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Oxygen Exchange in Leaves in the Light[1]

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ABSTRACT

Photosynthetic O_2 production and photorespiratory O_2 uptake were measured using isotopic techniques, in the C_3 species *Hirschfeldia incana* Lowe., *Helianthus annuus* L., and *Phaseolus vulgaris* L. At high CO_2 and normal O_2, O_2 production increased linearly with light intensity. At low O_2 or low CO_2, O_2 production was suppressed, indicating that increased concentrations of both O_2 and CO_2 can stimulate O_2 production. At the CO_2 compensation point, O_2 uptake equaled O_2 production over a wide range of O_2 concentrations. O_2 uptake increased with light intensity and O_2 concentration. At low light intensities, O_2 uptake was suppressed by increased CO_2 concentrations so that O_2 uptake at 1,000 microliters per liter CO_2 was 28 to 35% of the uptake at the CO_2 compensation point. At high light intensities, O_2 uptake was stimulated by low concentrations of CO_2 and suppressed by higher concentrations of CO_2. O_2 uptake at high light intensity and 1000 microliters per liter CO_2 was 75% or more of the rate of O_2 uptake at the compensation point. The response of O_2 uptake to light intensity extrapolated to zero in darkness, suggesting that O_2 uptake via dark respiration may be suppressed in the light. The response of O_2 uptake to O_2 concentration saturated at about 30% O_2 in high light and at a lower O_2 concentration in low light. O_2 uptake was also observed with the C_4 plant *Amaranthus edulis*; the rate of uptake at the CO_2 compensation point was 20% of that observed at the same light intensity with the C_3 species, and this rate was not influenced by the CO_2 concentration. The results are discussed and interpreted in terms of the ribulose-1,5-bisphosphate oxygenase reaction, the associated metabolism of the photorespiratory pathway, and direct photosynthetic reduction of O_2.

Both O_2 evolution and O_2 uptake take place in leaves of C_3 and C_4 plants in the light (4, 8, 17, 21, 23, 24, 27, 28). O_2 evolution is derived entirely from the water-splitting reaction of PSII, but three principal O_2 uptake processes are presently recognized. These are: the oxygenase reaction of ribulose bisP carboxylase-oxygenase and the associated metabolism of P-glycolate (2, 4, 5, 18, 20); the Mehler reaction (22), which results in the direct photoreduction of O_2 and may support ATP synthesis via pseudocyclic photophosphorylation (9, 13, 16); and the possibility that O_2 uptake associated with mitochondrial respiration continues in the light (18).

Volk and Jackson and their colleagues (17, 23, 24, 27, 28) have made substantial contributions to the study of O_2 exchange in intact leaves, but only a limited range of conditions were employed. In this paper, we describe experiments over a wide range of CO_2 and O_2 concentrations in which net CO_2 uptake, O_2 uptake, and O_2 evolution were measured. These show that, in C_3 plants but not in C_4 plants, O_2 uptake is depressed by high CO_2 and permits partial resolution of the alternative pathways of O_2 uptake in intact leaves.

MATERIALS AND METHODS

Leaves of Indian mustard (*Hirschfeldia incana* Lowe. syn. *Brassica genniculata* Desf.) were harvested from plants growing under natural conditions. Leaves of sunflower (*Helianthus annuus* L. var. Bronze Hybrid), *Amaranthus edulis* L. and bean (*Phaseolus vulgaris* L.) were harvested from plants growing in soil in a glasshouse.

Experiments were conducted with the closed gas exchange system of Berry *et al.* (4). The system had been modified by Berry and Badger (unpublished) who added a capillary arrangement to supply CO_2 to the system. Leaves of the above species were detached under water and placed in the plant chamber. After equilibration of the leaves in air at 400 μE m^{-2} s^{-1} illumination, the air in the system was replaced by flushing the system with argon. The flow of argon was stopped and, with the two-way valve open, the required amount of $^{18}O_2$ (99% ^{18}O, Norsk Hydro, Oslo, Norway) was injected into the system. The system was closed and the gas was circulated over the leaf with a metal bellows pump. Mass 32, mass 36, and mass 40 were monitored continuously with a GD 150/4 mass spectrometer.

O_2 uptake and O_2 evolution were calculated using the methods previously described (25, 27). CO_2 concentration was measured with an IRGA analyzer (UNOR-2, Maihak, Hamburg, Germany) included in the gas circuit, and CO_2 concentration during illumination could be controlled by varying the pressure of CO_2 on a capillary that bled pure CO_2 into the closed system. CO_2 uptake, at constant CO_2 concentration in the system, was calculated from the rate of CO_2 addition. Each measurement was averaged over an 8- to 10-min period of gas exchange after the rate of CO_2 uptake had reached a steady rate at each CO_2 concentration. The total gas pressure in the small system increased due to O_2 production and was equilibrated to atmospheric pressure between measurements. In this closed system, water vapor was condensed in a trap held at 5 C below the leaf temperature but it was not possible to measure stomatal responses, so that all CO_2 concentrations cited in the text refer to ambient, not intercellular, CO_2 concentration.

