ATOLL RESEARCH BULLETIN
NO. 376

CHAPTER 12

PHOTOSYNTHESIS OF THE SEAGRASS *THALASSODENDRON CILIATUM*: LEAF MORPHOLOGY AND CARBON METABOLISM

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ISSUED BY
NATIONAL MUSEUM OF NATURAL HISTORY
SMITHSONIAN INSTITUTION
WASHINGTON, D.C., U.S.A.
June 1992

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T.R. Pärnik*, K.Y. Bil'**, P.V. Kolmakov*** and E.A. Titlyanov***

ABSTRACT

The kinetics of the incorporation of ^{14}C into photosynthates during labeling and pulse-chase experiments has shown that the seagrass *Thalassodendron ciliatum* (Forskål) den Hartog is a typical C_3 plant. Both adaxial and abaxial leaf surfaces lack stomata and are covered with a thin layer of epidermal cells containing chlorophyll. 80% of the leaf volume is occupied by chlorophyll-free heterotrophic cells. Respiration of these unpigmented cells is equivalent to 66% of the gross photosynthetic rate. The molar rate determined from $^{14}CO_2$ fixation during a 10 min exposure is 0.2 m mol CO_2 per g dry weight per hour and exceeds the apparent O_2 release by 3 fold. Environmental conditions favorable for *Thalassodendron ciliatum* extend to the depth of 26 m. The half-maximum $^{14}CO_2$ uptake rate is achieved at a light level of 1.5 $W \cdot m^{-2}$ in plants from both deep (33 m) and shallow (0.5 m) waters. In plants growing 33 m deep, photoassimilation of CO_2 is relatively slow at all light intensities and tissues contain higher levels of flavonoids. The amino acid contents of mature leaves are identical in shallow and deepwater plants, whereas the younger leaves of deepwater plants are poorer in proline and cystein. In contrast to terrestrial plants, the pH in the sap of heterotrophic cells increases during the daytime and reaches a value of 6.1 by nightfall. Acidification of the sap reaches pH 5.6 from dawn into early morning due to the formation of malate from bicarbonate in the dark.

INTRODUCTION

The seagrass *Thalassodendron ciliatum* (Forskål) den Hartog [formerly known as *Cymodocea ciliata* (Hartog 1970)] is an inhabitant of the Red Sea, western areas of the Indian Ocean, coasts of Egypt, Sudan, Iran, Saudi Arabia, Somalia, Kenya, Tanzania, Mozambique, South Africa, Madagascar, Chagos Archipelago, Seychelles Islands and Comoro Islands. *Thalassodendron ciliatum* has also been found in waters of Indonesia, New Guinea, Philippines, Solomon Islands, Caroline Islands, China and the northwest to northeast coasts of Australia (Hartog 1970, 1990, Meñez et al. 1983, Walker and Prince 1987). *Thalassodendron ciliatum* occupies the upper sublittoral zone to the depth of 40 m and forms mats up to 30-40 cm in thickness. It normally grows at temperatures from 26 to 30°C, but in littoral basins it withstands temperatures up to 38°C. The average biomass of *T.*

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ciliatum reaches 6050 g fresh wt·m^{-2} (Aleem 1984).

Detailed descriptions of *Thalassodendron ciliatum* as well as information concerning its environmental requirements are available in the literature (Hartog 1970, Meñez et al. 1983, Aleem 1984, Johnstone 1984, Walker and Prince 1987). However, its physiology and biochemistry are poorly studied. This paper provides new data on the morphology of the leaf of *T. ciliatum* as well as the biochemistry of its photosynthetic carbon metabolism, which was investigated during an expedition to the southwest Indian Ocean from January to March 1989 on the R/V Akademik Aleksander Nesmeyanov.

MATERIALS AND METHODS

Thalassodendron ciliatum was sampled near the islands of Cõetivy, Farquhar, Aldabra and Desroche from shallow waters down to 33 m deep. The samples collected for experiments were kept in glass tanks flushed with running seawater. The plants used in biochemical studies were free of visible epiphytes.

Dependence of photosynthesis on light intensity was studied by the uptake of the radioactive carbon isotope, ^{14}C. Each experimental set included three replicate leaves and exposures were carried out in a chamber that measured 25x25x3 cm. Leaves were illuminated with a DRL-700 fluorescent lamp that provided a maximum intensity of white light of 80 W·m^{-2}. To convert illumination produced by the fluorescent lamp in the PAR region to quantum flux density, use the ratio 1 W·m^{-2} = 5μE·s^{-1}. Lower intensities were obtained by applying neutral density filters with transmission quotients of 0.2, 0.5, 1.4, 10.0, 14.0, 38.5 and 63%. Before exposure to $^{14}CO_2$, the leaves were flushed in the chamber for 20 min with seawater. Thereafter, $NaH^{14}CO_3$ was added for 20 min and the radioactive solution was removed, leaves were washed with seawater, killed at 100°C and dried to constant weight at 40-60°C. The samples were then ground in a micromill and their radioactivity was measured by means of the Geiger-Mueller counter method of Bykov and Koshkin (1969).

