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CO_2 and Water Vapor Exchange across Leaf Cuticle (Epidermis) at Various Water Potentials[1]

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Cuticular properties affect the gas exchange of leaves, but little is known about how much CO_2 and water vapor cross the cuticular barrier or whether low water potentials affect the process. Therefore, we measured the cuticular conductances for CO_2 and water vapor in grape (*Vitis vinifera* L.) leaves having various water potentials. The lower leaf surface was sealed to force all gas exchange through the upper surface, which was stoma-free. In this condition both gases passed through the cuticle, and the CO_2 conductance could be directly determined from the internal mole fraction of CO_2 near the compensation point, the external mole fraction of CO_2, and the CO_2 flux. The cuticle allowed small amounts of CO_2 and water vapor to pass through, indicating that gas exchange occurs in grape leaves no matter how tightly the stomata are closed. However, the CO_2 conductance was only 5.7% of that for water vapor. This discrimination against CO_2 markedly affected calculations of the mole fraction of CO_2 in leaves as stomatal apertures decreased. When the leaf dehydrated, the cuticular conductance to water vapor decreased, and transpiration and assimilation diminished. This dehydration effect was largest when turgor decreased, which suggests that cuticular gas exchange may have been influenced by epidermal stretching.

The waxy cuticle of leaves serves to inhibit water loss and thus to decrease the dehydration of the underlying cells (Scott, 1964, 1966; Norris and Bukovac, 1968; Leon and Bukovac, 1978). The waxes vary in thickness and composition, and the inhibition of water loss varies accordingly (Bengtson et al., 1978; O'Toole et al., 1979; O'Toole, 1982; Jordan et al., 1983, 1984; von Wettstein-Knowles, 1989; Jenks et al., 1994). Holmgren et al. (1965) found that water loss through the cuticle was 1.7 to 28.6% of that through open stomata, depending on the species. This indicates that gas exchange through the cuticle can be a small or substantial fraction of the exchange through open stomata. As stomata close, the fraction becomes larger and the control of gas exchange shifts increasingly to the cuticle. Leaves that are darkened or that dehydrate and close their stomata experience this shift from stomatal control to cuticular control.

As water loss becomes increasingly dependent on the cuticle, the exchange of CO_2 becomes similarly dependent on cuticular properties. Dugger (1952) found that $^{14}CO_2$ passes through the cuticle. Woolley (1967) observed that artificial films discriminate against CO_2 and predicted that the cuticle would show the same behavior. However, the CO_2-exchange properties have not been reported for cuticles. This issue is important because CO_2 in the intercellular spaces is the substrate for photosynthesis and the concentration is generally calculated from the diffusion of water vapor through the leaf (Moss and Rawlins, 1963; von Caemmerer and Farquhar, 1981). Similarly, the diffusive conductance for CO_2 is calculated from water vapor diffusion (Gaastra, 1959; von Caemmerer and Farquhar, 1981). The calculations assume that CO_2 and water vapor move along the same paths through the cuticle and stomata, but Kirschbaum and Pearcy (1988) and Meyer and Genty (1996) pointed out that the calculations do not include cuticular properties and could be affected if the diffusion properties differ from those in the stomata.

Because the cuticular properties for CO_2 are relatively unexplored in intact leaves, we measured the cuticular conductances for CO_2 and water vapor on the upper, astomatous surface of intact grape leaves and determined the effect of the cuticle on calculated levels of CO_2 inside the leaves. We also determined whether the cuticle changes its

