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Photorespiration and CO_2 compensation point in *Najas flexilis*[1]

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Abstract

The response of net photosynthesis, light:dark respiration ratio, and CO_2 compensation point to oxygen manipulation and to inhibitors of glycolate metabolism confirms earlier evidence that photorespiration occurs in *Najas flexilis* and can influence its productivity. Estimates of the CO_2 compensation point are similar to those for several other submersed angiosperms which cannot reduce total aqueous CO_2 concentration much below air equilibrium levels.

The process of photorespiration, oxygen uptake and CO_2 release in the light associated with glycolate metabolism, is a potentially significant factor in aquatic plant primary productivity (Hough 1974, 1976; Helder et al. 1974; Brown et al. 1974; Tolbert and Osmond 1976). We here report evidence to support the presumptive observations of photorespiration found earlier (Hough and Wetzel 1972; Hough 1974) in the freshwater angiosperm *Najas flexilis* (Willd.) Rostk. and Schmidt. In particular, we have examined the process as it relates to the carbon dioxide compensation point of this plant.

Photosynthesis generally is proportional to the available CO_2 concentration (up to a saturating level). The CO_2 compensation point is the environmental CO_2 concentration at which gross photosynthesis is limited by low CO_2 to the extent that it equals respiratory CO_2 release, and net photosynthesis becomes zero. Plants with low rates of respiration and photorespiration, and high rates of internal CO_2 refixation, have low CO_2 compensation points (0–10 ppm CO_2 in air for terrestrial C_4 plants), whereas less efficient plants have higher compensation points (50–70 ppm in air for terrestrial C_3 plants; e.g. *see* Zelitch 1971). Thus the CO_2 compensation point, while not normally actually reached in situ, at least in terrestrial plants, is often determined experimentally as an indication of plant photosynthetic efficiency. Although determination of the CO_2 compensation point is a simple and rapid process in air, it is less so for aquatic plants particularly because of slower CO_2 flux in water and bicarbonate interaction. Here we compare the CO_2 compensation point of *Najas* with the few known for other submersed plants and evaluate its significance relative to photorespiration and productivity.

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Materials and methods

Najas flexilis seedlings were grown axenically (Wetzel and McGregor 1968) in a simulated hard-water lake growth medium (Hough and Wetzel 1972), with addition of a trace metal mixture. Plants were used in experiments within 2 to 4 weeks of becoming green.

For measurements of photosynthesis and CO_2 compensation point, loose clumps of seedlings (about 20 mg total

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Table 1. Photosynthetic [^{14}C]CO_2 uptake in *Najas flexilis* (2-h incubations at 16 klux, 22°C, pH 8.2; low O_2 < 1 mg·liter^{-1}, high O_2 > 25 mg·liter^{-1}).

	cpm·g^{-1}·h^{-1} ± SD
Low O_2	11,849 ± 3,145
High O_2	4,887 ± 514
High O_2 + 10^{-4} M HMS*	9,735 ± 897

* Sodium hydroxymethanesulfonate.

Table 2. Light:dark respiration ratio in *Najas flexilis* (16 klux, 22°C, pH 8.2, 29 mg O_2·liter^{-1}).

	Ratio ± SD
control	0.88 ± 0.17
10^{-4} M HMS*	0.62 ± 0.07

* Sodium hydroxymethanesulfonate.

dry wt) were transferred aseptically to 10-ml flasks containing sterile experimental growth medium. Where applicable, total CO_2 concentrations in the medium were pre-established by air equilibration or known bicarbonate addition to autoclaved medium. Preliminary experiments indicated that high initial bicarbonate concentrations necessitated excessively long incubation to attain CO_2 compensation. To test for the presence of oxygen inhibition of net photosynthesis (a major aspect of photorespiration: Zelitch 1971) and its effect on compensation point, we varied dissolved oxygen concentrations from saturation level by N_2- or O_2-sparging. We also tested the involvement of photorespiration by attempting to inhibit it with the glycolate synthesis inhibitor potassium glycidate (Zelitch 1974) and the glycolate oxidase inhibitor sodium hydoxymethanesulfonate (HMS: Zelitch 1968) at concentrations of 10^{-2} M and 10^{-4} M. The pH was 8.1–8.2 in all experiments. Flasks were stoppered to exclude any air space and injected through the stopper (serum type) with aqueous [^{14}C]$NaHCO_3$ (1 μCi·μM CO_2^{-1}), placed in a controlled environment chamber at 16,000 lux ("cool white") and 25°C, and stirred slightly by magnetic buttons. Water samples were drawn through the stoppers initially and at intervals during the incubations and radioassayed by liquid scintillation in PCS solublizer-fluor (Amersham/Searle) to follow uptake of [^{14}C]CO_2 from the medium. The dry weight of plant material in each flask was determined at the end of each experiment.

