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Relations between K^+ uptake and photosynthetic uptake of inorganic carbon by aquatic plants

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Abstract

The uptake of K^+ by the leafy shoots of four submersed higher aquatic plants (*Elodea canadensis*, *Ranunculus aquatilis*, *R. trichophyllus*, and *Callitriche hamulata*) with different HCO_3^- affinity was measured in successive 2-h periods under the conditions of high or low photosynthetic rates (*i.e.* at pH 7.5 or 10). At pH 7.5 the uptake of K^+ by species with the higher HCO_3^- affinity (*E. canadensis*, *R. trichophyllus*) was significantly faster than that by species with a lower HCO_3^- affinity (*R. aquatilis*, *C. hamulata*). In the former group of species, the K^+ uptake rate at pH 7.5 was 1.7 - 3.5 times higher than at pH 10. At pH 10, the soft-water species, *R. aquatilis*, had the lowest net photosynthetic rate (P_N) of the three HCO_3^- users but, in contrast to the relative hard-water species, *R. trichophyllus*, showed a small K^+ efflux (47 nmol kg^{-1} s^{-1}). Thus, K^+ uptake by shoots was not strictly correlated with P_N. A significant K^+ efflux (73 - 86 nmol kg^{-1} s^{-1}) occurred from all HCO_3^- users in darkness. The relatively low K^+ uptake by the strict CO_2 user, *C. hamulata*, was quite independent of P_N and light or darkness. It may be suggested that uptake of K^+ by shoots of submersed plants depends on their HCO_3^- affinity.

Additional key words: *Callitriche hamulata, Elodea canadensis*, HCO_3^- affinity, hard- and soft-water species, leafy shoots, *R. aquatilis, R. trichophyllus.*

Introduction

Submersed aquatic plants take up dissolved inorganic carbon and also substantial amounts of certain mineral nutrients by their leaves. Furthermore, many submersed

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Abbreviations: FM - fresh mass; PAR - photosynthetically active radiation; P_N - net photosynthetic rate; TA - total alkalinity.

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plants can use HCO_3^- (besides free CO_2) as a carbon source for photosynthesis (Allen and Spence 1981, Maberly and Spence 1983). Of all chemical factors studied, the occurrence of higher submersed plants in standing waters usually correlates most positively with total alkalinity (TA), although the range is very wide in every species, and also with pH and Ca^{2+} concentration (Hellquist 1980, Kadono 1982, Arts and Leuven 1988). In standing waters, HCO_3^- users usually grow at medium to high levels of TA (1 - 5 meq dm^{-3}) whereas most species which use solely CO_2 are found in waters with low to medium TA (0.3 - 2.0 meq dm^{-3}; Hellquist 1980, Kadono 1982, Spence and Maberly 1985, Sand-Jensen 1987). However, naturally soft and hard waters differ not only in TA (*i.e.* HCO_3^- concentration) but also in concentrations of Ca^{2+}, Mg^{2+}, Na^+, and K^+ (Hutchinson 1975).

In rooted submersed macrophytes, the uptake of K^+, Ca^{2+}, Mg^{2+}, and Cl^- can be higher by leaves as compared to roots (Barko 1982, Waisel *et al.* 1982, Barko *et al.* 1991). It has been shown that the short-term uptake of K^+ by leaves was dependent on light and photophosphorylation (for a review see Jeschke 1976). It has been found in some HCO_3^- users that K^+ uptake occurs simultaneously with HCO_3^- use (Prins *et al.* 1982). Thus, K^+ uptake might differ in HCO_3^- users and non-users.

The aim of this paper was to investigate the relation between photosynthetic uptake of CO_2 and HCO_3^- and uptake of K^+ by leafy shoots of four submersed species showing different HCO_3^- affinity and partly different ecological requirements for TA. The main attention was paid to K^+ uptake at high pH which often occurs in dense stands of submersed macrophytes (Pokorný *et al.* 1984).

Materials and methods

The following species were used: *Elodea canadensis* Michx. (growing within a wide range of TA with its optimum at medium TA), *Ranunculus aquatilis* L. (growing in soft to medium-hard waters), *Ranunculus trichophyllus* Chaix (medium to hard waters), and *Callitriche hamulata* Kütz. (soft to medium-hard waters). All species, except for *C. hamulata,* were cultivated outdoors in two plastic 1.5 m^3 containers in pots with mixed sand and peat. Irradiance was reduced to about one-third of full-sun. TA in the container with *E. canadensis* and *R. trichophyllus* was kept within 1.4 ± 0.1 meq dm^{-3} by adding $NaHCO_3$. The pH values ranged from 8 to 9. *R. aquatilis* was grown in the other container, the TA of which was reduced to 0.30 meq dm^{-3} by adding HCl, with the pH 7 - 8. Totally submersed plants of *C. hamulata* were collected from the Golden Canal at Třeboň (South Bohemia, Czech Republic; TA 1.4 meq dm^{-3}, pH 7.5 ± 0.2).