RESULTS

The responses of net CO_2 fixation and the components of net O_2 exchange (gross O_2 production from water and gross O_2 uptake from the atmosphere) to ambient CO_2 concentration were measured in leaves of the C_3 plant *H. incana*. These responses at four quantum flux densities (light intensities) at 30 C are shown in Figure 1, A to D. In all of these experiments, net O_2 exchange measured by MS was within 5% of net CO_2 exchange which was

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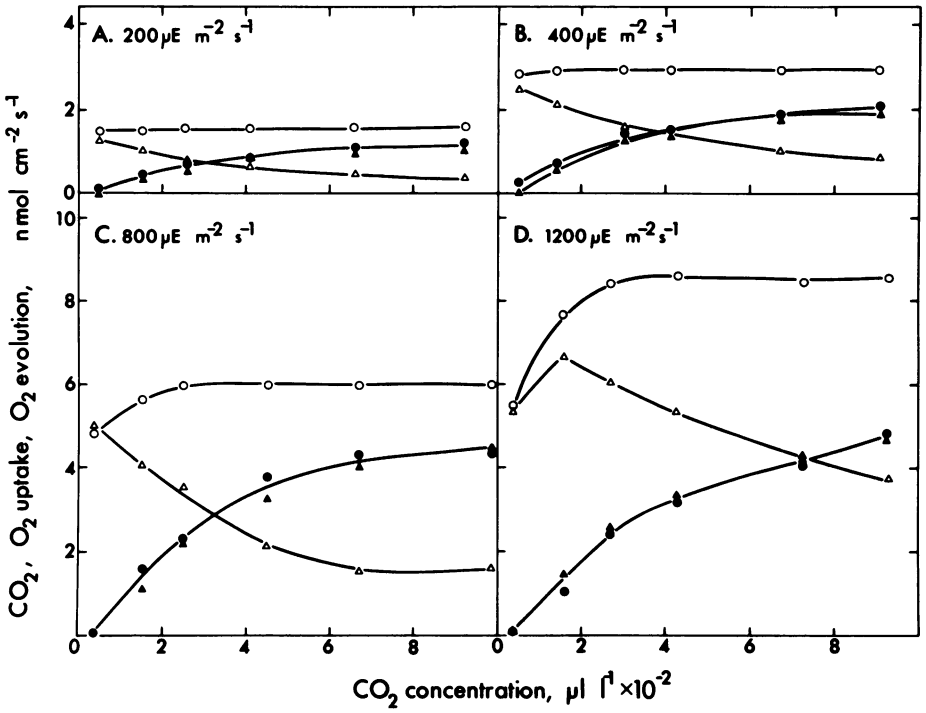


FIGS. 1. Effect of CO_2 and light intensity on O_2 exchange and CO_2 assimilation in leaves of *H. incana* L. Light intensity was as shown; temperature was 30 ± 1 C except for the three highest CO_2 concentrations of D where temperature was 34 to 36 C. Other conditions: A, 24 to 27% O_2; B, 25 to 28% O_2; C, 24 to 30% O_2; D, 25 to 30% O_2. ○, O_2 production; △, O_2 uptake; ●, net O_2 release; ▲, CO_2 uptake.

independently determined from the pressure *versus* flow calibration of the capillary used to add CO_2 to the closed system. Thus, the data of net O_2 evolution and CO_2 uptake are internally consistent. Increased CO_2 at each light intensity leads to an increase in CO_2 uptake. At the three lowest light intensities, either CO_2 or O_2 could apparently serve equally well and were mutually competitive as acceptors for electrons from the water-splitting reactions of photosynthesis. O_2 production was independent of CO_2 concentration at low light intensities but, at higher light intensities, CO_2 stimulated O_2 production (Fig. 1, C and D).

The inhibition of O_2 uptake by CO_2 is most clearly seen in Figure 2, which shows the data of Figure 1, A to C, normalized to the rate of O_2 uptake at the CO_2 compensation point at the appropriate light intensity and replotted as a function of CO_2 concentration. (The data of Fig. 1D, which will be discussed later, were omitted because of the complex change in O_2 uptake at the low CO_2 concentrations.) At the CO_2 compensation point, O_2 uptake was equal to the O_2 production (100%) and the relative rate of O_2 uptake fell to 28 to 35% of the rate of O_2 production at high CO_2 (900–1,000 μl l^{-1}). The decline of O_2 uptake with increasing CO_2 was hyperbolic. Half-inhibition occurred at about 400 to 450 μl l^{-1} CO_2 and inhibition did not appear to be saturated by 900 to 1,000 μl l^{-1}, the highest concentration of CO_2 used in these studies. The pattern of the inhibitory effect of CO_2 on O_2 uptake appeared to be similar at light intensities equal or below 800 μE m^{-2} s^{-1}.

