CSIRO PUBLISHING

AUSTRALIAN JOURNAL OF PLANT PHYSIOLOGY

AJPP

An international journal of plant function

www.publish.csiro.au/journals/ajpp

CSIRO
AUSTRALIA

Academy of Science

Variability in hydraulic architecture and gas exchange of common bean (*Phaseolus vulgaris*) cultivars under well-watered conditions: interactions with leaf size

Maurizio Mencuccini[A] and Jonathan Comstock

Boyce Thompson Institute for Plant Research at Cornell University, Tower Road, Ithaca, 14853, NY, USA.
[A]*Present address: Institute of Ecology and Resource Management, University of Edinburgh, The Kings Buildings,*
Mayfield Road, EH9 3JU, Edinburgh, Scotland, UK; corresponding author; email: m.mencuccini@ed.ac.uk

Abstract. In a greenhouse study, 12 common bean cultivars from a wide geographical range were compared for their morphological, gas exchange and hydraulic architecture characters. Cultivars bred for cultivation in hot and dry regions had significantly smaller leaves and crowns, but higher stomatal conductances and transpiration rates per unit of leaf area. Short-term variability in gas exchange rates was confirmed using leaf carbon isotope discrimination. A literature survey showed that, although previously unnoticed, the strong inverse coupling between leaf size and gas exchange rates was present in three other studies using the same set of cultivars. Several measures of 'leaf-specific hydraulic conductance' (i.e. for the whole plant and for different parts of the xylem pathway) were also linearly related to rates of water loss, suggesting that the coupling between leaf size and gas exchange was mediated by a hydraulic mechanism. It is possible that breeding for high production in hot regions has exerted a selection pressure to increase leaf-level gas exchange rates and leaf cooling. The associated reductions in leaf size may be explained by the need to maintain equilibrium between whole-plant water loss and liquid-phase hydraulic conductance.

Keywords: common bean, hydraulic conductance, gas exchange, leaf size, carbon isotope discrimination, leaf energy balance.

Introduction

Plants characterised by the small size of their leaves have frequently been reported to have higher stomatal conductances and net photosynthetic rates, especially so in crop species (e.g. Egli *et al.* 1970; Hiebsch *et al.* 1976). More recently, Van Den Boogard *et al.* (1997) showed that wheat (*Triticum aestivum* L.) cultivars characterised by small crowns had higher transpiration rates, stomatal conductances and assimilation rates per unit of leaf area than similar cultivars with larger crowns.

Using an historical sequence of Pima cotton (*Gossypium barbadense* L.) cultivars bred over the last 40 years, Zieger and co-workers showed that selection of high-yielding cultivars resulted in lines characterised by small leaves and high rates of stomatal conductance (Cornish *et al.* 1991; Radin *et al.* 1994). While the energy budget implications of high stomatal and boundary layer conductances have already been explored (Lu *et al.* 1994; Lu and Zieger 1994; Srivastava *et al.* 1995), little information is available about the consequences on whole-plant hydraulic balance. If the reduction in average leaf size of high-yielding Pima cotton cultivars was not associated with a parallel increase in the number of leaves produced, a smaller plant crown area would be predicted, possibly compensating for the increased rates of unit-leaf water losses.

Hydraulically, high rates of leaf water loss can be sustained in two ways, i.e. by a negative leaf water potential or by a large water transport capacity relative to crown size, as evident from the following form of the Ohm's law analogue:

$$E_L = g_{tot} * \Delta\omega = K_L^{pl} * (\Psi_s - \Psi_1), \qquad (1)$$

where E_L, g_{tot}, $\Delta\omega$, K_L^{pl}, Ψ_s and Ψ_1 are transpiration rate per unit leaf area (mmol m^{-2} s^{-1}), total leaf conductance (stomatal plus boundary layer, mmol m^{-2} s^{-1}), leaf-to-air vapour pressure difference (mmol mol^{-1}), plant hydraulic conductance per unit of leaf area (or 'leaf-specific' hydraulic conductance, mmol m^{-2} s^{-1} MPa^{-1}), soil and leaf water potentials (MPa), respectively.

Although not explicitly stated in Eqn (1), K_L^{pl} is itself a function of xylem Ψ. When Ψ declines, xylem cavitation may ensue, thereby reducing the number of functional elements through which water transport occurs (Tyree and Sperry 1989). It has been suggested that vulnerability to cavitation may determine the upper limit to E_L, a limit beyond which excessive xylem tension leads to runaway cavitation and plant death (e.g. Sperry *et al.* 1998).

If the reductions in leaf size associated with greater E_L and stomatal conductance (g_s, mmol m^{-2} s^{-1}), are to be explained hydraulically, i.e. with the need of maintaining equilibrium between transpiring surface area and supporting xylem tissues, then variability among plants or cultivars in stomatal behaviour must be associated with variability in hydraulic characteristics. We investigated the presence of this association for 12 pinto bean cultivars (*Phaseolus vulgaris* L.) selected to represent a wide range of growing conditions within the American continent. The cultivated common bean grows from the wet and relatively warm mid-elevations of the Andes to the hot and arid plains of northern Mexico and south-western USA. The native wild bean, predecessor of the modern cultivars, also occupies much of this range, although more limited to specific microsites (Gentry 1969). Adaptation to local habitats exploited by selection has resulted in a number of differences in habit and physiology, one of which is the size of the mature trifoliate leaflet (e.g. Singh *et al*. 1991).

In a previous investigation (Comstock and Ehleringer 1993), large differences were found in maximum stomatal opening at low $\Delta\omega$ for the same 12 cultivars, while subsequent closure at higher $\Delta\omega$ was very similar in percentage terms. The response was such that the maximum E_L characteristic of each cultivar was maintained over a large range of $\Delta\omega$ (Comstock and Ehleringer 1993). The findings were interpreted to indicate that a homeostatic mechanism may be operating to stabilise E_L (and hence Ψ_l) under a range of environmental conditions. Because maximum E_L varied largely among cultivars, we predicted that similar differences could be found in hydraulic transport properties.

Specifically, we tested the null hypotheses (i) that leaf gas exchange was not inversely related to leaf size, and (ii) that cultivar variability in leaf-level gas-exchange was not correlated with whole-plant water transport properties.

Materials and methods

Experimental material

Twelve common bean cultivars of the 'pinto bean' type were selected for analysis, based on previous work by Comstock and Ehleringer (1993).

Common bean does not have a unique known centre of origin and germoplasm is classified into two centres of diversity, a South American one, called Andean, and a Central-North American one, called Middle American. Each centre is subdivided into races characterised by specific morphological, physiological and genetic characters (Singh *et al*. 1991). A list of the 12 cultivars grouped by their centres of origin and race is given in Table 1. Durango cultivars are grown in the United States and Mexico, Nueva Grenada and Mesoamerica cultivars in Central-South America (i.e. their centres of domestication: Ehleringer *et al.* 1991). Some of the Durango cultivars have been selected for use in irrigated, others in rainfed conditions. Cultivars were ranked from 1 to 5 based on the length of the period necessary to reach final crop maturation (degree of maturity, Table 1). The ranks were as in Zacharisen *et al.* (1998), with the only difference that late-maturing cultivars were given a rank of 5 instead of 4 to improve linearity of response.

