

Apparent responses of stomata to transpiration and humidity in a hybrid poplar canopy

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ABSTRACT

It has been proposed that the stomatal response to humidity relies on sensing of the transpiration rate itself rather than relative humidity or the saturation deficit *per se*. We used independent measurements of stomatal conductance (g_s), transpiration (E), and leaf-to-air vapour pressure difference (V) in a hybrid poplar canopy to evaluate relationships between g_s and E and between g_s and V . Relationships between E , V and total vapour phase conductance or crown conductance (g_c) were also assessed. Conductance measurements were made on exposed and partially shaded branches over a wide range of incident solar radiation. In exposed branches, g_s appeared to decline linearly with increasing E and increasing V at both high and low irradiance. However, in a partially shaded branch, a bimodal relationship between g_s and E was observed in which g_s continued to decrease after E had reached a maximum value and began to decrease. The relationship between g_s and V for this branch was linear. Plots of g_c against E always yielded bimodal or somewhat variable relationships, whereas plots of g_c against V were invariably linear. It was not possible to derive a unique relationship between conductance and E or V because prevailing radiation partially determined the operating range for conductance. Normalization of data by radiation served to linearize responses observed within the same day or type of day, but even after normalization, data collected on partly cloudy days could not be used to predict stomatal behaviour on clear days and vice versa. An additional unidentified factor was thus also involved in determining operating ranges of conductance on days with different overall radiation regimes. We suggest that the simplest mechanism to account for the observed humidity responses is stomatal sensing of the epidermal or cuticular transpiration rate rather than the bulk leaf or stomatal transpiration rate.

Key-words: *Populus trichocarpa* × *P. deltoides* hybrid; poplar; humidity; stomata; transpiration.

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INTRODUCTION

Numerous laboratory and field studies have documented that stomatal conductance in a wide range of species decreases with increasing evaporative demand (Grantz 1990). Despite a general consensus concerning the occurrence of partial stomatal closure upon exposure to dry air, the most appropriate driving variable for describing so-called stomatal responses to humidity has remained somewhat controversial. Based on the good fit of experimental data to an empirical model relying on relative humidity (RH) and CO₂ concentration at the leaf surface to predict stomatal conductance, Ball, Woodrow & Berry (1987) concluded that stomata respond to RH. In contrast, Aphalo & Jarvis (1991) measured responses of stomatal conductance to either RH or saturation deficit at a constant leaf temperature, and to increasing temperature at either a constant RH, or constant saturation deficit, and concluded that water vapour saturation deficit is a more appropriate variable. In an elegant series of experiments carried out in air and in a helium–oxygen mixture (helox) Mott & Parkhurst (1991) demonstrated that the stomatal response to humidity was consistent with sensing of the transpiration rate itself rather than RH or saturation deficit. Meinzer & Grantz (1991) arrived at a similar conclusion, based on coordination of canopy stomatal and boundary layer conductances under field conditions.

In view of the poorly developed mechanistic basis for the dependence of stomatal conductance on RH or saturation deficit and of the experimental evidence presented by Mott & Parkhurst (1991), Monteith (1995) has recently proposed a reinterpretation of stomatal responses to humidity based on the dependence of conductance on transpiration rate. Monteith's re-analysis of 52 sets of measurements on 16 species revealed a negative, generally linear dependence of stomatal conductance on transpiration rate. However, in some cases Monteith was able to identify a 'regime B' in which stomatal conductance continued to decrease as transpiration remained either constant, or even began to decrease. Under this regime, transpiration would remain constant or begin to decrease despite increasing saturation deficit. Earlier, Farquhar (1978) proposed that a pattern in which transpiration first increases then begins to decline above a critical value of saturation deficit is evidence for a 'feed-forward' stomatal

response to humidity based on sensing of peristomatal transpiration, but Mott & Parkhurst (1991) and Monteith (1995) suggested that this behaviour may be associated with patchy closure of stomata. Dewar (1995) recently suggested that stomatal sensing of changes in the gradient of total water potential between guard cells and other epidermal cells may provide the mechanistic basis for both a linear decline in stomatal conductance with increasing transpiration and 'feed-forward' behaviour observed under regime B.

Most of the data sets reanalysed by Monteith (1995) were obtained in gas exchange cuvettes in which measurements of stomatal conductance and transpiration are not independent, thereby invalidating conventional statistical analyses of the relationship between conductance and transpiration. This constraint can be partially overcome by regressing the reciprocal of transpiration against the reciprocal of the water vapour saturation deficit (Monteith 1995). If the resulting plot is linear, the maximum conductance when transpiration is zero can be obtained from the slope, and the maximum transpiration as conductance approaches zero can be obtained from the intercept.