The rate of net photosynthesis (P_N) and dark respiration (R_D) were measured by the rate of O_2 increase or depletion in a closed system, which consisted of a leaf chamber (17.5x7x1.1 cm), a membrane pump and an oxygen detector. The concentration of O_2 was measured by means of the general-purpose oxygen meter Nr.5221 with oxygen sensor Nr.5972 (Zaklady Elektroniczne "ELWRO", Wroclaw) and recorded by means of a TZ 4100 potentiometer (Laboratorni Pristroje, Prague). The volume of the system was 200 cm^3 and the flow rate of seawater was 120 cm^3·min^{-1}. The sensor was calibrated at two O_2 concentrations before each experiment. An O_2 concentration of zero was obtained by adding a few crystals of dithionite ($N_2S_2O_4$). The other O_2 concentration for calibration corresponded to air-saturated seawater at the temperature of the experiment.

Exposure of leaves for the investigation of photosynthates was carried out in standard 50 ml beakers. Portions of leaves were weighed and placed into seawater for a 20 min pre-illumination period and then transferred to seawater containing $H^{14}CO_3^-$. After the period of illumination, the leaves were rinsed with seawater and killed in boiling ethanol acidified with formic acid. In pulse-chase experiments, the leaves were incubated under the expermental period of illumination with $H^{14}CO_3^-$ and thereafter rinsed and transferred to non-radioactive seawater and killed.

The fixed leaf material was ground in a mortar and its radioactivity was measured by the Geiger-Mueller counter. Ethanol- and water-soluble photosynthates were separated by means of two-dimensional paper chromatography (Benson et at. 1950, Värk et al. 1968, Bil' et al. 1981). The radioactivity of components was measured by means of a SL-30-300 scintillation counter

(Intertechnique, France) and a LS 100 C (Beckman, Austria). Amino acids were estimated by means of an AAA-339 amino acid analyzer (Mikrotechna, Czechoslovakia).

The pH of the sap of heterotrophic cells was measured over a 24 h period at 2.0 h intervals. Epidermal cells of the adaxial side of the leaf were removed by means of a razor blade and the pH of the cell sap was determined by means of Multiphan indicator paper (Chemapol, Czechoslovakia).

RESULTS AND DISCUSSION

Fig. 1 shows that both adaxial and abaxial surfaces of the leaf are covered with a thin layer of chlorophyll-containing epidermal cells that lack stomata. The leaf blade contains a number of parallel vascular bundles each surrounded by a layer of small unpigmented cells probably representing a mestome sheath. Vascular bundles occupy the middle part of the leaf blade and are connected to each other by means of cross veins. The vascular system is surrounded by large colorless vacuolized cells.

As can be seen from the photosynthesis vs. light curves (PI, Fig. 2), the previous conditions of illumination in the natural habitat have a strong influence upon the functioning of the photosynthetic apparatus. The diurnal kinetics of photosynthesis show that the maximum CO_2 uptake activity is observed at noon. The plateau region of the PI curves depends on the habitat, being reached at 20 to 30 $W \cdot m^{-2}$ in plants from deep waters and at 50 to 80 $W \cdot m^{-2}$ in plants from depths of 0.5 to 6 m. The initial slopes of the PI curves are also different (Fig. 2), with the half-maximum rate being reached at 1.5 $W \cdot m^{-2}$ and 5 $W \cdot m^{-2}$ in plants from deep and shallow waters, respectively. The greater sensitivity of the *T. ciliatum* photosynthetic apparatus in the range of lower light intensities facilitates efficient operation in natural low light habitats, analogous to macroalgae (Titlyanov et al. 1987). The increase in the photosynthetic capacity of *Thalassodendron ciliatum* as a function of depth to 26 m is distinct from other macrophytes and is difficult to explain. In plants from the deepest habitats, the slope of the PI curve is relatively small and at high light intensities assimilation of CO_2 is even inhibited. This is probably not a typical photoinhibitory reponse which occurs at high light intensities, because it takes place at very low light intensities of about 3 $W \cdot m^{-2}$. Rather, it appears to be a phenomenon of adaptation of these plants to unfavorable environmental conditions.

In order to get reliable values of radioactivity, the leaves of *Thalassodendron ciliatum* had to be exposed to $H^{14}CO_3^-$ for at least 20 min. During that time, a significant portion of the label is incorporated into intermediates of the glycolate pathway and into C_3 and C_4 acids. Since these products are decarboxylated, the calculated values of $^{14}CO_2$ influxes are less than the true rate of photosynthesis (P_T), but they exceed net photosynthesis (P_N) measured by $^{12}CO_2$. In the experiments with the isotope, the release of CO_2 at the expense of unlabeled end products of photosynthesis and from heterotrophic cells has not been taken into account.

Since heterotrophic cells make up to 80% of the volume of the leaf blade and photosynthetic cells only 20%, it is interesting to compare the $^{14}CO_2$ uptake and the concurrent O_2 release. The molar $^{14}CO_2$ uptake exceeded the apparent O_2 release by threefold (Table 1), apparently due to a very high respiration rate of the heterotrophic cells. Since the ratio of CO_2/O_2 during photosynthesis is unity, one may deduce that the respiration rate of the *Thalassodendron ciliatum* leaf is about 66% of the true photosynthetic rate and exceeds net photosynthesis by 1.5- to 2-fold. This agrees with the finding that the O_2 uptake rate immediately after switching off the light ("dark" respiration in the light) could be twice the rate of net photosynthesis. Therefore, application of the photosynthetic coefficient is not reasonable while the ratio of CO_2/O_2 is indefinite, due to the heterotrophic cells.

