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Ozone Concentration in Leaf Intercellular Air Spaces Is Close to Zero[1]

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ABSTRACT

Transpiration and ozone uptake rates were measured simultaneously in sunflower leaves at different stomatal openings and various ozone concentrations. Ozone uptake rates were proportional to the ozone concentration up to 1500 nanoliters per liter. The leaf gas phase diffusion resistance (stomatal plus boundary layer) to water vapor was calculated and converted to the resistance to ozone multiplying it by the theoretical ratio of diffusion coefficients for water vapor and ozone in air (1.67). The ozone concentration in intercellular air spaces calculated from the ozone uptake rate and diffusion resistance to ozone scattered around zero. The ozone concentration in intercellular air spaces was measured directly by supplying ozone to the leaf from one side and measuring the equilibrium concentration above the other side, and it was found to be zero. The total leaf resistance to ozone was proportional to the gas phase resistance to water vapor with a coefficient of 1.68. It is concluded that ozone enters the leaf by diffusion through the stomata, and is rapidly decomposed in cell walls and plasmalemma.

Atmospheric O_3 causes the reduction of the growth rate of plants (5, 8). Photosynthesis is reduced by the presence of O_3 before the symptoms of damage are visible in leaves (6). In Scotch pine the O_3 uptake rate was closely correlated with transpiration suggesting that the main route of O_3 into the leaf was through stomata (11). These data indicate that stomata are the main, if not the only, route of O_3 uptake into leaves (3). Obviously, the physical process of O_3 transport is diffusion, as it is for water vapor and CO_2. This makes it possible to calculate the actual concentration of O_3 in leaf intercellular air spaces.

THEORY

The method of calculating the intercellular CO_2 concentration is widespread, and we applied the same technique for calculating the intercellular O_3 concentration. It is based on the knowledge that in the intercellular air spaces, stomatal pores, and leaf boundary layer the diffusion pathways of CO_2 and water vapor coincide to a great extent. The measurements of the leaf transpiration rate reveal information about the diffusion resistance of the whole gaseous pathway from cell surfaces to ambient air:

$$E = \frac{w_i - w_a}{r_{gw}} \text{ or } r_{gw} = \frac{w_i - w_a}{E} \quad (1)$$

where E[2] is the transpiration rate (minus cuticular transpiration); r_{gw}, the diffusion resistance in the leaf gaseous phase to water vapor; w_i, the water vapor concentration at evaporating cell surfaces; and w_a, that in the ambient air.

CO_2 is a heavier gas ($M = 44$) than water vapor ($M = 18$); therefore, CO_2 moves more slowly than water vapor through the same diffusion pathway and at the same concentration difference. The ratio of the diffusion coefficients of H_2O and CO_2 in the leaf gaseous pathway was measured to be 1.62 (9).

We could not find a value of the diffusion constant for O_3 in air, D_z, in the literature. However, diffusion constants for various gas mixtures may be calculated using the molecular parameters of component gases (1)

$$D_{12} = \frac{0.43 \times \left(\frac{T}{100}\right)^{1.81} \times \left(\frac{1}{M_1} + \frac{1}{M_2}\right)^{1/2}}{P \times \left(\frac{T_{k1} \times T_{k2}}{10^4}\right)^{0.1405} \times \left[\left(\frac{V_{k1}}{100}\right)^{0.4} + \left(\frac{V_{k2}}{100}\right)^{0.4}\right]^2} \quad (2)$$

where M, T, T_k, V_k are, correspondingly, molecular weight, temperature (K), critical temperature (K), and critical volume ($cm^3\ mol^{-1}$) of a component gas, P is atmospheric pressure (bar). Indices 1 and 2 denote component gases. Using values $M_1 = 29$ g mol^{-1}, $T_{k1} = 132.3$ K and $V_{k1} = 87.88$ $cm^3\ mol^{-1}$ for air (4, p. 2121) and $M_2 = 48$ g mol^{-1}, $T_{k2} = 260.9$ K, $V_{k2} = 147.1$ $cm^3\ mol^{-1}$ for ozone (13, p. F65) we get at $P = 1$ bar and $T = 298$ K a value of $D_z = 0.137$ $cm^2\ s^{-1}$. Inserting parameters for water vapor $M_2 = 10$ g mol^{-1}, $T_{k2} = 647.1$ K, $V_{k2} = 55.56$ $cm^3\ mol^{-1}$ (13, p. F74) into the same formula yielded in $D_w = 0.229$ $cm^2\ s^{-1}$. Therefore, Eq. (2) gives the ratio of the diffusion coefficients of water vapor and O_3 $D_w/$