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Abbreviations: A, leaf assimilation rate per unit of projected leaf area (μmol m^{-2} s^{-1}); c_a, mole fraction of CO_2 outside of the leaf (μmol CO_2 mol^{-1} of air); c_i, mole fraction of CO_2 inside of the leaf (μmol CO_2 mol^{-1} of air); E_c, transpiration across the upper cuticle of a hypostomatous leaf, rate per unit of projected leaf area (mmol m^{-2} s^{-1}); E_l, leaf transpiration rate per unit of projected leaf area (mmol m^{-2} s^{-1}); g_c, cuticular conductance (mol m^{-2} s^{-1}); $g_{c(H_2O)}$, cuticular conductance to water vapor (mol m^{-2} s^{-1}); $g_{c(CO_2)}$, cuticular conductance to CO_2 (mol m^{-2} s^{-1}); g_l, leaf conductance (mol m^{-2} s^{-1}); $g_{l(H_2O)}$, leaf conductance to water vapor (mol m^{-2} s^{-1}); g_s, stomatal conductance (mol m^{-2} s^{-1}); IRGA, IR gas analyzer; Ψ_w, water potential (MPa); w_a, mole fraction of water vapor in the bulk air outside of the leaf (mmol H_2O mol^{-1} of air); w_l, saturated mole fraction of water vapor inside of the leaf at leaf temperature (mmol H_2O mol^{-1} of air).

conductance for water vapor as the leaves dehydrate sufficiently to lose turgor.

MATERIALS AND METHODS

Grape (*Vitis vinifera* L.) plants were grown individually in 20-L pots in about 18 kg of soil in a naturally lit greenhouse having temperatures in the range of 25 to 33°C during the day and 10 to 18°C during the night. The plants were watered daily and were provided with Hoagland nutrient solution weekly. Gas exchange was first measured on individual, fully expanded, attached leaves (unless otherwise noted) in an open gas-exchange system that indicated the activity of both surfaces (stomata plus cuticle). The leaves then were double-sealed on the underside by coating with silicone vacuum grease, to which was stuck a polyethylene sheet, and the gas-exchange measurements were repeated for the upper (stoma-free) cuticle alone. Finally, the upper surface was sealed similarly, and the gas-exchange measurements were continued on the completely sealed leaf to test the effectiveness of the double seal.

The gas exchange through the cuticle was very slow but could be measured by using the whole leaf with its large area (typically 0.015 m^2) and a slow flow of air through the system, so that the reliable lower limit of detection of transpiration was 0.005 mmol m^{-2} s^{-1} and for assimilation was 0.1 μmol m^{-2} s^{-1}. The system consisted of a large, anodized aluminum chamber with glass lid. The chamber was able to accommodate a leaf as large as 16 cm in width and 20 cm in length. A tangential fan was enclosed in the chamber and gave a boundary layer conductance to water vapor of 5 mol m^{-2} s^{-1}. Leaf temperature was controlled by circulating water from a cooling water bath to the water jacket of the leaf chamber. Leaf temperature was measured with a copper-constantan thermocouple. Illumination was provided by a metal-halide lamp (HQI-R, 250 W, Osram, Frankfurt, Germany). The UV and IR components were removed with a hot mirror (115, Schott, Cologne, Germany). Air was passed through the chamber at a rate between 0.5 and 2.0 L min^{-1}, monitored with a mass flowmeter (Brooks, Hatfield, PA). Air was obtained by mixing 6% CO_2 in air with CO_2-free air. The flows of both gases were controlled by two mass flow controllers (Tylan, San Diego, CA). Absolute CO_2 partial pressure of the inlet air was measured with an absolute IRGA (model ZAR, Fuji Electrics, Tokyo, Japan). The difference in partial pressure of CO_2 in the ingoing and outgoing air streams was measured directly with a differential IRGA (model 865, Beckman). The humidity of the air stream was controlled by passing CO_2-free air through a gas-washing bottle and then an anodized aluminum block condenser. The temperature of the condenser was controlled by a cooling water bath. The partial pressure of the water vapor was measured using a differential water-sensing IRGA (Binos 1, Leybold-Heraeus, Hanau, Germany). The outputs of all sensors were continuously logged by a computer.

The conductance for water vapor was measured for the unsealed leaf $g_{l(H_2O)}$ according to the equation of von Caemmerer and Farquhar (1981):

$$g_{l(H_2O)} = \frac{E_l}{w_l - w_a}. \quad (1)$$

After the lower surface was sealed, the cuticular conductance for water vapor $g_{c(H_2O)}$ was measured similarly according to:

$$g_{c(H_2O)} = \frac{2E_c}{w_l - w_a}. \quad (2)$$

The factor 2 corrects for the effect of sealing by assuming that the gas-exchange properties of the cuticles are identical for both of the epidermes, and thus the transpiration across the upper plus lower cuticle of an unsealed leaf would be double that of the sealed leaf.