Mechanisms of photosynthetic carbon metabolism are not as well investigated in marine plants as in terrestrial plants. Their characteristics do not always allow a plant to be classified unequivocally as belonging to the C_3 or C_4 type. For example, *Thalassia hemprichii* (Ehrenb.) Aschers, growing in the same area of the ocean as *Thalassodendron ciliatum,* has a coefficient of isotope discrimination $^{13}C/^{12}C$ characteristic of the C_4 type of photosynthesis; however, studies of its $^{14}CO_2$ metabolism reveal that it is a C_3 plant (Andrews and Abel 1979). We applied two different approaches to study the photosynthetic metabolism of *T. ciliatum*: (1) kinetics of the incorporation of ^{14}C from $^{14}CO_2$ into photosynthates and (2) the pulse-chase method.

Kinetic curves (Fig. 3) show that the initial rate of ^{14}C uptake is higher than the rate following a 2 min exposure at both limiting and saturating light intensities (respectively, 2 and 1.2 fold). Such a kinetic is characteristic of C^3 photosynthesis, as observed with terrestrial photorespiring plants. After a 35 sec labeling period, 72% of the ^{14}C was found in sugar phosphates and 12% in malate and aspirate (Table 2), which is also characteristic of C_3 metabolism. However, at a very low light intensity (0.15 $W \cdot m^{-2}$, the products of the PEP carboxylation, malate and aspartate, contained 41% ^{14}C incorporated during the 35 sec exposure. At longer exposures, the rate of the synthesis of sugars decreased and the relative rate of the synthesis of alanine increased. This was probably a result of a decrease in the PGA reduction rate and its conversion into pyruvate and alanine as an alternative route of metabolism. The relative rate of PEP carboxylation was also increased and led to an enhancement of the efflux of $^{144}CO_2$, which was released during the decarboxylation of malate and pyruvate (Pärnik et al. 1988). Such a loss of $^{14}CO_2$ at low light intensities was the reason why the CO_2 uptake rate obtained during short-pulse labeling was nearly twice that obtained during long exposures. At high light intensities, which favored the reduction of 3-PGA, the uptake of labeled bicarbonate was depressed by only 20%. At the low light intensity, a marked proportion of the label also appeared in serine, which can be formed via 3PGA and oxypyruvate by reversed reactions of the glycolate pathway (Lawyer et al. 1982). In strong light, serine and glycine could also be synthesized via the glycolate cycle which could have started with the transketolase reaction. Although the experiment was carried out at a high bicarbonate concentration (30.3 mM), oxygenation of ribulose-1,5 bisphosphate was ruled out.

After a 10 min pulse, leaves retained 45% of the tracer if illuminated, and 38% if kept in the dark. During a 2 min photosynthetic exposure, the label was incorporated mainly into 3 PGA, sugar phosphates and sugars (Fig. 4). Only 2 to 3% of the radioactivity of the water-and alcohol-soluble fraction appeared in C_4-dicarboxylic acid. During the subsequent pulse-chase, the label from 3PGA and sugar phosphates was incorporated into sugars and the radioactivity of glycine and serine also increased. Such kinetics might have been the result of the synthesis of these compounds in the glycolate pathway initiated by the transketolase reaction. The decrease in radioactivity of the leaves in the light was probably caused by decarboxylation of glycine. After the pulse-chase in the dark (Fig. 4b), an increase in the percentage of the label in sugars was discovered. This was due to a decrease in the total content of the tracer and the absolute radioactivity of the sugars remained unchanged. This indicates that $^{14}CO_2$ was released from intermediates of photosynthetic metabolism, not from end products. In the dark, $^{14}CO_2$ was produced mostly by decarboxylation of pyruvate (Pärnik et al. 1988) which is also a substrate for alanine synthesis. Radioactivity of this amino acid increased, both as a percentage of the total radioactivity and as an absolute amount of ^{14}C.

The experiments described above provided insights into the dynamic reversible regulation of the operation of the photosynthetic apparatus. Plants grown in the same habitat were subjected to varied environmental conditions during $^{14}CO_2$ exposure. We also compared plants adapted to different environments. Plants from different depths did not reveal any qualitative differences in their metabolism. During a 15 sec exposure under saturating light, ^{14}C was incorporated mainly into

3PGA and other intermediates of the reductive pentose phosphate cycle (Table 3). In a manner analogous to the short-term exposure experiments under low light intensity, the synthesis of sugars in deep-growing plants adapted to low levels of illumination decreased and incorporation of ^{14}C into alanine increased.