Greenhouse propagation

For each of the 12 cultivars, two cohorts of two individuals each were grown during October–November 1996 in the greenhouses of Boyce Thompson Institute (Ithaca, NY, USA). Planting of the second cohort was staggered 1 week after the first one, allowing time to perform measurements on each plant.

Seeds were germinated in seed trays under a shade cloth. After germination, seedlings were transplanted into 5 L pots and transferred into a new greenhouse bay. Soil was prepared by thoroughly mixing fritted clay (Turface), silica sand, pasteurised soil, vermiculite and peat (6:2:2:2:1, by volume) amended with dolomitic lime, gypsum, superphosphate and Micromax. Plants were watered twice a day and periodically fertilised throughout the experiment.

Environmental conditions within the greenhouses were controlled and continuously monitored. Plants received supplementary lighting using a combination of Na-vapour and metal halide lamps. Photoperiod was 12 h. Day/night time conditions were approximately 30/20 °C, 40/80% relative humidity, and 375/390 μmol mol^{-1} CO_2. A set of several rotating fans continuously stirred the air during growth. Stirring was strong enough to cause leaf fluttering. The greenhouse was equipped with extra fans and vents to

---

List of abbreviations used in the text

Leaf-level variables	*Definition*	*Units*
A_L	Average area of one leaflet	m^2
g_{tot}, g_s, g_{bl}	Total, stomatal and boundary layer conductance	mmol m^{-2} s^{-1}
$\Delta\omega, \Delta\omega_s$	Leaf-to-air and leaf-to leaf surface vapour pressure difference	mmol mol^{-1}
E_L	Transpiration rate per unit leaf area	mmol m^{-2} s^{-1}
$K_L^{pl}, K_L^{xyl}, K_L^{r}, K_L^{sh}, K_L^{e-xyl}$	Leaf-specific hydraulic conductance measured for the whole-plant hydraulic pathway, and separately for xylem, root and shoot xylem, extra-xylary pathway	mmol m^{-2} s^{-1} MPa^{-1}
Ψ_s, Ψ_l	Soil and leaf water potential	MPa
%N, [N]	Percentage leaf N per unit mass and N concentration per unit area	unitless, g m^{-2}
SLA	Specific leaf area	cm^2 g^{-1}
δ^{13}C, Δ^{13}C	Isotope ratio and C isotope discrimination	(‰)
Plant-level variables		
A^{pl}	Plant crown area	m^2
E^{pl}	Plant transpiration rate	mmol s^{-1}
$K^{pl}, K^{xyl}, K^r, K^{sh}, K^{e-xyl}$	Hydraulic conductance for the whole plant hydraulic pathway, and separately for xylem, root and shoot xylem, extra-xylary pathway	mmol s^{-1} MPa^{-1}
$R^{pl}, R^{xyl}, R^r, R^{sh}, R^{e-xyl}$	Equivalent resistances	MPa s mmol^{-1}

Table 1. Geographic provenance of the 12 cultivars used in this experiment

Cultivars are divided according to their centre of origin and race. Degree of maturity is ranked in classes from 1 to 5 according to Zacharisen *et al.* (1998) and indicates the length of the period from emergence to crop maturation

Cultivar	Centre of origin	Race	Type of agriculture	Degree of maturity
G4523	Andean	Nueva Grenada	Rainfed	2
G5201	Middle America	Mesoamerica	Rainfed	5
A54	Middle America	Mesoamerica	Rainfed	3
San Christobal	Middle America	Mesoamerica	Rainfed	5
CO 22625	Middle America	Durango	Irrigated	2
CO 33142	Middle America	Durango	Irrigated	1
Othello	Middle America	Durango	Irrigated	1
UNS 117	Middle America	Durango	Irrigated	2
Victor	Middle America	Durango	Irrigated	3
CZ18-13183	Middle America	Durango	Rainfed	2
San Juan Select	Middle America	Durango	Rainfed	3
Viva	Middle America	Durango	Rainfed	3

ensure high air turn-over rates, such that greenhouse air $[CO_2]$ was always within 5 μmol mol^{-1} of the outside air.

Measurement protocol

Measurements started when plants were less than 4 weeks old (after emergence), about 1 week before appearance of the first flowers. During Day 1 of the experiment, leaf stomatal conductance, plant transpiration rate, and transpiring leaf water potential were measured on one individual per cultivar (12 plants). On Day 2, plants were brought to the laboratory and destructively sampled for whole-root and whole-shoot xylem hydraulic conductance, and total leaf area. On Days 3 and 4, the experimental protocol was repeated for the second set of 12 plants of the same cohort. Since the two cohorts were staggered by 1 week at planting, the following week the protocol was repeated again from Day 1 to 4, giving a total of 48 plants for the 12 cultivars.

Stomatal conductance and transpiration rates

Plants were watered in the early morning and the excess water drained. The pots were subsequently tightly enclosed in plastic bags to prevent soil evaporation. A first measure of pot mass was taken. Stomatal conductance, g_s, was measured using a Li-Cor 1600 porometer, together with photosynthetic active radiation, leaf temperature and relative humidity. The porometer was brought into the greenhouse to equilibrate with ambient humidity at least 1 h before the measurement session. Three complete rounds of porometry measurements were taken for each individual, at 1000, 1130, and 1300 h local time. Leaf abaxial and adaxial conductances were sequentially measured on three labelled leaves per plant. Steady-state near-maximum stomatal conductances were obtained by periodic watering of the greenhouse floor. This reduced $\Delta\omega$ to about 17 mmol mol^{-1} on average. Average photosynthetic active radiation ($\pm$s.e.) was 775 μmol m^{-2} s^{-1} ($\pm$12).

Leaves used for porometry were healthy, fully expanded trifoliate leaves under no or limited shading by adjacent leaves. Normally, the first set of fully expanded trifoliate leaves from the top was selected. During analysis, a reduced light exposure effect became evident for some leaves. To eliminate this light-dependency, an empirical light response curve was fitted to the dataset (cf. Jones 1992) and values adjusted accordingly.

Between each porometry session and at the end, pot mass was measured again. From the recorded times, three measurements of plant transpiration rates E^{pl} (mmol s^{-1}) were obtained and the average taken. Transpiration rates per unit of leaf area, E_L, were then calculated from E^{pl} and plant crown area, A^{pl} (m^2). The plastic bags were removed from the pots at the end of the last porometry session.

Leaf water potentials

At the end of the gas-exchange session (normally around 1400), two of the three labelled leaves used for porometry measurements were sampled for transpiring leaf water potential, Ψ_l (MPa). Each leaf was inserted into a plastic bag lined with wet tissue, cut at the base and immediately stored in an insulated ice-filled box. In the laboratory, leaves were inserted into a Scholander-type pressure bomb (Plant Water Stress Inc., CA, USA), whose chamber was lined with wet paper tissue, and the balancing pressure recorded. Water potential measurements were made within 5 min of collection.

Carbon isotope discrimination

The two leaves used for stomatal conductance and water potential measurements were subsequently measured for their area and mass. They were then oven-dried, finely ground to a powder, pooled to produce a single sample per cultivar, and analysed for total N and $\delta^{13}C$. Isotope ratios and percentage nitrogen were determined in the Cornell Laboratory for Stable Isotope Analysis using continuous flow in a triple collecting gas-source Isotope Ratio Mass Spectrometer (Europa, model Geo 20:20, Crewe, England).