This study presents an analysis of independent, *in situ* measurements of stomatal conductance, transpiration, and leaf-to-air vapour pressure difference (V) obtained in a hybrid poplar canopy over a relatively wide range of atmospheric saturation deficit and solar radiation in the field. Concurrent measurements of foliage temperature, transpiration and air saturation deficit also allowed examination of relationships between total vapour phase conductance (stomatal plus boundary layer), transpiration and V . Variation in V and radiation resulted both from day-to-day variation in temperature and cloudiness, and from differences in exposure of foliage in the canopy. Our objectives were to assess the apparent dependence of stomatal conductance on transpiration, to determine the extent to which the behaviour observed was consistent with Monteith's proposed reinterpretation of stomatal responses to humidity, and to determine the frequency and implications of apparent feed-forward stomatal responses to humidity (Farquhar 1978; Regime B, Monteith 1995). Based on the entire range of responses observed, we also attempted to deduce whether sensing of stomatal or epidermal transpiration may be involved in stomatal responses to humidity.

MATERIALS AND METHODS

Plant material and site characteristics

Measurements were made at Washington State University's Farm 5, located near Sumner, Washington. *Populus trichocarpa* \times *P. deltoides* F₁ hybrid clone 50-194 from Replication I of the Phase II Trial (FT4-4) was used. Three replicates of this clone in 18-tree blocks were planted in the spring of 1989 along with 15 other hybrid clones. The trees were irrigated whenever soil-water suction measured with tensiometers at 0.3 m depth reached 0.05 MPa. The tree selected for this study was \approx 5 m tall at the time the measurements were carried out in late July and

early August 1992. A system of towers and scaffolding was constructed to gain access to the crown of the study tree and neighbouring trees. Three exposed upper branches and one partially shaded lower branch were selected for measurements of stomatal conductance, sap flow (transpiration) and leaf temperature. A diagram of the tower and scaffolding system, showing approximate positions of the branches, can be found in Hinckley *et al.* (1994).

Microclimate

Relative humidity (RH) and air temperature were measured with shielded sensors (HMP35C, Campbell Scientific, Logan, UT, USA) installed on one of the canopy access towers at approximately the average canopy height (c. 15 m). Ambient vapour pressure was subsequently calculated from the RH and temperature data. Leaf temperature on the branches fitted with sap flow gauges as described below was determined with fine wire (0.08 mm) copper-constantan thermocouples. One thermojunction was affixed to the abaxial surface of each of five leaves on each of the branches fitted with sap flow gauges. The five thermocouples on a given branch were connected in parallel in order to obtain an average leaf temperature for each branch. Average standard deviations of the leaf temperature measurements among the four branches fitted with sap flow gauges were approximately ± 1.0 °C on clear days and ± 0.3 °C on cloudy to partly cloudy days. RH and leaf and air temperature data were continuously recorded using a datalogger (CR21X, Campbell Scientific, Logan, UT, USA) equipped with a 32-channel multiplexer (AM 416, Campbell Scientific) and 20 min averages were stored in a solid-state storage module (SM 196, Campbell Scientific, Logan, UT, USA). The vapour pressure difference between the leaf interior and the bulk air (V) was calculated using saturation vapour pressure at leaf temperature and the ambient vapour pressure measured at the top of the canopy.

Solar radiation was measured with a pyranometer (LI-200, Li-Cor, Inc., Lincoln, NE, USA) and recorded at 0.5 h intervals by an automated weather station located in a clearing \approx 400 m from the site.

Transpiration

Transpiration (E) was measured as sap flow through individual terminal branches using a heat balance method as described by Baker & Van Bavel (1987). Sap flow gauges (SGA10, SG13 and SGB19, Dynamax Inc., Houston, TX, USA) consisting of a heating element, a thermopile and individual thermocouples were attached to three exposed upper terminal branches and one partially shaded lower terminal branch. The gauges and adjacent portions of the stem were wrapped with foam insulation, in addition to that supplied by the manufacturer, and the entire assembly was enclosed in an outer layer of reflective automobile windshield liner in order to minimize spurious temperature gradients caused by radiant heating of the stem (Gutierrez

et al. 1994). The heating elements were operated at constant power and the radial and longitudinal heat fluxes were evaluated with the thermopile and thermocouples, respectively. Data were recorded continuously and 20 min averages were stored using the data logger-multiplexer-storage module system described above for microclimate measurements. The sheath conductance for each sap flow gauge, a proportionality constant relating thermopile output to the radial heat loss at zero flow, was determined from the average values of sheath conductance calculated in the datalogger program between 03:00 and 05:00 hours local time when transpiration was assumed to be zero. Upon completion of the measurements, all the leaves were removed from the branches for determination of their area in an area meter (model 3100, LI-COR, Inc.). Total leaf area distal to the sap flow gauges was between 0.41 and 1.88 m² and average area per leaf was between 20 and 460 cm².