The cuticular conductance for CO_2 $g_{c(CO_2)}$ could also be measured for this leaf having a sealed undersurface:

$$g_{c(CO_2)} = \frac{2A}{c_a - c_i}. \quad (3)$$

The factor 2 has a meaning analogous to that in Equation 2. Of the three parameters necessary to determine $g_{c(CO_2)}$, A and c_a were measured in the gas-exchange system, and c_i was determined from the CO_2 compensation point with a slight correction for the CO_2 flux. With the lower surface sealed, c_i approached the CO_2 compensation point because the gas exchange was extremely slow through the cuticle. Small adjustments in c_i were made according to this slow rate of exchange.

The exact procedure was to seal a leaf in the gas-exchange chamber and determine the CO_2 compensation point by measuring the c_a at which no net gas exchange occurred. It should be noted that this is a direct measure of c_i. The c_i was close to this value when gas exchange was subsequently restricted to the cuticle. To correct for the small amount that c_i differed from this value, the c_i values near the compensation point were calculated using the equation:

$$c_i = c_a - 1.6\frac{A}{g_{l(H_2O)}}, \quad (4)$$

where $g_{l(H_2O)}$ was determined according to Equation 1, and the factor 1.6 is the ratio of the diffusivities of CO_2 and water vapor in air (McPherson and Slatyer, 1973; Nobel, 1983). The correction was 4 to 6 μmol CO_2 mol^{-1} when this equation was used and, thus, very small. After these measurements were completed, gas exchange was determined in the whole leaf when c_a was 350 or 1100 μmol CO_2 mol^{-1}. Then the cuvette was opened, the leaf was double-sealed on the lower surface, and the latter gas-exchange measurements were repeated. With the double seal, all of the stomata were prevented from gas exchange and c_i was essentially at the compensation point, except for the small correction described above. It is worth noting that a similar sealing occurs when the stomata close naturally, and it was demonstrated by direct measurement that a similar decrease in c_i occurs until c_i approaches near the compensation point (Lauer and Boyer, 1992). Finally, the leaf was double-sealed on both surfaces to prevent all gas exchange,

and the gas-exchange measurements were repeated once more to test the efficacy of the double seal.

In a separate experiment leaf gas exchange was measured similarly at various Ψ_w except that the vacuum grease in the double seal was replaced with petroleum jelly to increase the compatibility of the leaf tissue with the psychrometer used to measure Ψ_w (petroleum jelly is used in the psychrometer to coat instrument surfaces and decrease sorption of water vapor, see Boyer, 1995). There was no difference in gas-exchange behavior with the two sealants. The leaf with the coated lower surface was inserted into the gas-exchange cuvette, gas exchange was measured initially, the leaf was excised at the petiole, and the cut was covered with petroleum jelly. The leaf dehydrated for the next 2 d through the upper cuticle. At various times, the cuvette was opened, a leaf disc was removed and placed in a vapor chamber, and the disc Ψ_w was measured with an isopiestic thermocouple psychrometer (Boyer and Knipling, 1965; Boyer, 1995). The osmotic potential of the leaves was estimated from a similar psychrometer determination on discs that had been frozen and thawed. With separate leaves, the water content was measured by determining the fresh weight of the leaf, oven-drying at 70°C for 48 h, and then reweighing the leaf. The water content at any Ψ_w could be calculated by subtracting the amount of water loss measured in the cuvette from the estimated amount of water in the leaf at the beginning of the gas-exchange measurements.

