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A ^{14}C-Assay for Photorespiration in Aquatic Plants[1]

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ABSTRACT

The ^{14}C-assay developed by Zelitch to evaluate photorespiration was modified for use in submersed aquatic plants in the laboratory and *in situ*. Results in laboratory cultures of *Najas flexilis* suggest that photorespiration occurs but is limited in comparison to terrestrial C_3 plants and is related to the rate of diffusion of CO_2 from the plant and to the carrying capacity of water for dissolved oxygen.

Despite increasing progress in the physiology and biochemistry of photorespiration, the light-induced CO_2 production resulting from glycolate oxidation (2, 4, 5, 12, 18), most of this work involves terrestrial plants or planktonic algae and very little is known of this phenomenon in higher aquatic plants. A few studies have been made, such as glycolic acid oxidase activity, O_2 exchange and photosynthetic efficiency in *Typha latifolia* (7–9), O_2 effects on photosynthesis in *Elodea canadensis* (6), and compensation points in some marine macroalgae (1). The magnitude, distribution, and physiological ecology of photorespiration in higher aquatic plants are generally unknown, however.

Inasmuch as photorespiration in terrestrial plants is favored by high oxygen tension and high light intensity, one might expect a lack of photorespiration in submersed aquatic plants under natural conditions. This may not always be true, especially in view of the complexity and variability of the environment in littoral waters. If photorespiration does exist in some aquatic plants, its magnitude may vary in different species in relation to the C_3 and C_4 photosynthetic pathways and to environmental conditions with significant evolutionary and ecological implications particularly in terms of variations in CO_2 and O_2 stress. A method is needed for determining the presence, magnitude, and controlling factors of photorespiration in a variety of submersed plants relatively routinely, both *in situ* as well as in the laboratory. In this study, the basic ^{14}C technique developed by Zelitch (19) was modified to a totally aqueous system in which release of CO_2 and organic carbon from previously labeled plants is followed over time under various conditions.

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MATERIALS AND METHODS

Experiments were performed with axenic cultures of *Najas flexilis* (Willd.) Rostk. and Schmidt, a submersed freshwater angiosperm of the family Zosteraceae (for methods of axenic culture see ref. 17). In each case a loose clump of about 60 seedlings of about 60 mg total dry weight was incubated in growth medium containing 0.5 or 1 μc $NaH^{14}CO_3$ per milliliter in the light (5,500 lux) for 30 min to develop a ^{14}C-labeled cellular organic carbon pool. Plants were rinsed three times in distilled water and placed in tubular glass chambers (length: 15 cm; diam: 3 cm; vol: 110 ml) equipped with removable opaque jackets and situated between two fluorescent light tubes proving 2150 lux at the chambers. Aqueous media were allowed to flow unidirectionally through the chambers at constant rates controlled by an adjustable multi-channel peristaltic pump. The medium was collected in 5-min fractions for 30, 50, or 55 min and radioassayed for $^{14}CO_2$ and organic ^{14}C released by the plants.

Inasmuch as a CO_2-free medium is desirable for this method to avoid dilution of the labeled cellular carbon pool by ^{12}C fixed in the light during the experiment (19), media were prepared by omitting additions of carbonate, autoclaving to remove dissolved gases, and then allowing contact only with N_2, O_2, or CO_2-free air. Adjustments of pH were made with 1 N NaOH or HCl by syringe through the rubber stopper in the medium reservoir. Infrared CO_2 analyses (Beckman 215A) for inorganic carbon showed maximum levels of 0.20 mg C/liter. Dissolved oxygen concentrations in the media were determined by the unmodified Winkler method (3).