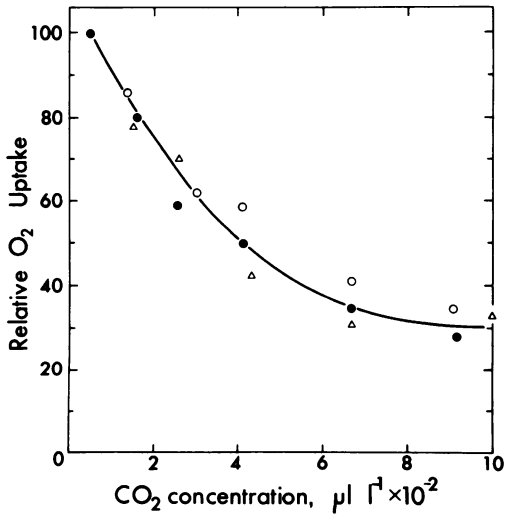
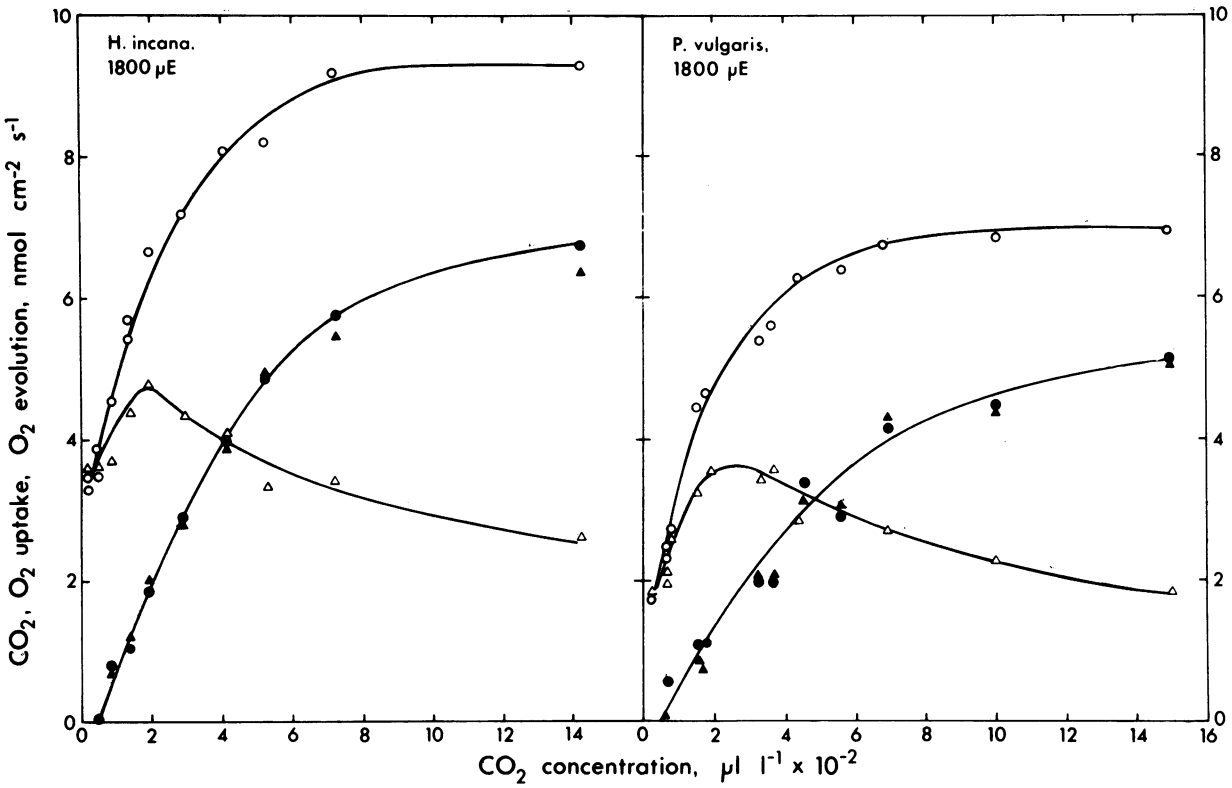


FIG. 2. Effect of CO_2 on O_2 uptake. O_2 uptake data of Figure 1, A (●), B (○), and C (△), are replotted as a percentage of the O_2 uptake that occurred at the CO_2 compensation point.

