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Photosynthetic and Stomatal Responses of the Grey Mangrove, *Avicennia marina*, to Transient Salinity Conditions[1]

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ABSTRACT

Measurements of gas exchange characteristics were made on intact, attached leaves of hydroponically grown seedlings of *Avicennia marina* (Forstk.) Vierh. var *australasica* (Walp.) Moldenke as the NaCl concentration of the culture solution was varied by step changes of 50 millimolar NaCl every 2nd day from 50 to 500 to 50 millimolar NaCl. The CO_2 assimilation rate, stomatal conductance, intercellular CO_2 concentration, and evaporation rate decreased at salinities above 250 millimolar NaCl and recovered substantially upon return to the original salinity.

The assimilation rate was measured as a function of the intercellular CO_2 concentration [$A(c_i)$ curve]. The lower linear portion of this curve was insensitive to variation in salinity, whereas the upper nonlinear portion declined with increasing salinity, indicating a reduction in the capacity for CO_2 assimilation which recovered upon return to the original salinity. Stomatal conductance changed such that the intercellular CO_2 concentration measured under normal atmospheric conditions occurred in the transition between the lower, linear and upper nonlinear portions of the $A(c_i)$ curve. Thus, stomatal conductance and photosynthetic capacity together co-limited the assimilation rate. The changes in gas exchange characteristics were such that water loss was minimal relative to carbon gain.

In the natural environment, halophytes experience ranges of salinities which fluctuate over different time scales. Previous studies have considered the changes in gas exchange characteristics in response to long term salinity conditions (1, 3, 5, 7, 10, 11), such as those which might characterize a habitat. However, responses to transient salinity conditions, such as those caused by tidal cycles and rainfall, may also be of considerable ecological importance. The present study describes the changes in gas exchange characteristics of the grey mangrove, *Avicennia marina*, in response to short term variation in salinity over the range from 10% to 100% seawater.

MATERIALS AND METHODS

Plant Material. Propagules of *Avicennia marina* (Forstk.) Vierh. var *australasica* (Walp.) Moldenke were collected from trees growing along Cullendulla Creek, New South Wales, Australia (35°42′S, 150°12′E). Propagules were cultivated in sand, subirrigated with 10% seawater in a growth cabinet adjusted to give day/night leaf temperatures of 25/20°C, a RH of ~70% to give a *vpd*[2] of 12 mbar, and a 12-h photoperiod with an average of 400 $\mu E\ m^{-2}\ s^{-1}$ incident at leaf height. Upon reaching a post-cotyledonary phase of development, the seedlings were transferred to 500-ml containers for hydroponic culture in Johnson's nutrient solution (8), which was made to approximate 10% sea water by addition of 50 mM NaCl. Four seedlings (three experimental seedlings plus a control) were then grown hydroponically until the next flush of leaves was fully developed before beginning the experiment. Solutions were changed weekly and the levels were maintained by addition of distilled H_2O every other day. The time required for leaf development under these conditions is approximately 6 weeks.

Experimental. Gas exchange characteristics were determined on the same leaf of each plant over a period during which the NaCl concentration of the nutrient solution was increased from 50 to 500 mM NaCl and then returned to the original salinity by step changes of 50 mM NaCl. Solutions were changed at the beginning of the dark period with an interval of 48 h between changes. Measurements of gas exchange characteristics were made while the plants were experiencing concentrations of 50, 150, 250, 350, 500, and 50 mM NaCl. The salinity of the nutrient solution of the control plant was maintained at 50 mM NaCl and the solution was changed at the same time as those of the salinity-treated plants.

Gas Exchange Measurements. Rates of CO_2 and water vapor exchange in intact, attached leaves were determined with an open system gas analysis apparatus as described by Wong *et al.* (17), with modifications as noted in Ball and Critchley (2). Calculations are according to von Caemmerer and Farquhar (16). Values in text are mean ± SE.

Dark respiration rates were measured before illumination and were followed by measurement of the assimilation rate as a function of the intercellular CO_2 concentration, c_i. Variation in the latter was obtained by changing the ambient CO_2 concentration in the sequence 330, 400, 500, 200, 100, and 50 $\mu l\ l^{-1}$. Other conditions were similar to those experienced in the growth chamber, *i.e.* leaf temperature of 25°C, quantum flux density of 500 $\mu E\ m^{-2}\ s^{-1}$, and a *vpd* of 12 mbar. Atmospheric pressure usually was 950 mbar. Boundary layer conductance to diffusion of water vapor was 0.4 $mol\ m^{-2}\ s^{-1}$.