Adaptation of *Thalassodendron ciliatum* to its habitat also was studied with respect to flavonoid compounds and bound amino acids. The plants were collected from the same habitats as those for investigation of photosynthetic metabolism. The content of flavonoids in mature leaves was 1.5 mg and 15.8 mg per g dry weight, respectively, at depths of 0.5 to 1 m and at 33 m. Chromatographic analysis (Margna and Margna 1969) and UV-absorption spectra of separated components (Mabry et al. 1970) suggested that the two major flavonoids present are probably glucoside derivatives of the flavonols quercetin and kaempherol. The roles of these compounds as well as their relation in the regulation of carbon metabolism is unclear.

The content of bound amino acids in adult leaves, analyzed following acidic hydrolysis of proteins, was identical in plants from the two habitats (Table 4). The young leaves of deep-water plants were markedly lower in proline and cystein. Differences in the total amino acid content at different depths were negligible. Young leaves contained 1.44 times more bound amino acids than old leaves (Table 4).

Thalassodendron ciliatum belongs to the group of C_3 plants. However, the presence of a large proportion of heterotrophic cells in the photosynthetic assimilator (Fig. 1) suggests coordination of autotrophic and heterotrophic cell functions and does not exclude carbon assimilation in the dark period and its storage in the form of organic acids in large vacuoles of the heterotrophic cells. This is in agreement with the diurnal kinetics of pH of the sap shown by the heterotrophic cells. Despite the alkalinity of the seawater (pH 8.2 at 29°C), the pH of the sap is in the range of 5.6 to 6.1. Acidification of the sap in *T. ciliatum* takes place at dawn and in early morning, but not during the night (Fig. 5), which is distinct from the pattern shown by terrestrial plants. The pH changes during the daytime were possibly due to conversion of labile keto acids (e.g., oxaloacetic acid) accumulated in vacuoles at night to the stable compound malate, rather than to an increase in the concentration of acids in vacuoles, because their formation during photosynthesis under saturating light levels was slow (Table 2, 3). To investigate this process, leaves were incubated for 10.5 h in the dark in seawater containing $H^{14}CO_3^-$, leaves were then killed and the content of the labeled malate was determined. After 18 min of illumination, the label in malate comprised 17%, following 30 min 22% and after 180 min 26% of the total radioactivity (Fig. 5). Malate was probably synthesized from some intermediate which was not identified; during a 180 min light period, its relative content decreased from 52 to 33%. Because the total radioactivity of the leaves did not change, the products of the dark CO_2 fixation did not appear to readily switch into respiratory metabolism. After a 180 min illumination period, only 8% ^{14}C occurred in sugars and 2% in serine and glycine, intermediates of the glycolate pathway. $^{14}CO_2$ taken up in the dark was probably metabolized via malate in the light, accompanied by an increase in pH up to 6.1, a value characteristic of the heterotrophic cells at night (Fig. 5).

CONCLUSIONS

Thalassodendron ciliatum is a typical C_3 plant exhibiting photorespiratory metabolism characteristic of this group of plants. As distinct from classical terrestrial C_3 plants, leaves of *T. ciliatum* lack stomata, and 80% of the leaf volume is occupied by chlorophyll-free heterotrophic cells which respire at a rate reaching about 66% of the gross photosynthetic rate. Light intensity curves of photosynthesis indicate that environmental conditions most favorable for the plant lay in the range of 6 to 26 m in depth. In plants growing 33 m deep, the CO_2 photoassimilation capacity is much

reduced at high as well as at low light intensities. Plants from deep waters contain more flavonoids, potential inhibitors of the electron transport chain (Allakhverdiev et al. 1989). A peculiarity of *T. ciliatum* is the synthesis of an intermediate from bicarbonate in the dark, which is converted into malate during subsequent illumination. This is accompanied by acidification of the cell sap, with pH values reaching as low as 5.6. During the daytime, the pH of the sap of heterotrophic cells increases and, by the evening, reaches the value characteristic of the dark period.

ACKNOWLEDGEMENTS

The authors are grateful to A. Lastivka for technical assistance, H. Ivanova for detailed analysis of the $^{14}CO_2$ assimilation products and amino acids and to Dr. U. Margna for kindly providing opportunities for flavonoid analysis in his laboratory. We thank Dr. J. Viil for useful comments on the manuscript.

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Table 1. $^{14}CO_2$ uptake and the concurrent O_2 release by *Thalassodendron ciliatum* leaves. $^{14}CO_3$-2.20 m, 6.0 Bq·nmol^{-1}, temperature 27°C, exposure time 10 min.

Habitat	$^{14}CO_2$ uptake mmol $CO_2 \cdot g^{-1} \cdot h^{-1}$	O_2 release mmol $O_2 \cdot g^{-1} \cdot h^{-1}$	Ratio $^{14}CO_2$
Cöetivy Island depth of 11m PAR 46 W·m^{-2}	0.18±0.03	0.060±0.010	3.0±0.9
Farquhar Island lagoon	0.19±0.03	0.072±0.010	2.6±0.8

Table 2. Incorporation of ^{14}C into photosynthates in *Thalassodendron ciliatum* leaves at low and high light intensity. $H^{14}CO_3$-30.2 mM, 880 Bq·nmol^{-1}, temperature 27°C, pre-illumination 30 min.