At 1,200 μE m^{-2} s^{-1} (Fig. 1D), O_2 uptake was stimulated by low concentrations of CO_2 and subsequently inhibited by higher CO_2 concentrations. With the detached leaf system, it was not possible to control leaf temperature or to avoid leaf wilting at higher light intensities. A modified chamber was developed which permitted studies with attached leaves and allowed exact control of leaf temperature at high light intensities. Experiments similar to those in Figure 1 were run at 1,800 μE m^{-2} s^{-1} using leaves of the C_3 plants *H. incana* and *P. vulgaris* (Fig. 3). At this light intensity, evidence to support the data of Figure 1D was obtained, as O_2 uptake was greatly stimulated by low concentrations of CO_2. Inhibition of O_2 uptake by higher CO_2 concentrations was much less than that observed at low light intensities (Fig. 2), as O_2 uptake rates at about 1,500 μl l^{-1} CO_2 were equal to, or only 25% less than, O_2 uptake rates at the CO_2 compensation point. It is also evident that O_2 production was severely limited at the CO_2 compensation point and that CO_2 greatly stimulated O_2 production.

The dependence of O_2 and CO_2 exchange on light intensity is shown in Figure 4, A to C. The rate of O_2 production equalled the rate of O_2 uptake at the CO_2 compensation point, and these were rate-saturated at about 800 μE m^{-2} s^{-1} (Fig. 4A). Presumably this saturation is due to a limitation on the supply of electron acceptors



FIGS. 3. Effect of CO_2 on O_2 exchange and CO_2 assimilation in leaves of *H. incana* and *P. vulgaris*. Light intensity was 1800 μE m^{-2} s^{-1}; temperature was 28 ± 0.5 C and 29 ± 0.5 C, respectively; the O_2 range was 18 to 25% and 20 to 27%, respectively. (○), O_2 production; (△), O_2 uptake; (●), net O_2 evolution; (▲), net CO_2 uptake.

under these conditions, rather than to a limitation of electron transport because, in the presence of 21% O_2 and concentrations of CO_2 of 300 μl l^{-1} or higher, the rate of O_2 production was linear to 1,200 μE m^{-2} s^{-1} (Fig. 4, B and C).

The slope of O_2 production (Fig. 4B) was 0.075 mol O_2 E^{-1} which is similar to the quantum yield for CO_2 fixation observed with C_3 plants in low O_2 concentrations. Net CO_2 uptake at 300 or 1,000 μl l^{-1} CO_2 increased with light intensity, but the relative rate of increase declined continuously so that uptake (Fig. 4, B and C) was saturated at about 800 μE m^{-2} s^{-1}. In contrast to CO_2 uptake, the rate of O_2 uptake increased with light intensity with a progressively greater rate of increase as the light intensity was increased (Fig. 4, B and C). These observations suggest that, as light intensity is increased, an increasing proportion of electron transport is diverted to O_2 as the terminal electron acceptor.

Another interesting feature of these data is that the light-response curves for O_2 uptake can be extrapolated to zero (Fig. 4). This indicates that dark respiration probably plays no significant role in the O_2 uptake measured here. O_2 uptake in the dark was 0.2 nmol cm^{-2} s^{-1}.

O_2 uptake as a function of O_2 concentration is shown in Figure 5. At the CO_2 compensation point and at the light intensity used (400 μE m^{-2} s^{-1}), uptake was rate saturated at 20 to 30% O_2. A similar response of O_2 uptake to O_2 concentration was also observed with sunflower (Fig. 6), soybean (23), and algae (26). At this light intensity, increased CO_2 concentration inhibited the rate of O_2 uptake (Fig. 5) but had little effect on the shape of the O_2 concentration dependence. The O_2 concentration required to saturate O_2 uptake in sunflower was lower at lower light intensity (Fig. 6). Similar data were obtained for mustard at 400 and 800 μE m^{-2} s^{-1}. These responses are similar to those observed for CO_2 uptake as a function of CO_2 concentration at different light intensities (10).

Figure 6 also shows a comparison between photorespiratory CO_2 release and O_2 uptake as a function of O_2 concentration. The CO_2 release into CO_2-free air (measured in a separate experiment but on an equivalent leaf) roughly parallels O_2 uptake and is consistent with the assumption that both processes are related to photorespiration. The ratio of CO_2 production to O_2 uptake was about 1:6 at O_2 concentrations above 10%.

Our studies also indicated a dependence of O_2 production on O_2 concentration. At the CO_2 compensation point, O_2 production equalled O_2 uptake over the entire range of O_2 concentration and decreased as the O_2 concentration decreased (Fig. 5). This would appear to be due to the inability of low concentrations of O_2, at the CO_2 compensation point, to support the maximum capacity for electron transport. The restriction of O_2 production at low O_2 was partially, but not completely, overcome at higher CO_2 concentrations (Fig. 7). At 400 μl l^{-1} CO_2 and 400 μE m^{-2} s^{-1}, O_2 production was still stimulated 25% between an O_2 concentration change from 4 to 21%. Over the same range of O_2 concentrations, O_2 uptake increased from nearly zero to close to saturation, whereas net CO_2 uptake decreased (Fig. 7). The increase in O_2 uptake was much larger than the decrease in CO_2 uptake. If the change in O_2 concentration only affected photorespiratory processes, one might have expected a much greater decrease in CO_2 fixation. Because CO_2 fixation did not decrease an amount equivalent to the increase in O_2 uptake and because O_2 production was stimulated by O_2, O_2 uptake processes other than oxygenase would seem to be involved.

