Phyton (Austria) Special issue: "P. J. C. Kuiper"	Vol. 40	Fasc. 3	(133)-(143)	31. 3. 2000
---	---------	---------	-------------	-------------

Elodea canadensis under N and CO₂ Limitation: Adaptive Changes in Rubisco and PEPCase Activity in a Bicarbonate User

By

LUCINA C. VAN GINKEL, INGEBORG SCHÜTZ & HIDDE B.A. PRINS¹⁾

K e y w o r d s : Aquatic plants, photosynthesis, fluorescence, bicarbonate assimilation, $Elodea \ canadensis$.

Summary

VAN GINKEL L.C., SCHÜTZ I. & PRINS H.B.A. 2000. *Elodea canadensis* under N and CO_2 limitation: Adaptive changes in Rubisco and PEPCase activity in a bicarbonate user. - Phyton (Horn, Austria) 40 (3): (133) - (143).

Diffusion of CO₂ in water is 10,000 times slower than in air. Because of this photosynthesis in submerged aquatic macrophytes is often limited by CO2 availability. Elodea canadensis shows HCO3 utilization under conditions of CO2 limitation. A closely related species, Hydrilla verticillata, which also belongs to the Hydrocharitaceae, shows a C4 like mechanism when grown under so-called summer conditions where low availability of CO₂ is expected. Here it is shown that a similar C₄-like mechanism is possibly also present in E. canadensis. To study the possible interference between the two processes, E. canadensis was grown at low and high CO2 and NO3, conditions expected to affect HCO3⁻ utilization. Moreover the effect was studied of these growth conditions on the occurrence of the C4 state. A low GCO2 was observed in E. canadensis when it was grown under low CO₂ conditions indicative for plants in the C₄-state. The low G_{CO2} was related to a change in the ratio Rubisco and PEPCarboxylase indicating a central role of these enzymes. The very same conditions induced HCO3⁻ utilization in this species. Both mechanisms thus operate in the same plant simultaneously. Growing E. canadensis at high NO₃, 0.75 mM, had a strong stimulatory effect on O₂ production in the light both in low and high CO₂ grown plants, suggesting that it has no direct relation with HCO3⁻ utilization. The actual cause for this stimulatory effect of high N on photosynthesis is unclear.

Introduction

Submerged aquatic macrophytes (SAM) are often faced with limitation of their photosynthesis by low availability of inorganic carbon, e.g. dissolved CO₂.

¹⁾ Department of Plant Biology, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands.

Diffusion of CO₂ in water is about 10,000 times slower than in air. Furthermore, the major component of the dissolved inorganic carbon (DIC) pool is HCO_3^- at pH 7-9, common for natural waters. Since only CO₂ diffuses freely over the plasmamembrane to the cytoplasm, other inorganic carbon species have to be converted into CO₂ or need an active transport system across the plasmamembrane to contribute to carbon fixation. To describe the inorganic carbon system, the following terms are used: CO₂, dissolved free CO₂ including H₂CO₃; HCO₃⁻, bicarbonate; CO₃²⁻, carbonate; DIC: sum of CO₂, H₂CO₃, HCO₃⁻ and CO₃²⁻.

The slow diffusion of gases in water also generates a high O_2 level in the photosynthesizing cells of submerged aquatic plants. The resulting low CO_2/O_2 ratio near the Rubisco site is favorable to photorespiration and accordingly would lead to a low rate of carbon fixation. In the course of evolution several SAM responded to this challenge by development of mechanisms to use HCO_3^- as an alternative carbon source and/or mechanisms to concentrate inorganic carbon (for review see BOWES & SALVUCCI 1989, PRINS & ELZENGA 1989). Mechanisms from the first category are associated with an increased potential for HCO_3^- utilization either by active uptake of HCO_3^- in the leaf cells or by conversion of HCO_3^- into CO_2 . The second category are CO_2 -concentrating mechanisms (CCM), based on a C_4 - or CAM-like metabolism. Often these mechanisms of enriching the cell with CO_2 are accompanied by an increased carbonic anhydrase (CA) activity in the apoplast of leaf cells.