RESULTS

Gas exchange was slow when it was restricted to the cuticle of the intact leaves, and low irradiances of 110 μmol photons m^{-2} s^{-1} were used in all of the experiments to prevent leaf overheating. Figure 1 shows that before sealing, a typical leaf had an A of about 4.5 μmol m^{-2} s^{-1} at

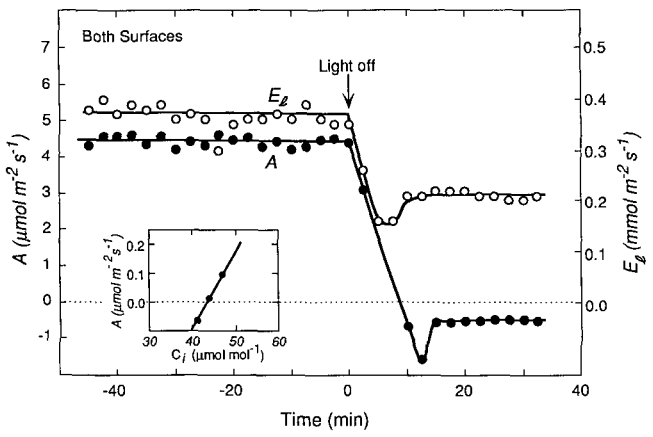

Figure 1. A and E_l of a grape leaf (leaf 1, Tables I–IV) in an atmosphere having a c_a of 1100 μmol CO_2 mol^{-1} under an irradiance of 110 μmol m^{-2} s^{-1}. The leaf was darkened at the arrow. Inset shows A versus c_i around the CO_2 compensation point. The CO_2 compensation point was directly measured, and the A versus c_i relation was determined according to Equation 4. Leaf and air temperatures were 25°C and vapor pressure deficit expressed as a mole fraction was 18.8 mmol H_2O mol^{-1}.

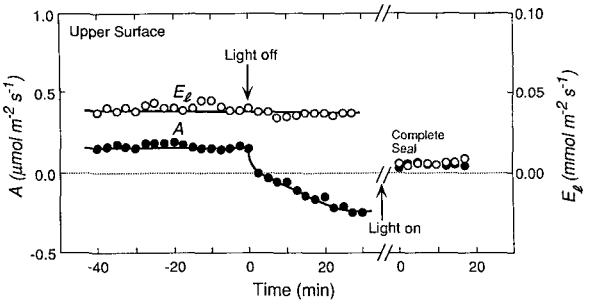

Figure 2. Same leaf and conditions as Figure 1, except the undersurface was double-sealed. Because of the slow A through the cuticle, the c_i was 50 μmol CO_2 mol^{-1} (taken from the inset in Fig. 1, according to A in this figure), which was close to the CO_2 compensation point (44 μmol CO_2 mol^{-1}, Fig. 1, inset). With the c_a at 1100 μmol CO_2 mol^{-1}, the concentration difference between the leaf exterior and interior was 1100 − 50 = 1050 μmol CO_2 mol^{-1}. At the break in the x axis, the double seal also was applied to the upper surface, the light was turned on, and gas exchange was measured to test the efficacy of the seal in the completely sealed leaf.

this irradiance when c_a was 1100 μmol mol^{-1} and the CO_2 compensation point was 44 μmol mol^{-1} (Fig. 1, inset). Transpiration was 0.37 mmol m^{-2} s^{-1}. When the leaf was darkened, assimilation immediately became negative and transpiration decreased. The leaf was several months old, and the decline in transpiration to about 60% of the rate in the light indicated that the stomata closed only partially. Younger leaves displayed more complete stomatal closure that brought transpiration to about 15% of the rate in the light. We used mostly older leaves to ensure that cuticle synthesis was minimal during the experiments.

When the stomata were closed by double-sealing the lower surface the upper surface became the only one exchanging gas, and transpiration was much less than in the unsealed leaf (compare Figs. 1 and 2). Assimilation also was much less, and the c_i became 50 μmol CO_2 mol^{-1} (CO_2 compensation point with small correction determined by reading c_i from Fig. 1, inset, at the assimilation rate in Fig. 2). Thus, the c_i was essentially at the CO_2 compensation point. When the light was turned off, assimilation became negative as respiratory activity slowly became detectable. Transpiration did not change when the light was turned off, indicating the absence of stomatal activity. Observations with a light microscope confirmed that there were no stomata on the upper leaf surface. When both surfaces of the leaf were sealed (Fig. 2, right), transpiration and assimilation became virtually zero, indicating that the double seal was an effective barrier against CO_2 and water vapor.