O_2 uptake in the C_4 species *A. edulis* was also observed. The dependence of O_2 uptake on O_2 concentration was similar to that observed with the C_3 species (Fig. 8A). However, O_2 uptake was not affected by the CO_2 concentration (Fig. 8B), and the maximum rate of O_2 uptake was only about 20% of that observed at the same light intensity with the C_3 species. These results indicate substantial differences in the O_2 exchange patterns of C_3 and C_4 plants.

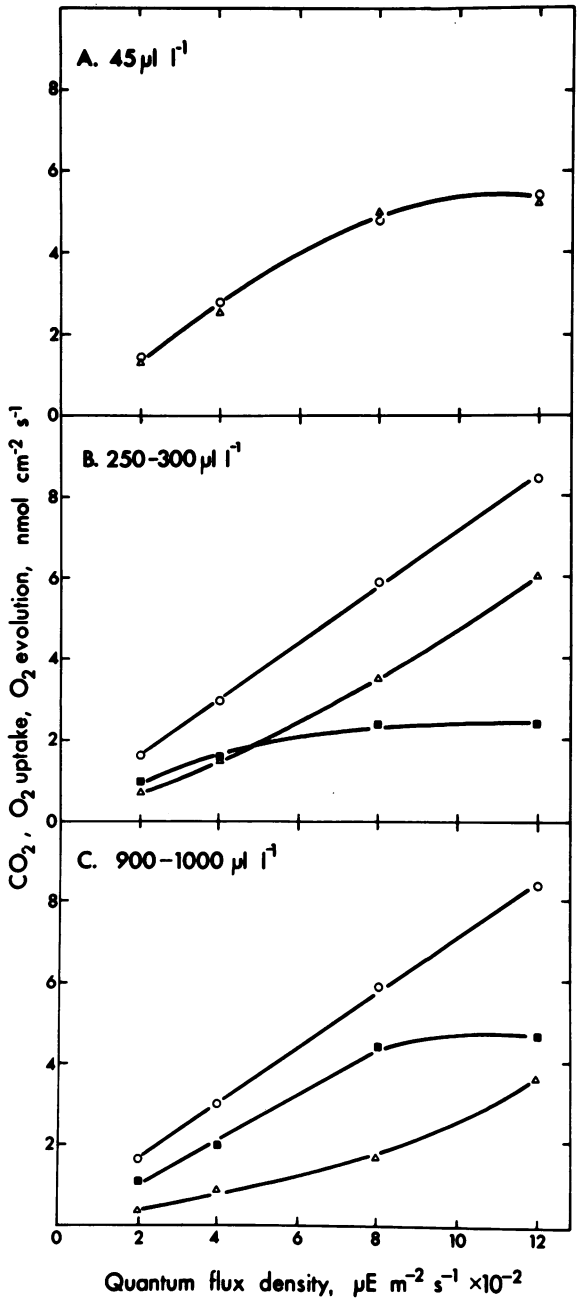


FIG. 4. Effect of light intensity on O_2 exchange in leaves of *H. incana* at the CO_2 compensation point (A), at 250 to 300 μl l^{-1} CO_2 (B), and at 900 to 1,000 μl l^{-1} CO_2 (C). ○, O_2 production; △, O_2 uptake; ■, CO_2 uptake. Measuring conditions were as in Fig. 1.

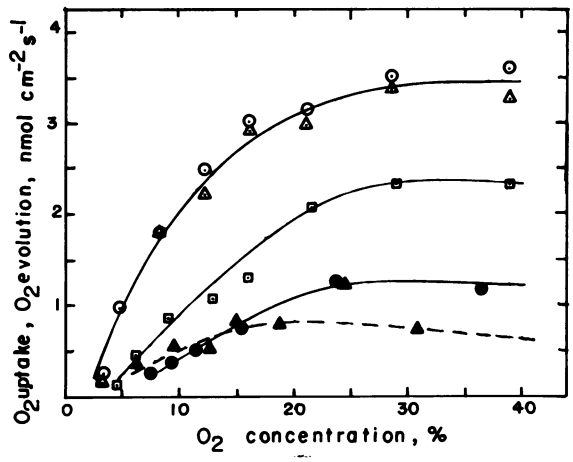


FIG. 5. Effect of O_2 and CO_2 on O_2 exchange in leaves of *H. incana*. Light intensity was 400 μE m^{-2} s^{-1}; temperature was 24.7 to 27.1 C. O_2 production (○) and uptake (△) at the CO_2 compensation point. O_2 uptake at 390 to 420 μl l^{-1} CO_2 (□), at 769 to 875 μl l^{-1} CO_2 (●), and at 1150 to 1240 μl l^{-1} CO_2 (▲).