Conductances

Stomatal conductance (g_s) was measured with a steady-state porometer (LI-1600, LI-COR, Inc.). The values reported represent averages on a single-sided area basis for both the ab- and adaxial leaf surfaces of this amphistomatic species (Ceulemans, Impens & Imler 1988). During each measurement cycle, conductances were measured in four to five leaves on each of the four branches fitted with sap flow gauges. The average standard deviation of these measurements was approximately $\pm 15\%$ of the mean value for each set of four to five leaves per branch.

Crown conductance (g_c) was calculated at 20 min intervals from the transpiration and microenvironmental data as:

$$g_c = \frac{EP}{V}, \quad (1)$$

where P is atmospheric pressure. All values of g_c are expressed on a unit leaf area basis. Crown conductance as defined here includes a stomatal component and boundary layer components, the magnitude of which is determined both by leaf dimensions and by attenuation of wind by surrounding leaves and vegetation. It can therefore be regarded as partially analogous to a canopy conductance, even though the latter is traditionally expressed on a unit ground area basis (Meinzer *et al.* 1993).

Data analysis

Stomatal and crown conductance were plotted in relation to the corresponding transpiration rate and also the corresponding V . Measurements of g_s , E and V were independent, but g_c was derived from measurements of E and V (see above). An additional analysis proposed by Monteith (1995) was also performed. This consisted of plotting $1/E$ against $1/V$. Extrapolated maximum values of conductance (g_m) and transpiration (E_m) were obtained from the inverse slope and y -intercept, respectively, of these plots. The

resulting estimates of g_m and E_m were then used in a model to predict instantaneous values of g_c :

$$g_c = g_m(1 - E/E_m), \quad (2)$$

where E is the measured transpiration rate (Monteith 1995).

In order to account for the influence of variation in solar radiation on relationships between conductance and transpiration or V , the data were either grouped based on prevailing radiation, or normalized by the corresponding radiation. Leaf-to-air vapour pressure difference rather than atmospheric saturation deficit is used throughout. Leaf temperature was frequently 3–4 °C below air temperature and ambient vapour pressure was measured at a reference point several metres away from the leaf surface, therefore V represents the appropriate driving force for calculating g_c and estimating g_m .

RESULTS

Courses of E , g_c , V and solar radiation for uppermost branches on representative clear and partly cloudy days are shown in Fig. 1. On clear days, E typically increased rapidly in the morning and abruptly reached a broad plateau at about 1000 h despite steadily increasing radiation and V (Fig. 1a). The abrupt decline in E at about 1700–1800 h took place well after radiation had begun to decline, and coincided roughly with the beginning of the decline in V at about 1800 h. On clear days, g_c rapidly attained its daily maximum value at about the same time the transpiration plateau was attained, but in contrast to E ,

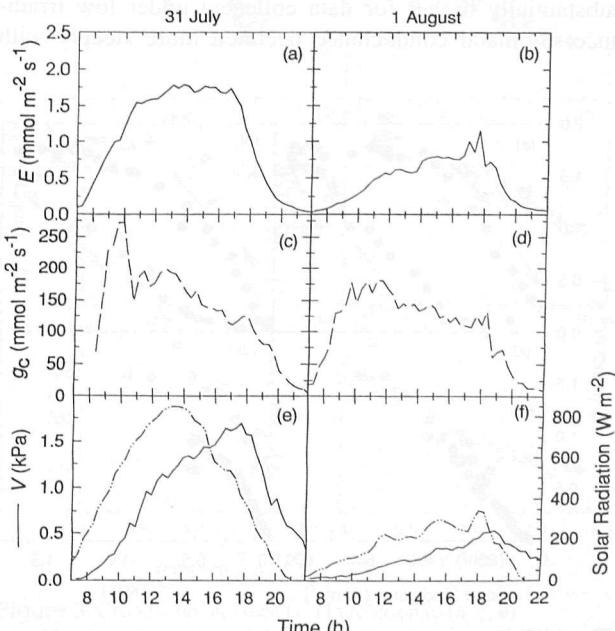


Figure 1. Daily courses of transpiration (E), crown conductance (g_c), leaf-to-air vapour pressure difference (V), and solar radiation for a clear (31 July) and a partly cloudy (1 August) day. Courses of E and g_c are averages of three terminal branches.

g_c began to decrease rapidly and continued to decrease throughout the rest of the day (Fig. 1c). Most partly cloudy days were characterized by gradual increases in V and radiation and maximum values were reached late in the day (1800–1900 h; cf. Figs 1e & f). On these days, E also increased gradually and attained a maximum value late in the day without reaching a noticeable plateau. In contrast with its behaviour on clear days, g_c remained relatively constant until late afternoon after attaining a morning maximum at about 1000 h (Fig. 1d). The relatively high values of g_c attained at low radiation on partly cloudy days suggested that low V , rather than low radiation, was the dominant factor governing maximum stomatal conductance on these days.