Initial analyses were made simultaneously with separate light and dark chambers attached in parallel to the same medium reservoir. The medium consisted of phosphate buffer (25 mM monobasic and dibasic potassium phosphate, pH 8.2, flow rate 20 ml/min). Dissolved O_2 was kept below 5 mg/l by allowing CO_2-free air to enter the space over the medium in the reservoir after autoclaving without aerating or agitating the medium. Alternately the medium was supersaturated by purging with O_2. The 5-min fractions were acidified to pH 2.5 and purged with N_2 in a closed system in which the gas was passed through 10 ml Hyamine-hydroxide to trap evolved CO_2. A 1-ml aliquot of Hyamine was then radioassayed in 14 ml of PPO/toluene (5 g/l) scintillation mixture with a Beckman LS-150 scintillation counter. Purged fractions were radioassayed for nonvolatile ^{14}C organic carbon by evaporating 3 ml aliquots on planchets and counting with a Nuclear-Chicago G.-M. counter (D-47) of known efficiency. Radioactivity of the plants was determined at the end of the experiment by combustion with a Packard tritum-carbon oxidizer and radioassay of CO_2 evolved in 15 ml of PPO/bis-MSB/toluene (15 g, 1 g/l) scintillation mixture.

In later experiments, duplicate 50-min analyses were made simultaneously with both chambers in the light for the first 25 min and in the dark for the remaining 25 min. A modified

synthetic lake water growth medium was used, containing in milligrams per liter: $CaCl_2$ (54); NH_4Cl (3.82); $MgSO_4 \cdot 7H_2O$ (100); KCl (30); K_2HPO_4 (0.56); nitrilotriacetic acid (20); and tris(hydroxymethyl) aminomethane (500) (pH 8.1; flow rate 20 ml/min). Dissolved O_2 was maintained at very low concentrations by introducing N_2 into the reservoir air space after autoclaving or was saturated by purging with O_2. Aliquots, 1 or 2 ml, of the 5-min fractions were radioassayed, before and after acidification and N_2 purging, in 6 ml of Triton X-100 and 7 ml of butyl PBD/PBBO/toluene (8.0 g/l, 0.5 g/l) scintillation mixture against standards of known specific radioactivity. In an additional experiment using the same medium, *Najas* in separate dark and light chambers were exposed first to a low O_2 concentration for 30 min immediately followed by a high O_2 concentration for the remaining 25 min.

RESULTS

CO_2 Release. In the initial experiments, cumulative $^{14}CO_2$ cpm at each time interval were converted to percentage of total radioactivity of the plants (sum of radioactivity at the end of the incubation and the total lost during the incubation) (Fig. 1). Cumulative cpm are presented directly for all other experiments (Figs. 2, 3). Rates of release of CO_2 were computed as percentage increments between 10 and 30 min in the initial experiments, and as cpm/min between 15 and 25 min and between 35 and 50 min in remaining experiments (Table 1).

At low concentrations of dissolved O_2 the rates of CO_2 release in light were much less than in the dark, while at high O_2 concentrations the rates of CO_2 release in the light were similar to those in the dark (Figs. 1, 2). This response to increased dissolved O_2 was rapidly inducible (Fig. 3). Rates of release of CO_2 in the dark were similar at both high and low O_2 levels. Light-dark ratios were below 0.30 at low O_2 and about unity at high O_2 (Table I).

Release of Organic Carbon. Cumulative release of organic carbon, presented as a percentage of total plant radioactivity in experiments in which plants were incubated separately (Fig. 4) and as cpm in those where the same plants were incubated in the light and dark (Fig. 5), demonstrated in all cases release in the dark was greater than in the light. Rates of secretion

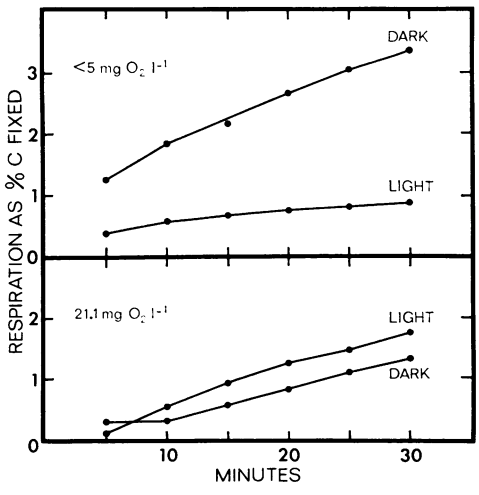

FIG. 1. Effect of dissolved oxygen concentration on respiration of *Najas flexilis* in the light and dark. Cumulative $^{14}CO_2$ cpm evolved into phosphate buffer flowing past prelabeled plants in tubular chambers, converted to percentage of total radioactivity of the plants. Light chambers: 2150 lux; all chambers 22 C, pH 8.2, flow rate 20 ml/min.

