Photosynthesis in Eurasian Watermilfoil (Myriophyllum spicatum L.)

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ABSTRACT

Gas exchange of Eurasian watermilfoil (Myriophyllum spicatum L.) indicated a near-zero CO₂ compensation point and a high temperature optimum for photosynthesis. These properties are characteristic of plants fixing CO₂ by a β-carboxylation mechanism. Operation of the Calvin cycle was shown and no evidence for β-carboxylation was obtained. These results indicate that near-zero CO₂ compensation points are not dependent on a β-carboxylation mechanism.

Plants seem to have either very low or fairly high CO₂ compensation points (11, 17). High compensation point is thought to be characteristic of plants assimilating CO₂ by the Calvin cycle, while plates with a low CO₂ compensation point possess an alternate fixation mechanism involving formation of a C₄-dicarboxylic acid (β-carboxylation) (7). The β-carboxylation mechanism is believed to recapture released CO₂ efficiently and thus be responsible for the low CO₂ compensation point (10, 12, 13).

In this paper, we examine the photosynthesis of the aquatic weed Eurasian watermilfoil, a circumboreal aquatic angiosperm (9, 15).

MATERIALS AND METHODS

Plants of Myriophyllum spicatum L. from a single clone, collected from Guntersville Reservoir, Alabama, in August 1966, were grown in pots containing greenhouse soil covered with sand and submersed in 3 feet of water in a concrete greenhouse tank. Vegetative apical fragments were used in all experiments reported here.

Gas exchange was measured by IRGA*. Two systems were used: in one the airstream passing over the experimental plant was recirculated in a closed system and in the other gas from a compressed air cylinder was passed over the plant to the IRGA, then exhausted to the room in an open system. The closed system employed a double walled Plexiglas plant chamber, with temperature controlled by circulating water through the outer chamber, and a Grubb-Parsons IRGA. Air passed through a humidifier then entered the inner chamber through a porous aerator near the bottom and exited above the water level near the top of the chamber. Fragments to be tested were placed in 50 ml of water in the inner chamber, and the Plexiglas lid of the chamber was sealed with silicon stopcock grease and tightly secured with brass bolts. The airstream was dried by passing through a condenser in a cold water bath and silica gel drying tubes before entering the IRGA. Light from seven 250-w photoflood bulbs was filtered through a heat trap (21) and entered the chamber from the top.

In the open system IRGA, Warburg flasks were the experimental chambers, and a Hartmann-Braun IRGA measured the CO₂ level. Gas flowed through a humidifier and a Matheson flowmeter, then entered the Warburg flask through the top of the manometer. The airstream was dried by passing through a condenser with a brine jacket inside a refrigerator before entering the IRGA. Light was provided by four 250-w photoflood bulbs through the glass side panel of a refrigerated, rectangular Aminco Warburg bath. Light was measured with a Yellow Springs Instrument Company radiometer, Model 65, or by a Clairex CL 704L photoresistor and a Simpson ohmm-amp-voltmeter calibrated with the radiometer. Intensities greater than 10⁵ ergs cm⁻² sec⁻¹ were obtained with both systems.

For metabolic studies, 4-cm apical fragments were exposed to ¹³C-bicarbonate by dropping them into the appropriate solution containing about 0.15 µc ¹³C per mole of inorganic carbon, incubating in the light or dark, rinsing, and plunging into 10 ml of 80% (w/v) ethanol-0.03% (w/v) 2,4-dinitrophenylhydrazine in test tubes bathed in ethanol and Dry Ice (8). Plants were ground with a chilled Kontes glass homogenizer, centrifuged, and re-extracted with 50% (w/v) ethanol. The combined extracts were taken to about 0.5 ml with a Buchler flash evaporator, made to 1 ml with H₂O, and extracted five times with 1 ml of diethyl ether (5, 6). The combined ether extracts were added to 1 ml of 95% (v/v) ethanol, taken to 0.5 ml with a fine airstream from a Pasteur pipette, and made to 1 ml with 95% ethanol.

The aqueous phase was separated into neutral sugars, amino acids, and nonamino acidic fractions using AG 50 × 8(H⁺) and AG 1 × 8 (formate) ion exchange resins (16, 20). Amino acids were eluted as a single fraction with 10 N HCl. Total radioactivity of each fraction was determined with a Baird-Atomic spectrometer, Model 530.

Keto acid, amino acid, and other organic acid fractions were separated into individual compounds by TLC on Cellulose MN 300 from Macherey, Nagel, and Company as follows: keto acid:1-butanol-NH₃-H₂O (8:1:1, v/v/v) (4); amino acids: 75% (v/v) phenol (3) and methanol-chloroform-NH₃-H₂O (4: 4:1:1, v/v/v) (3); and other organic acids:1-butanol-formic acid-H₂O (6:1:3, v/v/v) (18) and ethanol-NH₃-H₂O (7:2:1, v/v/v) (1, 14).

The developed chromatograms containing radioactivity were

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*Abbreviations: IRGA: infrared gas analysis; TLC: thin layer chromatography.
exposed to Kodak No-screen x-ray film. $R_F$ values of radioactive spots were measured and compared with those of standards. Standard methods of visualization were used as follows: phosphate esters: ammonium molybdate (1); amino acids: ninhydrin (19); and other organic acids: 0.04% (w/v) bromophenol blue adjusted to an intermediate tint (2). The keto acid 2,4-dinitrophenylhydrazone standards were visible on the TLC plates as yellow or yellow-orange spots.

**RESULTS**

The effect of temperature on photosynthesis was studied with both open and closed IRGA systems. Results were corrected for differences in CO$_2$ solubility and expressed as percentage of photosynthesis at 20°C for each set of conditions (Table I). Net CO$_2$ uptake increased with temperature to and including 35°C.

The relationship between CO$_2$ and net photosynthesis between 56 and 296 μl CO$_2$ 1·$^{-1}$ (Fig. 1) suggests a zero CO$_2$ compensation point. A photosynthetic rate of 112 μg CO$_2$ min$^{-1}$  g$^{-1}$ is indicated for equilibrium with atmospheric CO$_2$ and with 1.4 × 10$^{5}$ ergs cm$^{-2}$ sec$^{-1}$ light.

The nonketo nonamino organic acid fruction declined in percentage of radioactivity with time (Table II). Other fractions showed increasing percentage of radioactivity with longer time in the light (Table II). Chromatography of these organic acids revealed intense labeling of compounds for which $R_F$

![Image](https://www.plantphysiol.org/)

**DISCUSSION**

*M. spicatum*, a cool temperate dicotyledon, is photosynthetically similar to tropical grasses in that it has a low CO$_2$ compensation point and a high temperature optimum. These gas exchange characteristics are usually correlated with β-carboxylation (17). However, in this species, the C$_5$-dicarboxylic acid cycle is lacking. Thus, ability to assimilate CO$_2$ at a low level is not a unique function of β-carboxylation.

The high temperature optimum for photosynthesis by this species suggests that thermal enrichment will increase problems from this weed. Even if this watermilfoil has a low optimum for growth, possessing a high optimum for photosynthesis would give it a definite advantage in possible adaptation to environments with higher water temperatures.
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LITERATURE CITED