Substantial diurnal hysteresis was evident in plots of E against solar radiation and V on clear days (Figs 2a & b) but not on partly cloudy days (Figs 2c & d). Correlations between E and both V and solar radiation are generally positive because V represents the driving force for E and solar radiation stimulates stomatal opening. Although solar radiation and V tended to co-vary throughout the day, their variation was noticeably out of phase on clear days (e.g. Fig. 1e) and more in phase on partly cloudy days (e.g. Fig. 1f). The extent of diurnal hysteresis in the relationships between E and radiation and E and V was thus determined by the manner in which solar radiation and V co-varied throughout the day.

Measurements of g_s in uppermost exposed branches were divided into two groups according to the solar radiation regimes under which they were collected (radiation $\leq 500 \text{ W m}^{-2}$ or radiation $> 500 \text{ W m}^{-2}$). Stomatal conductance declined linearly with increasing E and increasing V under both radiation regimes (Fig. 3), but r^2 values were substantially higher for data collected under low irradiance. Stomatal conductance declined more steeply with

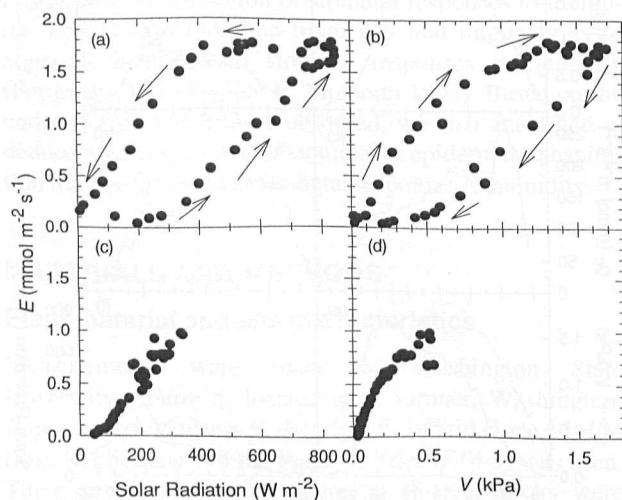


Figure 2. Transpiration (E) in relation to solar radiation and leaf-to-air vapour pressure difference (V) during a clear (a, b) and a partly cloudy (c, d) day. The direction of the arrows in (a) and (b) corresponds to the course of increasing and decreasing E throughout the day.

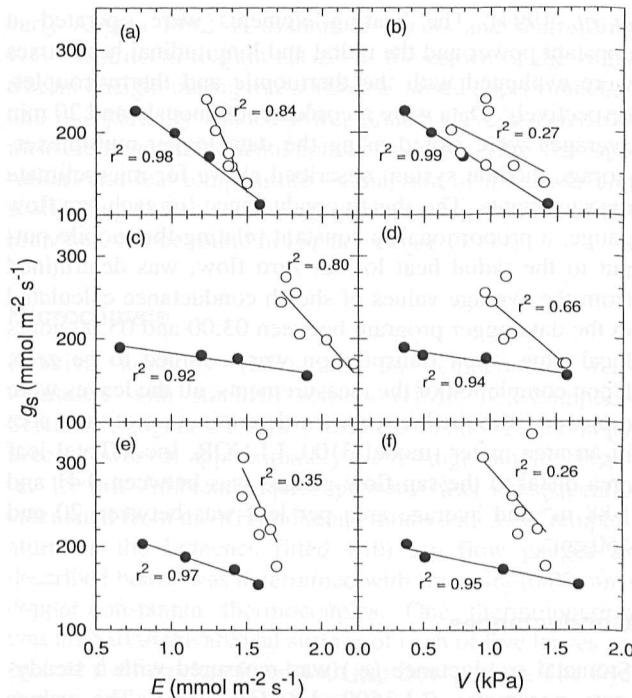


Figure 3. Stomatal conductance (g_s) in relation to (a, c, e) transpiration (E) and (b, d, f) leaf-to-air vapour pressure difference (V) for three exposed terminal *Populus* branches located in the upper canopy. Data collected during 4 d were pooled and then divided into two classes according to the solar radiation corresponding to each data point (● = solar radiation $\leq 500 \text{ W m}^{-2}$; ○ = solar radiation $> 500 \text{ W m}^{-2}$). Each point represents the average of measurements on four to five leaves on a given branch.

increasing E and V under high radiation. The four measurements of g_s when radiation was below 500 W m^{-2} include two partly cloudy days and one clear day. All measurements of g_s when radiation was above 500 W m^{-2} were obtained on two clear days. Data for the three individual replicate branches, rather than overall averages, are presented because the slope of the relationships between g_s and E and between g_s and V appeared to vary considerably from branch to branch.