Plant carbon isotope discrimination ($\Delta^{13}C$) was calculated from carbon isotope ratios (against the PDB standard) using a value for δ_{air} of -8‰, which is appropriate for our conditions, given the high turnover rates present in the greenhouse. $\Delta^{13}C$ represents a time-integrated measurement of photosynthetic gas exchange, since it is related to c_i/c_a, the ratio of CO_2 concentration in the leaf intercellular spaces to that in the atmosphere (Farquhar *et al.* 1989). The ratio c_i/c_a is mainly determined by the balance between the supply rate of CO_2 through the stomata and the rate of CO_2 uptake by the chloroplasts. Plant discrimination can formally be linked to c_i/c_a by the expression (Farquhar *et al.* 1982):

$$\Delta = a + (b - a)\frac{c_i}{c_a} \qquad (2)$$

where a is the fractionation occurring due to gas diffusion in air (4.4‰), and b is the net fractionation caused by carboxylation (mainly discrimination by RuBP carboxylase, about 27‰).

Measurements of xylary and extra-xylary components of native hydraulic conductance

On Days 2 and 4 of both weeks, a set of 12 plants was brought to the laboratory. All foliage was removed by cutting at the base of the leaves with a new sharp razor blade, leaving all petioles on the shoot. The base of the

shoot was then cut under water. While the shoot was being measured, roots were carefully extracted from the soil by hand, recut under water at the collar junction and kept damp until measured.

Native hydraulic conductance for entire root and shoot systems was measured using a vacuum canister system (Kolb *et al*. 1996). Shoots and roots were loaded into a cylindrical PVC canister, which could be sealed and linked to a vacuum pump. A separate valve was connected to a precision vacuum gauge measuring the canister vacuum depression. The cut stem of the plant was connected via rigid nylon tubing to a top-loaded balance. Under the depression created by the vacuum pump in the canister, water flowed from the container on the balance into the xylem in the normal direction of transpiration for shoots, and in the reverse direction for roots. Water had previously been distilled, deionised, filtered, degassed and acidified as in Mencuccini and Comstock (1997). Once the system had stabilised, near-constant flow rates could be maintained in well-watered plants for several hours. Preliminary tests had shown that applied pressure was linearly related to flow rates over most of the range of vacuum depression. Water flow was measured at atmospheric pressure (plus a gravity head of about 4 kPa and at two levels of atmospheric depression, i.e. 6.7 and 13.3 kPa) and hydraulic conductance, K, was calculated as the slope of the regression line. Since native conductance values were being measured, plants were not perfused.

Reverse flow into roots may potentially create unsteady and artificially low flow rates, due to the progressive accumulation of solutes at a putative osmotic barrier (e.g. the endodermis). However, manual root extraction from the soil likely removed all the very fine root tips. As a consequence, no such complication was apparent during measurement (cf. Kolb *et al*. 1996).

Total xylem hydraulic resistance (above- plus below-ground) was obtained from the sum of the shoot and root conductances in series:

$$R^{xyl} = \frac{1}{K^{xyl}} = \left(\frac{1}{K^{sh}} + \frac{1}{K^{r}} \right), \tag{3}$$

where K^{xyl}, K^{sh} and K^{r} are total xylem conductance and the two components of root and shoot conductance respectively (mmol s^{-1} MPa^{-1}), and R^{xyl} is the respective resistance (MPa s mmol^{-1}).

Whole-plant hydraulic resistance, R^{pl}, was estimated by the ratio of leaf water potential and whole-plant transpiration rate, assuming that soil water potential was zero, i.e.:

$$R^{pl} = \frac{\Psi_s - \Psi_1}{E^{pl}} \approx \frac{-\Psi_1}{E^{pl}}. \tag{4}$$

This allowed the calculation of the extra-xylary components of total resistance, R^{e-xyl}, by difference:

$$R^{e-xyl} = R^{pl} - R^{xyl}. \tag{5}$$

Extra-xylary resistance is likely to have been consistently overestimated because no account was taken of xylem resistance within leaf veins or due to a non-zero Ψ_s.

Leaf-specific hydraulic conductances K_L were calculated for each portion of the pathway by dividing the corresponding K by plant crown area.

Leaf area measurements

Trifoliate leaf blades were separated and counted, and their area was measured using a leaf area meter (Li-Cor 3000, NE, USA). To obtain an estimate of maximum leaflet size for each cultivar, the 10 largest leaflets per plant (cotyledons excluded) were selected and their width, length and area measured. To avoid confusion with the area of an individual leaf, the term 'crown area' is used to indicate total plant leaf area.

Statistical analyses

Differences among cultivars, centres of origin or domestication centres were analysed using a two-factor analysis of variance, with sampling date as the second factor. An LSD test was then used to test for differences among specific cultivars or centres.

Results

Morphological properties

Plant crown area, 4 weeks after emergence, varied between 0.18 and 0.35 m^2 for Othello and G4523, respectively. A multiple range test showed G4523 to have significantly greater plant crown area ($P<0.05$) than Othello, G5201, UNS117, San Christobal and CZ18-13183 (Table 2). Number of trifoliate leaves and leaflet area (total crown area divided by number of leaflets) also varied significantly among cultivars (Table 2). Differences in both parameters

Table 2.   ANOVA analyses for a range of morphological and physiological characters of 12 common bean cultivars.

(a) Plant leaf area

Main effects	SS	df	MS	F
Cultivar	0.149	11	0.0112	2.31*
Date	0.124	3	0.0104	2.14[ns]
Residual	0.146	30	0.0048	

(b) Leaflet size

Main effects	SS	df	MS	F
Cultivar	5.444×10^{-5}	11	4.95×10^{-6}	5.08***
Date	1.753×10^{-7}	3	5.84×10^{-8}	0.98[ns]
Residual	2.534×10^{-5}	26	9.75×10^{-7}	

(c) Number of leaves

Main effects	SS	df	MS	F
Cultivar	4904.64	11	445.88	2.61*
Date	1619.34	3	539.78	3.16*
Residual	4442.16	26	170.85	

(d) Stomatal conductance

Main effects	SS	df	MS	F
Cultivar	504785.8	11	45889.6	7.38***
Date	97928.2	3	32642.7	5.25**
Residual	186461.2	30	6215.4	

(e) Leaf transpiration rate

Main effects	SS	df	MS	F
Cultivar	0.00204	11	1.86×10^{-4}	1.43[ns]
Date	0.00165	2	8.26×10^{-4}	6.37**
Residual	0.00259	20	1.30×10^{-4}	

(f) Leaf transpiration rate

Main effects	SS	df	MS	F
Domestication centre	0.00092	1	9.19×10^{-4}	7.41*
Date	0.00162	2	8.08×10^{-4}	6.37**
Residual	0.00372	20	1.24×10^{-4}	

(g) Leaf water potential

Main effects	SS	df	MS	F
Cultivar	0.1289	11	0.0117	1.16[ns]
Date	0.2507	3	0.0836	8.28***
Residual	0.2185	30	0.0101	

were more highly significant when cultivars were grouped according to whether they belonged to the North American or to the Central–South American centres of domestication (data not given).