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[2] Abbreviations: E, transpiration rate; w_a, w_i, water vapor concentration in ambient air (a) and on evaporating cell surfaces (i); r_{gw}, r_{gz}, leaf gas phase diffusion resistance to water vapor (w) and to ozone (z); M, molecular weight; D_w, D_z, diffusion constant for water vapor (w) and for ozone (z); T, temperature, T_k, critical temperature; V_k, critical volume; P, atmospheric pressure; z_a, z_i, ozone concentration in ambient air (a) and in the leaf intercellular air space (i); Q, ozone uptake rate; v, gas flow rate; S, leaf area; g_z, total leaf conductance for ozone; r_z, total leaf resistance to ozone; g_{gw}, g_{gz}, leaf gas phase diffusion conductance for water vapor (w) and for ozone (z).

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$D_z = 1.67$. The diffusion resistance to ozone in the leaf gas phase can be calculated as

$$r_{gz} = r_{gw} \times D_w/D_z \quad (3)$$

where r_{gz} and r_{gw} are gas phase resistances to ozone and water vapor, correspondingly; D_w and D_z are diffusion constants for water vapor and O_3 in air. Now it is possible to calculate the O_3 concentration in intercellular air spaces from the measured O_3 uptake rate:

$$z_i = z_a - Q \times r_{gz} \quad (4)$$

where z is the O_3 concentration in ambient (a) and intercellular (i) air, Q is the O_3 uptake rate, r_{gz} is from Eq. (3). The O_3 uptake rate Q is measured as

$$Q = \frac{(z_1 - z_2) \times v}{S} \quad (5)$$

where z_1 and z_2 are O_3 concentrations at the inlet and outlet of the leaf chamber; v, gas flow rate; S, leaf area. Equations 1 to 5 are given in their basic form. The correction terms were included in practical calculations to account for the bulk flow of gas out of the leaf due to evaporation (7).

MATERIALS AND METHODS

Sunflower (*Helianthus annuus* L.) and *Perilla ocymoides* L. plants were grown in pots filled with soil on laboratory windows in summer. Upper full-grown leaves were used in experiments. The apparatus for measuring leaf CO_2 and water vapor exchange rates has been described by Oja (10). For present experiments, a self-made, corona-discharge O_3 generator was added to the system. In principle, the apparatus contains two open systems for measuring leaf gas exchange (below referred to as channels) in which the gas composition can be adjusted independently by means of gas mixers (MIX, Fig. 1). For adding CO_2 and O_3, capillaries are used, and the rate of injection of these gases into the carrier gas stream is controlled by pressure differences on those capillaries. A sandwich type one-sided leaf chamber LC (volume 4.4×4.4×0.3 cm^3, flow rate 20 $cm^3\ s^{-1}$) can be rapidly switched into the chain of either the first or the second channel by the 'channel exchange' valve. Infrared CO_2 analysers 'Infralyt IV' (GDR) are used for CO_2, self-made micropsychrometers for water vapor, and a Dasibi model 1003 AH analyser for ozone measurements. Volumes in the gas circuit are all reduced to the minimum. This guarantees a full-deflection response time of the system within 2.3 s (except for O_3).

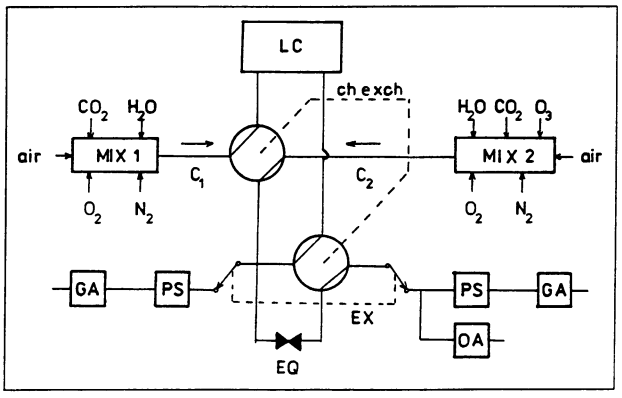

Figure 1. Basic circuit of the two-channel leaf gas exchange measurement system. LC, leaf chamber; MIX1 and MIX2, gas mixers; C_1 and C_2, different CO_2, O_2, O_3, and water vapor concentrations in the gas mixtures; CH EXCH, channel exchange valve for connecting the leaf chamber into the circuit of either channel 1 or channel 2; EQ, an equivalent resistance to the leaf chamber; EX, exit valves for flushing the leaf chamber after channel exchanges; GA, IR gas analysers; PS, psychrometers; OA, O_3 analyser.