In contrast with the linear relationships between g_s and E observed in upper branches, a bimodal relationship between g_s and E was obtained for a partially shaded lower branch on two clear days (Fig. 4a). However, when g_s of this branch was plotted against V , a significant, linear relationship emerged (Fig. 4b). Plots of g_c against E and V for this branch were consistent with those obtained for g_s , in that the relationship between g_c and E was bimodal (Fig. 4c) and the relationship between g_c and V was linear (Fig. 4d). Measurements of g_c are more numerous than those of g_s because unlike g_s , which was measured periodically, g_c was recorded continuously. Measurements of g_c therefore spanned a larger portion of the day.

When measurements of g_c for upper branches collected on two clear days were pooled and plotted against E , g_c exhibited a tendency to decrease with increasing E , but the relationships were variable (Figs 5a, c & e). In one case,

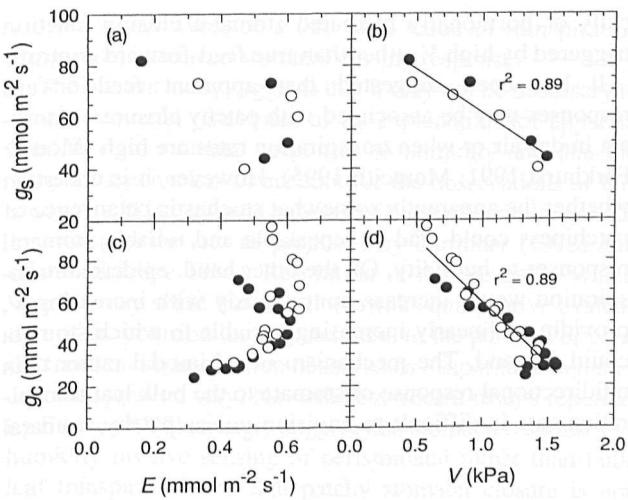


Figure 4. Stomatal (g_s) and crown (g_c) conductance of a lower, partially shaded *Populus* branch in relation to (a, c) transpiration (E) and (b, d) leaf-to-air vapour pressure difference (V). Data were collected during two clear days (● = 31 July; ○ = 3 August). Each g_s data point represents the average of measurements on four to five leaves and each g_c data point represents a 20 min average between 12:00 and 18:00 hours.

the relationship between g_c and E appeared to be bimodal (Fig. 5c), while in another case g_c appeared initially to change little with increasing E , then decline precipitously (Fig. 5a). In contrast, when g_c was plotted against V , it clearly declined with increasing V in a linear or slightly curvilinear fashion (Figs 5b, d & f). Similar behaviour was observed in measurements of g_c of upper branches collected on two partly cloudy days, although relationships between g_c and E were even more variable than those obtained on clear days (Figs 6a, c & e). Plots of g_c against V for cloudy days (Figs 6b, d & f) also exhibited more scatter than those obtained on clear days but g_c clearly declined continuously with increasing V .

The relationship between $1/E$ and $1/V$ for upper branches on a clear day is shown in Fig. 7a. Use of V rather than air saturation deficit in this type of plot ensured that the reciprocal of the slope could be used to estimate g_c , because *Populus* leaves were frequently 3–4 °C below air temperature (data not shown). It was apparent that the relationship between $1/E$ and $1/V$ was not unique and that at least two sets of linear relationships for each branch, differing dramatically in their slopes, could be discerned. The data points yielding the relationship with the steepest slope appeared to correspond with measurements obtained when radiation was low. Normalization of $1/V$ by solar radiation to account for diurnal hysteresis in the relationships between E and V and between E and solar radiation (Figs 2a & b) allowed the data collected for a given branch to be treated as a single linear relationship per branch (Fig. 7b).

On a partly cloudy day, the relationship between $1/E$ and $1/V$ was distinctly curvilinear, different for each branch, and showed some evidence of differing according to whether the data were collected under higher versus lower

radiation (Fig. 8a). Again, normalization of $1/V$ by solar radiation yielded a linear relationship with $1/E$ for each branch (Fig. 8b). As the slope of a plot of $1/E$ against $1/V$ represents total vapour phase resistance, the results shown in Figs 7 and 8 indicate that the relationship between resistance and V was linear when co-variation in radiation was taken into account.

When values of g_m and E_m obtained for the three upper branches on a clear day (day 213) were averaged and used in Eqn 2 to predict g_c on a subsequent clear day (day 216), the agreement between predicted and observed values of g_c was good (Fig. 9). However, values of g_m and E_m obtained on clear days proved to be poor predictors of g_c on partly cloudy days (data not shown).