Models for HCO_3^- utilization in fresh water macrophytes are generally based on active H^+ extrusion into the apoplast driven by a plasmalemma bound ATPase. Two mechanisms have been proposed. The first described mechanism is a light dependent acidification of the apoplast and unstirred layer near the leaf surface (PRINS & al. 1982). This acidification results in a shift of the equilibrium between HCO_3^- and CO_2 in the direction of CO_2 which then diffuses into the leaf cells. To balance the loss of H^+ there is an H^+ influx or OH⁻ efflux at another part of the leaf. There is no agreement if the alkaline zones result from H^+ influx or OH⁻ efflux or by another process. For convenience the expression H^+ influx is used here.

This mechanism has now been observed in a number of fresh water angiosperms. E.g., in *Elodea*, *Egeria*, and *Potamogeton* there generally is an acidification at the abaxial side of the leaf to pH values of 4-5 and an alkalization at the adaxial side to pH values or 9-11 (PRINS & ELZENGA 1989). A second mechanism has been proposed for a.o. the Characeae. The basis for this latter mechanism is $HCO_3^$ transport by symport with H⁺, driven by the proton motive force and accompanied by acid regions of H⁺ efflux and alkaline regions of H⁺ influx (LUCAS 1985).

A C₄-like metabolism has been proposed for *Hydrilla verticillata* and other SAM. Typical C₄-characteristics are induced in *Hydrilla* when grown under conditions normally expected to lead to high rates of photorespiration. SAM showing this C₄-like metabolism are, like their terrestrial counterparts, characterized by a low CO₂-compensation point (Γ_{CO2}), a photosynthesis relatively insensitive to O₂ and a low Rubisco/PEPCase (phosphoenolpyruvate) activity ratio (R/P), due to a high PEPCase activity (SALVUCCI & BOWES 1981, HOLADAY & al. 1983, BOWES &

(135)

SALVUCCI 1989). In this species, Rubisco is localized in the chloroplast and PEP-Case in the cytoplasm (REISKIND & al. 1989). After fixation of inorganic carbon by PEPCase in the cytoplasm, the concentrating of DIC occurs most likely in the chloroplast (REISKIND & al. 1997).

In *Elodea canadensis* acidification is induced by conditions of high light and low DIC in plants previously grown under conditions favorable to photorespiration. Acidification is absent in *Elodea* plants grown under high CO₂ conditions (ELZENGA & PRINS 1989). This observation led to the question: is acidification in *Elodea* accompanied by C₄-characteristics, such as a low Γ_{CO2} and a low R/P, as observed in *Hydrilla*. A low Γ_{CO2} could facilitate the diffusion of CO₂ into the leaf cells, following the conversion of HCO₃⁻ into CO₂ by the process of acidification (PRINS & al. 1982).

It is generally thought that the main physiological effect of HCO₃⁻ utilization is an increase of the rate of carbon fixation. It may also contribute to suppression of photorespiration through an increased availability of CO₂ and thereby, to a more efficient use of nitrogen (BEARDALL & al. 1982, RAVEN 1985, RAVEN & LU-CAS 1985). To answer the above question, experiments were set up to study the effect of low and high NO₃⁻, in combination with low and high CO₂ during the growth period, on acidification, Γ_{CO2} and R/P. It was hypothesized that high CO₂ and high NO₃⁻ during growth would lead to a relatively high Γ_{CO2} and a high R/P and the adverse conditions to low values for Γ_{CO2} and R/P. The combination of low and high CO₂ and low and high NO₃⁻ might also give insight in the mechanism, which induces acidification when CO₂ levels are low during growth. Under conditions of high NO₃⁻, low CO₂ will limit the rate of carbon fixation and thereby growth. Under conditions of low NO₃⁻, growth may be limited by N availability rather than by the rate of carbon fixation.

Materials and Methods

Elodea canadensis Michx. was grown in tanks on a clay substrate covered with sand (VAN GINKEL & PRINS 1998). For the present experiments sprouts of around 100 mm were transferred to 2-liter Erlenmeyer flasks containing an appropriate culture solution. Each flask contained about 15 sprouts, which were cultured for 1 to 3 weeks in 5% Hoagland growth medium. This solution contained 0.75 mM NO₃ (further referred to as high N) or 0.15 mM, one fifth of this concentration, (referred to as low N) at low or high CO₂. Low CO₂ was obtained by continuous aeration with normal air and high CO₂ by constant titration of the growth medium to pH 6.6 by bubbling with CO₂ (ELZENGA & PRINS 1989). The medium was changed every fourth day. The temperature was 21 °C (\pm 1 °C) and the fluence rate 60 µmol m⁻² s⁻¹ PAR with a photoperiod of 12 hours.