Replicate leaves showed nearly a 10-fold variation in conductance to water vapor when unsealed but only a 2-fold variation when sealed on the undersurface (Table I). The stomatal conductance thus was somewhat variable but the cuticular conductance was relatively reproducible, averaging about 5 mmol m^{-2} s^{-1}. As a consequence, the cuticular conductance was a variable fraction of the conductance of the whole leaf and accounted for up to 30% of the leaf transpiration in the oldest leaves (leaf 3, Table I).

The conductance of the cuticle for CO_2 was much less than for water vapor. Table II shows that for the leaves in

Table I. *$g_{l(H_2O)}$ for whole grape leaves when the stomata were open and $g_{c(H_2O)}$ for the cuticle of the same leaves when the lower stoma-containing surface was double-sealed*

Irradiance was 110 μmol m^{-2} s^{-1}. Conductances are in mmol m^{-2} s^{-1} and for $g_{c(H_2O)}$ are 2× the conductance of the upper surface.

Leaf	$g_{l(H_2O)}$	$g_{c(H_2O)}$	$g_{c(H_2O)}/g_{l(H_2O)}$
1	18.9	4.2	0.22
2	28.0	4.2	0.15
3	26.2	7.6	0.30
4	109	4.4	0.040
5	178	4.4	0.024
Average ± 1 SD	72.0 ± 69.7	5.0 ± 1.5	

Table I, the cuticular conductance for CO_2 was 2.5 to 13% of that for water vapor (average of 5.7%). A similar measurement with *Impatiens coralfutura,* which has stomata on only the lower surface, $g_{c(CO_2)}$ was too small to detect and $g_{c(H_2O)}$ was 4 mmol m^{-2} s^{-1} (two surfaces), confirming that the cuticular conductance for CO_2 was much smaller than for water vapor.

From these data, it was possible to determine the conductances of the stomata, cuticle, and total leaf for CO_2 and water vapor in grape. Table III shows that in a leaf with a small stomatal conductance (leaf 1), water vapor exchange was shared between the stomata and cuticle, but CO_2 exchange was almost entirely stomatal. In a leaf with a large stomatal conductance (leaf 5), almost all gas exchange was stomatal.

Leaf dehydration affected the cuticular properties. When attached leaves with double-sealed undersurfaces were excised from the plant, transpiration and assimilation decreased, as the leaf dehydrated through the upper cuticle alone. For a typical leaf, Figure 3A shows that transpiration was initially low in the double-sealed leaf, but it underwent a steep decrease with dehydration to about 25% of the initial rate and then a shallow decline that continued for the next 40 h. After 48 h the rate was about 10% of the initial rate. The decrease in assimilation was less than for transpiration, but the rate was so slow after 10 h that the measurement became variable (Fig. 3A).

Initially, the Ψ_w of the leaf was approximately −0.4 MPa and declined slowly during the dehydration to about −3.5 MPa at the end of 48 h (Fig. 3B). The leaf lost about 15 to 25% of its original water content during this time. The conductance for water vapor was about 4.6 mmol m^{-2} s^{-1} in these leaves initially, but it declined to 0.9 mmol m^{-2} s^{-1} by the end of the dehydration (Fig. 4). It was not possible to calculate the corresponding conductance to CO_2 because c_i probably changed during the dehydration (data not shown, but see Lauer and Boyer [1992] for change in c_i during dehydration).

Most of the decline in the conductance to water vapor occurred at Ψ_w between −0.4 and −2 MPa (Fig. 4), which was the range over which turgor was decreasing in the leaf. The osmotic potential was about −1.5 MPa in hydrated leaves having a Ψ_w of −0.4 MPa, which indicates an initial turgor of about 1.1 MPa and zero turgor at Ψ_w of −1.5 to −2 MPa. When turgor became zero at a Ψ_w of −2 MPa, the conductance had decreased to 25% of its initial value (Fig. 4).