The light:dark respiration ratio (L:D) was evaluated by the ^{14}C photorespiration assay of Zelitch (1968) as modified by Hough and Wetzel (1972) for submersed plants. Plants were labeled by incubation in the presence of [^{14}C] bicarbonate, placed in aqueous flow-through chambers in the light (16,000 lux; 25 min) and then dark (25 min), and effluent water was radioassayed at intervals for [^{14}C]CO_2 release from the plants. Plants were lyophilized, combusted, and radioassayed at the end of the experiments, and ^{14}C radioactivity released during the experiment was added to final plant radioactivity to give initial radioactivity. Respiration was calculated as % initial radioactivity released·h^{-1}·mg dry wt^{-1}, from which L:D was calculated as rate in light divided by rate in dark.

Results

In an initial examination of the relationship of oxygen concentration to net photosynthesis in *N. flexilis*, high oxygen (ca. 300% of air saturation) depressed net photosynthesis to about 40% of the rate at low oxygen (Table 1). Glycolate oxidase inhibitor HMS at a high O_2 concentration restored net photosynthesis to within 80% of the rate at low oxygen. The L:D respiration ratio at high oxygen (Table 2) was reduced by about 30% in the presence of HMS.

In long term incubations for CO_2 compensation point evaluations (Fig. 1), ^{14}C levels in the closed chambers generally came to equilibrium after several hours. The percent of initial [^{14}C]CO_2 remaining at equilibrium was converted to concentration of total CO_2 remaining in the medium (compensation point: Table 3). In addition to compensation points, we estimated initial net photosynthetic rates using the initial data points (Table 3). When oxygen concentration was manipulated (Fig. 1B), the compensation point

Table 3. Photosynthesis ($\mu g\ CO_2 \cdot g^{-1} \cdot h^{-1}$) and CO_2 compensation point ($mg \cdot liter^{-1}$) in *Najas flexilis* (16 klux, 25°C, pH 8.2).

Initial ΣCO_2	Dissolved O_2	Inhibitor	Photosynthesis initial rate ± SD	CO_2 comp. pt.
($mg \cdot liter^{-1}$)				
0.50	8.0	Control	13.4 ± 1.9	—
		HMS*	17.5 ± 5.9	—
0.73	8.0	—	82.8 ± 34.3	0.26
0.81	>25	—	87.9 ± 32.1	0.24
	<1	—	156.7 ± 20.7	0.16
1.34	9.0	Control	152.9 ± 22.3	0.08
		HMS	226.9 ± 27.6	0.08
3.33	8.0	Control	124.8 ± 14.0	0.50
		Glycidate†	237.4 ± 45.6	0.27

* Sodium hydroxymethanesulfonate (10^{-4} M).
† Sodium glycidate (10^{-2} M).

at low oxygen was about ⅓ less than at high oxygen; initial photosynthetic rate at low oxygen was nearly twofold that at high oxygen. The presence of HMS resulted in a 30–50% increase in initial photosynthetic rate over controls (Table 3) but did not appear to influence final compensation point (Fig. 1C). Sodium glycidate increased initial photosynthetic rate nearly twofold over controls, and apparently decreased compensation point to nearly half of the control level (Fig. 1D; Table 3). Compensation point estimates from curves of initial photosynthetic rates vs. initial CO_2 concentration (Fig. 2) extrapolated to zero net photosynthesis, rather than from the long term equilibrations, provided alternative estimates of slightly <0.5 mg $CO_2 \cdot liter^{-1}$ (ppm).