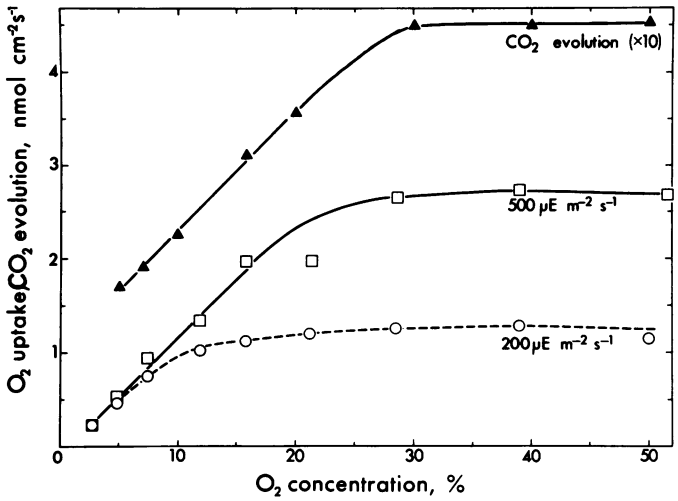


FIG. 6. Effect of O_2 concentration and light intensity on O_2 uptake at the CO_2 compensation point and CO_2 evolution into a CO_2-free atmosphere by sunflower leaves at 420 μE m^{-2} s^{-1}, at 26 to 30 C. O_2 uptake at the light intensities shown (□, ○); CO_2 evolution (× 10) into a CO_2-free atmosphere (▲).

DISCUSSION

At normal O_2 concentrations, reactions involving atmospheric O_2 apparently consume a large fraction (20–100%) of the electrons produced in the photosynthetic oxidation of water by illuminated intact leaves of C_3 and C_4 plants. There is now abundant evidence that the biochemical pathway of photorespiration (18, 20) occurs with the fixation of O_2 by Ru-P_2[5] carboxylase-oxygenase and the metabolism of the product of that reaction, P-glycolate, to 3-PGA and CO_2 (2, 4, 5, 8, 20). Rapid O_2 uptake has also been observed with algae (25, 26) and here it was attributed to direct photosynthetic reduction of O_2 that was independent of photosynthetic carbon metabolism. It is important to consider which of these two mechanisms of O_2 uptake should be applied to the O_2 uptake reported here, but, as with previous studies (23, 28), the results of O_2 exchange in intact leaves of C_3 and C_4 plants are difficult to interpret unequivocally. The data, however, may allow a partial resolution of the question and some issues which arise from the data should be considered. These include (*a*) the mechanisms of O_2 uptake, (*b*) the basis of the differences of the O_2 exchange properties of C_3 and C_4 plants, (*c*) the relative abilities of O_2 and CO_2 to support photosynthetic electron transport (act as electron acceptors) *in vivo*, (*d*) the basis for the inhibition of O_2 uptake by increased concentrations of CO_2, (*e*) the apparent kinetics of O_2 exchange reactions *in vivo* in comparison to measurements which have been made *in vitro* of possible O_2 uptake mechanisms, and (*f*) the apparent stimulation of photosynthetic O_2 production by O_2 or CO_2.

Mechanisms of O_2 Uptake. Either CO_2 or O_2 may serve as acceptors for electrons from photosynthetic O_2 production. To

[5] Abbreviations: Ru-P_2, ribulose 1,5-bisphosphates; 3-PGA, 3-phosphoglyceric acid.

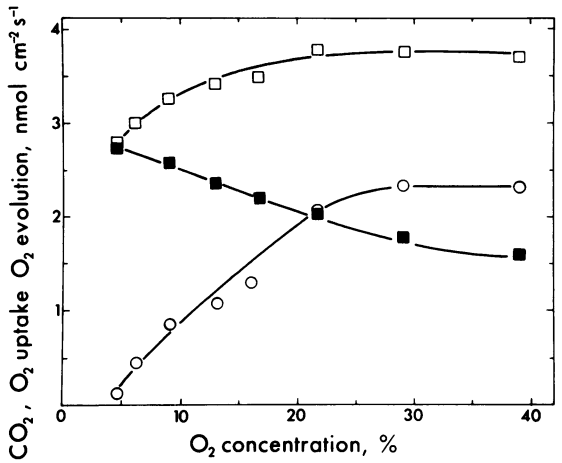


FIG. 7. Effect of O_2 concentration on O_2 production (□), O_2 uptake (○), and CO_2 uptake (■) by leaves of *H. incana* at 390 to 420 μl l^{-1} CO_2, 30 C, and 400 μE m^{-2} s^{-1}.

