1}$.

Increasing $E$ and decreasing $A$ combined to cause a very large decrease in instantaneous water-use efficiency ($A/E$) as $v$ increased under cuvette conditions (Fig. 2*A*). Simulated values of $E$ and $A/E$ (Fig. 2*A*, *B*) were examined for a hypothetical leaf having the same $g_{max}$ and $A$–$c_i$ relationship as the study leaf, but without a decrease in $g$ with increasing $v$. Since leaf temperatures were held constant in the cuvette, a linear increase in simulated $E$ occurs throughout the range in $v$. The $c_i$ values (not shown) were predicted to decline by an average of 4 $\mu L\ L^{-1}$ between $v = 10$ and 35 mbar $bar^{-1}$, simply due to the changing interaction between $CO_2$ and water vapour during the diffusion process. This caused a very slight decline in simulated $A$ (values not shown) even when $g$ was held constant. Stomatal closure was most effective in controlling the increase in $E$, resulting in the stable $E_{max}$ observed in almost all leaves, and, at $v = 35$ mbar $bar^{-1}$, reducing $E_{observed}$ by 50·8% relative to $E_{constant\ g}$ (Fig. 2*B*). Stomatal closure was slightly less effective at stabilising $A/E$, and, again at $v = 35$ mbar $bar^{-1}$, the observed $A/E$ was only 36·8% higher than that simulated for constant $g$ (Fig. 2*A*). The reduced impact of $g$ on $A/E$ was because $A$ also declined to

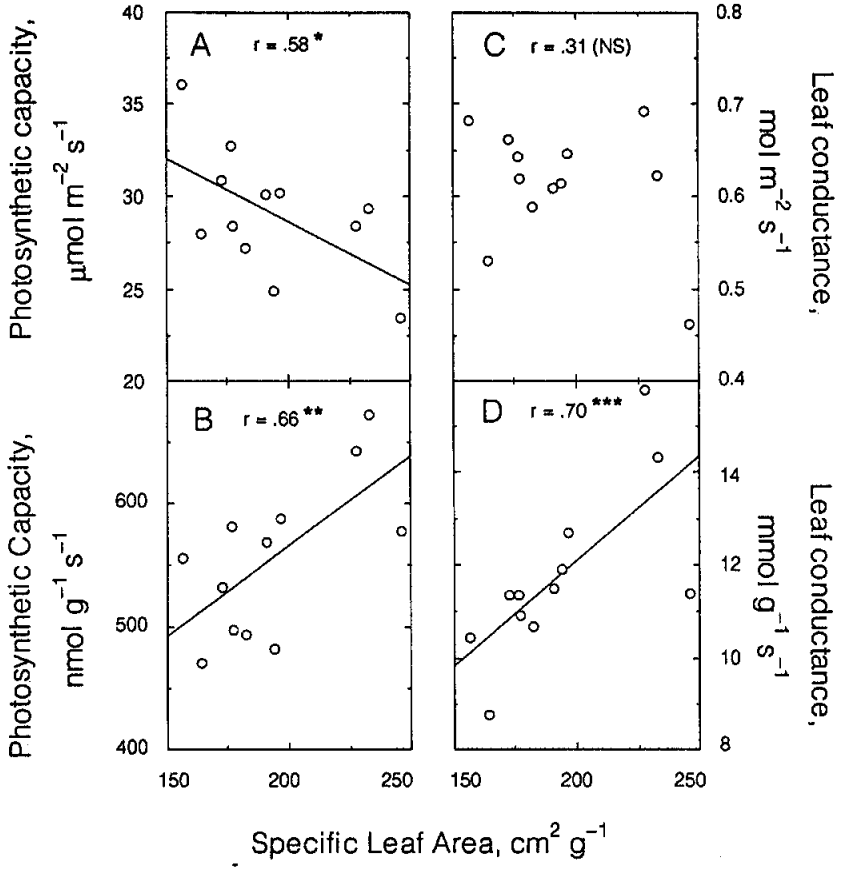


**Fig. 4.** The relationships between maximum photosynthesis and maximum leaf conductance, expressed on both leaf area and leaf dry weight basis, and specific leaf area. Gas exchange parameters are those measured at $v = 15$ mbar $bar^{-1}$. Each point represents the mean of six leaves measured on one cultivar. The lines shown represent the least squares best fits, and are significant at $P < 0{\cdot}05$, 0·02, and 0·01, for panels *A*, *B*, and *D*, respectively.

some extent as stomatal closure caused a reduction in $c_i$; consequently, water-use efficiency continued to decline even after $E$ had reached its maximum. The hyperbolic shape of the $A/E$ response was not dependent on stomatal closure, but occurred because, at constant leaf temperature, $A/E$ reduces to $\sim(c_a - c_i)/1{\cdot}6\nu$ and thus it asymptotically approached infinity and 0 at low and high $\nu$, respectively.

The relationships of $A$ and $g_{max}$ to specific leaf area were quite complex. Photosynthetic capacity, defined as $A$ at a fixed $c_i$ of 250 $\mu$L $L^{-1}$, had either a negative or a positive correlation with specific leaf area, depending on whether $A$ was expressed on the basis of surface area or unit dry weight, respectively (Fig. 4*A*, *B*). Leaf nitrogen contents plotted against specific leaf area mirrored the patterns in $A$ (Fig. 5), and $A$ was correlated with leaf nitrogen content whether expressed on a mass basis or an area basis (Fig. 6). Photosynthetic nitrogen-use efficiency (A, nmol (mg N)$^{-1}$ $s^{-1}$) did not correlate with nitrogen content on either an area or a mass basis. These patterns among cultivars are similar to those seen in contrasting native species (Field and Mooney 1986), and are consistent with the interpretation that RuBP carboxylation capacity was linearly related to protein and pigment content of the leaf mesophyll. The increased photosynthetic rate per unit mass in thin leaves was apparently made possible by increased nitrogen content per gram dry weight, and may have permitted fuller use of the displayed surface area. Despite this greater efficiency per unit mass in thin leaves, however, thicker leaves had higher photosynthetic rates expressed on a surface area basis, possibly through increased numbers of chlorenchyma cells and chloroplasts per unit surface area (Louwerse and Zweerde 1977; Nobel 1991). While