K^+ uptake was investigated in a pH-statted glass chamber. The chamber was magnetically stirred and the temperature was kept at 22.0 ± 0.1 °C. Eighty cm^3 of an experimental solution was placed in the chamber and vigorously bubbled (5 cm^3 s^{-1}) with humidified CO_2-free air through a fine capillary to remove CO_2 continuously and keep dissolved O_2 concentration low $\leq$ 0.40 mM) during photosynthesis. The pH of the solution was continuously monitored with a single pH electrode and a separate reference Ag/AgCl electrode. The porous tip of the latter was connected to the experimental solution through a narrow 5-cm long salt bridge filled with the same solution to prevent contamination from KCl. The electrodes were connected to a pH meter (*M 110, Mikrotechna*, Prague, Czech Republic) which was connected to a pH-stat (*M 120, Mikrotechna*).

In light (at CO_2-free air), the plants raised the pH value in the solution. When the set-point pH value was slightly exceeded, a fine electro-magnetic valve was briefly opened. This step released very small CO_2 bubbles from a CO_2 cylinder through a fine glass capillary into the solution which resulted in a pH decline within 20 s. In this way, the set-point pH value was kept constant with a precision of ± 0.04. The CO_2-gaseous pH-stat was advantageous for the experiments used as it ensured a constant volume of the solution over the whole exposure (*cf.* Denny *et al.* 1983). However, it did not enable measurement of the rate of photosynthesis. The chamber was loosely covered by a plexiglas lid. O_2 concentration in the solution was occasionally measured by an O_2 sensor. Irradiance in the chamber was 70 ± 3 W m^{-2} of PAR (400-700 nm; *ca.* 320 μmol m^{-2} s^{-1}) provided by a halogen lamp.

Experimental procedures: Healthy apical 6 - 8 cm plant segments were cleaned of organisms and marl encrustation by hand. These were used for the study of K^+uptake. Before each experiment, the plant segments were exposed in 1 mM $NaHCO_3$, 0.1 mM $CaCl_2$, 0.1 mM $MgSO_4$, 0.3 mM KNO_3 and 0.25 mM Na_2HPO_4 to low irradiance of 2 W m^{-2} for 2 h. The plants were gently blotted and 1.6 - 2.4 g (FM) was inserted into the pH-stat chamber with the same solution. The plants were exposed in the light either at medium pH 7.5, allowing predominantly a high uptake of CO_2, or at high pH 10.0, allowing rather low use of HCO_3^-. A pH 9.0 was used for *E. canadensis* and 8.8 for *C. hamulata* as the high pH values. The plants were also exposed at the pH of 7.5 in darkness.

After the initial 1-h exposure, 20 cm^3 of the solution was sampled and 20 cm^3 of fresh solution was quickly added. Thus, the solution used was partly renewed. The uptake of K^+ was measured as the change in K^+ concentration in the solution between the beginning and end of the 2-h sampling periods. The initial concentration of K^+ did not fall below 70 % during the 2-h sampling period. The uptake of K^+ was not taken into account during the initial 1-h period. Generally, the plants were exposed in light at a high pH of 10.0 (9.0 or 8.8) for 7 h followed by 4 h of darkness at a medium pH of 7.5. Other plants were used in the same experiment but exposed at the medium pH in the light. In other series of experiments, a 5-h exposure in light at high pH was followed by a 4-h exposure at medium pH and by 2-h exposure in darkness at medium pH. Every two hours, the solution in the chamber was sampled and partly renewed by the fresh one. K^+ concentrations in the samples were measured by flame emission photometry (*AAS 1N, Zeiss*, Jena, Germany). The fresh mass of the plants was determined. K^+ uptake (and/or efflux) was expressed in nmol kg^{-1}(FM) s^{-1}. The values of K^+ uptake in single 2-h sampling periods of the same pH and light/dark regime were pooled (n = 5 - 15) and mean values are presented. At least three parallel runs of experiments were performed with each species.

Photosynthetic measurements: For all species, the basic photosynthetic data were determined according to Allen and Spence (1981) in pH-drift experiments in a closed chamber (for the details see Adamec 1993). The plants were exposed in the closed chamber under the same conditions (irradiance, temperature, TA) as in the ion uptake experiments. The solution contained 1 mM $NaHCO_3$ and 1 mM KCl. The initial pH was 7.4, whereas the final pH was close to the calculated final maximum pH when photosynthesis is stopped. pH was recorded and the carbon-based net photosynthetic rate (P_N) and HCO_3^- compensation concentration were calculated from the decrease in inorganic carbon over time. The intercept of linear P_N plotted against pH at zero P_N indicated the final maximum pH. The mean of two measurements is presented for all species. Carbonate equilibria were calculated from TA and pH according to Helder (1988).