Rubisco and PEPCase were assayed in a crude extract made from newly formed shoots. The extraction buffer contained 60 mM HEPES, 24 mM MgCl₂, 30 mM KHCO₃, 0.24 mM NaEDTA and 6 mM DTT. The pH was set to pH 8.0 by KOH for Rubisco and to pH 7.1 for PEP-Case (adapted from REISKIND & BOWES 1991). The shoots were first frozen in liquid N₂ and thereafter ground in the presence of 5% (w/w) Polyclar-AT^(TM). The ground plant material was suspended in extraction buffer of pH 7.1 or 8.0. The ratio plant material/extraction buffer was 1:10 (w/v). Three aliquots of 50 µl were taken and added to 950 µl 85% acetone for chlorophyll determination (LICHTENTHALER 1987). Thereafter the plant debris was spinned down in an Eppendorf centrifuge (14,000 rpm, 10 min.). Aliquots of 100 µl supernatant were tested for enzyme activity. The

©Verlag Ferdinand Berger & Söhne Ges.m.b.H., Horn, Austria, download unter www.biologiezentrum.at (136)

assay for both enzymes was spectrophotometrically and based on a decrease in NADH concentration, measured as absorption at 340 nm. The Rubisco assay was as described by BESFORD 1983. The PEPCase assay was based on a method described by STITT & al. 1989. The buffer used for this assay contained 50 mM HEPES, 20 mM MgCl₂, 25 mM KHCO₃, 0.2 mM NaEDTA, 5 mM DTT and was brought at pH 8.0 with KOH. The reaction was started by adding phosphoenolpyruvate (PEP, final concentration 8 mM) after a preincubation at 30 °C. The enzyme activity was determined after 10 minutes incubation at 30 °C. The Γ_{CO2} (at 21 % O₂) was determined with an infra red gas analyzer (HOLADAY & al. 1983). The experimental solution was a 5% Hoagland medium buffered with 50 mM MES brought at pH 5.5 with KOH. This pH was chosen so that CO₂ was the only carbon source and to prevent any contribution by HCO₃⁻ (VAN & al. 1976). During the experiment the pH remained constant, the fluence rate was 300 µmol m⁻² s⁻¹ PAR and the temperature 21 °C. The light induced pH-polar reaction was measured in artificial pond water, APW, containing 5 mM CaCl₂, 1.5 mM KCl, 1 mM NaCl and KHCO₃ as indicated (PRINS & al. 1982).



Fig. 1. Light induced O_2 production rate, corrected for dark respiration, of *E. canadensis* leaves plotted against pH using either a strongly buffered (25 mM MES/BTP) or an unbuffered APW solution containing 1 mM HCO₃⁻.

Photosynthetic O_2 production at different pH's was measured using an O_2 electrode. The experimental solution, consisting of APW and 25 mM MES/BTP buffer, was rapidly stirred. The combination of stirring and strong buffering should effectively prevent any use of HCO₃⁻ as carbon source, as the build up of an acid region by polarity was prevented. To test this, net O_2 production was measured in a strongly buffered and an unbuffered experimental solution of APW. With buffering, O_2 production stopped completely at pH 8.5, while in APW it continued till pH 9.5 (Fig. 1).

Results

The water in the tanks, wherein the plants originally were grown before the low and high CO_2 culture period, was weakly aerated by normal air and has to be considered 'low CO_2 '. Accordingly the plant material directly taken from the tanks showed pH-polarity, with abaxial acidification under CO_2 limiting conditions. Transferred to the Hoagland growth medium the *Elodea* sprouts grew vigorously,

especially in the high CO_2 solutions, and new sprouts (and roots) developed. The pH of the low CO_2 growth medium increased to values of around 10 in the light and returned in the dark to around 7, as shown in the inset of Fig. 2 for *Elodea* grown at low N. This drift of the pH, while the solution was aerated with normal air, indicated a high capacity of *Elodea* for extracting DIC from its surrounding medium (ADAMEC 1993, SAND-JENSEN & GORDON 1986). In accordance herewith *Elodea*, grown in 5% Hoagland under low CO_2 showed the typical pH polar reaction, when transferred to APW + 1 mM KHCO₃ pH 8, light 60 µmol m⁻² s⁻¹, (Fig. 2) as described earlier (ELZENGA & PRINS 1989). When *Elodea* was grown under high instead of low CO_2 conditions no pH polarity was induced and there was only a small a-polar increase of the pH near both leaf sides and the change to adaxial alkalization and abaxial acidification did not occur (Fig. 2). At this stage no difference between low and high N plants was observed.