RESULTS

Figure 2 shows the time course of CO_2, water vapor, and O_3 exchange of the physiologically lower side of an attached sunflower leaf taken from dim light and exposed to saturating PAR in the chamber. To minimize the effects of O_3 on the leaf, it was exposed to O_3 for 3 min periods only (B–H). It could be seen that during a light-induced increase in transpiration and in CO_2 uptake rates, the O_3 uptake rate (proportional to the depth of breaks B–H on the z-line) also increased. From break A it follows that the walls of the empty chamber absorbed less than 3% of incoming O_3.

Figure 3 displays the dependence of O_3 uptake rate on the O_3 concentration derived from Figure 2 (upper line) and from an experiment with another sunflower leaf with less open stomata (lower line). The proportionality indicates that O_3 degradation in leaf cells is a simple first-order reaction over the range of concentrations used.

The first-order kinetics of O_3 uptake justifies our application of the resistance/conductance approach and the calculation of total leaf conductance to O_3

$$g_z = \frac{Q}{z_a} \quad (6)$$

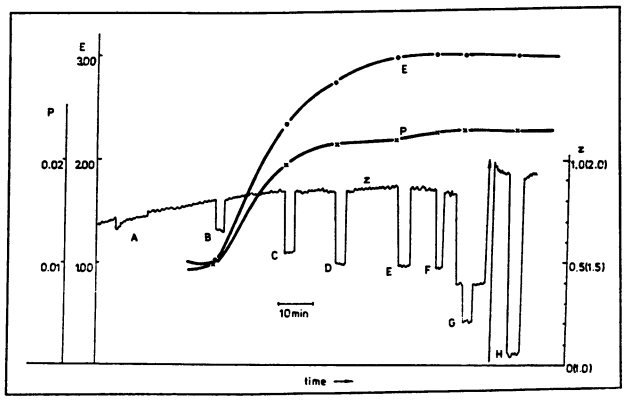

Figure 2. Parallel measurement of photosynthesis, transpiration, and O_3 uptake in a sunflower leaf. E, transpiration rate, mmol $m^{-2}\ s^{-1}$; P, CO_2 uptake rate, mmol $m^{-2}\ s^{-1}$; z, ozone concentration, $\mu L\ L^{-1}$, recorded by the ozone analyser. In A to H the leaf chamber was connected for about 3 min into the gas stream of the channel containing O_3. In A the chamber was empty, before G the inlet O_3 concentration was decreased to 0.4 $\mu L\ L^{-1}$, before H it was increased to 1,930 $\mu L\ L^{-1}$ (note that at H the range of the analyser extends from 1.0 to 2.0 $\mu L\ L^{-1}$). Leaf temperature 23.1° C, PAR 30 mW cm^{-2}, CO_2 concentration 320 $\mu L\ L^{-1}$.

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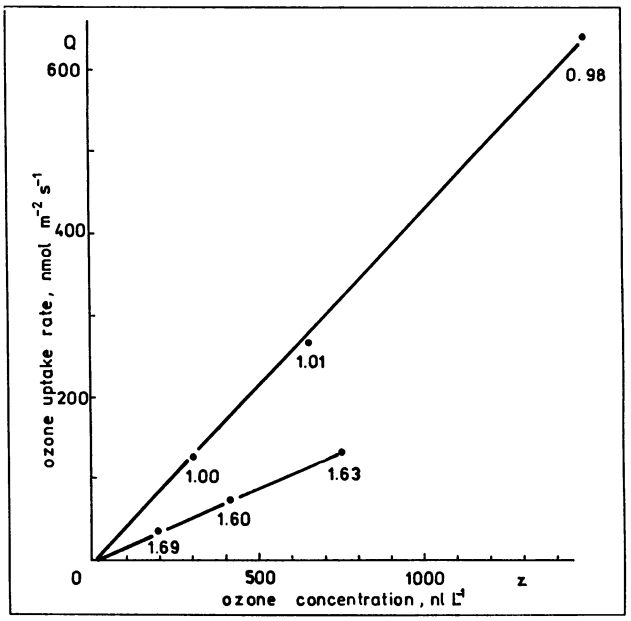