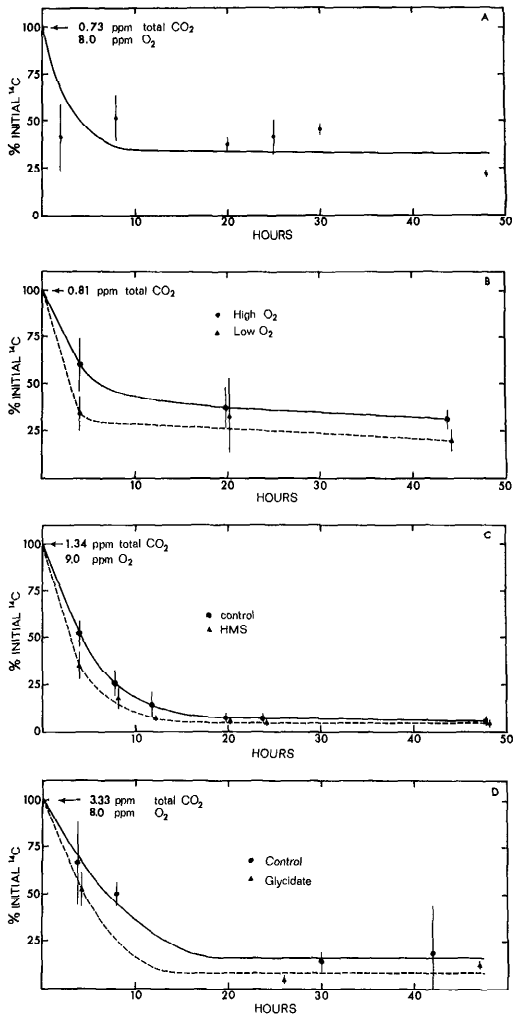

Fig. 1. Removal of [^{14}C]CO_2 from water by *Najas flexilis* (16 klux; mean ± SD, $n = 3$): A—0.73 ppm initial total CO_2; B—0.81 ppm initial total CO_2; high O_2 >25 $mg \cdot liter^{-1}$, low O_2 <1 $mg \cdot liter^{-1}$; C—1.34 ppm initial total CO_2; hydroxymethanesulfonate at 10^{-4} M; D—3.33 ppm initial total CO_2; sodium glycidate at 10^{-2} M.

Discussion

The photosynthetic response of *N. flexilis* to high oxygen concentration constitutes the classical Warburg effect, for which photorespiration has been implicated as a causal factor (e.g. Zelitch 1971). The action of HMS in decreasing the oxygen effect on both net photosynthesis and L:D respiration ratio and the enhancement of net photosynthesis by sodium glycidate are consistent with the results of Zelitch (1968, 1974) with these photorespiration inhibitors in other plants and support earlier suggestions (Hough and Wetzel 1972; Hough 1974) that photorespiration is involved in the oxygen effects in *N. flexilis*. The apparent decreases in estimated CO_2 compensation points in the presence of potassium glycidate and low dissolved oxygen also suggest repressible photorespiration activity. Similar responses of compensation

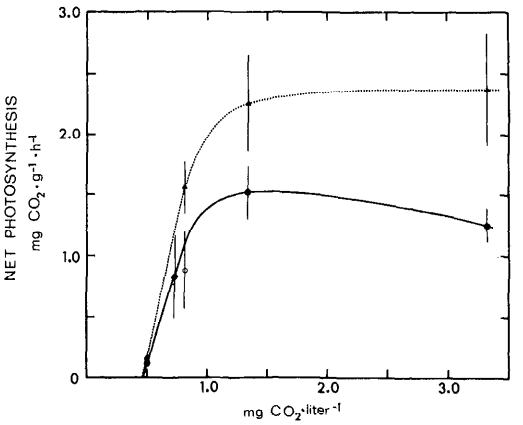

Fig. 2. Net photosynthesis of *Najas flexilis* vs. total CO_2 concentration (initial photosynthetic rates from Table 3; ●—controls, ▲—photorespiration inhibiting factors, ○—photorespiration enhancement by high O_2).

point to low O_2 have been reported for the submersed angiosperms *Vallisneria spiralis* (Prins and Wolff 1974), *Elodea canadensis*, *Egeria densa*, and *Lagarosiphon major* (Brown et al. 1974), and three marine macroalgae (Tolbert and Garey 1976). While inhibited photorespiration was indicated by an increase in initial photosynthetic rate in the presence of HMS, the lack of an expected corresponding decrease in compensation point (Fig. 1C) is contradictory. However, a long term general inhibition of photosynthesis by HMS has been reported (e.g. Smith et al. 1976), and in our long incubations an early decrease in photorespiration may have been nullified by eventual HMS toxicity. Also inconsistent with the above indications of lower compensation points associated with photorespiration inhibition was the lack of discernible difference between compensation points extrapolated from initial photosynthetic rates in the presence and absence of photorespiration inhibition (Fig. 2).

The high dissolved oxygen concentrations necessary to demonstrate photorespiration-related responses by these methods can occur in nature in dense aquatic plant populations in nonturbulent water at high light intensity. Such high concentrations are unusual for natural waters, and it may therefore be that significant rates of photorespiration are not common in nature. However, oxygen buildup within the plants (e.g. Hartman and Brown 1967), especially in the gas lacunae, may well induce photorespiration at rates greater than would be predicted on the basis of the oxygen content of the surrounding water (Hough 1974).

A mean estimated CO_2 compensation point for control and nonmanipulated experiments (Table 3) of about 0.3 mg $CO_2 \cdot liter^{-1}$ compares roughly with the extrapolated value from Fig. 2 of just below 0.5 mg $CO_2 \cdot liter^{-1}$. These levels are low relative to normal total inorganic carbon concentrations in most lakes, particularly those in which *N. flexilis* grows (i.e. often in excess of 150 mg $CO_2 \cdot liter^{-1}$). However, the values are close to the dissolved free CO_2 concentration expected in water in equilibrium with air, which would be viewed as a very high compensation point for a terrestrial plant. On this basis even the lowest estimate (0.08 mg $CO_2 \cdot liter^{-1}$: Table 3), corresponding to nearly 100 ppm in air, is a high compensation point.