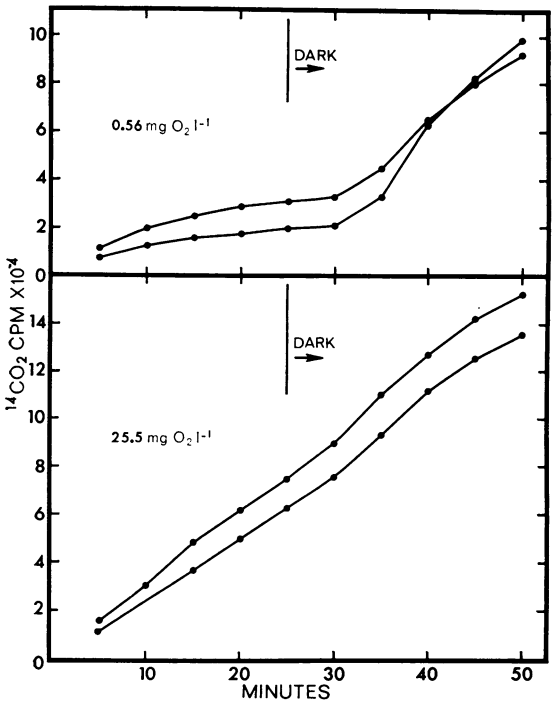

FIG. 2. Effect of dissolved oxygen concentration on respiration of *Najas flexilis* in the light and dark. Cumulative $^{14}CO_2$ cpm evolved into growth medium flowing past prelabeled plants in tubular chambers. All chambers 2150 lux for 25 min, then dark; 22 C, pH 8.1, flow rate 20 ml/min. Two lines in each graph represent duplicates.

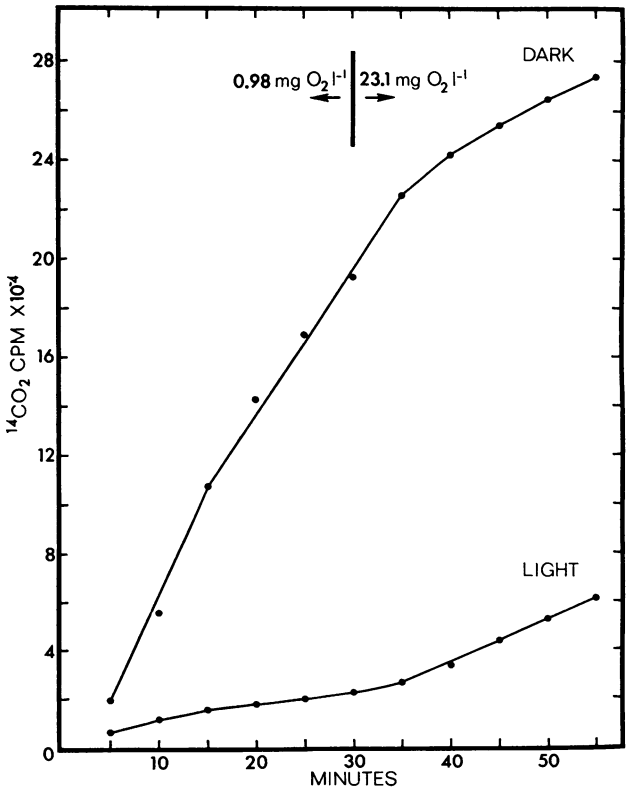

FIG. 3. Effect of dissolved oxygen concentration on respiration of *Najas flexilis* in the light and dark. Cumulative $^{14}CO_2$ cpm evolved into growth medium flowing past prelabeled plants in tubular chambers. Light chamber: 2150 lux; both chambers 22 C, pH 8.2, flow rate 20 ml/min, low O_2 for 30 min, then high O_2.

were greatest immediately after onset of darkness and then leveled off at rates higher than occurred in light. No consistent differences in secretion rates were found with respect to variations in concentration of dissolved O_2.