RESULTS

Gas Exchange Characteristics under Normal Atmospheric Conditions. The effects of short term changes in salinity on the

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[1] This work was presented at the International Botanical Congress, Sydney, 1981, and was submitted in partial fulfillment of the requirements for a Ph.D. degree by M. C. B.

[2] Abbreviations: *vpd*, leaf to air vapor pressure difference; *A*, photosynthetic CO_2 assimilation rate; $A(c_i)$, assimilation rate as a function of the intercellular CO_2 concentration; c_i, intercellular CO_2 concentration; *E*, evaporation rate; *g*, leaf (primarily stomatal) conductance to water vapor; g_t, total (boundary layer plus leaf) conductance to CO_2.

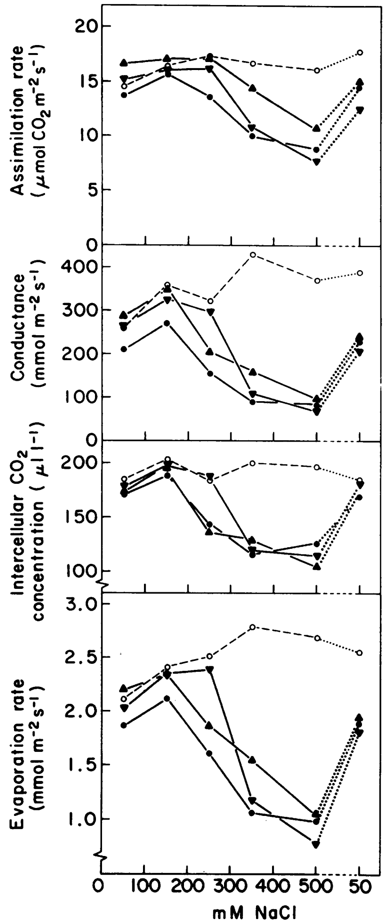

FIG. 1. Gas exchange characteristics of mature leaves of *Avicennia marina* in response to transient changes in salinity. Symbols identify the control plant (○) and three plants receiving salinity treatments (●, ▲, ▼). Measurements were made during increase of salinity from 50 to 500 mM NaCl (points joined by solid lines) and at the conclusion of decrease in salinity from 500 to 50 mM NaCl (points joined by dotted lines). All measurements were made under normal atmospheric conditions, *i.e.* 330 μl l^{-1} CO$_2$ and 210 ml l^{-1} O$_2$.

gas exchange characteristics of *Avicennia marina* under normal atmospheric conditions are shown in Figure 1. The CO$_2$ assimilation rates at the start of the experiment (*i.e.* when the salinity was 50 mM NaCl) averaged 14.9 ± 0.7 μmol CO$_2$ m^{-2} s^{-1} at a mean intercellular CO$_2$ concentration (c_i) of 177 ± 3 μl l^{-1}. With increase in salinity above 250 mM NaCl, the assimilation rates decreased 37% to average rates of 9.1 ± 1.0 μmol CO$_2$ m^{-2} s^{-1} at 500 mM NaCl. The decline in assimilation rate was accompanied by decrease in both stomatal conductance and c_i. The evaporation rate was related directly to stomatal conductance because the *vpd* remained constant; the evaporation rate decreased from 2.05 ± 0.08 to 0.94 ± 0.08 mmol m^{-2} s^{-1} with 10-fold increase in salinity from 50 to 500 mM NaCl, respectively. These responses were substantially reversed upon return to the original salinity. The rates of dark respiration of the leaves were not obviously affected by changes in salinity and averaged 1.53 ± 0.22 ($n = 5$) and 1.41 ± 0.09 ($n = 18$) μmol CO$_2$ m^{-2} s^{-1} in the control leaf and in the leaves of plants receiving salinity treatments, respectively, during the whole study.