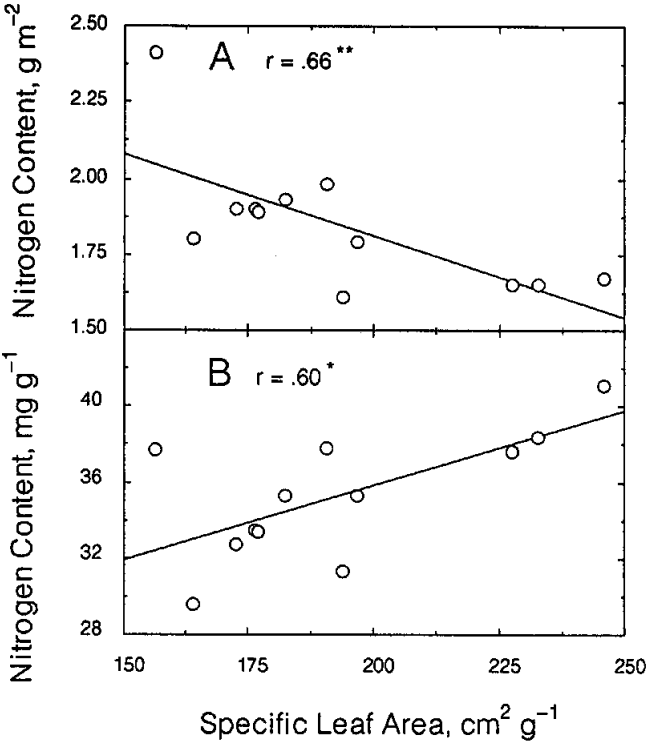


**Fig. 5.** Variation in leaf nitrogen content on the basis of either surface area or dry weight as a function of specific leaf weight (area per unit mass). Each point represents the mean of six leaves measured on one cultivar. The lines represent the least squares best fits and are significant at $P<0{\cdot}01$ and $0{\cdot}02$ for panels *A* and *B*, respectively.

increased leaf thickness may imply increased investment in carboxylation capacity of the mesophyll, leaf conductance is not mechanistically linked to specific leaf area, but only to the characteristics and behaviour of the epidermal cells themselves. Thus it was not too surprising that leaf conductance, expressed per unit surface area, did not significantly correlate with specific leaf area (Fig. 4*C*). It is true that, if the relationship between $A$ and specific leaf area (Fig. 4*A*) were very strong, this would be expected to cause indirectly a relationship between $g_{max}$ and specific leaf area due to the necessary scaling of $A$ and $g_{max}$ required to meet the diffusion limitations to $CO_2$ assimilation. The correlations between $A$ at $c_i = 250$ $\mu$L $L^{-1}$ and $g_{max}$ in this dataset were $r = 0{\cdot}69$ and $r = 0{\cdot}81$ ($P \leqslant 0{\cdot}02$) when both parameters were expressed either per unit surface area or per unit leaf dry weight, respectively. Despite this significant correlation between $A$ and $g$, however, the observed relationship in Fig. 4*A* was weaker, and failed to drive a relationship between $g_{max}$ and specific leaf area. A relatively strong, positive correlation was seen between leaf conductance expressed per unit leaf dry weight and specific leaf area (Fig. 4*D*). This was probably the result of two factors. The first is the necessary correlation between $A$ and $g_{max}$ as mentioned above, and the positive correlation between $A$ per unit mass and specific leaf weight (Fig. 4*B*), the second, and probably more important, is that if no significant relationship existed when $g_{max}$ was expressed per unit surface area, dividing each point by leaf weight/area necessarily results in thin leaves having higher conductance values.

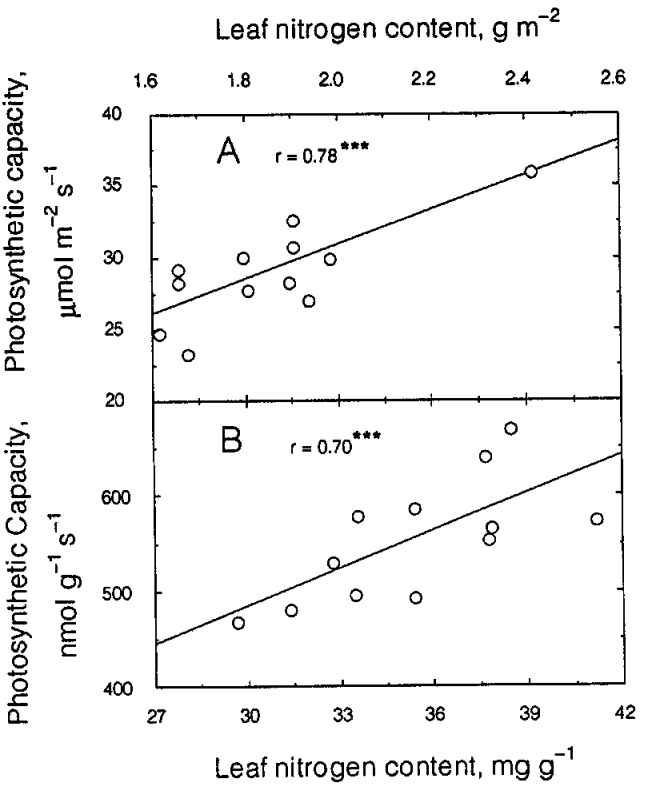


**Fig. 6.** The dependency of photosynthetic capacity, defined as net photosynthetic rate at an experimentally controlled $c_i$ of 250 $\mu$L $L^{-1}$, on leaf nitrogen content. Each point represents the mean of six leaves measured on one cultivar. The lines represent the least squares best fits, and are significant at $P < 0{\cdot}007$ and $0{\cdot}02$ for panels $A$ and $B$, respectively.