DISCUSSION

The results indicated that respiration in the light was more O_2-sensitive than that in the dark. The greater release of CO_2 in the light at high O_2 than at low O_2 suggests that photorespiration was induced. Contrary to the response commonly found in photorespiring terrestrial plants, however, photorespiration in *Najas* was never appreciably greater than dark respiration.

Presence of small amounts of inorganic carbon in the media may have caused some dilution of the cellular ^{14}C pool by ^{12}C in the light, resulting in reduced specific radioactivity of CO_2 released and corresponding underestimation of photorespiration. However, our medium contained two orders of magnitude less CO_2 than does air, and if at high O_2 our plants had been truly photorespiring to the extent that typical terrestrial C_3 plants do, the relatively minimal ^{12}C dilution in our system probably would not have caused observed ratios to be as low as unity.

Table I. *Effects of Dissolved Oxygen Concentration on $^{14}CO_2$ Released from Najas flexilis in the Light and Dark*

Light and dark periods: experiments 1 and 2: 30 min; experiments 3 and 4: 25 min; experiment 5 (low O_2): 30 min; experiment 5 (high O_2): 25 min; 2150 lux, 22 C, pH 8.1 or 8.2. Rates of release of CO_2 were computed as percentage increments between 10 and 30 min in experiments 1 and 2, and as cpm/min between 15 and 25 min and between 35 and 50 min in the remaining experiments.

Experiment	O_2 Concn in Medium	O_2 Saturation[1]	$^{14}CO_2$ Released in Light	$^{14}CO_2$ Released in Dark	Light-Dark Ratio
	mg/l	%			
1	<5 g	<60	0.015% min^{-1}	0.075% min^{-1}	0.21
2	21.1	257	0.060	0.052	1.16
3	0.56	6.8	1305 cpm/min	4423 cpm/min	0.29
	0.56	6.8	735	3249	0.23
4	25.5	310	2909 cpm/min	2826 cpm/min	1.03
	25.5	310	2540	2809	0.90
5	0.98	11.8	464 cpm/min	6171 cpm/min	0.08
	23.1	282	1720	2600	0.66

[1] Percentage saturation of O_2 dissolved in water at experimental temperature and pressure.

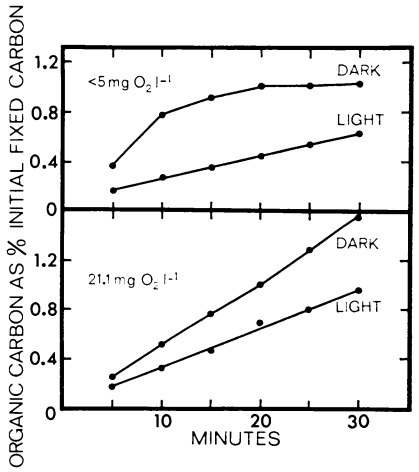

FIG. 4. Release of organic carbon by *Najas flexilis* in the light and dark at low and high dissolved oxygen concentrations. Cumulative organic ^{14}C cpm evolved into phosphate buffer flowing past prelabeled plants in tubular chambers, converted to percentage of total radioactivity of the plants. Light chambers: 2150 lux; all chambers 22 C, pH 8.2, flow rate 20 ml/min.

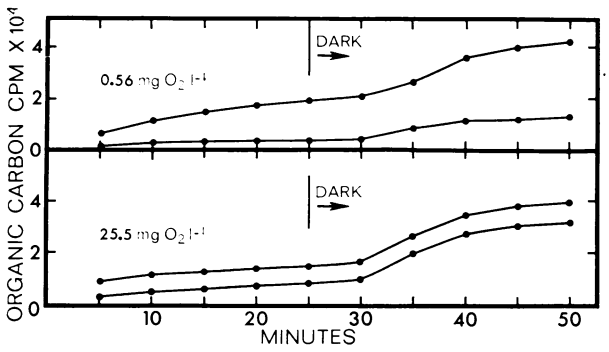

FIG. 5. Release of organic carbon by *Najas flexilis* in the light and dark at low and high oxygen concentrations. Cumulative organic ^{14}C cpm evolved into growth medium flowing past prelabeled plants in tubular chambers. All chambers 2150 lux for 25 min, then dark; 22 C, pH 8.1, flow rate 20 ml/min. Two lines in each graph represent duplicates.