DISCUSSION

Behaviour of stomata in a hybrid poplar canopy was consistent with a humidity response based on sensing of the transpiration rate itself. However, the sometimes contrasting apparent stomatal responses to transpiration and to leaf-to-air V observed (e.g. Figs 4–6), raise the question of whether stomata may have been sensing and responding to the stomatal or bulk leaf transpiration rate, or to the epidermal transpiration rate. Relationships between both g_s and g_c and V were always essentially linear, regardless of prevailing irradiance or time of day. In contrast, relationships

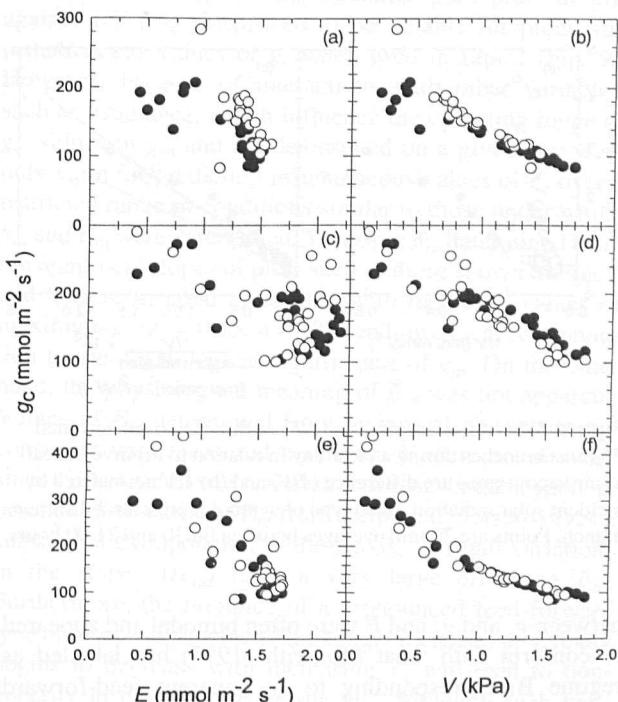


Figure 5. Crown conductance (g_c) in relation to (a, c, e) transpiration (E) and (b, d, f) leaf-to-air vapour pressure difference (V) for three exposed terminal *Populus* branches located in the upper canopy. Data were collected during two clear days (● = 31 July; ○ = 3 August). Each point represents a 20 min average between 09:00 and 18:30 hours.

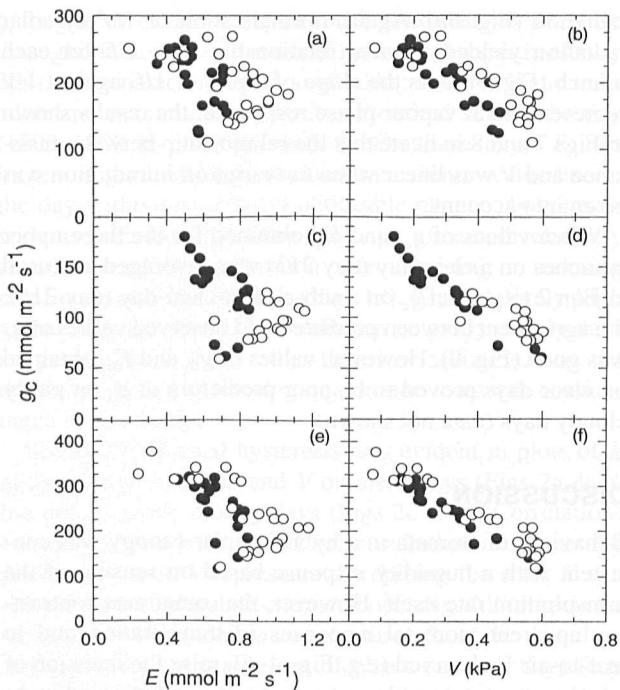


Figure 6. Crown conductance (g_c) in relation to (a, c, e) transpiration (E) and (b, d, f) leaf-to-air vapour pressure difference (V) for three exposed terminal *Populus* branches located in the upper canopy. Data were collected during two partly cloudy days (● = 1 August; ○ = 4 August). Each point represents a 20 min average between 11:30 and 18:30 hours.

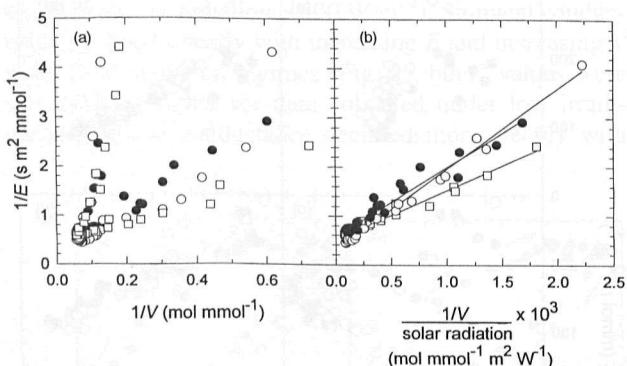


Figure 7. Inverse transpiration ($1/E$) of three exposed, terminal *Populus* branches during a clear day in relation to (a) inverse leaf-to-air vapour pressure difference ($1/V$) and (b) $1/V$ normalized by incident solar radiation. Each type of symbol represents a different branch. Points are 20 min averages between 08:30 and 21:00 hours.

between g_s and g_c and E were often bimodal and appeared to conform with what Monteith (1995) has labelled as regime B, corresponding to an apparent feed-forward stomatal response to humidity (Farquhar 1978) characterized by a decrease in conductance with an increase in the stomatal transpiration rate. More recent data presented by Franks, Cowan & Farquhar (1997) suggest that apparent feed-forward responses of stomata in some species are irreversible in the short term and may reflect a metabolically

or hormonally mediated stomatal closing reaction triggered by high V rather than true feed-forward control.