Cultivars with large leaflets had significantly larger crowns ($P < 0.05$); however, some large-leafed slow-growing cultivars appeared to fall on different regression lines. When degree of maturity was taken into account as a second variable, this effect disappeared and the relationship between leaf and crown size became highly significant ($P < 0.01$). Similar relationships were found when leaflet size was estimated as average area of the 10 largest leaflets.

Gas exchange properties

Average g_s varied significantly among sampling dates and among cultivars (Table 2). Average g_s ranged from 441 to 845 mmol m^{-2} s^{-1} for G4523 and for Viva, respectively. When grouped by their domestication centre (but not when considered individually), cultivars differed significantly also for their transpiration rates per unit of leaf area. Stomatal conductance was strongly and negatively associated with leaflet size, expressed as average leaflet area (Fig. 1, $P < 0.01$), or as average area of the 10 largest leaflets ($R^2 = 0.62$, $P < 0.01$). While E_L also varied inversely with leaflet area ($P < 0.01$, Fig. 2A), whole-plant water loss E^{pl} showed a positive relationship with crown size ($P < 0.01$, Fig. 2B).
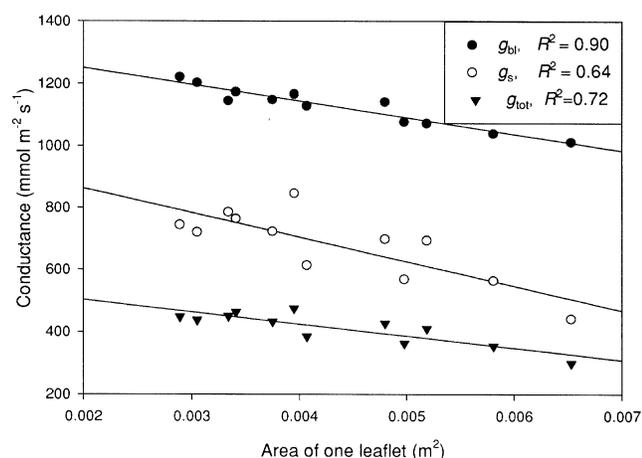

Fig. 1. Relationships between average area of individual leaflets and vapour-phase conductances g of 12 common bean cultivars ($n = 4$ per cultivar). Boundary-layer conductance (g_{bl}, ●) and total conductance (stomatal plus boundary layer, g_{tot}, ▲) are plotted alongside stomatal conductance, g_s (○). R^2, proportion of variance explained by the regressions. Stomatal conductance was measured at three times (1000, 1130 and 1300 h) and the average is plotted. Boundary layer conductances were estimated from leaf characteristic dimensions d and a wind speed of 1 m s^{-1}, which is close to the average wind speed in our greenhouses. Leaf d values were estimated assuming a rhomboidal leaf shape and wind blowing from all directions (i.e. $d = 0.866 \times$ leaf width), as appropriate for our greenhouse conditions. Very similar regressions were obtained when the average size of the 10 largest leaflets was used, instead of average leaflet area.
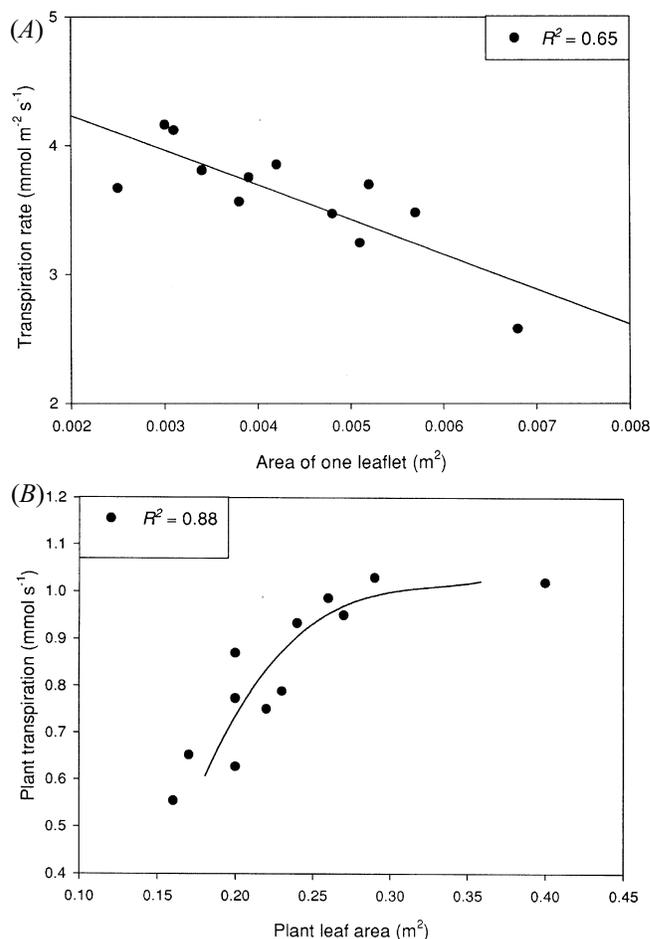

Fig. 2. *(A)* Relationship between average area of individual leaflets and transpiration rate per unit of leaf area of 12 common bean cultivars ($n = 3$ per cultivar). R^2 is the proportion of variance explained by the regression. A very similar regression was obtained when average area of the 10 largest leaflets was used, instead of the average area of one leaflet.
(B) Relationship between plant leaf area and whole-plant water loss for 12 bean cultivars ($n = 3$ per cultivar). Plants with large crowns were also characterised by large leaflets. The reduction in slope with increasing leaf area indicates that transpiration per unit leaf area declines in large-leafed cultivars. R^2 is the proportion of variance explained by the regression. Plant water loss was measured by periodically weighing the pots. Transpiration rate was determined from plant water loss and leaf area.

Hydraulic properties and their relationships with gas exchange

Whole plant and xylem K varied between 0.64 and 1.27 mmol s^{-1} MPa^{-1} for Othello and G4523 and between 1.88 and 4.01 mmol s^{-1} MPa^{-1} for G5201 and San Juan, respectively. Root and shoot contributed almost equally to xylem resistance, while xylem resistance accounted for about 30–50% of total plant resistance, this percentage being positively related to plant crown area ($P < 0.01$).

