PHOTOSYNTHETIC CO_2 AFFINITY OF AQUATIC CARNIVOROUS PLANTS GROWING UNDER NEARLY-NATURAL CONDITIONS AND *IN VITRO*

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Keywords: chemistry: photosynthesis, *Aldrovanda*, *Utricularia*.

Abstract

Net photosynthetic rate of aquatic carnivorous plants in standing waters can sometimes be limited by low concentration of free CO_2. As net photosynthetic rate of terrestrial plants growing *in vitro* is greatly reduced, as compared to the same plants grown naturally, it could be assumed that photosynthetic CO_2 affinity in aquatic carnivorous plants growing *in vitro* will be reduced. The aim of this study was to compare values of CO_2 compensation point of photosynthesis in several strains of *Aldrovanda vesiculosa* and in 13 aquatic *Utricularia* species, both in plants growing under nearly-natural conditions in containers or aquaria and *in vitro*. The dependence of CO_2 compensation point on growth conditions is discussed.

Introduction

About 50 species of the genera *Aldrovanda* and *Utricularia* are submerged aquatic or amphibious carnivorous plants (Juniper *et al.* 1989; Taylor 1989; Guisande *et al.* 2007). Aquatic carnivorous plants (ACPs) usually grow in shallow standing dystrophic (humic) waters which are usually poor in inorganic N and P, but commonly also in K (Adamec 1997a). They are rootless and take up all necessary nutrients through their shoots, either directly from water or from prey. Very rapid growth of ACPs in nutrient-poor habitats requires ecophysiological adaptations that enable the plants to gain limiting mineral nutrients. These adaptations include carnivory, efficient nutrient re-utilization (recycling) from senescent shoots, and very high affinity for mineral nutrient uptake from water (Kamiński 1987; Adamec 2000; Englund & Harms 2003).

ACPs in their typical habitats may also face shortage of light (only 2-20% of incident PAR irradiance) and sometimes also free CO_2 (below 0.02-0.05 mM) though CO_2 concentration ($[CO_2]$) is commonly high in their habitats, >0.1 mM (*e.g.*, Hough & Fornwall 1988; Adamec 1997b, 2007; Adamec & Kovářová 2006). Therefore, net photosynthetic rate of ACPs in standing waters, like that of other submerged non-carnivorous plants generally, is limited by unfavourable physical and chemical factors which are attributes of the aquatic environment: low diffusion rate of CO_2, variable $[CO_2]$ strongly dependent on water pH and total alkalinity (TA), and, also, shortage of light (*e.g.*, Maberly & Madsen 2002). The maximum net photosynthetic rate of ACPs (per unit biomass) is usually higher than that known in aquatic non-carnivorous plants (Adamec 1997b, 2006). It has been shown that *Aldrovanda vesiculosa* and several aquatic *Utricularia* species use only CO_2 for photosynthesis (Moeller 1978; Adamec 1995, 1997b; Adamec & Kovářová 2006). For these species, estimated values of CO_2 compensation point of photosynthesis (CP CO_2; *i.e.*, CO_2 concentration at which net photosynthetic rate is zero and gross photosynthetic rate equal to respiration) range between 1.5-13 μM. Similar values within 1.4-11 μM have also been reported for aquatic non-carnivorous plants (Maberly & Spence 1983). However, it has recently been found in ecologically very plastic *U. australis* that growth conditions of a very high pH (or negligible $[CO_2]$) can even induce a weak HCO_3^- use (Adamec 2009).

Most species of ACPs grow very rapidly, due to both rapid apical growth and frequent branching (Friday 1989; Adamec 1999, 2000, 2007; Adamec & Kovářová 2006). Their doubling time of biomass can be only within 8-20 days and their apical growth rate within 1-3.5 new leaf whorls or nodes per day. Obviously, their high net photosynthetic rate is a prerequisite for their very rapid growth (Adamec 1997b) as this growth pattern is associated with a significant loss of carbohydrates in senesced shoot segments (Adamec 2000). Therefore, high [CO_2] in the water is one of the most important ecological requirements for rapid growth of ACPs (Adamec 1999).

Besides growing in nutrient-poor waters in containers or aquaria, ACPs of both genera can also be grown easily *in vitro* in mineral-rich liquid media supplemented with 2-3% sucrose as an energy source (Adamec & Pásek 2000; Adamec & Kondo 2002). Up to now, 14 *Aldrovanda* strains and 14 aquatic *Utricularia* species have been successfully grown *in vitro* (Pásek & Adamec, unpubl.). It is well-known that net photosynthetic rate of terrestrial plants grown *in vitro* on agar is greatly decreased, as compared to the same plants grown naturally, and that a significant part of this effect is caused by sucrose uptake from media (*e.g.*, Fuentes *et al.* 2005; Fila *et al.* 2006). It can therefore be assumed that net photosynthetic rate and CO_2 affinity in ACPs growing *in vitro* in liquid media will also be significantly lower than those in the plants growing under nearly-natural conditions. The aim of this study was to compare values of CP CO_2 in several strains of *A. vesiculosa* and in 13 aquatic *Utricularia* species, both in plants growing under nearly-natural conditions in containers or aquaria and *in vitro*. The dependence of CP CO_2 on growth conditions (pH, [CO_2]) is discussed.

Materials and Methods

Experimental ACPs were grown under nearly-natural, dystrophic conditions outdoors either in two large plastic containers of 350 or 700 litres (Adamec 1997c) or in 3-30 l aquaria standing in cooling water in a 1.5-m^3 plastic container, or were grown in a greenhouse in 3-20 l aquaria floating in a 300-l plastic container. Litter of robust sedges (*Carex*) was used as a standard substrate (Adamec 1997c, 1999) and all cultures were partly shaded (usually 20-50% of incident irradiance on the level of plants) and occasionally fed on fine prey. The species grown under these conditions at the beginning of August 2007, to measure CP CO_2, are given in Table 1.