Considerable variation has been seen previously in carbon isotope discrimination ($\Delta$) between these bean cultivars, and this variation was confirmed in this greenhouse material (Fig. 1). We sought to test whether the variation between cultivars in $\Delta$ was related to specific gas exchange parameters, and, particularly, if it was attributable to differences in $\partial \ln(g)/\partial \nu$, the stomatal sensitivity of $g$ to $\nu$. High sensitivity of $g$ to $\nu$ would cause a rapid decrease in $g$ as $\nu$ increased between morning and afternoon under typical field conditions. This could result in a greater midday depression of $c_i$, and lower mean $c_i$ over the course of a day, and thus result in lower $\Delta$.

Consistent with previous field studies, $\Delta$ did not significantly correlate with either specific leaf area (White *et al.* 1990), or leaf nitrogen content (Ehleringer 1990). A weak but statistically significant correlation was observed between $\Delta$ and $\partial g/\partial \nu$ (Fig. 7*B*). The slope of this relationship, however, was opposite in sign to that which it would have had had the relationship been causal with midday depression of $g$, lowering mean daily $c_i$ and decreasing $\Delta$. Furthermore, in determining $c_i$, the absolute magnitudes of $A$ or $g$ which are not important, but the ratio between them is. Thus, if variation in $A_{max}$ and $g_{max}$ (respective values under near-optimal environmental conditions) existed between cultivars, but their ratio was constant, variation in $\Delta$ would be proportional, not to the absolute slopes of

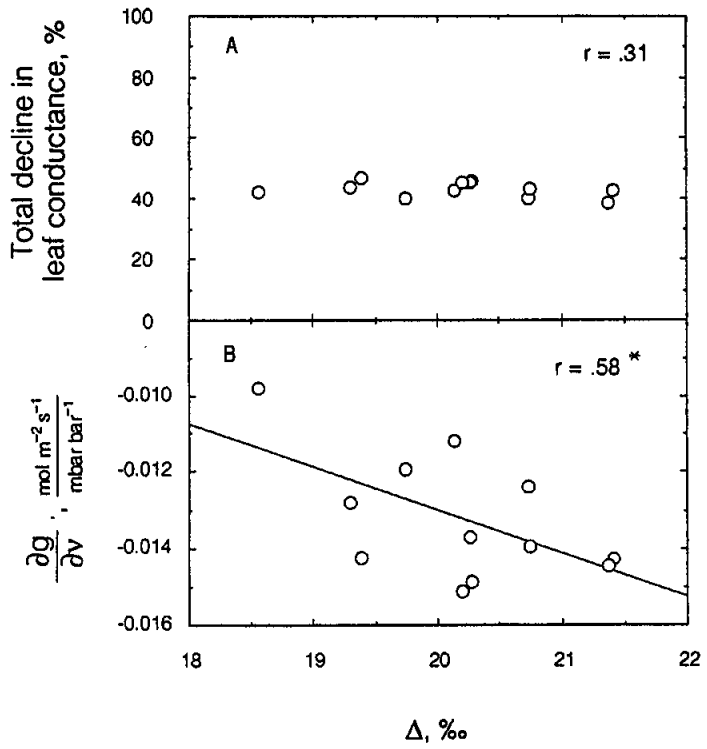


**Fig. 7.** The relationship in the cultivars between stomatal closure in response to increasing $\nu$, and leaf carbon isotope discrimination ($\Delta$). (*A*) Percentage difference between leaf conductance at $\nu = 15$ and 35 mbar bar$^{-1}$. (*B*) Absolute slope of linear regression of $g$ on $\nu$ for data spanning the range $\nu = 15$ to 35 mbar bar$^{-1}$. The $r^2$ for each individual regression was $>0{\cdot}90$. Each point represents the mean of six leaves measured on one cultiver. The relationship in panel *A* is not statistically significant. The line in panel *B* is the least squares best fit and significant at $P<0{\cdot}05$.

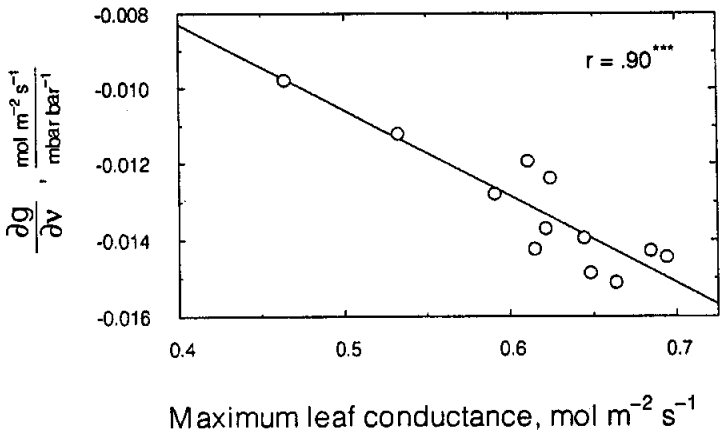


**Fig. 8.** The relationship in the cultivars between the rate of change in leaf conductance with increasing $v$ between 15 and 35 mbar bar$^{-1}$, and the initial leaf conductance at $v = 15$ mbar bar$^{-1}$. Each point represents the mean of six leaves measured on one cultivar. The line represents the least squares best fit: slope $= 0{\cdot}0008 - 0{\cdot}228 * g_{initial}$ ($r = 0{\cdot}90$; $P < 0{\cdot}001$), and the $y$-intercept is not significantly different from 0.