Figure 3. Dependences of O_3 uptake rates Q on O_3 concentration z in two sunflower leaves. Circles on the upper line are calculated from breaks F, G, H. in Figure 2; circles on the lower line—from a similar experiment with an another leaf. Leaf gas phase resistance to water vapor, r_{gw} in s cm^{-1} shown at each point.

where z_a is the ambient O_3 concentration and Q is the uptake rate. In the linear-flow chamber the ambient O_3 concentration

$$z_a = \frac{z_1 + z_2}{2} \tag{7}$$

where z_1 and z_2 are concentrations at inlet and outlet ports. Combining Eq. (6) with Eqs. (5) and (7), we find

$$g_z = 2 \times \frac{z_1 - z_2}{z_1 + z_2} \times \frac{v}{S}. \tag{8}$$

In our chamber the actual profile of concentration is closer to exponential than to linear. Nevertheless, the error caused by the use of Eq. (7) does not exceed 3% of g_z calculated from Eq. (8) (V Oja, personal communication). The calculated values of $r_z = 1/g_z$ corrected for nonlinearity of concentration profile are given in Table I.

The O_3 uptake rate increased in parallel with the transpiration rate (Fig. 2). The total conductance for O_3 g_z is plotted against the gas phase conductance $g_{gw} = 1/r_{gw}$ in Figure 4. The relationship is fully linear up to the highest stomatal openings observed. The conductance for water vapor extrapolates to zero at a small value for O_3 conductance. This residual conductance was mainly caused by the absorption of O_3 by chamber walls and tubing (Fig. 2, A). After subtracting this conductivity (0.035 cm s^{-1}) from the total, the slope of the line does not change, but the intercept moves very close to the origin. Evidently, there was very little or no cuticular uptake of O_3 in our experiments with sunflower leaves. The proportionality between the conductances for water vapor and O_3 suggests that the diffusion paths for both gases fully coincide, and that there is no additional resistance to O_3 in the liquid phase of cells. Evidently, O_3 actively reacts with chemical substances in cell walls and plasmalemma, and it undergoes rapid degradation. This means that the O_3 concentration in leaf intercellular air spaces must be close to zero.

Table I lists the values of the intercellular O_3 concentration z_i calculated from transpiration and O_3 uptake rates and Eq. (4). Though there is some scattering of data, one can see that at different stomatal openings z_i stays quite close to zero independent of the ambient O_3 concentration applied in the range up to 1500 nL L^{-1}.

We used Oja's (9) method for determining the concentration of a gas in the intercellular spaces to verify independently the concentration of O_3 inside the leaf. If an amphistomatous leaf is exposed to O_3 from one side, some of the O_3 molecules should diffuse out from the leaf through the stomata of the other side if the O_3 concentration in intercellular spaces is different from zero. To carry out this experiment, the two-channel system (Fig. 1) was rearranged for use with a two-sided leaf chamber. One side of the chamber was connected to the circuit of one channel and the other side to the other channel. O_3 was supplied to the gas stream at the physiologically lower side of a sunflower or *Perilla* leaf while O_3 analyser was switched between the channels. Stomata on both sides of the leaf were open enough to allow a sufficient O_3 exchange. There occurred a significant O_3 uptake from the lower leaf side but no O_3 evolution through the upper epidermis was detected in sunflower (Table II) as well as in a Perilla leaf (data not shown). This shows that the O_3 concentration is negligibly low in the intercellular air spaces of leaves.

DISCUSSION

We conclude that O_3 enters the leaf through stomata by diffusion. These results confirm more indirect evidence (2, 11) and are in accordance with conclusions drawn by Heath in his review (3). The O_3 concentration in intercellular spaces is extremely low irrespective of the ambient concentration of O_3 applied (up to 1500 nL L^{-1}). This means that O_3 is absorbed and rapidly decomposed in the cell walls or plasmalemma; it does not penetrate into the deeper layers of cells. The O_3 uptake rate, Q, can be calculated from the conductance for water vapor and the ratio of the measured diffusion rates for water vapor and O_3 as

$$Q = z_a \times g_{gw}/1.68 \tag{9}$$

where z_a is the ambient O_3 concentration, g_{gw} is the gas phase conductance to water vapor. Moldau and Söber (personal communication) found a significant component of transpiration in bean leaves that was not accompanied by the proportional O_3 uptake, and they identified it as the cuticular transpiration. In our experiments with sunflower, the cuticular transpiration of sunflower leaves was either very small, or it was accompanied by O_3 uptake. Probably, long exposures under high O_3 concentrations damage the cuticule causing increased cuticular transpiration.