Fig. 2. pH-polar reaction of *E. canadensis* leaves grown at either low or high CO₂. Medium: 5% Hoagland low N, fluence rate 60 μ mol m⁻²s⁻¹ PAR. Inset: Culture solution pH of low CO₂ culture, one light-dark cycle (24 hours).

A shift to a C₄-like state was observed in *Elodea* when the plants were cultured under low CO₂ conditions. The Γ_{CO2} was low for the low CO₂ plants compared to the Γ_{CO2} of plants cultured at high CO₂. In the low CO₂ plants R/P shifted from Rubisco to PEPCase (Table 1). Fig. 3 shows the actual Rubisco and PEPCase activity for plants after being transferred to the Hoagland culture solutions of low and high N and C for 1 to 2 weeks. The results for high and low N are described below.

Table. 1. Γ_{CO2} in (μ l $\Gamma^1 \pm$ SEM), chlorophyll content in mg g FW⁻¹ \pm SEM, c_a/c_b and R/P of *E. canadensis* grown in 5% Hoagland, normal or 1% NO₃⁻ (high or low N) for 2-3 weeks, under high and low CO₂ availability. ^{*}) The Γ_{CO2} for low CO₂ plants at either high or low N was not significantly different.

	High CO ₂ High N	Low CO ₂ High N	High CO ₂ Low N	Low CO ₂ Low N
Chlorophyll	2.53 ± 0.34	3.27 ± 0.36	2.31 ± 0.16	2.61 ± 0.18
Ca/Cb	2.77 ± 0.02	2.69 ± 0.05	2.79 ± 0.03	2.68 ± 0.02
$\Gamma_{\rm CO2}$	70-90	20-30*)	70-90	30-40*)
R/P	2.09 ± 0.35	1.25 ± 0.41	3.10 ± 0.33	1.88 ± 0.17

The Rubisco activity remained constant in the low CO₂, but increased in the high CO₂ plants. The PEPCase activity in contrast, remained nearly constant in the high as well as in the low CO₂ plants (Fig. 3). As a result the R/P was relatively high in these high N plants grown at high CO₂, but low in those grown at low CO₂ (Fig. 3; Table 1). This corresponded with a high Γ_{CO2} in the high CO₂ plants and a low Γ_{CO2} in the low CO₂ plants (Table 1).

The Rubisco activity increased in both low and high CO₂ grown plants (Fig. 3). The PEPCase activity increased in the low CO₂ plants, while it showed only a slight, probably not significant, downward tendency in the high CO₂ plants (Fig. 3). As a result the activity ratio of Rubisco/PEPCase (R/P) of the low N plants showed the same tendency as in high N plants, R/P was high in high CO₂ grown plants and low in those grown at low CO₂ (Fig. 3). As with the high N plants this corresponded with a high Γ_{CO2} in the high CO₂ plants and a low Γ_{CO2} in those grown at low CO₂ (Table 1).

Net O_2 production by Elodea was measured, using a strongly buffered experimental solution with a constant DIC concentration of 1 mM and a varying pH. Under these conditions photosynthesis depends entirely dependent on the CO₂ with no contribution of HCO₃⁻ (Fig. 4). Under these conditions no difference was ob served between the low and high CO₂ plants, of either low or high N grown plants. Therefore the low and high CO₂ data were pooled. In contrast there was a very marked difference between the low and high N plants. The rate of O₂ production of the high N plants at the highest external [CO₂] was twice that of the low N plants. In the low N plants photosynthetic O₂ production was saturated between 0.1 and 0.2 mM CO₂ while in the high N plants saturation was not yet reached at 0.5 mM CO₂.



Fig. 3. Rubisco and PEPCase activity of *E. canadensis* shoots grown in 5% Hoagland solution for 1-2 weeks, at high or low N and low or high CO_2 , fluence rate 60 µmol m⁻² s⁻¹ PAR, photoperiod 12 h, 21 °C. 1-2 weeks.