Similar to the case for crown size, K^r, K^{sh}, K^{xyl}, K^{e-xyl} and K^{pl} were all positively related to leaflet size and inversely related to degree of maturity (all $P < 0.01$).
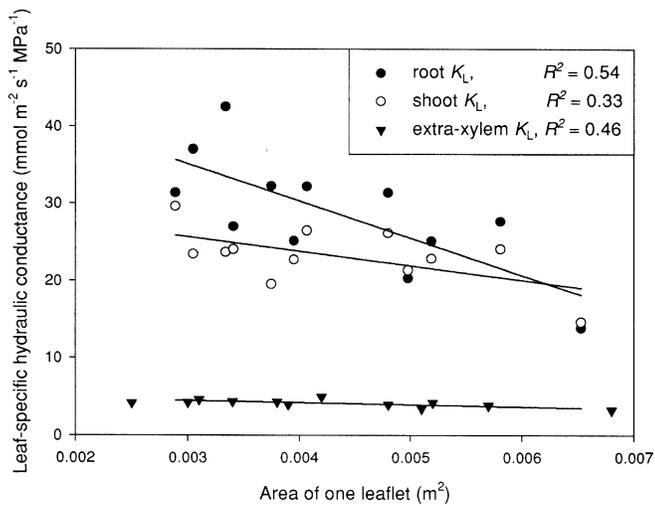
Fig. 3. Relationships between leaflet area and 'leaf-specific' hydraulic conductance K_L of the xylary (roots and shoots separately) and extra-xylary portions of the water-flow pathway. Leaf-specific hydraulic conductance K_L of the extra-xylary portion of the pathway was almost constant among cultivars, but K_L of the xylary portion declined with increasing leaflet area, particularly so in the root system. Xylem hydraulic resistance (sum of root and shoot xylem resistance in series) accounted for 30–50% of total hydraulic resistance and was significantly greater in large-crowned cultivars (see text).

As expected, the above conductances were all significantly related to whole-plant water loss E^{pl}, and none of the log–log regression exponents was different from 1, i.e. the relationships were linear (Table 3). Isometric scaling between plant hydraulic conductance and water loss suggests that constant water potential gradients were maintained in the different portions of the soil–plant system (see Eqn 1). This was confirmed by direct measurement. Midday leaf water potentials varied significantly among the 4 days of measurements, but were not significantly different among cultivars or among centres of domestication (Table 2).

Similar to the cases for g_s and E_L (Figs 1 and 2), values of 'leaf-specific' hydraulic conductance K_L^r and K_L^{sh}, as well as their sum in series, K_L^{xyl}, declined with increasing leaflet area (Fig. 3). Conversely, K_L^{e-xyl} showed only limited variability (Fig. 3).

Since both hydraulic and gas-exchange properties varied with leaflet area, not surprisingly they were also significantly related to one another. Stomatal, total leaf conductance and transpiration rate were all significantly related to various expressions of 'leaf-specific' hydraulic conductance, the best regressions being with root K_L (Table 4 and Fig. 4A and 4B). With E_L however, interpretation is more difficult because both variables were calculated by dividing by plant crown area. To confirm the presence of significant correlations, the relationships between E^{pl} and various expressions of total hydraulic conductance (K^{pl}, K^{xyl}, K^{e-xyl}, K^r, K^{sh}) were tested, with plant crown area used as a second independent variable. After accounting for crown area, changes in E^{pl} could largely

Table 3. Allometric scaling of various measures of hydraulic conductance K with whole-plant water loss

K was measured separately for roots (K^r), shoots (K^{sh}), xylem (root + shoot, K^{xyl}) and for the whole plant (K^{pl}) (n=12). The extra-xylary component (K^{e-xyl}) was obtained by difference (see text). Regressions have the form: $\ln(K) = a + m \times \ln(E^{pl})$, where E^{pl} is plant transpiration rate; m, F and Sign. are regression coefficient (i.e. slope), F-test and significance level for the regression. Regression coefficients for the log–log regression with plant crown area are also given in parentheses. Note that, for K^{pl} and K^{e-xyl}, values are inflated by autocorrelation. If the regression coefficient is followed by the letter [a], it is significantly lower than one ($P < 0.05$); if by the letter [b], it is not significantly different from 1; ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

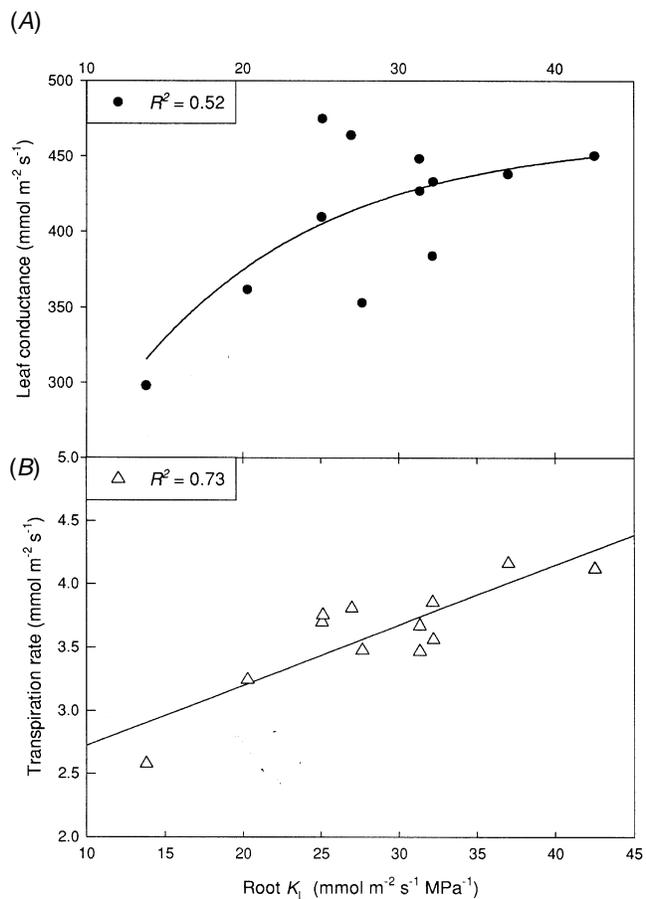| Property	m		F	Sign.
K^r	0.895[b]	(0.285[a])	11.5	**
K^{sh}	0.523[b]	(0.421[a])	5.7	*
K^{xyl}	0.666[b]	(0.308[a])	14.6	**
K^{e-xyl}	1.309[b]	(1.105[b])	40.8	***
K^{pl}	1.067[b]	(0.813[a])	98.1	***

Fig. 4. Relationships between root leaf-specific hydraulic conductance K_L and (A) leaf conductance (sum of stomatal plus boundary layer) and (B) leaf transpiration rate. R^2, proportion of variance explained by the regressions. Note that in (B) the coefficient of the regression is likely to be inflated by autocorrelation, since both variables share the same denominator. See text and Table 4 for further details and an alternative treatment of the data.

Table 4. **Regressions between gas exchange properties (stomatal conductance in (*a*), transpiration rate in (*b*) and 'leaf-specific' hydraulic conductance K_L of different portions of the hydraulic pathway for 12 common bean cultivars**

(*a*) Regressions had the form: $\ln(g_s) = a + b \times \ln(K_L)$, where K_L, 'leaf-specific' hydraulic conductance. R^2, proportion of variance explained by the regression; F, F-test for the regression; Sign., significance level of the regression; ns, not significant; *, $P < 0.05$; **, $P < 0.01$

(*b*) To avoid autocorrelation, regressions had the form: $\ln(E^{pl}) = a + b \times \ln(K) + c \times \ln(A^{pl})$, where E^{pl}, whole-plant water loss, K hydraulic conductance and A^{pl}, plant leaf area. R^2, proportion of variance explained by the multiple regression; t, t-test for the significance of K alone; P, significance level of K alone: ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

(*a*) Stomatal conductance

Property	R^2	F	Sign.
K_L^r	0.47	8.91	*
K_L^{sh}	0.20	2.53	ns
K_L^{xyl}	0.35	5.42	*
$K_L^{e\text{-}xyl}$	0.07	0.83	ns
K_L^{pl}	0.45	8.34	*