	Radioactivity of photosynthates (% total)					
	0.15 W·m^{-2}			20 W·m^{-2}		
	Exposure time (sec)					
	35	105	500	35	105	500
3-PGA+sugar						
phosphates	18.7	30.0	17.3	72.5	61.5	29.3
Sugars	-	4.5	7.7	3.8	14.4	41.9
Glycine	4.1	5.7	4.6	1.5	2.4	4.6
Serine	14.3	12.3	12.1	2.2	3.2	5.9
Malate	14.0	9.0	14.0	3.5	3.3	3.8
Aspartate	27.4	17.0	22.8	8.9	6.6	4.3
Alanine	3.6	5.0	4.0	1.5	2.0	2.6
Glutamate	3.2	4.0	3.8	0.4	2.0	2.6
Organic acids	6.8	2.5	1.7	1.7	1.1	1.3
Remainder	7.9	9.2	11.0	4.0	4.8	4.4

Table 3. Incorporation of ^{14}C into photosynthates in *Thalassodendron ciliatum* leaves growing at different depths. $H^{14}CO_3$-4,5 mM, 540 Bq·nmol^{-1}, temperature 26°C, PAR 28 W·m^{-2}, pre-illumination 30 min.

	Radioactivity of photosynthates (% total soluble fraction)							
	Aldabra Is.			Farquhar Is.		Cöetivy Is.		
	Exposure time (sec)							
	15	60	300	15	120	17	60	120
PGA+sugar phosphates	76.9	39.1	5.1	94.5	62.8	95.7	75.4	70.3
Sugars	8.3	37.7	73.2	2.2	25.1	-	8.9	13.5
Serine+Glycine	1.1	2.5	9.8	-	1.1	-	1.6	0.6
Malate	-	3.1	-	1.0	0.9	-	-	-
Aspartate	6.5	6.1	4.3	2.3	1.8	3.0	3.3	2.1
Alanine	1.1	3.3	3.3	-	1.3	1.3	10.7	12.9
Glutamate	-	5.4	0.7	-	2.5	-	-	0.6
Unidentified	6.1	2.6	3.6	-	4.6	-	-	-

Table 4. Content of bound amino acids in leaves of *Thalassodendron ciliatum* from shallow (0.5 m) and deep (33 m) water.

Amino acid	Concentration, mg per g dry weight ± S.D.			
	Young leaves		Mature leaves	
	0.5 m	33 m	0.5 m	33 m
Aspartic acid	6.29±0.39	6.10±0.18	4.34±0.16	4.36±0.18
Threonine	2.60±0.16	2.62±0.09	1.75±0.03	1.68±0.02
Serine	2.70±0.10	2.90±0.10	1.99±0.13	1.83±0.03
Glutamic acid	9.27±0.27	8.46±0.33	6.39±0.27	5.42±0.11
Proline	6.44±0.73	3.35±0.26	0.32±0.09	3.03±0.31
Cysteine	2.49±0.31	0.98±0.05	1.51±0.16	1.19±0.11
Glycine	3.93±0.10	4.16±0.17	3.27±0.17	2.61±0.09
Alanine	3.70±0.13	3.64±0.12	2.49±0.09	2.48±0.01
Valine	4.43±0.17	1.09±0.14	2.85±0.06	3.07±0.15
Methionine	1.74±0.31	1.08±0.02	0.69±0.13	0.81±0.02
Isoleucine	2.81±0.02	3.10±0.08	2.07±0.05	2.18±0.17
Leucine	4.72±0.01	5.13±0.16	3.43±0.06	3.34±0.04
Tyrosine	2.12±0.14	2.15±0.10	1.74±0.22	1.85±0.10
Phenylalanine	3.83±0.13	3.69±0.03	2.62±0.33	0.91±0.29
Histidine	2.02±0.01	1.81±0.04	1.30±0.10	1.43±0.09
Tryptophane	- -	0.37±0.37	0.21±0.21	- -
Lysine	4.17±0.41	4.22±0.04	4.13±0.25	3.74±0.16
Arginine	4.81±0.17	4.22±0.04	4.13±0.25	3.74±0.16

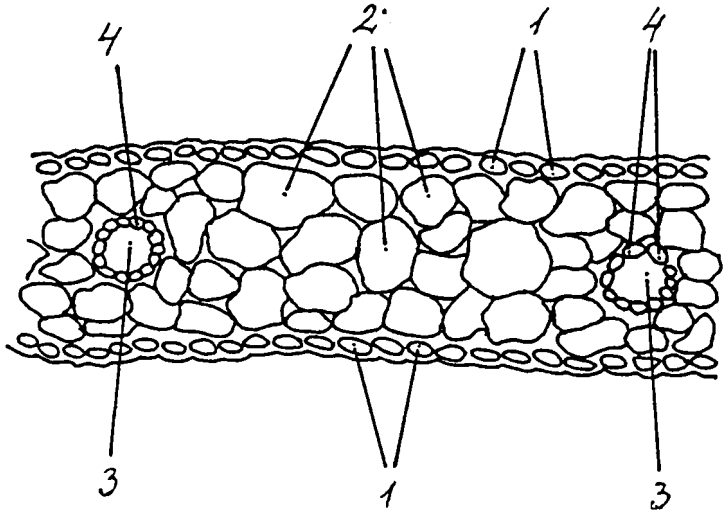

Figure 1. Cross-section of the leaf of seagrass *Thalassodendron ciliatum*.