It is evident that this plant refixes respired CO_2 rather efficiently. Preliminary chromatographic results of 10-second ^{14}C fixation products (Hough, unpublished data) indicate no labeling in malate and aspartate, and hence little likelihood of significant refixation by P-enolpyruvate carboxylase. The diffusive resistance of water to CO_2, causing relatively slow diffusion of CO_2 out of the plant, probably was responsible for most of the refixation. Stomata, important in gas exchange in terrestrial plants, are nonfunctional in *Najas*, as in all submersed vascular plants (11). In view of this CO_2 refixation, the present method obviously measures apparent, rather than true, photorespiration; the latter may have been greatly underestimated in this study. Apparent or net photorespiration is the event of importance to the plant in terms of overall photosynthetic efficiency, however.

Twenty-five milligrams of O_2/l can occur in littoral zones of productive lakes, but it approaches the limit of O_2 saturation of water. This concentration is low in relation to air, however. The concentration of dissolved O_2 in water is governed by solubility characteristics (temperature, pressure) (10) and is not equivalent to the concentration in air or in any other gas mixture in contact with water; this of course includes experimental aqueous systems which are purged for the purpose of varying O_2. Consequently, the magnitude of photorespiration likely was limited by O_2 concentration in this study. If so, under natural conditions this species probably would never be exposed to O_2 concentrations sufficiently high to cause apparent photorespiration to exceed dark respiration appreciably. Oxygen concentrations in freshwater normally range from 0 to about 15 mg/l (11).

Light probably also limits photorespiration in aquatic plants, depending on the depth at which they grow. The light intensity used in these experiments was relatively high, but not maximal in relation to natural underwater conditions, especially near the surface.

Data on release of organic carbon show that this information is easily acquired by this method. This aspect of the path of carbon may be unique to aquatic plants; excretion or secretion of dissolved organic matter by these plants is important in community metabolism in littoral zones of lakes (15, 16). A

comparison of respiratory and organic release in terms of percentage carbon fixed under various conditions is a useful aspect of the method.

The present data demonstrated that O_2 concentration did not affect release of organic substrates, although release in dark exceeded that in light. The release in the dark may represent a postillumination burst of photosynthetic intermediates which, having been maintained at a given pool size, are no longer carried through the pathway. Qualitative analysis of released organic compounds, an on-going project in this laboratory, should assist in the interpretation of the relationship of organic carbon release to photorespiration, particularly if glycolic acid is found. It is conceivable that a balance between glycolate oxidation and excretion exists depending on environmental conditions.

Of primary interest is the extent to which photorespiration occurs in naturally growing aquatic plants, environmental controlling factors and effects of photorespiration on primary productivity and its measurement. The present method was designed for adaptation to *in situ* studies in which naturally growing plants are prelabeled in the field and the analyses carried out with the chamber in the water at the site of natural growth. Regardless of whether apparent photorespiration ever actually exceeds dark respiration, it is ecologically important that respiration rates in the light can vary with O_2 concentration, since efficiency of primary productivity would vary markedly. For example, observations of daily fluctuation of carbon fixation in natural aquatic plant communities in which fixation rate rises through the morning, falls during midday, and then rises again prior to falling at the end of the day (13), might partly be explained by onset of photorespiration at midday when O_2 concentration rises with increasing photosynthetic activity. Also of special interest is the role of photorespiration in aquatic plants growing in highly alkaline lakes in which free CO_2 can be at limiting levels (14). In moderately buffered lakes, the night-time accumulations of CO_2 can easily be reduced to zero in a few hours by active photosynthesis of the littoral flora. It is under such severe environmental conditions that photosynthetic efficiency is of greatest adaptive value.

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