There occurred no detectable O_3 flux through the leaf, which may mean that O_3 is rapidly decomposed in stomatal pores or substomatal cavities. The rapid decomposition of O_3 may damage cell walls and the plasmalemma. The reflection

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Table I. *Calculated Resistances and Intercellular O_3 Concentrations for the Experiment in Figure 2*

Q is the O_3 uptake rate; r_{gw}, leaf gas phase resistance to water vapor; r_{gz}, gas phase resistance to O_3 calculated from r_{gw} (Eq. 4); r_z, total resistance to O_3, calculated from the O_3 uptake rate; z_i, calculated O_3 concentration in intercellular air space using r_{gz}.

Point	O_3	O_3	Q	r_{gw}	r_{gz}	r_z	z_i
	$nL L^{-1}$	$\mu mol\ m^{-3}$	$nmol\ m^{-2}\ s^{-1}$		$s\ cm^{-1}$		$\mu mol\ m^{-3}$
A	723	29.0	10.58			27.5	
B	733	29.42	61.3	4.15	6.93	4.83	−4.59
C	691	27.77	123.6	1.55	2.59	2.28	−1.32
D	665	26.32	148.1	1.22	2.04	1.85	−1.66
E	675	27.12	165.1	1.07	1.78	1.69	−0.35
F	660	26.52	165.1	1.01	1.69	1.66	0.43
G	306	12.30	79.6	1.00	1.67	1.59	−0.16
H	1492	63.98	370.3	0.98	1.64	1.67	−7.55

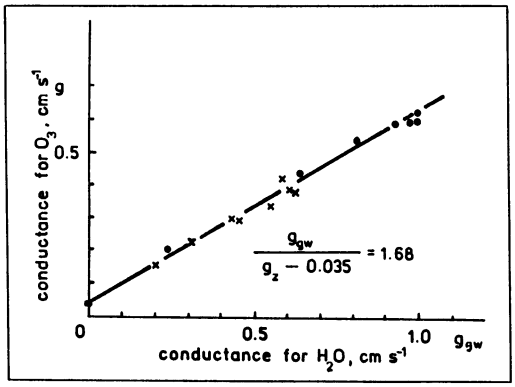

Figure 4. Relationship between the leaf gas phase conductance to water vapor g_{gw} and the total conductance for ozone g_z in sunflower leaves. Circles are from the experiment in Figure 2; crosses are from other experiments; square denotes ozone absorption by chamber walls.

Table II. *Measured O_3 Fluxes and Concentrations in a Sunflower Leaf*

O_3 was supplied to the leaf from the lower side, and the evolution of O_3 through the upper epidermis was measured. Abbreviations: r_{gw}, leaf gas phase resistance to water vapor; Q, ozone exchange rate

Lower Side of the Leaf			*Upper Side of the Leaf*		
r_{gw}	O_3	Q	r_{gw}	O_3	Q
$s\ cm^{-1}$	$nL\ L^{-1}$	$nmol\ m^{-2}\ s^{-1}$	$s\ cm^{-1}$	$nL\ L^{-1}$	$nmol\ m^{-2}\ s^{-1}$
2.36	709	89.3	5.30	0	0
2.96	1790	179.6	4.17	0	0

coefficients of plastid membranes were reduced in the presence of ozone; the introduction of O_3 into a culture of *Chlorella* cells caused a 15- to 20-fold increase in the efflux of potassium (reviewed in 3). Söber (12) has shown that the properties of the cell walls or plasmalemma of bean leaves changed after a rather short exposure to O_3. The elasticity modulus of the walls increased, the stretchability of cells decreased.

The tolerance of plants to O_3 has two different mechanisms. One is based on the stomatal closure in response to higher O_3 concentrations and operates on the basis of suppressing the O_3 flux into the leaf. This brings along a considerable reduction in the CO_2 uptake rate and a decrease in the growth rate and plant yield. This mechanism inpractice did not operate during our short-time exposures. The other way to withstand higher O_3 concentrations is to develop a mechanism for the neutralization of the damage caused by O_3 for living cells. Evidently, this mechanism involves the resynthesis of damaged enzyme molecules or membrane fractions, and it causes increased maintenance energy costs accompanied by higher respiration rates (3, 11, and references therein). Studies of the effect of O_3 on plants should lead to the establishment of a repair cost of damage caused by one O_3 molecule in a number of respired CO_2 molecules.

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