DISCUSSION

CO_2 and water vapor moved slowly across the cuticle despite a complete sealing of the stomata. This indicates that leaf gas exchange always occurred no matter how tightly the stomata were closed. However, the cuticle was a much more effective barrier against CO_2 than against water vapor. The CO_2 conductance was only 5.7% of that for water vapor in grape on average and was undetectable in *I. coralfutura.* This comparatively large discrimination against CO_2 by the cuticle may have been caused by the different diffusion paths for the two gases. CO_2 reaching the mesophyll cells through the cuticle must move through the entire epidermal cell layer. Water vapor diffusing in the opposite direction can originate in the epidermis without passing through the epidermal layer, thus giving a shorter path and a larger conductance than for CO_2. Also, it is possible that a simple ultrafiltration occurs because the CO_2 molecule is larger than the H_2O molecule. There could be special molecular features of the waxes that contribute to this discrimination, as suggested by Woolley (1967) from data for other polymers. Regardless of the mechanism, however, the net effect was that the diffusion path for CO_2 was strongly stomatal, whereas the path for water vapor involved both the stomata and the cuticle, and thus the paths differed for the two gases.

The effect of this discrimination varied with the leaf. Stomatal conductances differed among leaves, but cuticular conductances were similar among the same leaves and in comparison with the leaves of other species (van Gardingen and Grace, 1992; Kerstiens, 1995). Especially in older leaves, conductances were generally low and the cuticle contributed a large fraction of the conductance for water vapor. Because of the cuticular component, water loss would not be a good indicator of CO_2 uptake. Other leaves had high conductances and the cuticle contributed relatively less. In these leaves, water loss would give a reasonable estimate of CO_2 movement. However, even in these leaves, the cuticular contribution would become a factor as the stomata closed. CO_2 uptake would diminish more than water loss, inhibiting photosynthesis more than transpira-

Table II. *$g_{c(H_2O)}$ and $g_{c(CO_2)}$ for the cuticle of grape leaves*

Conductances were measured after double-sealing the lower surface and are 2× the conductance of the upper surface in mmol m^{-2} s^{-1}.

Leaf	$g_{c(H_2O)}$	$g_{c(CO_2)}$	$g_{c(CO_2)}/g_{c(H_2O)}$
1	4.20	0.274	0.067
2	4.20	0.112	0.027
3	7.60	0.190	0.025
4	4.40	0.400	0.091
5	4.40	0.160	0.036
6	6.26	0.346	0.055
7	1.90	0.254	0.13
8	3.96	0.382	0.096
Average ± 1 SD	4.62 ± 1.68	0.265 ± 0.106	

Table III. *g_l, g_s, and g_c for two grape leaves*

Numbers in parentheses are percentages of g_l. Data are for leaves 1 and 5 of Tables I and II. For water vapor, the g_l and g_c were directly measured (Tables I and II), and g_s was calculated as $g_l - g_c$. The g_s for CO_2 was 0.63 g_s for water vapor because of the different diffusivities of the two gases in air. The g_c for CO_2 was directly measured (Table II), and g_l for CO_2 was then $g_s + g_c$. Conductances are for both surfaces in units of mmol m^{-2} s^{-1}. Irradiance was 110 μmol m^{-2} s^{-1}.

Leaf	Gas	g_l	g_s	g_c
1[a]	Water vapor	18.9 (100)	14.7 (78)	4.20 (22)
	CO_2	9.53 (100)	9.26 (97.2)	0.274 (2.8)
5[b]	Water vapor	178 (100)	173.6 (97.5)	4.40 (2.5)
	CO_2	109.5 (100)	109.3 (99.8)	0.160 (0.2)

[a] Measured at c_a = 1100 μmol CO_2 mol^{-1}. [b] Measured at c_a = 350 μmol CO_2 mol^{-1}.

tion, and causing less photosynthesis per unit of water used.