It has been suggested that apparent feed-forward responses may be associated with patchy closure of stomata in dry air or when transpiration rates are high (Mott & Parkhurst 1991; Monteith 1995). However, it is uncertain whether the apparently somewhat stochastic occurrence of patchiness could lead to repeatable and reliable stomatal responses to humidity. On the other hand, epidermal transpiration would increase continuously with increasing V , providing a linearly increasing variable to which stomata could respond. The mechanism of a bimodal rather than unidirectional response of stomata to the bulk leaf transpiration rate is difficult to envision unless patchy stomatal

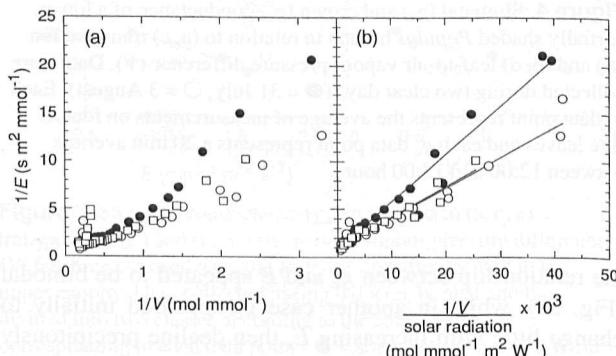


Figure 8. Inverse transpiration ($1/E$) of three exposed, terminal *Populus* branches during a partly cloudy day in relation to (a) inverse leaf-to-air vapour pressure difference ($1/V$) and (b) $1/V$ normalized by incident solar radiation. Each type of symbol represents a different branch. Points are 20 min averages between 08:30 and 21:00 hours.

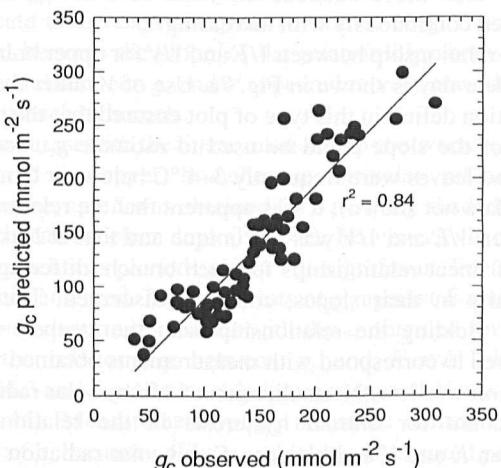


Figure 9. Predicted crown conductance (g_c) in relation to measured g_c on a clear day (3 August; mean solar radiation = 483 W m^{-2}). Measurements of E and V collected on 31 July (mean solar radiation = 481 W m^{-2}) were used to generate plots such as those shown in Fig. 7, from which values of E_m and g_m required in Eqn 2 were estimated.

closure is involved, or a threshold value of transpiration triggers an enhanced stomatal closing response.

The present study suggests that it may not be necessary to invoke stomatal patchiness as an explanation for apparent feed-forward stomatal responses to humidity and that the simplest mechanism to account for the observations of this study would be stomatal sensing of the epidermal or cuticular transpiration rate as proposed by Farquhar (1978) and others (Schulze 1986). Resolution of the means by which stomata may sense transpiration awaits quantitative evaluation of the contribution of patchiness to the patterns of conductance and transpiration observed as evaporative demand and transpiration vary. Nevertheless, recent results reported by Bunce (1997) strongly suggest that stomatal responses to humidity involve sensing of peristomatal rather than bulk leaf transpiration and that patchy stomatal closure is not involved in apparent feed-forward responses when V is high.

The results indicate that, as might be expected, there was a strong interaction between the prevailing solar radiation and the apparent responsiveness of conductance to transpiration and evaporative demand. When radiation was low, either because of cloudiness or branch position within the canopy, g_s and g_c at a given value of V were lower than when radiation was high. Prevailing radiation thus partially determined the operating range for conductance, making it impossible to derive a unique relationship between conductance and V or transpiration. Because of this and other interactions, it is probably more realistic to regard stomatal responses to humidity as a family of relationships corresponding to varied solar radiation and other set points. The interactive influence of radiation and evaporative demand on conductance could be partially, but not completely, taken into account by normalizing V by solar radiation (Figs 7 & 8). This procedure worked well in accounting for variation in radiation within the same day or type of day (Figs 7 & 9). Nevertheless, even after normalizing by radiation, data collected on partly cloudy days could not be used to predict behaviour on clear days, and vice versa. An additional unidentified factor was thus also involved in determining operating ranges of conductance on days with different overall solar radiation regimes. The influence of radiation on apparent stomatal responsiveness to humidity is consistent with numerous reports that stomatal conductance is closely linked to photosynthetic capacity (Schulze & Hall 1982). It is likely that irradiance-induced variation in intercellular CO_2 concentration played a role in determining operating ranges of conductance under different light regimes (Farquhar & Sharkey 1982). It has also been suggested that the linkage between conductance and photosynthesis, and therefore between conductance and light, relies on a chemical signal transported from the mesophyll to the guard cells, depending on the rate of photosynthesis (Wong, Cowan & Farquhar 1979).