(139)



Fig. 4. Light induced O_2 evolution of *E. canadensis* leaves at 60 µmol m⁻² s⁻¹ versus [CO₂], [DIC] was constant, 1 mM. [CO₂] was varied by changing the pH, see *inset*.

The chlorophyll content and the chlorophyll a/b ratio, c_a/c_b , depended on the growth conditions (N, CO₂) of the plants. High CO₂ plants grown at either low or high N, did not significantly differ in their chlorophyll content. When plants were grown at low CO₂ high N plants contained far more chlorophyll than low N plants, and also more than the plants grown at high CO₂, either at low or high N. A higher c_a/c_b was observed in plants grown at high CO₂ compared to plants grown at low CO₂ (Table 1). The different growth conditions resulted in plants with similar dry/fresh weight ratios of around 8%. Furthermore both, low and high CO₂ plants (leaves) had a similar protein content. The low N plants, both low and high CO₂, had a lower protein content, 3-4 µg protein g FW⁻¹, than high N plants, 7-8 µg protein g FW⁻¹.

Discussion

Light induced pH polarity is suppressed in *Elodea* grown at high CO₂ in both low (Fig. 2) and high N cultured plants (data not shown, but see ELZENGA & PRINS 1989), in agreement with the inhibition of bicarbonate utilization (ADEMEC 1993). At 0.4 mM external [CO₂] the rate of O₂ production of high N grown plants was twice that of low N plants. No difference was observed between low and high

(141)

 CO_2 plants. Strong pH buffering makes acidification by pH-polarity ineffective and therefore, CO_2 was the only C source in these experiments (PRINS & al. 1982). The stimulation of O_2 production by high N growth conditions did not result from an increased chlorophyll content as the rate of O_2 production is expressed on a chlorophyll basis.

High CO₂ growth conditions induced a high Γ_{CO2} , in both high and low N plants (JAHNKE & al. 1991, ADAMEC 1993). The compensation point of low CO₂ grown *Elodea* seemed somewhat higher in high than in low N grown plants. This may be related to a higher rate of net O₂ production observed in high N grown plants and a twice as high protein content of these leaves.

The data on Rubisco and PEPCase activity show that R/P was higher in the high than in low CO₂ plants. The underlying changes in Rubisco and PEPCase activity differ between low and high N *Elodea* plants. The growth condition in the tanks, from which the original starting plant material was collected, was low CO₂. Accordingly the Rubisco activity increased under high CO₂ in high N plants conditions while it remained the same in low CO₂ plants. The PEPCase activity of the high N plants did change however, neither in the low CO₂ nor in the high CO₂ plants. As a result R/P increased when plants were grown under high N, high CO₂ conditions. In the low N plants the changes are less clear although they lead to a similar change in R/P: Rubisco activity increased under both low and high CO₂ conditions; PEPCase activity of low N plants only increased in the low CO₂ grown plants. No significant change was observed in high CO₂ grown plants. An observed increase of both Rubisco and PEPcase activity under low CO₂ conditions in low N plants indicates a more specific effect of low N during growth.

E. canadensis is taxonomically closely related to H. verticillata, both species belong to the Hydrocharitaceae family. The species are morphologically very similar, both are fresh water submerged macrophytes and both show photosynthetic HCO₃⁻ utilization and pH polarity (ELZENGA & PRINS 1986, 1989, REISKIND & al. 1997). In Hydrilla C4-like characteristics were induced by so-called summer growth conditions: high light and temperature and low CO₂ (HOLADAY & al. 1983, BOWES & SALVUCCI 1989). Typical for C₄ like *Hydrilla* are a low Γ_{CO2} and a low R/P activity ratio due to a relatively high PEPCase activity compared to C3 like Hydrilla. In Elodea pH polarity and the C₄ characteristics of a low Γ_{CO2} and a relatively high R/P ratio were all induced by growing the plants under low CO₂ conditions, while fluence rate and temperature were kept constant. This effect of high CO₂ growth conditions may seem comparable to some extent to the effect of winter and summer conditions on Hydrilla. One of the characteristics of winter conditions, which lead to C₃ plants characterized by a high Γ_{CO2} , is a higher availability of CO₂ compared to summer conditions which lead to C₄ plants with a low Γ_{CO2} , (SAL-VUCCI & BOWES 1981, HOLADAY & al. 1983, BOWES & SALVUCCI 1989). However, winter growth conditions did not suppress pH-polarity in Hydrilla. The observation of local acidification, leading to the conversion of HCO3⁻ into CO2, and C_4 -like characteristics induced in both species by low CO_2 , raises the question how these two processes cooperate or interact. Given the similarities between both spe-