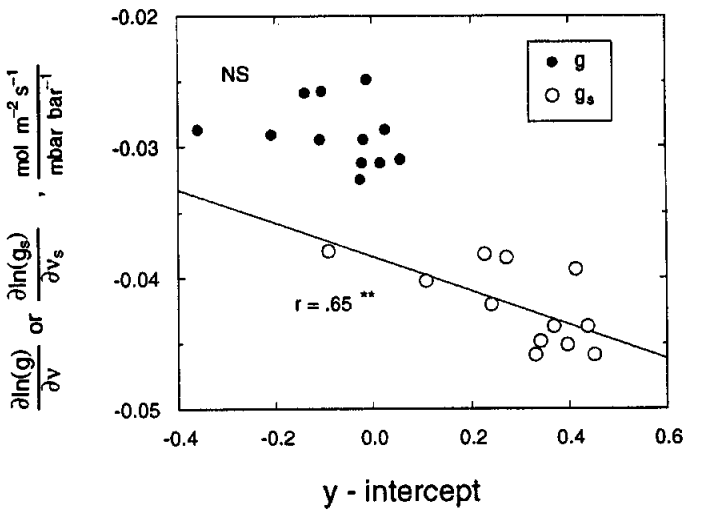


**Fig. 9.** The relationship between slopes (sensitivity) and intercepts (maximum values) for regressions of ln(conductance) versus the humidity gradient. Closed circles refer to the regression of ln(total conductance) on the leaf-air humidity gradient, and open circles refer to the regression of ln(stomatal conductance) on the humidity gradient across the stomatal pore. Since the conductances are ln-transformed, all differences in maximum conductances are incorporated into the $y$-intercepts, and variation in the slopes represent different proportional rates of reduction (different sensitivities). Only for stomatal conductance is there a significant correlation between sensitivity and the maximum conductance value.

$g_{max}$–$\nu$, but to differences in the percentage change in $g_{max}$ with $\nu$. For different bean cultivars, $\partial g/\partial \nu$ was directly proportional to $g_{max}$ (Fig. 8). Although cultivars did show significant differences in the percentage decline in $g$ (Table 1), the actual range of values was very small, and no significant correlation existed between percentage decline in $g$ and $\Delta$ (Fig. 7*A*). The slope of $\ln(g)$–$\nu$ did not positively correlate with $g_{max}$, and, again, this slope showed a small range of values, indicating that all cultivars had a similar sensitivity to $\nu$ (Fig. 9, closed symbols). This pattern was only possible, however, because a positive correlation did exist between the $\nu_s$ sensitivity of $g_s$ and $g_{max}$ (Fig. 9, opened symbols). Cultivars with high $g_{max}$ were more limited by $g_b$ (Eqn 6), and needed much higher maximum values of $g_s$ (Fig. 2*B*). Associated with different limitations by $g_b$, cultivars with high maximum $g$ appeared to have very high initial $g_s$ values, and greater sensitivity of $g_s$ to $\nu_s$.

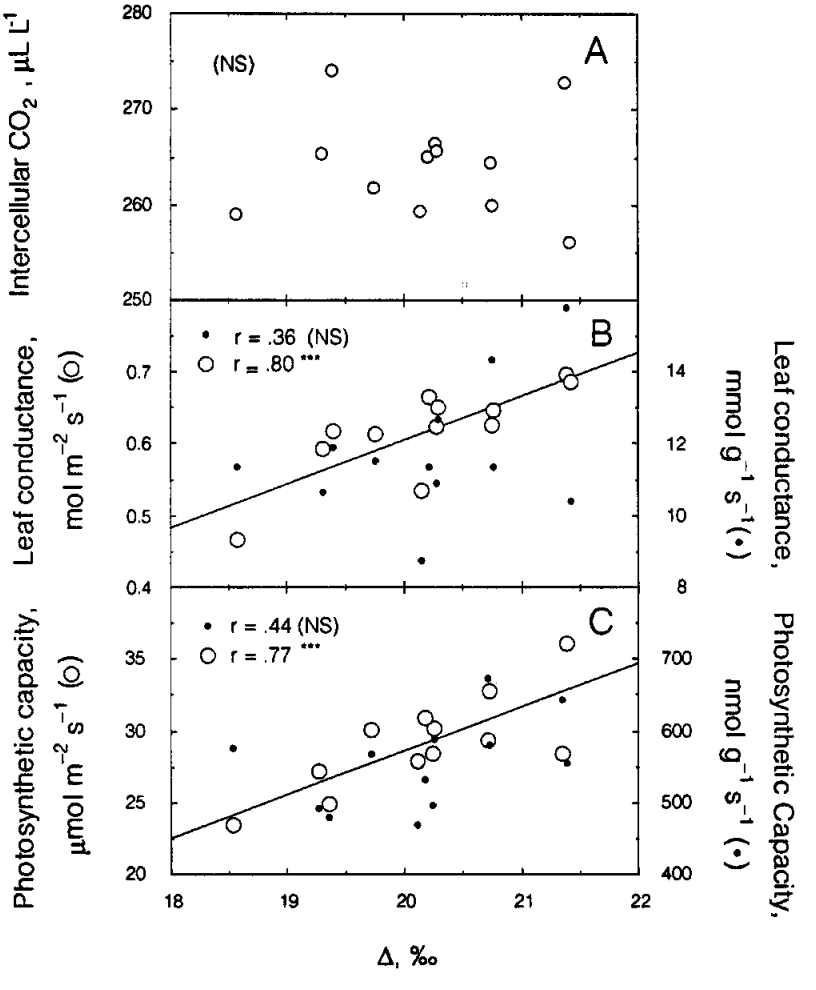


**Fig. 10.** The relationship in the cultivars between leaf carbon isotope discrimination ($\Delta$) and selected gas exchange parameters. (*A*) Intercellular $CO_2$ ($c_i$), relationship not significant. (*B*) Leaf conductance on both the basis of leaf area (open circles) and leaf dry weight (filled circles): the relationship to $\Delta$ is significant only with $g$ expressed on an area basis ($P<0{\cdot}002$). (*C*) Net photosynthesis on basis of both leaf area (open circles) and leaf dry weight (filled circles): the relationship to $\Delta$ is significant only with net photosynthesis expressed on an area basis ($P<0{\cdot}003$).

