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EFFECTS OF LIGHT AND TEMPERATURE ON DUCKWEED PHOTOSYNTHESIS*

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ABSTRACT

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Rates of photosynthesis of *Lemna minor* L. and *Spirodela punctata* (G.F.W. Meyer) Thompson, two aquatic angiosperms, were measured at different temperatures and light intensities. Photosynthesis was measured both as oxygen evolution and $^{14}CO_2$ fixation. At temperatures ranging from 15 to 35° C, light saturation of photosynthetic O_2 evolution of *Lemna* occurred from 300—600 $\mu E\ m^{-2}\ s^{-1}$, while in *Spirodela* photosynthetic O_2 evolution was light saturated at 600—1200 $\mu E\ m^{-2}\ s^{-1}$. Photosynthetic O_2 evolution of both species was photoinhibited at light intensities greater than 1200 $\mu E\ m^{-2}\ s^{-1}$. The optimal temperature for *Lemna* photosynthetic O_2 evolution was 30° C, while the optimal temperatures for $^{14}CO_2$ fixation were from 20 to 30°C. For *Spirodela* maximum photosynthetic O_2 evolution occurred at 35° C, while maximum $^{14}CO_2$ fixation was at 30°C.

INTRODUCTION

Terrestrial C_3 and C_4 plants often may be distinguished from each other by their photosynthetic responses to increasing light and temperature; C_3 plants become photosynthetically saturated at one-third to one-half full sunlight (Moss, 1963) while C_4 plants are not saturated even at full sunlight (Björkman et al., 1968). Most C_3 plants also show an optimal temperature for photosynthesis near 20° C, while C_4 plants show decreasing photosynthesis only when the temperature exceeds 40° C (Hew et al., 1969b). Aquatic macrophytes are not easily classified as C_3 or C_4 plants. Like C_4 plants, many aquatic plants have high light and temperature optima (Stanley and Naylor, 1972), but like C_3 plants many aquatic plants show inhibition of photosynthesis with high oxygen levels (Van et al., 1976). Recent work of

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Holaday and Bowes (1980) suggests that aquatic plants such as *Hydrilla* may have a modified form of Crassulacean acid metabolism (CAM), and they suggest that this type of photosynthesis may be common among aquatic macrophytes.

This controversy indicates that further work on the photosynthetic parameters of aquatic plants is required. Because of this, a study was undertaken to determine the light and temperature optima of *Lemna minor* L. and *Spirodela punctata* (G.F.W. Meyer) Thompson. These plants are floating aquatic angiosperms of the family Lemnaceae. They are found at the surface of shallow ponds, often forming mats several layers thick. The family is worldwide in distribution and is found in both temperate and tropical climates. With their small size (the frond is less than 1 cm in length) and rapid asexual reproduction, they are ideal laboratory plants. Therefore, Lemnaceae, and *Lemna* in particular, have been widely used in laboratory experiments ranging from the effect of red light on respiration (Hillman, 1970) to enzyme assay work (Goto, 1979). However, to date little work has been done on the photosynthetic physiology of these plants. Because of their small size and aquatic habitat whole plants of *Lemna* and *Spirodela* may be used in photosynthetic studies which employ techniques similar to those used in studying algae. In particular, the effects of temperature and light on photosynthesis can be examined by placing the plants in an oxygen electrode and measuring oxygen evolution and/or $^{14}CO_2$ uptake. In our experiments the photosynthetic responses of *Lemna* and *Spirodela* to changes in light and temperature were measured and are reported in this paper.

MATERIALS AND METHODS

The duckweed, *Spirodela punctata*, was obtained for culturing from the pond in the greenhouse of Buckhout Laboratory, The Pennsylvania State University. The duckweed, *Lemna minor*, was cultured from a wild population growing on the fish ponds at Fisherman's Paradise, Bellefonte, Pennsylvania. These duckweeds were grown in Jacob's medium enriched with 1% sucrose (McLay, 1976). When growth was vigorous the plants were sterilized according to the methods outlined by Hillman (1961). Individual fronds were then placed in 300-ml Erlenmeyer flasks equipped with cotton stoppers and containing approximately 50 ml of sterile Jacob's medium without sucrose. The pH of the medium varied slightly between batches, but was usually in the range of 6.0—6.5. Cultures were maintained by reinoculating new flasks on a weekly basis. Cultures were grown under cool white fluorescent lights at 20° C on a 13/11 h light/dark cycle in an Environator Culture Chamber at a light intensity of 140 μE m^{-2} s^{-1}. All experimental runs conducted on the same day used duckweed fronds which were growing in the same culture flask. This reduced the chances of experimental variability caused by using physiologically different fronds.