cies it is assumed that the mechanism of CCM in Hydrilla is also active in Elodea. Activity of such a C4-like CCM could result in a lowering of cytoplasmic CO2 producing a steeper gradient for CO₂ between apoplast and cells leading to a higher photosynthetic rate. Although this seems an attractive hypothesis it is not entirely supported by the experiments on photosynthetic O_2 production. The O_2 production as determined in these experiments reflected CO₂ fixation without HCO₃ utilization. Under conditions of an active CCM therefore, one would expect a higher rate of CO₂ fixation and thus a higher rate of O₂ production by low CO₂ plants. This was not observed, neither in the low N nor in the high N plants. Assuming that CCM also occurred in *Elodea* it did not result in a higher rate of O₂ production, in contrast to the use of HCO3⁻ via acidification, which leads to an increased O2 production (PRINS & al. 1982). Clearly the two mechanisms, CCM and pH-polarity, contribute in different ways to photosynthesis in SAM like Elodea and Hydrilla. CCM activity seems to result in suppression of photorespiration but has only a marginal effect, if any, on the net rate of photosynthesis when diffusion of CO_2 into the leaf is limiting.

The cause of the stimulatory effect of high N during growth on photosynthetic O_2 production in both low and high CO_2 plants remains unclear. Possibly low N during growth in low CO_2 , but not in high CO_2 , induces a C₄-like state, i.e. it lowers the Γ_{CO2} and the R/P ratio, which may indicate that suppression of photorespiration is related to a more efficient use of N (RAVEN 1985). MADSEN & BAAT-TRUP-PEDERSEN 1995 showed that in *Elodea* Rubisco activity was directly related to tissue-N level, and activity was substantially higher than could be expected on the basis of photosynthetic capacity. Furthermore, the relation between initial slope of the CO_2 response curve and Rubisco activity was linear. So clearly Rubisco had a regulatory effect under CO_2 -limiting circumstances and a similar conclusion can be drawn from the present results.

Acknowledgement

We thank Prof. Dr. Ir. P.J.C. KUIPER for critically reading of the text and for his many helpful suggestions.

References

- ADAMEC L. 1993. Rapid inhibition of HCO₃⁻ use by high concentrations of free CO₂ in *Elodea canadensis*. Aquatic Bot. 45: 311-324.
- BEARDAL J., GRIFFITH H. & RAVEN J.A. 1982. Carbon isotope discrimination and the CO₂ accumulating mechanism in *Chlorella emersonii*. J. Exp. Bot. 33: 729-737.
- BESFORD R.T. 1983. Some properties of ribulose biphosphate carboxylase extracted from tomato leaves. – J. Exp. Bot.35: 495-504.
- BOWES G. & SALVUCCI M.E. 1989. Plasticity in the photosynthetic carbon metabolism of submersed aquatic macrophytes. - Aquatic Bot. 34: 233-266.

(143)