To determine the range of TA at which the species used occur in naturally standing waters, water samples were collected at sites in the south and north Bohemia). Total alkalinity of filtrated samples was estimated by Gran titration with 0.01 M HCl (Talling 1973).

Results and discussion

Although TA was measured in a limited number of sites, its range was rather wide for all species (Table 1). *E. canadensis* was found to grow in standing waters of a very wide range of TA whereas *R. aquatilis* and *C. hamulata* did not occur in hard waters. However, the median (0.84 - 1.00 meq dm^{-3}) as well as the lowest TA values (0.12 - 0.16 meq dm^{-3}) were nearly the same for these three species. *R. trichophyllus* was not found in soft waters. The lowest TA in which *E. canadensis* was found (0.13 meq dm^{-3}) approaches an ecological limit for its growth, as proposed by Madsen and Sand-Jensen (1987).

Table 1. Total alkalinity (TA) in standing waters in natural sites of the plant species studied. Median values and extremes are shown; *n* - number of sites.

Species	TA [meq dm^{-3}] *n*	median	range
Elodea canadensis	24	0.89	0.13 - 4.20
Ranunculus aquatilis	14	1.00	0.12 - 2.20
Ranunculus trichophyllus	6	1.94	1.94 - 3.14
Callitriche hamulata	21	0.84	0.16 - 2.20

Of the four species, only *C. hamulata* was unable to use HCO_3^- (Table 2). The final pH of 8.81 corresponded to its CO_2 compensation concentration 3.3 μM. This finding agrees with that for the related species *C. cophocarpa* (3.6 μM CO_2; Madsen and Maberly 1991) and *C. stagnalis* (2.5 μM; Sand-Jensen 1983). *E. canadensis, R. aquatilis,* and *R. trichophyllus* were able to photosynthesize even at pH values above 10.0 and their apparent HCO_3^- compensation concentration ranged from 0.28 to 0.40 mM (*cf.* Maberly and Spence 1983). Of the three HCO_3^- users, *R. aquatilis* exhibited the lowest HCO_3^- affinity and its P_N at pH 10 was only 2.2 % of that at pH 7.5. At pH 7.5, P_N values were comparable for all species.

Table 2. Photosynthetic characteristics of the studied plants: final pH calculated (pH_{fin}); compensation point (CP) [μM] for CO_2 or HCO_3^- and net photosynthetic rate (P_N) [μmol kg^{-1}(FM) s^{-1}] at pH 7.5 or 10 (pH 8.8 for *C. hamulata*). Values in parentheses indicate the ratio between P_{N10} and $P_{N7.5}$ in % of $P_{N7.5}$.

Species	pH_{fin}	CP CO_2	CP HCO_3^-	$P_{N7.5}$	P_{N10}
Elodea canadensis	10.22		0.35	13.9	2.10 (15)
Ranunculus aquatilis	10.15		0.40	12.5	0.28 (2.2)
Ranunculus trichophyllus	10.34		0.28	16.7	1.60 (9.3)
Callitriche hamulata	8.81	3.3		10.8	0.08 (0.8)

K^+ uptake was significantly different ($P = 0.01$) under the different irradiance and pH (P_N) in all three HCO_3^- users (Table 3). The uptake rate at pH 7.5 was 1.7 - 3.5 times higher than that at high pH. In *E. canadensis*, K^+ uptake was the same at pH 9 and 10 and corresponded to that in *R. trichophyllus* at pH 10. At pH 10, the soft-water species, *R. aquatilis*, had the lowest P_N of the three HCO_3^- users but, in contrast to the relative hard-water species, *R. trichophyllus*, showed a small K^+ efflux (47 nmol kg^{-1} s^{-1}). However, K^+ uptake by the two species was comparable at medium pH (high P_N). Thus, K^+ uptake by shoots was not strictly correlated with P_N (Fig. 1). A significant K^+ efflux ($P = 0.01$; 73 - 86 nmol kg^{-1} s^{-1}) occurred from all HCO_3^- users in darkness. However, the relatively low K^+ uptake by the strict CO_2 user, *C. hamulata*, was quite independent on P_N and light or darkness. It may be suggested that uptake of K^+ by shoots of submersed plants either depends on their HCO_3^- affinity (*i.e.* HCO_3^- compensation concentration) or cannot take place when P_N is lower than a threshold.