(*b*) Transpiration rate

Property	R^2	t	P
K^r	0.93	74.65	**
K^{sh}	0.74	16.88	ns
K^{xyl}	0.89	43.61	**
$K^{e\text{-}xyl}$	0.77	19.17	ns
K^{pl}	0.89	44.68	***

Fig. 5. Results of a leaf energy-balance model used to assess the independent effects of changes in g_s (stomatal conductance) and g_{bl} (boundary-layer conductance) on the leaf-to-air temperature differences ΔT. Results are plotted as a function of the leaf characteristic dimension of each cultivar. g_{bl} was calculated as for Fig. 1. For one set of simulations, g_s was assumed constant for all cultivars at the level of G4523 (continuous line with no circles) and only leaf size (hence g_{bl}) was allowed to vary. Small-leafed cultivars tended to have lower leaf T but the effect was small. The second set (continuous line with black circles) represents a similar case, but now a stomatal response to $\Delta\omega_s$ was introduced. This set shows the interaction between changes in leaf size and stomatal behaviour, due to altered conditions at the leaf surface. In the third set (empty circles and broken-line curve), g_s was allowed to vary alongside g_{bl}, as measured. This produced a much greater range of temperature responses among cultivars. These results are typical of other simulations, but the magnitude of the leaf-to-air ΔT would change accordingly. Other conditions: air temperature, 30°C; relative humidity, 60%; wind speed, 0.6 m s^{-1}; net radiation, 400 W m^{-2}.

be explained by changes in hydraulic conductance (Table 4). This implies a significant relation between K_L and E_L, i.e., there was a balance between unit-leaf water loss and 'leaf-specific' hydraulic conductance. Similar to the case for g_s, regressions on shoot K and extra-xylary K were not significant (Table 4). Compared with g_s, E_L was always more linearly related to measures of liquid-phase transport capacity.

Carbon isotope discrimination and specific leaf area

Short-term variability among cultivars in gas-exchange was confirmed using $\Delta^{13}C$ measurements. $\Delta^{13}C$ varied between 19.25‰ and 21.38‰ for G4523 and Othello, respectively. $\Delta^{13}C$ was positively associated with g_s, E_L, K_L^{xyl} and K_L^{pl}, and negatively related to A_L and A^{pl}. $\Delta^{13}C$ was not significantly related to $K_L^{e\text{-}xyl}$, degree of maturity, Ψ_l, leaf N concentration (whether on a leaf mass or a leaf area basis) or specific leaf area (Table 5).

Discussion and conclusions

Variability in gas-exchange properties among common bean cultivars has already been reported (e.g. Peet *et al*. 1977; Hidalgo 1978). The differences in g_s and E_L among these 12 cultivars largely correspond with previous work on stomatal responses to $\Delta\omega$ (Comstock and Ehleringer 1993), with cultivars from the Nueva Grenada and the MesoAmerica races showing lower rates than cultivars from the Durango
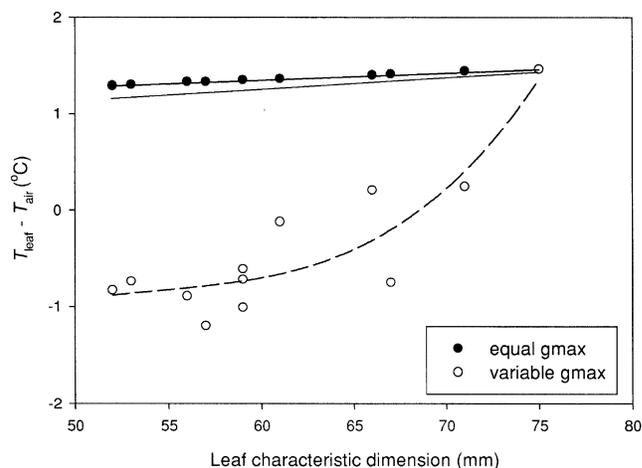
race. Using a different set of cultivars, Ehleringer *et al.* (1991) were able to show significant differences in long-term water use efficiency (so-called transpiration efficiency) and in $\Delta^{13}C$ between cultivars developed for use in Central-South America and those developed for use in North America. Finally, the positive association between $\Delta^{13}C$ and g_s has already been shown for common bean by Ehleringer (1990) and Comstock and Ehleringer (1993), who also reported non-significant correlations between Δ, leaf nitrogen concentration and specific leaf area (see also White *et al.* 1990).

It is not surprising that differences among cultivars for g_s were larger than for E_L, since the sampling protocol specifically aimed at increasing stomatal conductance by raising ambient humidity at the time of sampling (obtained by watering the greenhouse floor). While this increased the likelihood of finding significant differences in g_s among cultivars, it also reduced transpiration rates. Because of the shape of stomatal response to $\Delta\omega$, this reduction was particularly large for cultivars with high values of g_s, which probably explains why whole-plant water losses were lower for them (Fig. 2*B*).

The inverse relationships between g_s, E_L, $\Delta^{13}C$ and leaf size (Figs 1 and 2; Table 5) have not been reported before for

Table 5. Correlation coefficients between $\Delta^{13}C$ (leaf carbon isotope discrimination), gas exchange and hydraulic properties of twelve common bean cultivars

g_s, stomatal conductance; E_L, transpiration rate per unit of leaf area; E^{pl}, whole-plant transpiration; A^{pl}, plant crown area; A^L, average area of one leaflet; K_L^{pl}, plant leaf-specific hydraulic conductance; K_L^{xyl}, xylem leaf-specific hydraulic conductance; K_L^r, root leaf-specific hydraulic conductance; K_L^{sh}, shoot leaf-specific hydraulic conductance; K_L^{e-xyl}, extra-xylary leaf-specific hydraulic conductance; Ψ_l, leaf water potential; degree of maturity, number of days from emergence to crop maturity (grouped in ranks from 1 to 5); %N and [N], percentage leaf nitrogen (per unit mass) and N concentration (per unit area); SLA, specific leaf area. Sign., level of significance:

ns, not significant; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$

Property	Correlation coefficient	Sign.
g_s	0.64	*
E_L	0.89	***
E^{pl}	−0.53	ns
A^{pl}	−0.85	**
A_L	−0.78	**
K_L^{pl}	0.75	**
K_L^{xyl}	0.91	***
K_L^r	0.77	**
K_L^{sh}	0.73	*
K_L^{e-xyl}	0.14	ns
Ψ_l	0.36	ns
Degree of maturity	−0.17	ns
% N	−0.16	ns
[N]	0.26	ns
SLA	−0.59	ns

common bean. However, they are likely to have been present in other studies as well, although unnoticed. To demonstrate the point, we looked in the literature for $\Delta^{13}C$ values for the same 12 cultivars. We found data for a field trial in Cortez (CO, USA, Comstock and Ehleringer 1993), for a greenhouse study at the University of Utah (Salt Lake City, UT, USA, Comstock and Ehleringer 1993) and for a field trial at the SouthWest Research Center (Yellow Jacket, CO, USA, Zacharisen *et al.* in press). Significant relationships were found between the ranked measures of leaflet size of this experiment and all $\Delta^{13}C$ ($r=-0.64$, -0.58, -0.71 respectively, all $P<0.05$, Spearman correlation coefficients), in the same direction as the one found in this work (Table 5). Coefficients were all highly significant ($P<0.01$) when a parametric test was used. The presence of a significant negative association between long-term gas exchange properties and leaf size under four largely different environmental conditions (two greenhouse experiments and two field trials) suggests that a genetic link exists between leaf morphology and time-integrated physiological behaviour. This interpretation may help explain why leaf size is so strongly correlated with molecular markers of adaptive significance for a large range of cultivars (Singh *et al.* 1991).