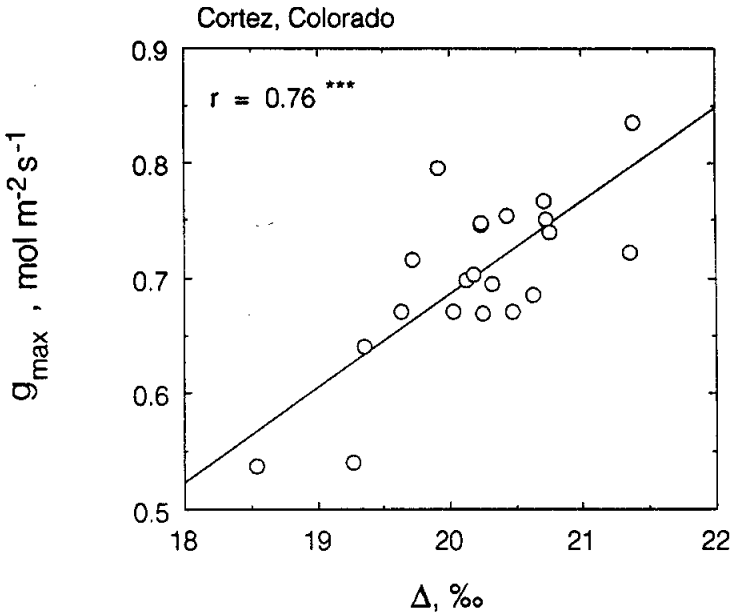


**Fig. 11.** The relationship between maximum stomatal conductance ($g_{max}$) measured in the field and dry matter carbon isotope discrimination ($\Delta$) values for 21 cultivars of common bean in Cortez, Colorado. The correlation is significant at the $P<0{\cdot}01$ level.

$\Delta$ was positively correlated with variation in $g_{max}$, but only when $g$ was expressed on a surface area basis (Fig. 10*B*). This correlation is in the right direction to postulate a causal relationship based on theory if photosynthetic capacity were constant and variation in $g$ resulted in variation in $c_i$. However, a positive correlation also existed between $\Delta$ and $A$ at a fixed $c_i$ of 250 $\mu$L $L^{-1}$ for data shown in Fig. 10*C*; the correlation was significant at any fixed $c_i$. These two parallel trends in $g$ and $A$ largely cancel out in their expected influence on $c_i$, which depends on the ratio of $A/g$. Thus $c_i$ measured under cuvette conditions did not correlate with either field $\Delta$ (Fig. 10*A*) or greenhouse $\delta^{13}C$ (data not shown) of bulk leaf tissues.

A broader evaluation of common bean cultivars under field conditions further supported a linkage between $\Delta$ and $g_{max}$. These data consisting of non-overlapping cultivars exhibited the same highly significant correlation between $g_{max}$ and $\Delta$ (Fig. 11). Contributing to the changes in leaf conductance were significant increases in the stomatal conductance on the adaxial surface. Over the range of stomatal conductance data in Fig. 11, the fraction of the total conductance on the adaxial surface increased from 20% to 37% ($r=0{\cdot}653$, $n=21$, $P<0{\cdot}01$). While adaxial conductances accounted for only about 30% of the total stomatal conductance, it appeared that increased diffusion through the upper surface was necessary in order to minimise decreases in $c_i$ in leaves of cultivars that would be exhibiting higher $A$ values.

## Discussion

Leaf conductance of dry beans showed a strong sensitivity to $v$, and, given the range in maximum stomatal conductance, a rather surprising consistency in the percentage decline in the different cultivars. The linear relationship between the slope of $g$–$v$ and $g_{max}$ (Fig. 8)

was one of the strongest correlations observed among any of the gas exchange parameters, and the $y$-intercept was not significantly different from zero. This leads to relatively fixed sensitivity to $v$ across all cultivars despite the wide range in $g_{max}$, and it is worthwhile asking whether this particular level of sensitivity is of special importance. If $E = gv$, and $g = mv + b$, then $E = mv^2 + bv$. Differentiating with respect to $v$ we find that $\partial E/\partial v = B + 2mv$, and setting this equal to zero we find the value of $v$ at which the maximum $E$ will occur: $v = -B/2m$. A zero intercept in Fig. 8 means that this ratio will be constant for all beans, and, regardless of their $g_{max}$, they will all reach their maximum $E$ at the same value of $v$. It was mentioned that a ln-transformation of $g$ gives a slightly better fit to the data for beans. If the above analyses is repeated using $\ln(g) = m^*v + B^*$, the maximum value of $E$ is predicted to occur at $v = -1/m^*$ (see the Appendix). Bear in mind that for ln-transformed data, the slope $m^*$ represents a constant proportional rate of change in $g$, and, if values for $m$ are equal, then differences in $g_{max}$ are incorporated into the $y$-intercept. Thus, this analysis also tells us that the maximum value of $E$ is reached at a particular value of $v$ dependent only on the relative rate of decline in $g$, and independent of the absolute value of $g_{max}$. The main difference between the linear and ln models is that if the $\ln(g)$ rather than $g$ is declining linearly with $v$, then the maximum $E$ will be approached more gradually and with a broader curve. The shaded region of Fig. 12 shows the prediction of what value of $v$ is associated with