Table 3. K^+ uptake [nmol kg^{-1}(FM) s^{-1}] by leafy shoots in light (L) at pH 7.5, 9.0, or 10.0 or in darkness (D). In *C. hamulata*, pH 8.8 was used instead of 9.0. Means of 5 - 15 single 2-h sampling periods (number in parentheses) ± S.E. are shown. Negative values indicate K^+ efflux from the plants.

Conditions	*E. canadensis*	*R. aquatilis*	*R. trichophyllus*	*C. hamulata*
L - pH 7.5	231 ± 25 (15)	578 ± 37 (9)	453 ± 30 (9)	40 ± 18 (8)
L - pH 9.0	134 ± 26 (6)	-	221 (3)	23 ± 40 (9)
L - pH 10.0	130 ± 19 (6)	165 ± 21 (9)	-47 ± 46 (9)	-
D - pH 7.5	-86 ± 23 (8)	-73 ± 19 (5)	-77 ± 23 (5)	43 ± 25 (6)

It is obvious that a certain amount of K^+ was released during the light-dark transition (Prins *et al.* 1982). As shown by Brammer and Wetzel (1984) isolated submersed leaves of *Stratiotes aloides* were not able to take up K$^+$ in darkness but neither did they release K$^+$. A very high K$^+$ efflux occurred from the emergent-type leaves in the light in the two days after they had been submersed. Regardless of light conditions, K$^+$ efflux from shoots and roots of aquatic plants was closely correlated with NH$_4^+$ uptake producing redundant protons (Beck and Feller 1991, Roelofs 1991). As reviewed by Jeschke (1976) short-term uptake of K$^+$ (and Cl$^-$) by leaves of aquatic plants depended strongly on light conditions and photosynthetic electron flow, but it did not occur during long-term uptake and/or when the leaves were pretreated in a higher concentration of K$^+$ (or Cl$^-$).

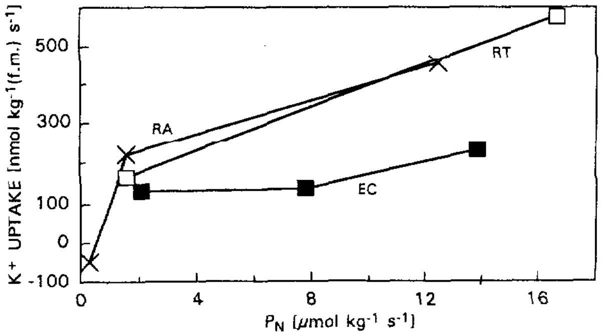

Fig. 1. Relationship between K$^+$ uptake by the three HCO$_3^-$ users at light and P$_N$ at different pH. K$^-$ uptake is expressed in nmol kg^{-1}(FM) s^{-1}. EC - *Elodea canadensis*, RT - *Ranunculus trichophyllus*, RA - *R. aquatilis*.

In this study, rootless apical shoot segments were used for simplicity although all the species form roots. The shoots usually have a similar affinity for K$^+$ as roots (Waisel *et al.* 1982, Roelofs 1991) and K$^+$ is taken up mostly by shoots from water (Barko *et al.* 1991). Therefore, it is possible that the different rates of K$^+$ uptake in the four species are not only the result of their different growth rates (Nielsen and Sand-Jensen 1991) but also due to the relative contribution of shoots to the total uptake by the whole plant. As found by mineralization, K$^+$ content in shoots was similar in all the species used (in % of dry mass it was 2.9 in *E. canadensis*, 2.5 in *R. trichophyllus*, 3.1 in *R. aquatilis*, and 2.8 in *C. hamulata*; *cf.* Dykyjová 1979)

The uptake of K$^+$ in light and efflux of K$^+$ in darkness (Table 3) may indicate natural diurnal rhythmicity of K$^+$ uptake by aquatic plant shoots which may lead to conspicuous daily oscillations of K$^+$ concentration in waters. A profound K$^+$ uptake by dense aquatic vegetation can markedly reduce K$^+$ concentration in K$^+$-poor water and thus, inhibit the growth of phytoplankton (Brammer 1979). Comparison of K$^+$ uptake in the two ecologically different *Ranunculus* species at high pH and in light (Table 3; Fig. 1) suggests that competition for inorganic carbon among aquatic species might be secondarily strengthened by competition for K$^+$. In *Najas flexilis*, photosynthesis was considerably inhibited at Ca^{2+} concentrations above 0.25 mM or at a higher (Ca+Mg):(K+Na) ratio (Wetzel and McGregor 1968). Such an inhibition could also occur after an extraction of K$^+$ in water. Since the ratio of

(Ca+Mg):(K+Na) is usually higher in harder waters (*e.g.* Hutchinson 1975) it might be one of the possible factors eliminating soft-water species from these habitats.

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