To study photosynthesis, oxygen evolution was measured with a Clark-

type O_2 electrode (Rank Bros., Cambridge, England). The duckweed fronds were placed in the electrode chamber in a small wire basket which prevented damage to the fronds by holding them above the stirring bar. The fronds were completely immersed, and there were no air spaces in the O_2 electrode chamber which was sealed on the top with a lucite plug. Although some self shading of the fronds occurred, it was equally distributed, as the relative positions of the fronds were constantly changed as they were moved by the active stirring of the water in the chamber. Photosynthesis was determined at temperatures ranging from 10 to 40° C and at light intensities ranging from 150 to 3000 μE m^{-2} s^{-1}. Temperatures were regulated with a Haake water circulating bath. Light was provided by a Kodak slide projector with a 500 W bulb. The light intensity was measured with a LiCor Quantum meter and was expressed as the average of light incident on the front of the chamber and the light which penetrated through to the rear of the chamber.

Uptake of $H^{14}CO_3^-$ was also used as a means of measuring photosynthesis by the duckweed. One μCi of ^{14}C as $NaH^{14}CO_3$ (final specific activity = 2 mCi/mmol inorganic carbon) was added to the duckweed suspension in the oxygen electrode. At a specified time the fronds were removed, rinsed in distilled water and killed in a liquid scintillation vial containing 0.2 ml of concentrated HCl. The samples were then dried under a fume hood, liquid scintillation fluid was added (toluene:triton X-100:2,5-diphenyloxazole:*p*-bis-(*o*-methylstyryl)benzene;2000 ml:1000 ml:16.5 g:0.3 g) and the samples were counted. Use of the oxygen electrode allowed simultaneous determination of both photosynthetic oxygen evolution and $^{14}CO_2$ uptake. Inorganic carbon concentrations were determined by Gran titrations following the methods of Stumm and Morgan (1970) and Gieskes and Rogers (1973).

RESULTS

Light effects

The effects of light intensity and temperature on photosynthetic oxygen evolution by 2-week-old cultures of *Lemna* plants are illustrated in Fig. 1. Photosynthesis was light-saturated at 600 μE m^{-2} s^{-1} for all temperatures, except 30° C where saturation was at 300 μE m^{-2} s^{-1} (full sunlight was measured as 1400 μE m^{-2} s^{-1}). At light intensities higher than 1200 μE m^{-2} s^{-1} photosynthesis was inhibited. Similar experiments (data not shown) were performed with 6-week-old cultures of *Lemna* and photosynthesis was again saturated at 300--600 μE m^{-2} s^{-1}, but photoinhibition did not occur until at least 2000 μE m^{-2} s^{-1}.

The effects of light intensity and temperature on photosynthetic oxygen evolution of 2-week-old cultures of *Spirodela* appear in Fig. 2. Photosynthesis was light-saturated at 600—1200 μE m^{-2} s^{-1}. In spite of the occasional higher light levels required for saturation of *Spirodela* compared to *Lemna*, photoinhibition followed a similar pattern, that is, photoinhibition occurred

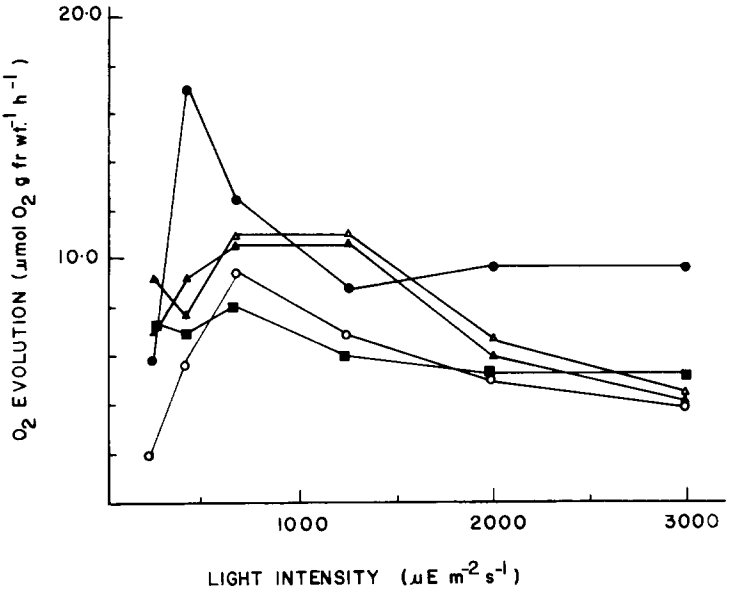

Fig. 1. The effect of light intensity and temperature on oxygen evolution by 2-week-old *Lemna minor* plants. ○, 15° C; △, 20° C; ▲, 25° C; ●, 30° C; ■, 35° C. Standard deviations for each temperature are as follows: 15° (0.7); 20° (1.0); 25° (1.5); 30° (1.8); 35° (1.7).