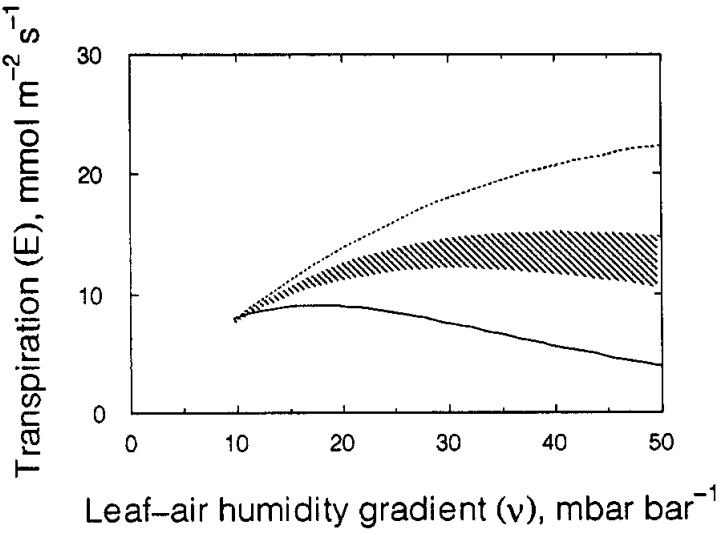


**Fig. 12.** The influence of the sensitivity of $g$ to $v(\partial \ln(g)/\partial v)$ on the stability of transpiration rates at high $v$. The shaded region represents the full range of sensitivities rates in the twelve cultivars studied. The dashed and solid lines represent the potential rates of transpiration if sensitivities had been either one-half or twice the sensitivity of the mean cultivar, respectively. All data are simulated. All leaves are assumed to have the same initial $g$ at $v = 10$ mbar bar$^{-1}$. This substantially reduces the variation in $E_{max}$ indicated between cultivars compared to that actually observed, but will have no effect on the shape of the $E$–$v$ response curve in terms of (1) the $v$ at which $E_{max}$ occurs, or (2) the stability of $E_{max}$ between $v = 25$ and 50 mbar bar$^{-1}$. The analysis shown thus evaluates the importance of one factor, the relative rate of stomatal closure, on $E$. The response of $g$ to $v$ was taken to have the form $\ln(g) = mv + b$, and transpiration was therefore $E = vg = ve^{(m^*v + B^*)}$. $m$ was evaluated from the data on each cultivar and had a grand mean of $-0{\cdot}029$, and a range of $-0{\cdot}025$ to $-0{\cdot}033$ in the cultivars.

$E_{max}$ for all the bean cultivars in this study. The actual variation in $g_{max}$ will cause considerable variation in the magnitude of $E_{max}$, but will have no effect on the value of $\nu$ at which it will occur, or the shapes of the curves. Fig. 12 assumes a constant value of $g_{max}$ for purposes of graphical illustration. Two points are of considerable interest: (1) all cultivars reach their absolute maximum $E$ between $\nu = 25$ and 40 mbar bar$^{-1}$; and (2) variation in $E$ is negligible between $\nu = 25$ and 50 mbar bar$^{-1}$, which encompasses the full range of potentially stressful midday $\nu$ to be experienced by beans during a summer growing season. The dashed and solid lines show the expected behaviour of $E$ if the slope of $\ln(g)$–$\nu$ were one-half or twice the mean value for bean cultivars, respectively. The importance of this apparent homeostasis in $E$ at high $\nu$ cannot be definitely assessed from these data alone, but some interesting points may be suggested. The fact that $E$ does decline slightly over the experimental range of $\nu$ in at least some cultivars suggests that feed-forward mechanisms of stomatal control are probably operative in beans as would be required for models of optimal diurnal patterns of stomatal opening (Cowan and Farquhar 1977; Farquhar 1978). These models, however, are not related in any specific way to $E_{max}$, and they could be consistent with any of the curves in Fig. 12 depending on the specific values given to optimisation parameters. The unique range of sensitivities measured for beans is one that generates strong homeostasis of $E$ over the range of potentially stressful midday conditions, and could thus help to stabilise minimum leaf water potentials ($\Psi_{min}$).

Several recent studies suggest that hydraulic limitations to water transport may limit transpiration rates (Tyree and Sperry 1988, 1989), and the observed stomatal sensitivity could help ensure that stomata remain as open as possible while holding $\Psi_{min}$ at a fixed value over a wide range of conditions. The actual variation in $g_{max}$ between cultivars would cause a wide range in actual transpiration rates per unit leaf area at $E_{max}$. However, this variation could well be balanced at the whole-plant level by a suite of other characters such as leaf area index, root/shoot ratio, and conducting efficiency of the xylem, so that a balance was always achieved between hydraulic conductance of the liquid-phase transport system and $E_{max}$ at the whole plant level. In such a system, the important role of stomatal control illustrated in Fig. 12 could be the homeostasis of $E_{max}$ over a wide range of environmental conditions. Thus if $g$ were plotted against $\Psi$ from several diurnal courses, stomatal closure could appear to occur in response to a threshold value of $\Psi$, while in fact stomatal response was mechanistically linked to atmospheric humidity and not $\Psi$. Limiting $\Psi_{min}$ may indeed be important to avoid catastrophic cavitation in the xylem, but it need not be achieved solely by hydraulic feedback acting through $\Psi$.

Considerable controversy currently exists concerning the nature of the direct humidity response of $g$ (Bunce 1985, 1988; Woodrow *et al.* 1987; Grantz 1990; Aphalo and Jarvis 1991). Bunce (1985) has presented data and a cogent argument suggesting that during short-term variation in $g_b$, which changes the relationship between $\nu_s$ and $\nu$, $g_s$ responds directly to $\nu_s$ and not to $\nu$. This is consistent with the view that the leaf can only directly sense the humidity inside the leaf boundary layer and not true ambient humidity. It is interesting to note that in the data for bean cultivars, however, the variation in the sensitivity of $g_s$ to $\nu_s$ occurs in a compensatory fashion so that a constant sensitivity of total conductance is maintained despite large differences in $g_{max}$ (Fig. 9). It cannot be determined from these data whether this variation in the $g_s$ response to $\nu_s$ was genetically pre-determined, or a consequence of the regulatory acclimation processes which maintain a balance between $A$ and $g$ over wide ranges of photosynthetic capacity. In either case, however, it indicates that the $g_s$–$\nu_s$ response is modulated over the long term to produce an appropriate response in $g$–$\nu$, which, though it may not be the primary mechanism of stomatal behaviour, nonetheless represents the functionally important response of the plant to its environment.