These results for *N. flexilis* agree generally with those that have been found for other aquatic plants. Data from a similar technique with *Elodea* sp. (Donaldson and Tolbert unpublished) show compensation at levels corresponding approximately to air saturation. Also in similar experiments (differing primarily in method of CO_2 analysis), Tolbert and Garey (1976) found compensation points near or at air saturation levels in three marine macroalgae. Steemann Nielsen (1947) concluded from studies of net photosynthesis (dissolved O_2 technique) vs. CO_2 and bicarbonate concentration that the compensation point of *Myriophyllum spicatum* would be no lower than air saturation level.

The compensation points found by Brown et al. (1974) and Prins and Wolff (1974) by sparging gas through plant containers and measuring CO_2 uptake from the gas phase by infrared spectrometry were somewhat below air saturation levels (but still within the range reported

here for *N. flexilis*). Prins and Wolff also reported a compensation point of 30–40 ppm CO_2 in gas phase for *Hydrilla verticillata*, which is substantially lower than those described above but not within the low range (0–10 ppm) characteristic of the highly efficient terrestrial C_4 plants. With a similar technique, Van et al. (1976) found compensation points of 44 and 41 ppm CO_2 for *H. verticillata* and *Ceratophyllum demersum*; *Myriophyllum spicatum* reached a level of 19 ppm. Stanley and Naylor (1972) also found a very low apparent compensation point for *M. spicatum* (reported as zero) using the sparging method. These low values for *M. spicatum* conflict substantially with the results of Steemann Nielsen (1947) for this species, probably as a result of differences in technique and experimental conditions.

Experiments involving sparging gas may result in somewhat lower CO_2 compensation point estimates than those in which there is little or no atmospheric contact. Under conditions of the sparging, the gas diffusion path is greatly reduced, and CO_2 presumably is maximally available to the plant leaf surface; the necessity of using low pH to facilitate CO_2 exchange with the gas phase likewise facilitates CO_2 exchange by the plant. Also, low pH favors low photorespiration (e.g. Andrews et al. 1973) which could reduce the compensation point of a plant normally growing in water of high pH. Most of the very low (near zero) compensation points reported for algae (*see* Tolbert 1974) have been obtained with sparging systems. Relative to macrophytes, unicellular algae probably benefit even in nonsparged water from maximal exposure to dissolved CO_2 because of their morphology and their generally constant motion (passive or otherwise) in the water.

The apparent inability of most aquatic macrophytes to reduce the total CO_2 content of water much below air saturation levels (at least without the aid of sparging) may be related in part to photorespiration, but the principal factor probably is the slow diffusion of CO_2 in water, and it is clear that maximal photosynthetic rates cannot always be supported by atmospheric equilibrium alone. The significance of these generally similar compensation levels, in spite of such differences in carbon utilization as the requirement for free dissolved CO_2 (e.g. *N. flexilis*: Wetzel 1969) vs. ability to use bicarbonate (e.g. *M. spicatum*: *see* Hutchinson 1975), is not clear. At air saturation levels, diffusion limitation may be paramount regardless of the CO_2 form used. At high bicarbonate concentrations, even species requiring free dissolved CO_2 are provided with a CO_2 source from equilibrium reaction (within pH limits) which obviously is more available than that from the atmosphere (without sparging). In addition to slow CO_2 diffusion, Van et al. (1976) concluded that low carboxylating enzyme activity and low enzyme affinity for CO_2 contributes to the relatively low photosynthetic rates of the aquatic plants they investigated. Whether this situation can be generalized remains to be seen.

Although the CO_2 compensation point is a property primarily used as an indicator of comparative photosynthetic efficiency and is presumed not to be reached normally in situ by either terrestrial or aquatic plants (Tolbert and Garey 1976), plants requiring free CO_2 in hard-water lakes apparently can reach compensation when pH exceeds about 9.0 and only atmospheric CO_2 is available. Plants in soft-water lakes can perhaps reach compensation during intensive photosynthesis and low turbulence. It is under such circumstances that photorespiration would be most influential in the competitive photosynthetic capabilities of these plants.

Added in proof (27 June 1978): Using a water vapor saturated atmosphere, Lloyd et al. (1977, Can. J. Bot. 55: 3001) have found relatively high CO_2 compensation points (31–75 ppm) in *Myriophyllum spicatum* and *Potamogeton amplifolius*.

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