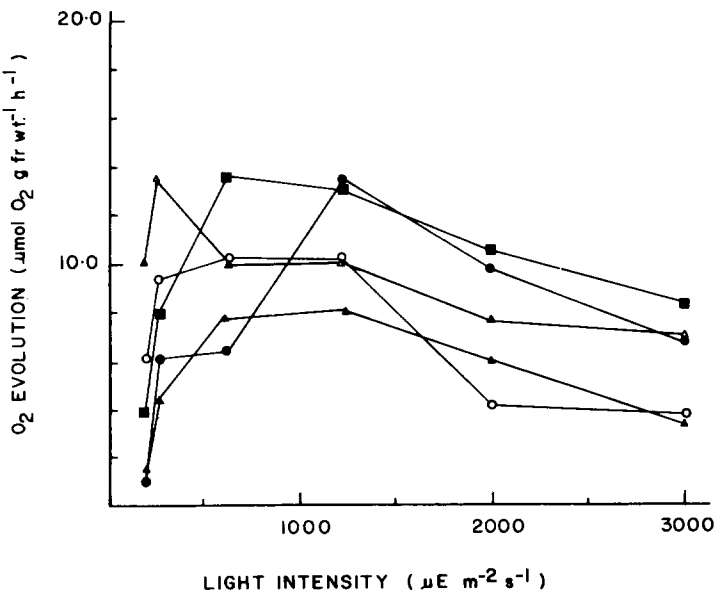

Fig. 2. The effect of light intensity and temperature on oxygen evolution by 2-week-old *Spirodela punctata* plants. ○, 15° C; △, 20° C; ▲, 25° C; ●, 30° C; ■, 35° C. Standard deviations for each temperature are as follows: 15° (2.6); 20° (1.1); 25° (1.6); 30° (1.3); 35° (0.9).

at light levels greater than 1200 μE m^{-2} s^{-1}. Six-week-old cultures of *Spirodela*, usually light-saturated at 1200 μE m^{-2} s^{-1}, and the older fronds were more resistant to photoinhibition.

Temperature effects

The effects of temperature (15—35° C) on oxygen evolution are presented in Fig. 1 for 2-week-old cultures of *Lemna*, while the effects of a wider range of temperatures on $^{14}CO_2$ fixation at low light intensities by young (1—3 weeks old) cultures of *Lemna* appear in Fig. 3. An optimum temperature for photosynthesis of 30°C was observed for oxygen evolution, with plants at 20 and 25° C having somewhat lower values and plants at 15 and 35° C having even lower values (Fig. 1). These oxygen results in general are confirmed in $^{14}CO_2$-uptake experiments, but a rather broad temperature optimum from 20 to 30° C was observed, and above and especially below this optimum substantial inhibition was observed. Six-week-old cultures had similar oxygen evolution patterns, except photosynthesis at 35° C was often as high or higher than photosynthesis at 30° C.

Spirodela was examined under the same environmental conditions as *Lemna* (Figs. 2 and 3). Maximum photosynthesis for 2-week-old cultures of plants was usually at 35° C, although at 1200 μE m^{-2} s^{-1} oxygen evolution