- ELZENGA J.T.M. & PRINS H.B.A. 1986. ATPase activity and redox reactions in polar leaves of the submerged macrophyte *Elodea canadensis*. - In: BEILBY M.J., WALKER N.A. & SMITH J.R. (Eds.), Proceedings 7th international workshop on plant membrane transport, Sydney, 24-29 August 1986, pp. 327-331.
- & 1989. Light-induced polar pH changes in leaves of *Elodea canadensis*. I. Effects of carbon concentration and light intensity. Plant Physiology 91: 62-67.
- HOLADAY A.S., SALVUCCI M.E. & BOWES G. 1983. Variable photosynthesis/photorespiration ratios in *Hydrilla* and other submersed aquatic macrophyte species. – Can. J. Bot. 61: 229-236.
- JAHNKE L.S., EIGHMY T.T. & FAGERBERG W.R. 1991. Studies of *Elodea nuttallii* grown under photorespiratory conditions. I. Photosynthetic characteristics. - Plant Cell Environ. 14: 147-156.
- LICHTENTHALER H.K. 1987. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. - In: PARKER L. & DOUCE R. (Eds.), Methods in enzymology 148, pp. 350-382. -Acad. Press Inc. New York, London.
- LUCAS W.J. 1985. Bicarbonate utilization by Chara: A re-analysis. In: LUCAS W.J. & BERRY J.A. (Eds.), Inorganic carbon uptake by aquatic photosynthetic organisms, pp. 229-254. ASPP, Rockville, MD.
- MADSEN T.V. & BAATTRUP-PEDERSEN A. 1995. Regulation of growth and photosynthetic performance in *Elodea canadensis* in response to inorganic nitrogen. – Funct. Ecol. 9: 239-247.
- PRINS H.B.A. & ELZENGA J.T.M. 1989. Bicarbonate utilization: Function and mechanism. Aquatic Bot. 34: 59-83.
 - , SNEL J.F.H., ZANSTRA P.E. & HELDER R.J. 1982. The mechanism of bicarbonate assimilation by the polar leaves of *Potamogeton* and *Elodea*. CO₂ concentrations at the leaf surface. - Plant Cell Environ. 5: 207-214.
- RAVEN J.A. 1985. The CO₂ concentrating mechanism. In: LUCAS W.J. & BERRY J.A. (Eds.), Inorganic carbon uptake by aquatic photosynthetic organisms, pp. 67-82. - ASPP, Rockville, MD.
 - & LUCAS W.J. 1985. Energy costs of carbon acquisition. In: LUCAS W.J. & BERRY J.A. (Eds.), Inorganic carbon uptake by aquatic photosynthetic organisms, pp. 305-324. -ASPP, Rockville, MD.
- REISKIND J.B. & BOWES G. 1991. The role of phosphoenolpyruvate carboxykinase in a marine macroalga with C₄-like photosynthetic characteristics. - Proc. Natl. Acad. Sci. USA 88: 2883-2887.
 - BERG R.H., SALVUCCI M.E. & BOWES G. 1989. Immunogold localization of primary carboxylases in leaves of aquatic and a C₃-C₄ intermediate species. - Plant Science 61: 43-52.
 - MANANAN T.V., VAN GINKEL L.C. & BOWES G. 1997. Evidence that inducible C₄-type photosynthesis is a chloroplastic CO₂-concentrating mechanism in *Hydrilla*, a submersed monocot. - Plant Cell Environ. 20: 211-220.
- SALVUCCI M.E. & BOWES G. 1981. Induction of reduced photorespiratory activity in submersed and amphibious aquatic macrophytes. Plant Physiol. 67: 335-340.
- SAND-JENSEN K. & GORDON D. 1986. Variable HCO₃⁻ affinity of *Elodea canadensis* Michaux in response to different HCO₃⁻ and CO₂ concentrations during growth. - Oecologia 70: 426-432.
- STITT M., LILLEY R.McC., GERHARDT R. & HELDT W. 1989. Metabolite levels in specific cells and subcellular compartments of plant leaves. - In: FLEISCHER S. & FLEISCHER B. (Eds.), Methods in enzymology, 174, pp. 518-552. - Academic Press Inc. New York, London.
- VAN K.T., HALLER W.T. & BOWES G. 1976. Comparison of the photosynthetic characteristics of three submersed aquatic plants. Plant Physiology 58: 761-768.
- VAN GINKEL L.C. & PRINS H.B.A. 1998. Bicarbonate utilization and pH polarity. The response of photosynthetic electron transport to carbon limitation in *Potamogeton lucens* L. leaves. -Canadian Journal of Botany 76: 1018-1024.

ZOBODAT - www.zobodat.at

Zoologisch-Botanische Datenbank/Zoological-Botanical Database

Digitale Literatur/Digital Literature

Zeitschrift/Journal: Phyton, Annales Rei Botanicae, Horn

Jahr/Year: 2000

Band/Volume: 40_3

Autor(en)/Author(s): Van Ginkel Lucina C., Schütz Ingeborg, Prins Hidde B. A.

Artikel/Article: Elodea canadensis under N and CO2 Limitation: Adaptive Changes in Rubisco and PEPCase Activity in a Bicarbonate User. 133-143