1 - epidermal cells
2 - colorless vacuolized cells
3 - vascular bundles
4 - mestome sheath cells.

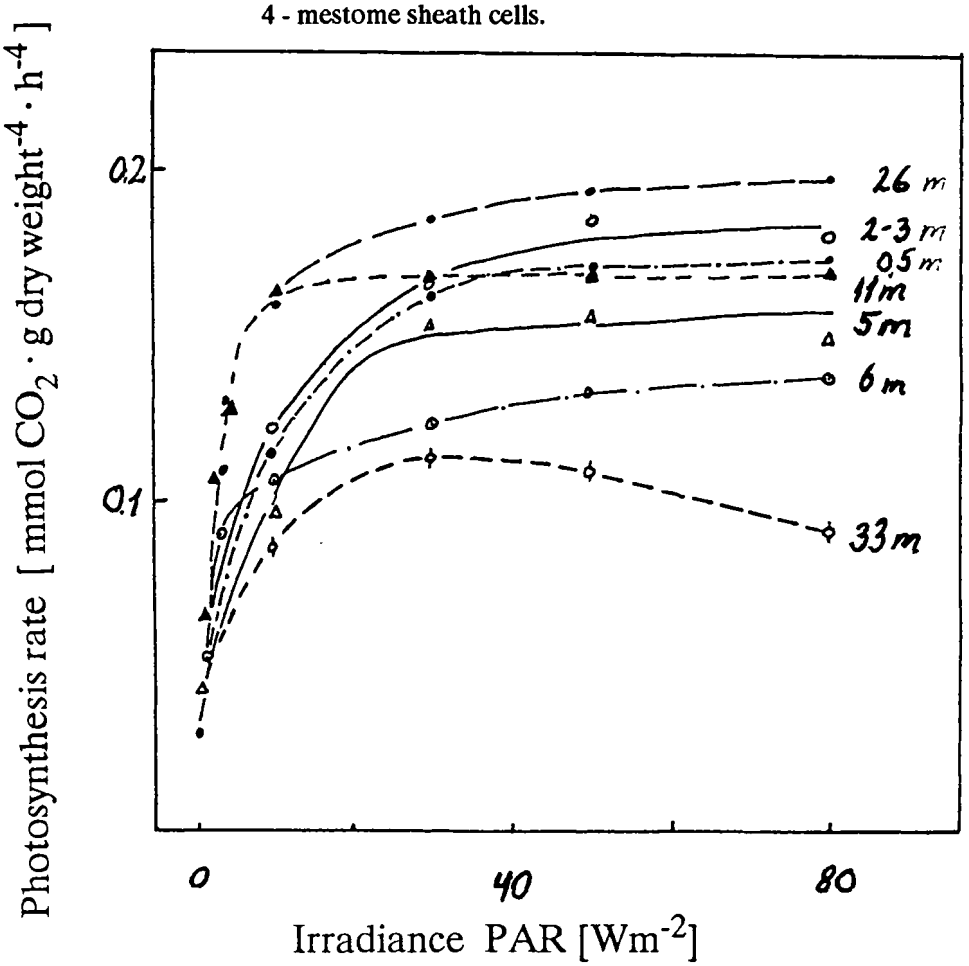

Figure 2. Dependence of photosynthesis on irradiance in *Thalassodendron ciliatum* leaves growing at different depths. $H^{14}CO_3^-$ 2.02 mM, 6.0 Bq·nmol^{-1}, temperature 27°C, exposure time 20 min.