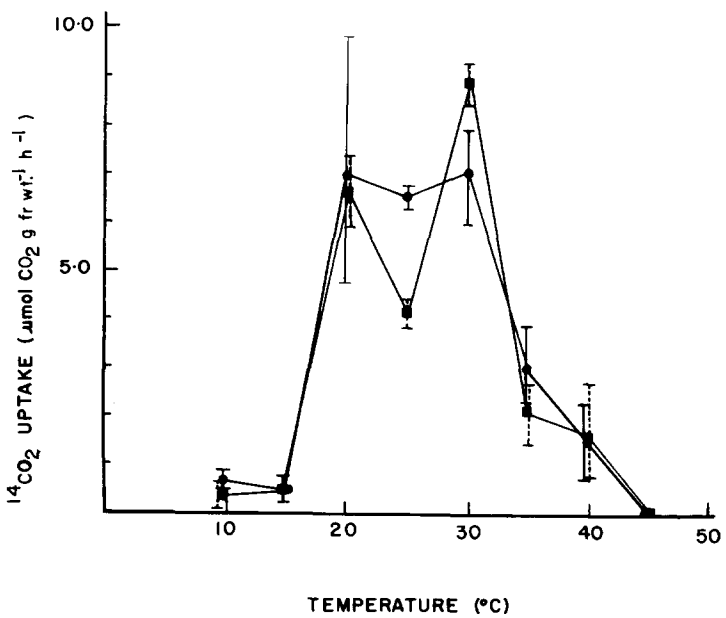

Fig. 3. The effect of temperature on $^{14}CO_2$ uptake by 1 to 3-week-old *Lemna minor* and *Spirodela punctata* plants. Light intensity was 300 μE m^{-2} s^{-1}. •, *Lemna minor*; ▪, *Spirodela punctata*.

was greatest at 30°C (Fig. 2). Surprisingly, temperatures of 25°C depressed photosynthesis below the rates of 15 and 20°C. $^{14}CO_2$ uptake at different temperatures and a constant low light intensity (Fig. 3) were different than the oxygen evolution results, as a maximum photosynthesis was at 30 and 35°C. Why a depression in photosynthesis should occur at 25°C is unknown. Six-week-old cultures of *Spirodela* also had the highest photosynthetic oxygen evolution rates at 35°C, although at 15°C photosynthesis was higher than in the younger plants.

DISCUSSION

The effects of temperature and light intensity on photosynthesis of the two species of duckweed should give some indication of whether they are C_3 or C_4 plants. Moss (1963) determined that the photosynthesis of C_4 plants was not saturated at full sunlgiht, whereas C_3 plants were saturated at approximately one-third full sunlight. For 2-week-old cultures of *Lemna* and *Spirodela* plants, saturation generally occurred at light intensities from 600 to 1200 $\mu E\ m^{-2}\ s^{-1}$, intensities less than the 1400 $\mu E\ m^{-2}\ s^{-1}$ of full sun. These results would place the duckweeds somewhere between C_3 and C_4 plants with respect to their responses to light intensity. Our results differ somewhat though from those of Ashby and Oxley (1935) who found light saturation of *Lemna minor* to occur at approximately 1000 ft-candles, a value lower than our saturation point, and closer to the value of a typical C_3 plant. The sensitivity of the young duckweed plants to photoinhibition by high light intensities (greater than 1200 $\mu E\ m^{-2}\ s^{-1}$) may indicate though that they are closer to C_3 plants than C_4 plants, as C_4 plants are not photoinhibited at light intensities less than full sunlight. This C_3 photoinhibitory response would support the results of Bauer et al. (1976) who found that the photosynthetic ^{14}C-labeling pattern of *Lemna minor*, following a short term exposure to $^{14}CO_2$, to be that of a typical C_3 plant.

The responses of *Lemna* and *Spirodela* photosynthesis to temperature are not typical though of C_3 plants. For example, in *Helianthus annuus* L., sunflower, a C_3 plant, a temperature optimum of 20°C was observed at both low (300 $\mu E\ m^{-2}\ s^{-1}$) and high (1800 $\mu E\ m^{-2}\ s^{-1}$) light intensities, and temperature increasing above 20°C decreased photosynthesis (Hew et al., 1969a, b), whereas in our experiments temperatures above 20°C did not decrease photosynthesis. Ashby and Oxley (1935) also found for *Lemna* that higher temperatures did not depress CO_2 assimilation rates, with CO_2 assimilation being rather constant from 18—29°C. More significantly, the duckweeds do not follow the usual pattern of a C_3 plant's photosynthetic response to temperature and light, that is, temperature and light optima are usually similar to the conditions at which the plants are grown (Chollet and Ogren, 1975). The duckweeds instead seem to have the higher temperature optima characteristic of C_4 plants. For example, *Zea mays* L., maize, a C_4 plant, showed a photosynthetic optimum at 30°, with rates at this

temperature almost double those observed at 20° at a light intensity of 6000–10000 ft-candles (Moss, 1963). The duckweeds had a temperature optimum in the 30—35° C range with lower photosynthesis at 20° C, although the difference in photosynthesis between 20 and 30° C was much less pronounced than for the C_4 plant.

Based on our experiments and those of other investigators (Ashby and Oxley, 1935; Bauer et al., 1976) the duckweeds appear to be C_3 plants. Their high temperature optima and light intensities for saturation of photosynthesis make them atypical C_3 plants though, worthy of further study.

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