Assimilation Rate as a Function of the Intercellular CO$_2$ Concentration. The effects of short-term changes in salinity on photosynthetic metabolism were assayed by measuring the assimilation rate as a function of c_i (Fig. 2). Two regions of the $A(c_i)$ curve can be distinguished, one insensitive and the other sensitive

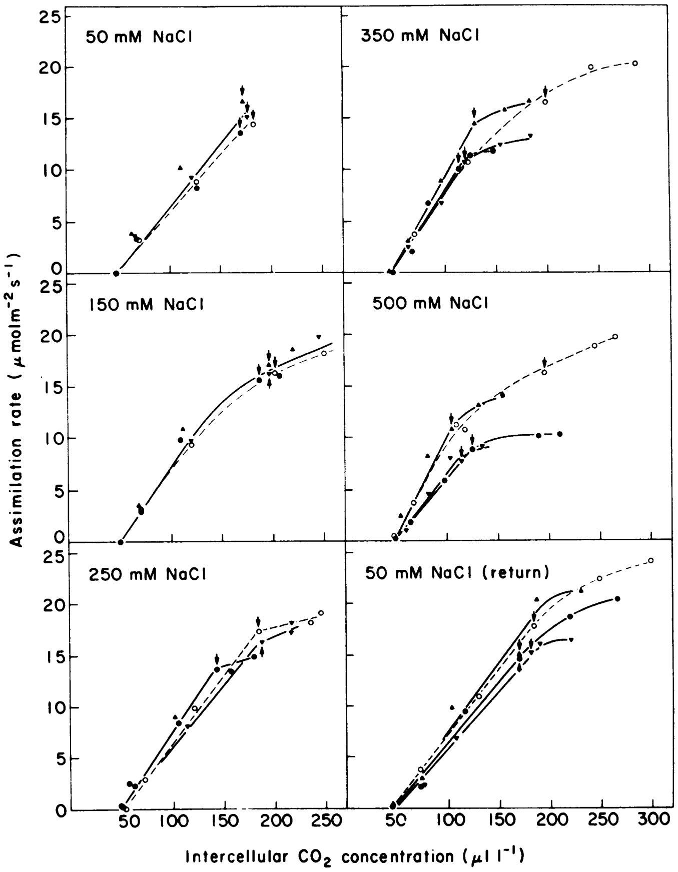

FIG. 2. Assimilation rate as a function of the intercellular CO$_2$ concentration in leaves of *A. marina* in response to transient salinity conditions. Arrows indicate measurements made under normal atmospheric conditions, *i.e.* the operational points, and are the same set of measurements shown in Figure 1. Symbols as in Figure 1. Lines drawn by eye.

to temporary changes in salinity. The insensitive region encompasses the lower, linear portion of the $A(c_i)$ curve in which neither the CO$_2$ compensation point nor the initial slope was affected by changing salinity. There was some variation in the latter which was neither consistent nor obviously related to salinity. The upper, nonlinear portion of the curve was sensitive to changing salinity although there was considerable variability between individuals in the extent of the effect and in the salinity at which it became obvious. Stomata often closed so much with increase in the CO$_2$ concentration above normal ambient levels that it was not possible to determine more of the plateau region of the $A(c_i)$ curve than is shown in Figure 2. The general trend was for curvature from the initial linear slope to commence at progressively lower values of c_i with increments in salinity beyond 250 mM NaCl. The net effect of these changes in the $A(c_i)$ curves was a lowering of the photosynthetic capacity with increasing salinity, and the effects were substantially reversible.

DISCUSSION

Rapid and reversible changes in the photosynthetic capacity of *Avicennia marina* were induced by short term variation in salinity (Fig. 2), and similar results have been obtained with another mangrove species, *Aegiceras corniculatum* (1). In these species, the decline in photosynthetic capacity with increasing salinity was shown by changes in the shapes of $A(c_i)$ curves, such that the assimilation rates declined at high c_i, but were unaffected at lower c_i (Fig. 2). The relative insensitivity at low c_i (Fig. 2) contrasts with the decrease in the initial slope of the $A(c_i)$ curve with increase in the salinity in which either *A. marina* (3) or *A. corniculatum* (3) are grown. Similarly, 'mesophyll resistance' (which is probably equivalent to the inverse of the initial slope of the $A(c_i)$ curve as discussed later) is reported to increase in several halophytes grown at sub- or supra-optimal salinities (5, 7, 10, 11). Clearly, the responses to short and long term exposure to salinity are quite different.

The changes in gas exchange characteristics of *A. marina* in response to transient changes in salinity (Figs. 1 and 2) are similar to those observed in glycophytes in response to short term water stress, *e.g.* that obtained by sudden changing of the water potential of a culture solution, by rapid desiccation of detached leaves, or by allowing the plant to rapidly deplete the soil water content of a pot. For example, measurements of the $A(c_i)$ characteristics of *Phaseolus vulgaris* (15) and *Xanthium strumarium* (14) showed that only the upper plateau of the curve declined with rapid imposition of water stress. Similarly, mesophyll resistance was unaffected by rapid decrease of the leaf water potential in several species (12, 13). Thus, transient variation in salinity may change leaf water relations in a way which interferes, directly or indirectly, with photosynthetic metabolism.