Comstock and Ehleringer (1993) proposed that the stability of maximum E_L over a large range of $\Delta\omega$ could be inter-

preted as indicating the presence of a homeostatic mechanism regulating stomatal sensitivity to $\Delta\omega$ so that excessively negative Ψ_l were avoided under stressful atmospheric conditions. If that is the case, then variability in E_{max} among cultivars must be associated with variability in water transport capacity. Also, no difference in Ψ_l should be apparent (see Eqn.1). Both predictions are supported by our data and give credit to the idea that stomatal behaviour and whole-plant hydraulic characters are functionally coupled.

Root pressurisation experiments have recently shown the existence of direct short-term control of stomatal conductance by Ψ_l or some other signal linked to Ψ_l (Saliendra *et al.* 1995; Fuchs and Livingston 1996; Comstock and Mencuccini 1998). The same result has also been recently found for 2 of these 12 cultivars (Mencuccini and Comstock, unpublished data). Whether or not Ψ_l is directly involved in controlling stomatal activity, coupling of leaf-level properties to plant hydraulic capacity will ensure that excessive levels of xylem tensions are avoided, reducing the risks of runaway cavitation. Because we measured only native hydraulic conductance, we cannot determine whether these cultivars operated so that xylem embolism was completely avoided or whether some limited level of cavitation was tolerated in order to optimise gas exchange. Analyses of the vulnerability to cavitation for these cultivars will help to clarify this point.

Among all regressions between K_L and gas-exchange parameters (g_s and E_L), the ones based on root K_L always explained the largest proportion of variance (Table 4 and Fig. 4). This result agrees with previous reports on the importance of root systems in common bean (e.g. White *et al.* 1990). A more efficient root water transport system may also be crucial during drought and may explain the differences in E_L previously attributed to a variable root system depth (White *et al.* 1990).

It is noteworthy, that xylem hydraulic resistance always accounted for at least about 30% of the plant viscous flow resistance. Based mainly on studies of water transport of individual roots and of young seedlings, the conclusion is often made that in crop plants axial xylem resistance is negligible compared to resistance to radial flow (i.e. extra-xylary) in roots and leaves (e.g. Passioura 1988; Frensch and Steudle 1989). Our work is the first to directly partition whole-plant resistance between these two components (Eqn 4) and supports the contention that xylem resistance may play a significant role also in some crop species. In cultivars characterised by large crowns, xylem resistance accounted for about half of total resistance, largely because xylem conductance did not scale proportionally to crown size. It is possible that, when a large fraction of dry matter is allocated to leaves, a trade-off arises with the need for more transport tissue in the xylem. It is also possible that xylem structure is optimised in relation to the plant's biomechanical requirements, not the hydraulic ones. The cultivar with the largest fraction of hydraulic resis-

tance in the xylem (G4523) was also the one displaying the least tendency to climbing and a more clearly developed bushy appearance. A self-supporting habit may require a greater fraction of xylem tissue to be devoted to mechanical support rather than to hydraulic transport (e.g. Gartner 1991).

We cannot separate what we bulked into extra-xylary hydraulic resistance into its root and leaf components. However, it is interesting to note that K_L^{e-xyl} was almost constant among cultivars (Fig. 3), indicating an almost perfect isometric scaling between plant crown area and absolute K^{e-xyl}. If the extra-xylary hydraulic resistance was primarily located within leaves, one would expect that an increase in crown size would lead to an equivalent increase in the number of parallel pathways for water flow, thereby leading to proportional increases in K^{e-xyl}.

Under a greenhouse, conditions at the leaf surface were likely different for each cultivar, depending on the average size of their leaves. Reductions in leaf size will tend to increase $\Delta\omega_s$ (i.e. the gradient across the leaf surface, now closer to $\Delta\omega$) and to decrease leaf T (as a consequence of the increased E_L). As far as g_s is concerned, these effects would partially oppose one another but together they would tend to slightly reduce g_s. Therefore, had the experiment been performed under constant conditions at the leaf surface, greater differences in stomatal aperture would have likely been found across cultivars.

The covariation of leaf size, g_s and E_L must have important implications for leaf and crown energy balance. In order to gain some insights into the relative importance of these properties in affecting leaf temperature, a leaf energy-balance model (cf. Comstock and Ehleringer 1993) was parameterised using the data on leaf size and stomatal conductance. Simulations were conducted under different scenarios. In the first case (Fig. 5, continuous line with no dots), it was assumed that all cultivars had the same value of g_s (set at the level of G4523) and only differed by the size of their leaves (i.e. g_{bl}). The second case was similar, but a stomatal response to $\Delta\omega_s$ was introduced (line with black dots). In the final case, both maximum g_s and g_{bl} were allowed to vary among cultivars (empty dots with broken-line curve). Reductions in leaf size did decrease leaf T, but only marginally, and even less so when a response to humidity was introduced, as a consequence of the increased $\Delta\omega_s$ and subsequent stomatal closure in small leaves. By contrast, changes in maximum g_s strongly affected leaf T.

The negligible effect of a twofold change in leaf size on boundary layer conductance was not unexpected. Since the leaf characteristic dimension d roughly scales with leaflet area A_L as $d \propto A_L^{0.5}$, while the boundary layer thickness w varies with d and wind speed v as (p. 284, Gates 1980) $w \propto d^{0.5}/v^{0.5}$, it follows that $w \propto A_L^{0.25}/v^{0.5}$.

Consequently, although average leaflet area changed twofold among cultivars, leaf g_{bl} probably changed only 20%, or even less if scaling coefficients appropriate for tur-

bulent heat flow over flapping leaves are used for w (Gates 1980; Van Gardingen and Grace 1991). Although the observed changes in leaf size are not likely to have a large direct impact on leaf temperature, before drawing final conclusions other factors such as leaf paraheliotropic movements (Sato 1988; Fu and Ehleringer 1991) should be tested in relation to leaf size.

Cultivar differences in g_s and E_{max} may strongly affect crop growth, both directly by varying the degree of stomatal limitation to photosynthesis and indirectly, by adjusting the leaf thermal environment. In a field trial of 96 common bean cultivars, leaf-to-air temperature differences were strongly associated with final yield (Anon. 1982).

Our analysis points to the same conclusions reached by previous authors (e.g. Lu et al. 1994; Lu and Zieger 1994; Srivastava et al. 1995). Crop species bred for maximum yield under hot and dry climates may have increased rates of leaf water loss to allow a better control of leaf temperatures. Our data also show that such a strategy must be associated with the selection of favourable traits at the whole-plant level (i.e. high 'leaf-specific' water transport capacity). In the case of common bean, this appears to be largely mediated by co-ordinated changes in single-leaf and whole-plant crown area.