Boundary layer conductance will vary in magnitude as wind speed changes under field conditions. This will affect the shapes of the curves shown in Fig. 12, by stretching or contracting the $x$-axis (Fig. 13) but will have no effect on the values of $E_{max}$. From the Appendix, Eqns A6 and A7, it is apparent that (1) $\nu$ is always larger than $\nu_s$, as must

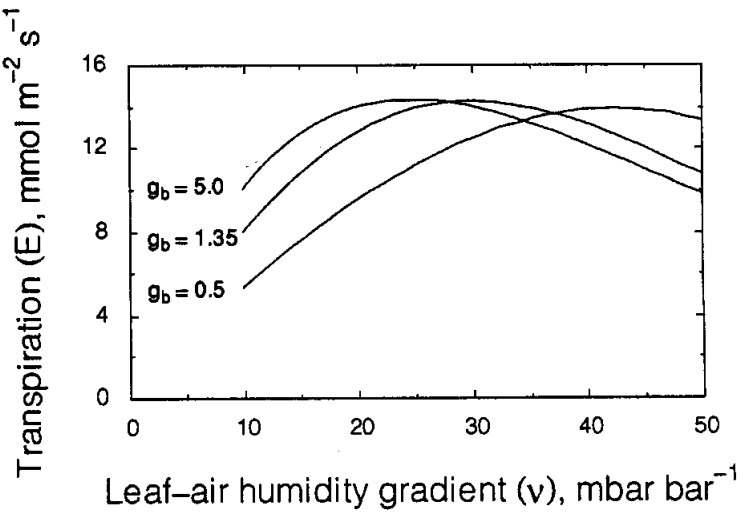


**Fig. 13.** Simulated effects of variation in the boundary layer on $E_{max}$. The boundary layer values of 0·5, 1·35, and 5 mol m$^{-2}$ s$^{-1}$ for each side of the leaf correspond to a bean leaflet with a mean width of 5 cm in wind speeds of 0·2, 1·0, and 20 m s$^{-1}$, respectively. Although Eqn A3 of the Appendix predicts that $E_{max}$ has no dependency whatsoever on $g_b$, a very subtle effect of high $g_b$ slightly decreasing $E_{max}$ is present in this simulation. This was due to the amphistomatous nature of the simulated bean leaves which, having unique $B_{upper}$ and $B_{lower}$, reached $E_{max}$ at slightly different values of $v$ on the upper and lower surfaces consistent with the predictions of Eqn A7. The degree of offset between the two surfaces depended in some degree on $g_b$, resulting in slight differences in the maximum summation of $E$ as $g_b$ varied, but this effect was very small.

indeed be the case since $v_s$ represents only a subportion of the pathway of $v$, and (2) that $v$ will be very similar to $v_s$ when $g_b$ is very large relative to $g_s$. These effects are rather intuitive, but it must be stressed that these are the *only effects* of $g_b$ on the $E$–$v$ relationship. If the $\ln(g_s)$ is a linear function of $v_s$, then the value of $v_s$ at which $E_{max}$ will occur is $v_s = -1/m_s^*$ and $E_{max} = 1/m_s^* e^{(B_s^* - 1)}$ (Appendix, Eqns A3 and A4). $g_b$ does not appear in these expressions, and has no influence on $E_{max}$. $g_b$ has a strong influence only on the value of $v$ at which $E_{max}$ will occur.

Previous studies have reported that the net effect of increasing wind speed in growth studies of *P. vulgaris* was to decrease transpiration rate (Kalma and Kuiper 1966). The simulations illustrated in Fig. 13 suggest that this result may be dependent on air humidity. At high humidity (low $v$) increasing $g_b$ should increase $E$ because of increased total leaf conductance (Eqn 6), and at low atmospheric humidity it should decrease transpiration due to increased stomatal closure. This interaction of atmospheric humidity and wind speed is to be expected for any model of stomatal behaviour which produces a discrete maximum followed by declining $E$ at a specified value of $v_s$ (e.g. $g_s$ or $\ln(g_s)$ decreasing linearly with increasing $v_s$). Other observed stomatal responses, such as a linear increase is stomatal resistance ($1/g_s$) with increasing $v_s$, do not imply a defined $E_{max}$ and would be expected to result in $E$ increasing with wind speed at any atmospheric humidity.

The variation between cultivars of photosynthetic rates at a constant $C_i$ (thus removing the influence of stomatal behaviour) was quite substantial (Table 1). In describing a mechanistic relationship between mesophyll characteristics and photosynthetic capacity, different authors have variously stressed a positive correlation between carboxylation capacity and protein content with little specific reference to tissue structure (Field and Mooney 1986; Evans 1989), or alternatively, the importance of the number of mesophyll

cells per unit surface area of leaf (Nobel 1991). Both of these concepts appear to be important in explaining the variation in photosynthetic capacity between bean cultivars. Very thin leaves have higher than average rates of photosynthesis when expressed on a dry weight basis (Fig. 5), and this appears to be related to high nitrogen investment per unit mass of leaf tissue (Fig. 6), which implies higher nitrogen contents per mesophyll cell, although anatomical work was not performed. However, despite the high carboxylating capacity of thin leaves per unit mass, thick leaves had higher carboxylation rates per unit surface area. Previous work with *P. vulgaris* supports the interpretation that this was related to increased numbers of mesophyl cells and chloroplasts per unit leaf area in thick leaves (Louwerse and Zweerde 1977).