The effects of changes in stomatal conductance (Fig. 1) and photosynthetic metabolism (Fig. 2) on the assimilation rates (Fig. 1) can be evaluated from the $A(c_i)$ measurements with a graphical method devised by Jones (9). In this method, the slope of a line connecting the operational point on the $A(c_i)$ curve with the point on the x axis where c_i equals the ambient CO_2 concentration is equal to minus the total conductance to diffusion of CO_2, g_t under those conditions (Fig. 3). Variations in g_t are due primarily to changes in stomatal conductance because the boundary layer conductance is held constant in the gas exchange system. Using these graphical relationships, the gas exchange characteristics which would occur with change in the $A(c_i)$ characteristics and/or stomatal conductance can be calculated from the intersection of the lines describing these functions. In the present study, stomata sometimes closed so much with increasing ambient CO_2 concentration when the plants were experiencing high salinities that it was not possible to obtain a c_i high enough for the $A(c_i)$ curve to intersect a particular g_t line. In this case, the intersection was estimated by extrapolation.

The present study follows this method and includes the modifications recommended by Farquhar and Sharkey (6) to correct for the effects of water vapor on the inward diffusion of CO_2 through stomata. This correction is minor because the *vpd* was only 12 mbar. An implicit assumption in this analysis is that changes in stomatal conductance and hence in evaporation rate do not cause changes in leaf temperature and water potential. Thus, it is assumed that the only effect of variation in stomatal conductance on the assimilation rate occurs through effects on c_i, although other data (1) have shown that changes in evaporation rates may affect the plateau region of the $A(c_i)$ curve.

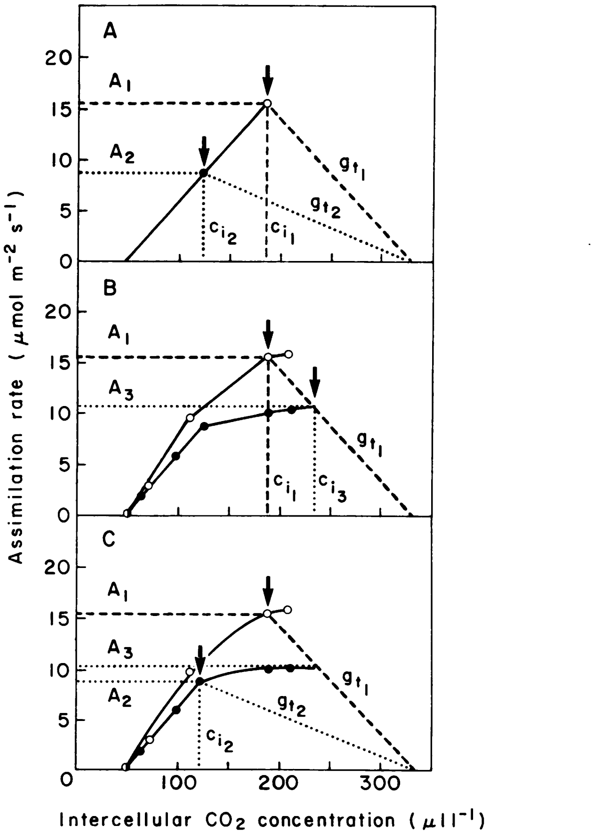

FIG. 3. Effect of changes in stomatal conductance and photosynthetic metabolism on the assimilation rate with change in salinity from 150 (○) to 500 (●) mM NaCl. Graphs are drawn according to Farquhar and Sharkey (6). Data are from the plant labeled (●) in Figures 1, 2, and 3. A_1 and A_2 are the assimilation rates that occurred at 150 and 500 mM NaCl, respectively. The corresponding conductances to diffusion of CO_2 are g_{t_1} and g_{t_2}, respectively, and the corresponding intercellular CO_2 concentrations are c_{i_1} and c_{i_2}. The arrows in A and C, and the upper one in B, indicate normal atmospheric conditions. The lower arrow in B indicates the assimilation rate, A_3, and the corresponding c_{i_3} which would occur in the leaf when the plant was experiencing 500 mM NaCl if the conductance had remained at the value g_{t_1}.