References

Anonymous (1982). Bean Program. Annual Report 1982. International Center for Tropical Agriculture, Cali Colombia.

Comstock, J., and Ehleringer, J. (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.

Comstock, J., and Mencuccini, M. (1998). Control of stomatal conductance by leaf water potential in Hymenoclea salsola (T. and G.), a desert subshrub. *Plant, Cell and Environment* **21**, 1029–1038.

Cornish, K., Radin, J.W., Turcotte, E.L., Lu, Z., and Zieger, E. (1991). Enhanced photosynthesis and stomatal conductance of Pima cotton (*Gossypium barbadense* L.) bred for increased yield. *Plant Physiology* **97**, 484–489.

Egli, D.B., Pendleton, J.W., and Peters, D.B. (1970). Photosynthetic rate of three soybean communities as related to carbon dioxide levels and solar radiation. *Agronomy Journal* **62**, 411–414.

Ehleringer, J.R. (1990). Correlations between carbon isotope discrimination and leaf conductance to water vapor in common beans. *Plant Physiology* **93**, 1422–1425.

Ehleringer, J.R., Klassen, S., Clayton, C., Sherrill, D., Fuller-Holbrook, M., Fu, Q.-N., and Cooper, T.A. (1991). Carbon isotope discrimination and transpiration efficiency in common bean. *Crop Science* **31**, 1611–1615.

Farquhar, G.D., O'Leary, M.H., and Berry, J.A. (1982). On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. *Australian Journal of Plant Physiology* **9**, 121–137.

Farquhar, G.D., Ehleringer, J.R., and Hubick, K.T. (1989). Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* **40**, 503–537.

Frensch, J., and Steudle, E. (1989). Axial and radial hydraulic resistance to roots of maize (*Zea mays* L.). *Plant Physiology* **91**, 719–726.

Fu, Q.A., and Ehleringer, J.R. (1989). Heliotropic leaf movements in common beans controlled by air temperature. *Plant Physiology* **91,** 1162–1167.

Fuchs, E.E., and Livingston, N.J. (1996). Hydraulic control of stomatal conductance in Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) and alder (*Alnus rubra* (Bong.)) seedlings. *Plant, Cell and Environment* **19**, 1091–1098.

Gartner, B.L. (1991). Is the climbing habit of poison oak ecotypic? *Functional Ecology* **5**, 696–704.

Gates, D.M. (1980). 'Biophysical Ecology.' (Springer-Verlag: New York.)

Gentry, H.S. (1969). Origin of the common bean, *Phaseolus vulgaris*. *Economic Botany* **23**, 55–69.

Hidalgo, R. (1978). Screening for drought tolerance in dry beans (*Phaseolus vulgaris* L.). M.Sc. Thesis, Cornell Univ., Ithaca, NY, USA.

Hiebsch, C.K., Kanemasu, E.T., and Nickell, C.D. (1976). Effects of soybean leaflet type on net carbon dioxide exchange, water use and water-use-efficiency. *Canadian Journal of Plant Science* **56**, 455–458.

Jarvis, P.G., and McNaughton, K.G. (1986). Stomatal control of transpiration: scaling up from leaf to region. *Advances in Ecological Research* **15**, 1–49.

Jones, H.G. (1992). 'Plants and Microclimate.' (Cambridge University press: Cambridge.)

Kolb, K.J., Sperry, J.S., and Lamont, B.B. (1996). A method for measuring xylem hydraulic conductance and embolism in entire root and shoot systems. *Journal of Experimental Botany* **47**, 1805–1810.

Lu, Z., Radin, J.W., Turcotte, E.L., Percy, R.G., and Zieger, E. (1994). High yields of advanced lines of Pima cotton are associated with higher stomatal conductance, reduced leaf area and lower leaf temperature. *Physiologia Plantarum* **92**, 266–272.

Lu, Z., and Zieger, E. (1994). Selection for higher yields and heat resistance in Pima cotton has caused genetically determined changes in stomatal conductance. *Physiologia Plantarum* **92**, 273–278.

Mencuccini, M., and Comstock, J. (1997). Vulnerability to cavitation in populations of two desert species, *Hymenoclea salsola* and *Ambrosia dumosa*, from different climatic regions. *Journal of Experimental Botany* **48**, 1323–1334.

Nobel, P.S. (1991). 'Physicochemical and Environmental Plant Physiology.' (Academic Press: London.)

Passioura, J.B. (1988). Water transport in and to roots. *Annual Review of Plant Physiology and Plant Molecular Biology* **39**, 245–265.

Peet, M.M., Bravo, A., Wallace, D.H., and Ozbun, J.L. (1977). Photosynthesis, stomatal resistance and enzyme activities in relation to yield of field-grown dry bean varieties. *Crop Science* **17**, 287–293.

Radin, J.W., Lu, Z., Percy, R.G., and Zieger, E. (1994). Genetic variability for stomatal conductance in Pima cotton and its relation to improvements in heat adaptation. *Proceedings of the National Academy of Sciences USA* **91**, 7217–7221.

Saliendra, N.Z., Sperry, J.S., and Comstock, J. (1995). Influence of leaf water status on stomatal response to humidity, hydraulic conductance, and soil drought in *Betula occidentalis*. *Planta* **196**, 357–366.

Sato, H. (1988). Studies on leaf orientation movements in kidney beans (*Phaseolus vulgaris* L.): II. Differences among cultivars and their inheritance. *Japanese Journal of Crop Science* **57**, 340–345.

Singh, S.P., Gepts, P., and Debouck, D.G. (1991). Races of common bean (*Phaseolus vulgaris*, Fabaceae). *Economic Botany* **45**, 379–396.

Sperry, J.S., Adler, F.R., Campbell, G.S., and Comstock, J. (1998). Limitation of plant water use by rhizosphere and xylem conductance: results from a model. *Plant, Cell and Environment* **21**, 347–359.

Srivastava, A., Lu, Z., and Zieger, E. (1995). Modification of guard cell properties in advanced lines of Pima cotton bred for higher yield and heat resistance. *Plant Science* **108**, 125–131.

Tyree, M.T., and Sperry, J.S. (1989). The vulnerability of xylem to cavitation and embolism. *Annual Review of Plant Physiology and Plant Molecular Biology* **40**, 19–38.

Van Den Boogard, R., Alewijnse, D., and Lambers, H. (1997). Growth and water-use efficiency of 10 *Triticum aestivum* cultivars at different water availability in relation to allocation of biomass. *Plant, Cell and Environment* **20**, 200–210.

Van Gardingen, P., and Grace, J. (1991). Plants and wind. *Advances in Botanical Research* **18**, 189–253.

White, J.W., Castillo, J.A., and Ehleringer, J.R. (1990). Associations between productivity, root growth and carbon isotope discrimination in *Phaseolus vulgaris* under water deficit. *Australian Journal of Plant Physiology* **17**, 189–198.

Zacharisen, M.H., Brick, M.A., Fisher, A.G., and Ehleringer, J.R. (1998). Relationships between carbon isotope discrimination and field productivity of dry beans under two soil moisture regimes. *Crop Science* (in press).