Between a $\nu$ of 15 and 20 mbar bar$^{-1}$, the observed values of $A$ were often slightly higher than expected based on the $A$–$c_i$ response measured at an initial $\nu$ of 10 mbar bar$^{-1}$ (Fig. 3). It is possible that slightly non-uniform stomatal behaviour occured even at extremely low $\nu$, but an alternative explanation can also be suggested. Leaf water potential varied from 0·28 to 0·85 MPa, responding to plant transpiration rate predawn or at midday. As water potential drops, large pore spaces in the leaf apoplast should drain of water. The pores which should drain of water in response to the observed drop in $\Psi$ are those with diameters of 1·0–0·3 $\mu$m (Zimmerman 1983; Tyree and Ewers 1991). These diameters are much larger than would be expected for pores within cell walls, and should not have caused large changes in wall hydration. They would, however, correspond to the radii of crevices formed at contact points between leaf mesophyll cells. If these intercellular spaces were partially flooded at very low $\nu$, and then drained at higher $\nu$ and higher $E$ (lower $\Psi$), this could have decreased the liquid-phase resistance to $CO_2$ diffusion between the intercellular spaces and the sites of carboxylation, and could have caused an increase in the apparent $A$–$c_i$ response.

At $\nu = 35$ mbar bar$^{-1}$, the highest treatment value used, $A$ was clearly falling below that predicted by the initial $A$–$c_i$ curve (Fig. 3), but this deviation had not yet had a major impact on the absolute value of $A$. Under most growing conditions, the maximum $\nu$ experienced by beans will not be greater than 40 mbar bar$^{-1}$. We conclude that under most conditions, gas exchange in beans will respond in a manner accurately predicted by assumptions of uniform stomatal closure and a constant $A$–$c_i$ response. This may not hold, however, under sudden (e.g. ABA applications) or extreme (low humidity or very high soil moisture deficit) stress levels.

The correlation reported here between $g_{max}$ and $\Delta$ has also been observed for field-grown beans. This study was intended to test whether variation in stomatal sensitivity to $\nu$ was the precise mechanism of this relationship. We conclude that it was not, but the data do suggest another mechanistic interpretation. The fact that carboxylation capacity co-varied with $g_{max}$ tended to hold the ratio of $A/g$ (and therefore $c_i$) constant under cuvette conditions with uniform leaf temperatures. Furthermore, when compared under constant conditions of temperature and $\nu$, there was no correlation between instantaneous measurements of $c_i$ and the $\Delta$ of bulk leaf material, even though bean photosynthesis is specifically known to conform to theoretical relationships between these parameters (Ehleringer *et al.* 1991). Finally, not only did the variation in $c_i$ among cultivars not correlate with $\Delta$ when compared at constant $\nu$, but the total variation in $c_i$ was insufficient to account for the level of variation in $\Delta$ based on theoretical relationships (Eqn 4). These observations are difficult to reconcile if it is assumed that all beans experienced the same $\nu$ during growth. This, however, need not have been the case. Different bean cultivars may have had different leaf temperatures under similar field or greenhouse conditions by virtue of different leaf sizes, orientations,and transpiration rates per unit leaf area. Leaves with higher $g$, whether or not this correlated with higher photosynthetic capacity, would have had higher transpiration rates and therefore lower leaf temperatures. Thus plants with lower $g_{max}$ would always experience higher $\nu$ than leaves with high $g$. The higher $\nu$ would exacerbate this difference by inducing further stomatal closure in the plant with intrinsically low initial $g$. These differences in

transpirational cooling would result in cultivars with lower gas-exchange rates per unit area operating on a different part of the $c_i$–$\nu$ response curve (Fig. 2*C*), and this could be reflected in different $\Delta$. Consistent with this interpretation, a strong correlation was found between $\Delta$ and $E_{max}$ for this set of cultivars ($r = 0{\cdot}86$). Leaf-energy-budget simulations incorporating the observed regressions of $\ln(g_s)$ on $\nu_s$ for each cultivar resulted in a spread of leaf temperatures over about three degrees, and the variation in percentage reduction in $g$ significantly correlated with $\Delta$ ($r = 0{\cdot}68$). Nonetheless, the range of variation in per cent reduction of $g$ was only 33–45% under midday conditions, and this is still insufficient to explain the magnitude of the range in $\Delta$. Thus, the lack of correlation between $\Delta$ and $c_i$ in this dataset is not fully explained, though variation in leaf temperature through variation in $g_{max}$ appears to play a major role.

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## Appendix

Let stomatal conductance ($g_s$) be related to the leaf–leaf surface humidity gradient ($v_s$) according to the linear function:

$$\ln(g_s) = m_s^* v_s + B_s^*. \tag{A1}$$

Then

$$E = v_s g_s = v_s e^{(m_s^* v_s + B_s^*)} \tag{A2}$$

and the rate of change in $E$ with change in $v_s$ is

$$\frac{\partial E}{\partial v_s} = e^{(m_s^* v_s + B_s^*)}(1 + m_s^* v_s).$$

Setting $\partial E/\partial v_s$ equal to zero to find the value of $v_s$ at which $E_{max}$ occurs gives

$$v_s = -\frac{1}{m_s^*}. \tag{A3}$$

Substituting (A3) into (A2) gives an expression for the actual value of $E_{max}$:

$$E_{max} = v_s e^{(B_s^* - 1)} = -\frac{1}{m_s^*} e^{(B_s^* - 1)}. \tag{A4}$$

Equation A2 describes the diffusion process through the stomatal pore, but at steady state this must also be equal to the rate of diffusion of water vapour through the leaf boundary layer. If the total diffusion gradient between the inside of the leaf and the ambient air is $\nu$, then the gradient across the boundary layer is equal to the difference between the total gradient and the fraction of the gradient occurring across the stomatal pore giving:

$$E = g_b(\nu - \nu_s). \tag{A5}$$

Setting (A2) equal to (A5) and solving for $\nu$ gives

$$\nu = \nu_s\left(1 + \frac{g_s}{g_b}\right)$$

and, in the specific case of $E_{max}$ (substituting from (A1) for $g_s$ and (A3) for $\nu_s$),

$$\nu = \nu_s\left(1 + \frac{e^{(B\xi - 1)}}{g_b}\right) = -\frac{1}{m_s^*}\left(1 + \frac{e^{(B\xi - 1)}}{g_b}\right). \tag{A7}$$

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