The three parameters necessary to determine the cuticular properties for CO_2 appeared to be quite accurate. A and c_a were measured when the leaf was sealed on the underside, and c_i was measured from the CO_2 compensation point before sealing, with a slight correction for gas exchange through the cuticle. The data for A were above the detection limit for the instrument and clearly could be distinguished from zero A in the completely sealed leaf. The c_a was high and readily measured. The compensation point also was easily measured, and c_i was nearly at the CO_2 compensation point in each leaf.

This method is in contrast to determinations of c_i that depend on water vapor diffusion. Since the work of Gaastra (1959) and Moss and Rawlins (1963), the leaf conductance to CO_2 and the c_i have been calculated by assuming that CO_2 and water vapor diffuse along identical paths (with minor corrections for ternary effects; von Caemmerer and Farquhar, 1981). The cuticular contribution is usually disregarded. For example, the c_i calculated from Equation 4 makes this assumption and, as long as the leaf conductance is large so that the cuticular contribution is relatively small, the assumption does not seriously affect the calculation. Calculated c_i and directly measured c_i are comparable in this situation (Sharkey et al., 1982). When stomata close, however, the larger conductance of the cuticle to water vapor than to CO_2 causes the water-based calculation of c_i to appear to increase, whereas the actual c_i decreases.

The effect can be seen in Table IV when the stomata were completely closed by sealing the underside of the leaf. The calculated c_i increased nearly to c_a, but the actual c_i decreased nearly to the CO_2 compensation point (Table IV, compare open and completely closed stomata). This marked effect represents an important inaccuracy in calculated values of c_i. It applies to all leaves when the stomata close and to those leaves having inherently low stomatal conductances (e.g. leaf 1 in Tables I–IV).

In principle, the error can be corrected with a modification of Equation 4 by subtracting the cuticular transpiration from the leaf transpiration to give the vapor diffusion through the stomata alone:

$$c_i = c_a - 1.6\frac{A}{E_l - 2E_c}(w_l - w_a) = c_a - 1.6\frac{A}{g_{s(H_2O)}}, \quad (5)$$

where $(E_l - 2E_c)$ is the rate of leaf transpiration (E_l) minus that through the cuticle ($2E_c$) when E_c is measured through the upper cuticle of hypostomatous leaves. However, al-

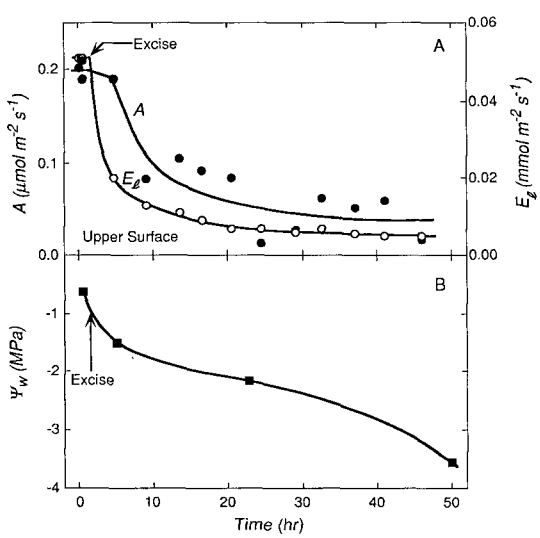

Figure 3. A, A (●) and E_l (○) in a grape leaf during dehydration while the undersurface was double-sealed. B, Ψ_w at various times in the leaf in A. The petiole was excised at the arrow to initiate dehydration through the upper cuticle. Other conditions are as in Figure 1 in the light, except the vapor pressure deficit was 22.6 mmol H_2O mol^{-1}.

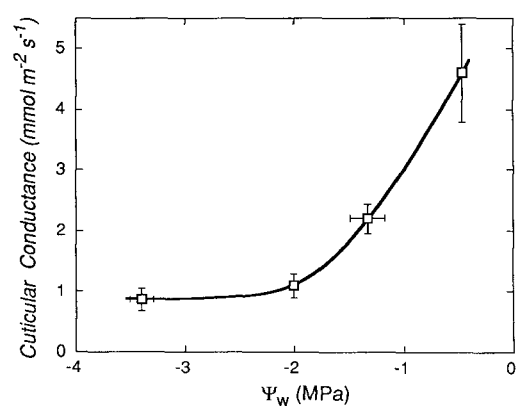

Figure 4. $g_{c(H_2O)}$ (□) at various Ψ_w in grape leaves. Data are means, and vertical and horizontal bars are 1 ± SE (n = 3).