Variation in behaviour among the upper, fully exposed branches studied indicated that other factors in addition to solar radiation were involved in determining the characteristics of the relationships between conductance, transpiration and evaporative demand; for example, on clear days

maximum g_c ranged from about $200 \text{ mmol m}^{-2} \text{ s}^{-1}$ to over $400 \text{ mmol m}^{-2} \text{ s}^{-1}$ in different branches (Fig. 5). Similarly, maximum E ranged from about $1.5 \text{ mmol m}^{-2} \text{ s}^{-1}$ to $2.25 \text{ mmol m}^{-2} \text{ s}^{-1}$. On partly cloudy days, differences in conductance among upper branches were of similar magnitude, but differences in E were smaller (Fig. 6). The considerable degree of autonomy with regard to transpiration and stomatal behaviour displayed by these branches suggests that each may have been exposed to significantly different microenvironmental conditions. The possible basis of this partial branch autonomy with respect to operating ranges for physiological processes was not determined. Nevertheless, it was previously reported that maximum values of g_s and leaf-specific hydraulic conductivity among *Populus* branches varied and appeared to be determined by the light environment in which the branches had developed rather than the current light environment (Hinckley *et al.* 1994). This is consistent with numerous studies of hydraulic architecture showing that leaf-specific hydraulic conductivity varies substantially within trees (Tyree & Ewers 1991) and that stomatal conductance is often closely coupled to water transport efficiency (Kuppers 1984; Meinzer & Grantz 1990; Sperry & Pockman 1993; Meinzer *et al.* 1995). Development of physiological models and scaling from field data thus require careful attention to spatial variation in physiological behaviour within complex canopies as well as to the range of environmental conditions over which data are collected.

Estimates of g_m and E_m obtained from plots of $1/E$ against $1/V$ (Fig. 7) proved to be reliable for predicting instantaneous values of g_c when used in Eqn 2 (Fig. 9). However, because of interactions with other variables, such as irradiance, which influence the operating range of g_c , values of g_m and E_m determined on a given day were only valid for predicting instantaneous values of g_c over a restricted range of conditions similar to those under which g_m and E_m were determined. Values of g_m determined from the reciprocal slopes of plots such as those shown in Figs 7 and 8 were in good agreement with measured values of maximum g_c ($r^2 = 0.75$, $n = 18$), lending a clear interpretation to the physiological significance of g_m . On the other hand, the physiological meaning of E_m was not apparent. Values of E_m determined from reciprocal plots were not consistent with measured maximum E (data not shown), since E_m is a mathematical abstraction never achievable in practice. Estimation of E_m from reciprocal plots involves a substantial extrapolation to the y-axis, so small variations in the slope ($1/g_m$) have a very large effect on E_m . Furthermore, the presence of a pronounced feed-forward type of response, in which E no longer increases or actually begins to decrease with increasing V , will lead to non-linearity in plots of $1/E$ versus $1/V$. Although such non-linearity may be difficult to detect visually or as a reduced r^2 value for the relationship, its effect on the estimate of the y-intercept, and therefore on E_m may be significant. Thus, although E_m can be used along with g_m to analyse stomatal responses to evaporative demand, it does not seem desirable to ascribe a specific physiological interpretation to E_m .

In the present study, g_s was measured independently of both E and V , while g_c was derived from both E and V . Although conclusions about relationships between a derived variable and its parent variables should be drawn with caution, our interpretation of relationships between g_c , E and V are reinforced by their complete consistency with those observed for the independently measured g_s . Most of the data re-analysed by Monteith (1995) were gathered in gas-exchange cuvettes under laboratory or growth chamber conditions where differences between the atmospheric environment experienced by enclosed and exposed foliage may alter stomatal responses (Turner, Schulze & Gollan 1984). Despite the disadvantage of co-variation of environmental variables in the field, it may thus be preferable to assess relationships between stomatal conductance and transpiration there because the entire plant is exposed to the same bulk atmospheric conditions. If the range of environmental conditions under which such measurements are made is sufficiently broad, then it should be possible to extract data that allow relationships between g_s , E and V to be evaluated while other interacting variables are held relatively constant.

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