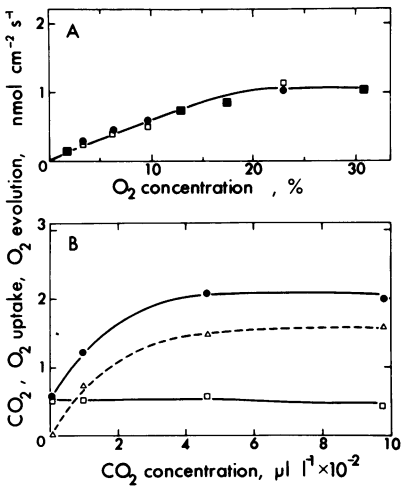


FIG. 8. A, O_2 production (●) and uptake (□) of a leaf of *A. edulis* L. at the CO_2 compensation point. B, O_2 production (●), O_2 uptake (□), and net O_2 release (△) of a leaf of *A. edulis* L. as a function of CO_2 concentration. Light intensity was 400 μE m^{-2} s^{-1}; temperature was 26 to 28 C. O_2 concentrations for B were 26 to 28%.

some extent, one may substitute for the other (Fig. 1) but in other cases (Figs. 3 and 8) the two acceptors appear to be additive. CO_2 acts as an ultimate acceptor because its fixation and metabolism in photosynthesis regenerates ADP and $NADP^+$. O_2 acts as an electron acceptor because the reactions of photorespiration regenerates ADP and $NADP^+$ or it may react directly with a component of PSI and be reduced in a reaction termed the "Mehler reaction" (22). As mentioned earlier, both O_2 uptake reactions have been shown to occur *in vivo* and it is not possible to distinguish them by only following total O_2 uptake.

Some estimate of each reaction may be obtained by considering the stoichiometry of the photorespiratory reactions at the compensation point. In a previous study, the balanced photorespiratory reactions require that 3.5 O_2 should be taken up for every CO_2 released (4). In this study, CO_2 release into CO_2-free air over a wide range of O_2 concentrations was approximately one-sixth of the O_2 uptake at the compensation point (Fig. 6). By applying the expected stoichiometry, about 60% of the O_2 uptake may be attributed to photorespiration. This estimate is lower than that reported previously (4), but the estimate may be low due to refixation of CO_2. The balance of the O_2 uptake could be due to a Mehler reaction and dark respiration. Many workers have argued that a Mehler reaction may be involved in regulating phosphorylation *in vivo* (9, 16, 19), but the exact requirement for this reaction is unknown.

O_2 Exchange in C_3 and C_4 Plants. The rate of O_2 uptake by the C_4 plant *A. edulis* was only about 20% of that of a C_3 species under the same conditions, and this rate was apparently unaffected by the CO_2 concentration (Figs. 1 and 8). It is generally accepted that C_4 plants possess a mechanism for concentrating CO_2 at the site of the Ru-P_2 carboxylase-oxygenase and that this mechanism leads to a diminished rate of photorespiration (3, 14). Oxygenase activity is not completely eliminated, however, as judged from $^{14}CO_2$ fixation experiments and ^{18}O incorporation into glycine and serine (8, 14). Thus, some of the O_2 uptake in C_4 plants may be due to photorespiration and the remaining O_2 uptake may be associated with a Mehler reaction. The ATP requirements of C_4 photosynthesis are greater than those for C_3 photosynthesis (14) so that the need for a mechanism to balance ATP formation to NADPH use may be more essential in C_4 plants than in C_3 plants. Recent experiments (7) show that the rate of O_2 uptake in isolated bundle sheath cells of maize is too low to provide for the pseudocyclic generation of the additional ATP requirements. Thus, direct O_2 uptake in C_4 plants may also only fulfill a role of "poising" the reduction state of the electron transport chain as has been proposed for C_3 plants (16).

Capacity of O_2 and CO_2 as Acceptors. At low light intensities, O_2 uptake at the CO_2 compensation point supports the same rate of O_2 evolution observed at CO_2 saturation (Fig. 1). With increasing light intensity, O_2 evolution is severely limited by low CO_2 concentration (Figs. 1 and 3). O_2 evolution is stimulated by increasing CO_2 concentration up to that required to saturate CO_2 uptake. At near full sunlight intensity (1800 μE m^{-2} s^{-1}), O_2 alone supports only 25 to 35% of the O_2 evolution observed at saturating CO_2 (Fig. 3). A similar dependence of O_2 evolution upon CO_2 concentration has been found in *Chlamydomonas* at 400 μE m^{-2} s^{-1} and at ambient O_2 concentration (Fock and Badger, in preparation), in isolated spinach chloroplasts (4, 21), and in spinach cells (21). Studies with *Scenedesmus* (25, 26) show that O_2 was able to substitute completely for CO_2 as an electron acceptor. With higher plants, however, such substitution appears to be only complete at low light intensities.