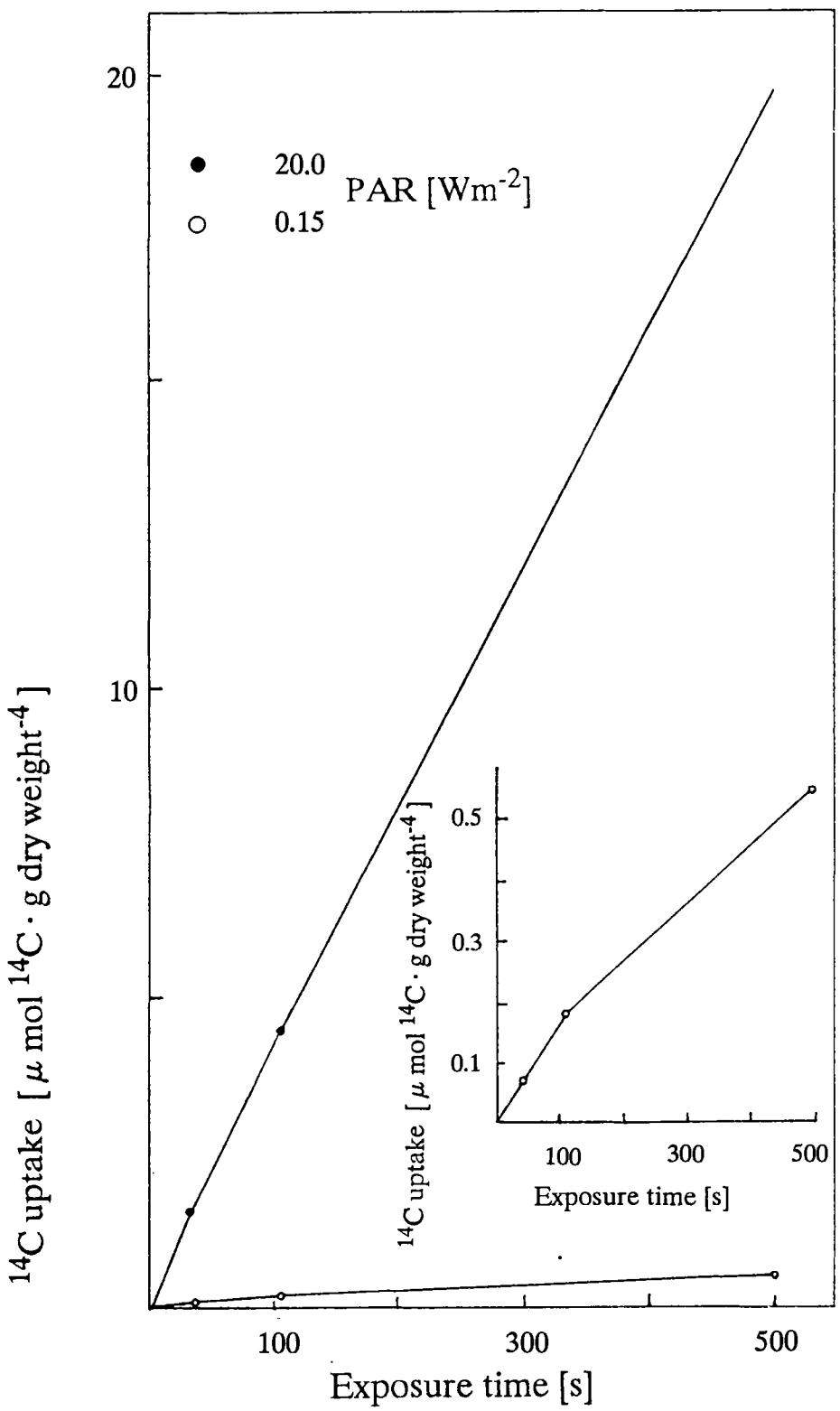

Figure 3. Kinetics of the ^{14}C uptake by *Thalassodendron ciliatum* leaves under limiting (PAR 0.15 $W \cdot m^{-2}$) and saturating (PAR 20 $W \cdot m^{-2}$) irradiance. $H^{14}CO_3^-$ 30.2 mM, 880 $Bq \cdot nmol^{-1}$, temperature 27°C, pre-illumination 30 min.