The following discussion will examine the reduction in assimilation rate of one leaf from 15.6 to 8.8 μmol CO_2 m^{-2} s^{-1} with increase in salinity from 150 to 500 mM NaCl (Fig. 3). The data obtained at the initial salinity of 50 mM NaCl have been omitted from this analysis because gas exchange measurements were not made at ambient CO_2 concentrations greater than 330 μl l^{-1}. In Figure 3A, the $A(c_i)$ characteristics occurring during exposure to 150 and 500 mM NaCl are approximated by connecting the points measured under normal atmospheric conditions with their respective CO_2 compensation points, a method used for calculation of mesophyll resistance. The decline in stomatal conductance with increase in salinity from 150 to 500 mM NaCl (Fig. 1) is shown by the decline in slope from g_{t_1} to g_{t_2}, respectively (Fig. 3A). The decrease in stomatal conductance was associated with a decline in c_i from 196 to 123 μl l^{-1}. Further, the decrease in assimilation rate from 15.6 to 8.8 μmol CO_2 m^{-2} s^{-1} was proportional to the decline in c_i. It might be concluded from these calculations that stomatal closure in response to increasing salinity restricted the gaseous diffusion of CO_2 into the leaf, causing a decline in c_i which resulted in a decrease in the assimilation rate. However, this calculation is incomplete since it ignores the actual shapes of the $A(c_i)$ curves at high c_i as pointed out by Farquhar and Sharkey (6).

The shapes of the $A(c_i)$ curves measured when the plant was experiencing 150 and 500 mM NaCl are shown in Figure 3B. Photosynthetic capacity declined with increase in salinity as shown by the large decrease in the upper plateau and slight decline in the initial slope of the $A(c_i)$ curves. If stomata had been insensitive to the increase in salinity and g_t remained constant, then c_i would have increased from 196 to 234 μl l^{-1}, but the assimilation rate would have declined from 15.6 to 10.2 μmol CO_2 m^{-2} s^{-1} because of the decline in photosynthetic capacity. This decrease in the assimilation rate is almost as great as that which occurred with the accompanying decline in stomatal conductance as shown in Figure 3C. Thus, it cannot be concluded that if stomatal conductance were to increase to its original level that the original assimilation rate would be restored; this would be ignoring the reduction in capacity for photosynthesis. In this sense, the stomatal limitation is relatively small.

Conversely, it might be concluded from these calculations that reduction in the photosynthetic capacity of the mesophyll cells in response to increasing salinity largely caused the decrease in assimilation rate. However, it would again be incorrect to assume that if photosynthetic capacity were to be restored to its original level that the original assimilation rate would also be restored; this would be ignoring the reduction in stomatal capacity for diffusion of CO_2. In this sense, the biochemical limitation is also relatively small. In summary, even though the decline in either stomatal conductance or photosynthetic capacity could singly account for the decrease in the assimilation rate, the actual limitation imposed by either factor, individually, on the assimilation rate is relatively small because both stomatal conductance and photosynthetic capacity decline in the same sense with increasing salinity. The assimilation rate is therefore co-limited by stomatal conductance and photosynthetic capacity.

The effect of changing stomatal conductance in response to variation in salinity can also be considered in terms of its influence on the evaporation rate relative to the rates of photosynthetic CO_2 assimilation. The decline in stomatal conductance with increase in salinity from 150 to 500 mM NaCl was accompanied by a nearly proportional decrease in both c_i and the assimilation rate; the evaporation rate varied in direct proportion to stomatal conductance because *vpd* was constant (Fig. 1). Under these conditions, the ratio of water lost to carbon gained, E/A, decreased with increasing salinity because the evaporation rate was reduced to a greater extent than the c_i with decrease in stomatal conductance. In contrast, the ratio E/A remained nearly constant in *A. marina* exposed to long term salinity conditions because the changes in stomatal conductance and photosynthetic metabolism were such that c_i was also nearly constant (3).