Table IV. *Calculated and actual c_i in leaves 1 and 5 of Tables I to III*

Calculated c_i was estimated in the usual way from water vapor diffusion according to Equation 4 using leaf conductances to water vapor from Table III (g_l = open stomata, g_c = completely closed stomata). Actual c_i was corrected for cuticular conductances to water vapor according to Equation 5 when stomata were open (by using g_s for water vapor from Table III) or was directly measured in the same leaves near the CO_2 compensation point when stomata were completely closed using the methods in this paper. Leaves with open stomata were exchanging CO_2 on both surfaces, and the same leaves were double-sealed on the undersurface to completely close the stomata.

Leaf	Stomatal Aperture	Calculated c_i	Actual c_i
		μmol mol^{-1}	
1[a]	Open	660	534
	Completely closed	990	50
5[b]	Open	257	254
	Completely closed	341	50

[a] Measured at c_a = 1100 μmol CO_2 mol^{-1}; A = 5.2 and 0.288 μmol m^{-2} s^{-1} with open and completely closed stomata, respectively. [b] Measured at c_a = 350 μmol CO_2 mol^{-1}; A = 10.4 and 0.025 μmol m^{-2} s^{-1} with open and completely closed stomata, respectively.

though this equation minimizes the contribution to gas exchange by the cuticle and more closely approximates the diffusion path of CO_2 through the stomata than in Equation 4, cuticular transpiration may not be constant. The data show that E_c and the conductance to water vapor became markedly less in grape as turgor decreased during dehydration. Because the CO_2 flux also decreased, the cuticle appeared to become tighter for both gases.

Leaves shrink as they lose water content (Kramer and Boyer, 1995) and the smaller epidermis may have tightened the structure of the waxes, suggesting that the conductance may be affected by stretching of the wax layer on the leaf surface. van Gardingen and Grace (1992) reported a similar but more modest decrease in cuticular conductance in *Fagus sylvatica* when leaves dehydrated or were exposed to low humidities, and they suggested that hydration of the cuticle itself may affect its conductance properties. Moreshet (1970) used an argon porometer to explore the conductance of the cuticle and reported a similar decrease at low humidities, but it is uncertain whether the porometer was specific enough for stomata to allow a straightforward conclusion. Schönherr and Schmidt (1979) and Schönherr et al. (1980) found that isolated cuticles displayed humidity- and temperature-dependent permeabilities to liquid water and suggested that altered composition or arrangement of the waxes may play a part (Schönherr et al., 1980). Thus, the cuticle appears to form a dynamic barrier to gas exchange, which depends on the condition of the underlying cells and the waxy layer.

In practical terms, evaluating the cuticular contribution to gas exchange has been difficult because of the presence of stomata in many epidermes. Even when stomata close, leaks may exist that prevent accurate estimation of cuticular properties (Kerstiens, 1995). However, as shown here, when stomata are not present, the variation may be small between cuticles of replicate leaves, but the cuticle changes as conditions change within the leaf. Using Equation 5 to correct for cuticle properties thus remains difficult.

Means for improving the accuracy of calculated parameters such as c_i have been sought by accounting for nonuniform stomatal closure (Terashima et al., 1988; Mansfield et al., 1990; Terashima, 1992; Meyer and Genty, 1996) and internal gradients in CO_2 (Parkhurst et al., 1988). Kirschbaum and Pearcy (1988) and Meyer and Genty (1996) also suggested that attention should be paid to cuticular effects, and our results agree that accounting for them could markedly improve the accuracy of calculations relating to CO_2. However, because of the practical problems of determining E_c, more reliance may need to be placed on directly measuring the c_i and conductance to CO_2 with methods similar to those of Sharkey et al. (1982), Mott and O'Leary (1984), Parkhurst et al. (1988), and Lauer and Boyer (1992).

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