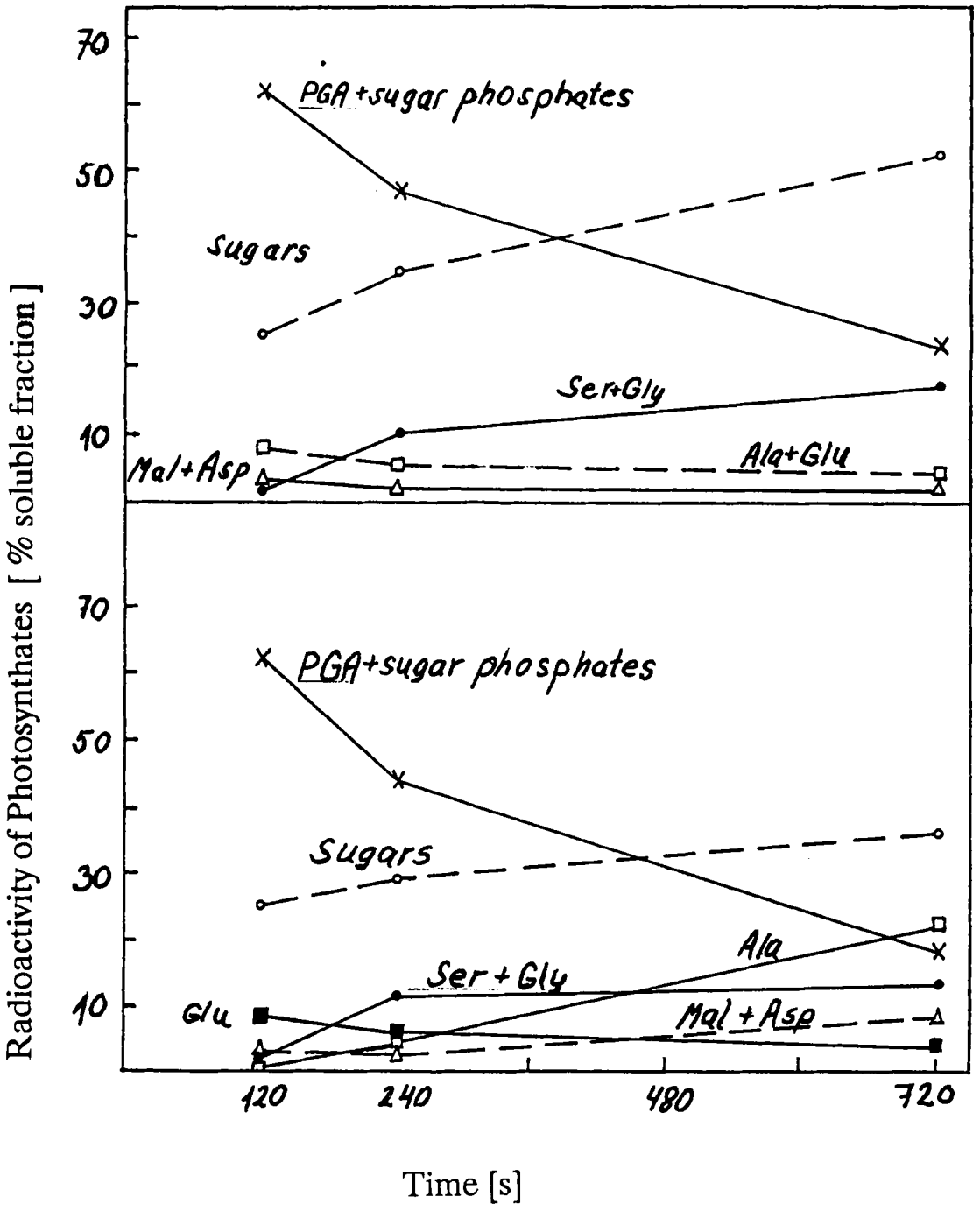

Figure 4. Pulse-chase of ^{14}C assimilated during a 120 sec exposure to $H^{14}CO_3^-$ in the light (A) and in the dark (B). $H^{14}CO_3^-$ 4.5 mM, 540 Bq·nmol^{-1}, temperature 26°C, PAR 28 W·m^{-2}.

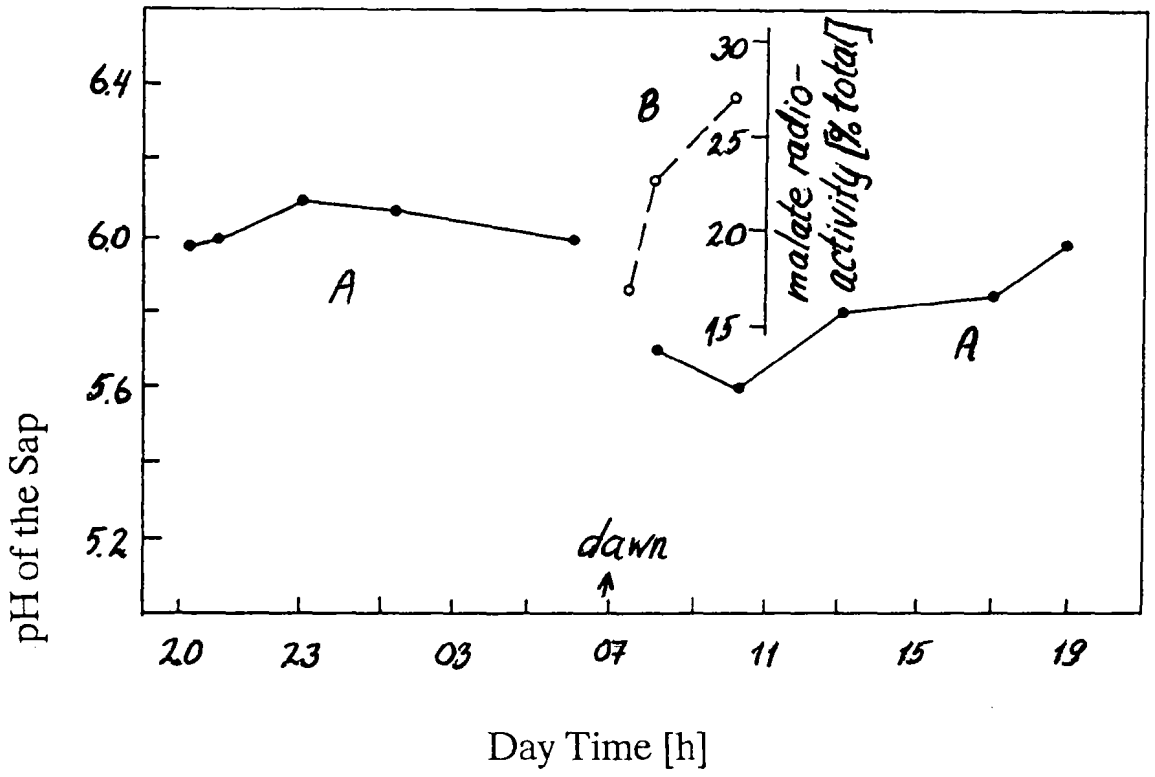

Figure 5. Diurnal kinetics of sap pH of the heterotrophic cells (A) in *Thalassodendron ciliatum* leaves and of ^{14}C incorporation into malate (B) from $H^{14}CO_3^-$ assimilated in the dark during 10.5 h.