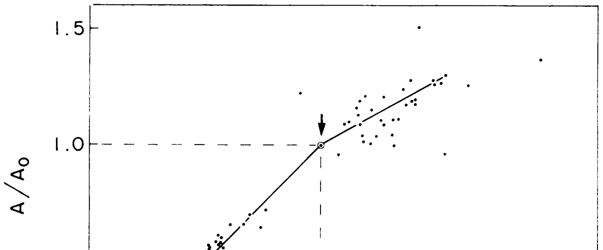

FIG. 4. Normalization of all $A(c_i)$ curves measured in *A. marina* during short term changes in salinity to emphasize the position of the operational points, *i.e.* those obtained with an ambient CO_2 concentration of 330 $\mu l\ l^{-1}$, relative to the shapes of the $A(c_i)$ curves. The assimilation rate, A, is expressed relative to A_0 and $c_i - \Gamma$ is expressed relative to $c_{i_0} - \Gamma$ where (c_{i_0}, A_0) is the operational point and Γ is the CO_2 compensation point for each leaf. These operational points coincide at (1, 1). Lines drawn by eye.

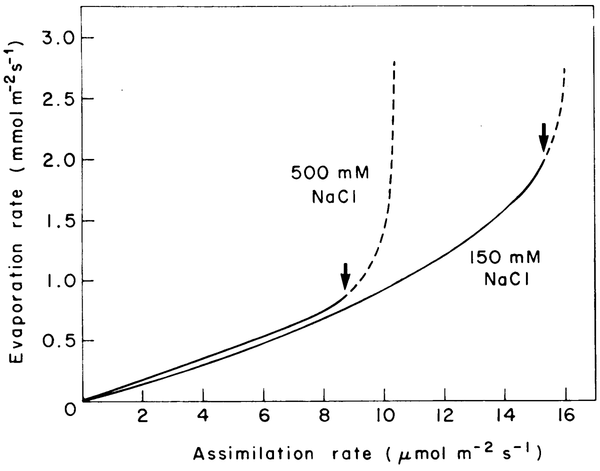

FIG. 5. Relationship between evaporation rate and assimilation rate if stomatal conductance were to vary independently. Values are calculated from the $A(c_i)$ curves measured when the plant was experiencing 150 and 500 mM NaCl. Arrows indicate the operational points. Data from the leaf labeled (●) in Figures 1, 2, and 3. Calculations according to Farquhar and Sharkey (6).

In spite of the differences noted above, the changes in stomatal conductance with either long (3) or short term exposure of *A. marina* to different salinity treatments were such that the operational c_i, *i.e.* that c_i obtaining under normal atmospheric conditions, occurred in the region of transition between the lower linear and upper plateau of the $A(c_i)$ curves. For this reason, the concept of mesophyll resistance, taken as the inverse of the initial slope of the $A(c_i)$ curve, is a useful device for calculating the assimilation rate if c_i is reduced because of a reduction in either the ambient CO_2 concentration or stomatal conductance. This virtual resistance can be thought of as that controlling the flux from the intercellular spaces to an imaginary site where the CO_2 concentration equals the compensation point concentration. However, the concept breaks down if it is used to predict the effect of increased c_i caused by increase in either the ambient CO_2 concentration or stomatal conductance.

The position of the operational points relative to the shape of the $A(c_i)$ curves measured during short term salinity treatments is shown in Figure 2 but demonstrated more clearly in Figure 4. As discussed in the accompanying paper (3), the marginal water cost of carbon assimilation, $\partial E/\partial A$ (4) shows large changes in the transition region of the $A(c_i)$ curve (3, 6, 16). The simultaneous changes in the rates of evaporation and assimilation which would have occurred when the plants were experiencing 150 and 500 mM NaCl if stomatal conductance were to vary are estimated in Figure 5. The dashed lines correspond to the plateau regions of the respective $A(c_i)$ curves and are so drawn to emphasize uncertainty, as discussed in the accompanying paper (3). This graph shows that the decline in stomatal conductance with increase in salinity was such that the slope, $\partial E/\partial A$, at the operational points was approximately maintained near 250 mol H_2O mol^{-1} CO_2. This is similar to the values obtained in *A. marina* grown under long term salinity conditions (3).

Avicennia marina adjusts to long term salinity treatments with a complex array of changes ranging from the biochemical level to the structural organization of the plant (1). It is not surprising that there are differences in the gas exchange characteristics of *A. marina* receiving short and long term (3) salinity treatments. However, the occurrence of the operational c_i in the transition region of the $A(c_i)$ curve under either short or long term (3) salinity conditions indicates that the stomata remain coordinated with metabolism when challenged by salinity. With short term treatments, stomatal functioning appears consistent with a strategy for allowing minimal water loss relative to carbon gain, even as the leaf metabolism is affected.

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