Effect of CO_2 Concentration on O_2 Uptake. As shown previously (11), O_2 uptake at low light intensities was suppressed by increased CO_2 concentration (Figs. 1 and 2). Studies of Ru-P_2 carboxylase-oxygenase *in vitro* show that the oxygenase activity is competitively inhibited by CO_2 (2, 5). The CO_2 concentration for half-inhibition of O_2 uptake by intact leaves in 21% O_2 was about 450 μl l^{-1} (Fig. 2), which is similar to that for half-inhibition of the oxygenase *in vitro* (2). At 900 to 1000 μl l^{-1} CO_2, O_2 uptake was 28 to 35% of that which occurred at the compensation point.

Based on *in vitro* kinetic properties of the oxygenase (2), O_2 uptake by this means would still be expected at that CO_2 concentration and ^{18}O incorporation into glycine was also observed at the same CO_2 levels (Lorimer, Badger, and Berry, in preparation). Of course, the Mehler reaction may also be suppressed by CO_2 because, if it depends on the $NADP^+$ level (25), CO_2 fixation would be expected to alter the level of that compound. It appears, though, that if CO_2 is affecting both reactions, the response of each reaction to increased CO_2 concentrations would appear to be similar.

If the inhibition of O_2 uptake *in vivo* by increased CO_2 concentration is due to inhibition of Ru-P_2 oxygenase, we would expect it to be correlated with a decrease in CO_2 evolution. The data available indicates that photorespiratory CO_2 release is insensitive to CO_2 concentrations (6). Although there is some contradiction here, we point out that, at high light intensities (Fig. 3), the response of O_2 uptake to increasing CO_2 concentration is complex

and that the rate of O_2 uptake at high CO_2 concentration is not greatly different from that at the CO_2 compensation point. If CO_2 release is dependent on O_2 uptake, then there may not be much effect of CO_2 concentration on CO_2 release at high light intensities. Photorespiratory CO_2 release at limiting light intensities should be re-examined.

Effect of O_2 Concentration on O_2 Uptake. O_2 uptake by Ru-P_2 oxygenase has a K_m (O_2) of 20 to 33% O_2 *in vitro* (2), whereas the Mehler reaction of isolated chloroplasts is half-saturated by less than 5% O_2 (1, 15). In principle, it should be possible to distinguish between the two mechanisms by studies of the O_2 concentration dependence of O_2 uptake by intact leaves. In the studies presented here, O_2 uptake of C_3 and C_4 plants was saturated by 20 to 30% O_2 (Figs. 5–8). The response was, in fact, similar to the O_2 uptake that was observed in algae (26) and attributed to a Mehler reaction. The O_2 uptake observed here might be attributed to either or both mechanisms on the basis of the O_2 concentration dependence alone. It would seem that a comparison of *in vivo* and *in vitro* O_2 uptake responses to O_2 concentration is not possible because of constraints *in vivo* that may alter the responses. Constraints such as ATP turnover, if the Mehler reaction is coupled to complete electron transport, or light intensity, which could limit the supply of Ru-P_2 would alter the O_2 uptake response pattern to reflect these factors rather than allowing the O_2 uptake to be purely a response to O_2 concentration, as would occur when these other factors were nonlimiting.

Effects of O_2 and CO_2 Concentration. Two responses observed here are difficult to fit into the general conceptual framework of O_2 exchange and should be commented upon. At normal concentrations of CO_2, CO_2 uptake increased when the O_2 concentration was decreased from 21 to 4% (Fig. 7). Over the same O_2 concentration change, however, O_2 production decreased (Fig. 7) and the increase in net CO_2 uptake was less than that anticipated from the decrease in O_2 uptake. It seems that, at normal CO_2 levels, O_2 may be required for maximum O_2 production.

The other response that was unusual was the complex pattern of O_2 exchange at high light intensities (Figs. 3 and 10). As the CO_2 concentration was increased from the CO_2 compensation point, O_2 uptake was first stimulated and then inhibited (Fig. 3). The increase in the CO_2 concentration also greatly stimulated O_2 production (Fig. 3). A stimulation of O_2 uptake by CO_2, observed previously (23), was attributed to a CO_2-dependent increase in substrate for O_2 uptake. The capacity for O_2 to act as an acceptor may also be stimulated, however, because increased CO_2 may activate Ru-P_2 oxygenase *in vivo*. The concentration of activating sites in the chloroplast is in excess of 3 mM and, at the CO_2 compensation point in C_3 plants, intercellular CO_2 concentration is only 2 μM. In these circumstances, less than fully active enzyme is at least feasible. The effect of inactivation would be most pronounced at high light intensities when O_2 exchange and CO_2 uptake is carboxylase-oxygenase-limited, rather than electron-transport-limited.

Yet another alternative is that increased uptake follows from increased O_2 production as it is known from studies with isolated chloroplasts that CO_2 will stimulate PSII activity (12), but this seems less likely as there is no effect of CO_2 observed at low light intensity.

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