The following species growing *in vitro* were used in another run of the experiment: *Aldrovanda vesiculosa* (three strains from E Poland, Hungary, Botswana), *U. stygia* (from the Czech Rep.), *U. bremii* (from NW Russia), *U. reflexa* (from Zambia), *U. inflata* (from NJ, USA), *U. floridana* (from FL, USA), *U. striata* (from NJ, USA), *U. aurea* (from Malaysia), *U. breviscapa*, and *U. hydrocarpa* (both from Nicaragua). *in vitro* plants were grown aseptically in *ca.* 150 ml of a medium in 0.5-l serological flasks in white fluorescent light (15-40 μmol m^{-2} s^{-1} PAR) at a light:dark regime of 14/10 h and at 19±1°C. A half-strength Gamborg B5 liquid medium with 500 mg l^{-1} KNO, microelements, vitamins, and 2.5% sucrose, but without other organic substances (see Adamec & Pásek 2000), was used for growing the plants for 3-5 weeks. The initial pH of the medium before autoclaving was *ca.* 5.5. Each species of ACPs was usually available in 2-3 flasks. Before the experiment, the bulk of the medium was more or less loosly overgrown by plant biomass. In species forming two types of shoots (carnivorous and photosynthetic; *U. stygia*, *U. striata*, *U. floridana*), the proportion of greenish carnivorous shoots with traps was very low, by guess <5-10%. In most species but *U. hydrocarpa* and *U. breviscapa*, the shoots were freshly green, while in these two species, shoot bases were yellowish.

The plants from containers or aquaria or *in vitro* cultures were washed thoroughly by tap water and cleaned of sessile organisms. For estimation of CP CO_2 using the final-pH method (Maberly & Spence 1983), apical parts of 1-3 shoots about 6-7 cm long (or 3-15 shoots 3-8 cm long in *in vitro* plants) were put in 10-ml test-tubes in the solution of 1 mM $NaHCO_3$ + 0.1 mM KCl (pH *ca.* 7.65; Adamec 1995; Adamec & Kovářová 2006). The whole internal volume was filled evenly with the plants. Air volume of about 1 ml was let in the closed tubes to reduce the final [O_2] in the solution. The tubes with plants were exposed to natural light in water at 21-25°C and *ca.* 300-450 μmol m^2 s^{-1} PAR for 5 h and final-pH values were measured. Values of CP CO_2 were cal-

culated from pH and TA after Helder (1988). All measurements were performed in five replicates.

For a calculation of $[CO_2]$ in culture waters, pH and TA were measured in each aquarium or container at the time of plant sampling. pH was also measured in used *in vitro* media from which the experimental plants were taken. pH values were used as such and were not transformed. Mean values ±1SE intervals are shown. Statistically significant differences between the same species were evaluated by a two-tailed Student t-test. Linear regression models were used to find statistically significant meaningful relationships between variables. Three important linear regressions at $p<0.05$ are shown in Table 2. For this aim, results for various *Aldrovanda* strains and *Utricularia* species were pooled together as both groups behave ecologically in the same way (*e.g.*, Adamec & Kovářová 2006).

Species (accession)	Plants from containers or aquaria				Plants *in vitro*	
	pH	TA ($meq\ l^{-1}$)	$[CO_2]$ (μM)	CP CO_2 (μM)	pH *in vitro*	CP CO_2 (μM)
A. vesiculosa, E Poland	7.72	1.51	67	6.25±0.47	3.68-3.73	4.24±0.15*
A. vesiculosa, Hungary	8.67	0.71	3.4	1.90±0.15	3.11-3.28	5.15±0.29*
A. vesiculosa, SE Australia	8.56	0.78	4.9	2.87±0.19	--	--
A. vesiculosa, Botswana	7.66	0.61	31	3.09±0.19	3.83-4.04	5.95±0.47*
U. stygia, Czech Rep.	8.11	1.49	27	3.08±0.20	3.21-3.35	5.72±0.33*
U. vulgaris, Czech Rep.	8.11	1.49	27	7.09±0.61	--	--
U. intermedia, Czech Rep.	7.49	0.79	60	3.60±0.17	--	--
U. bremii, NW Russia	7.49	0.79	60	4.38±0.28	--	--
U. bremii, S Bohemia	8.33	1.25	13	2.45±0.16	3.19-3.21	8.49±0.34*
U. reflexa, Botswana	7.66	1.02	52	4.04±0.09	--	--
U. reflexa, Zambia	7.91	0.54	15	4.57±0.23	3.11-3.50	8.75±0.61*
U. inflata, NJ, USA	7.66	1.02	52	8.31±0.81	3.08-3.25	4.52±0.25*
U. purpurea, FL, USA	8.57	1.55	9.4	3.70±0.34	--	--
U. floridana, FL, USA	7.86	1.23	40	13.6±1.94	6.13	2.53±0.08*
U. striata, NJ, USA	6.79	0.54	205	7.38±0.62	5.14-5.41	3.07±0.08*
U. aurea, Malaysia	7.26	0.79	102	4.66±0.67	3.08-3.17	$3.67±0.25^{NS}$
U. dimorphantha, Japan	6.60	0.76	449	9.74±0.54	--	--
U. breviscapa, Nicaragua	--	--	--	--	3.36-3.37	5.04±0.75
U. hydrocarpa, Nicaragua	--	--	--	--	4.35-5.07	147±35

Table 1: Comparison of compensation points of CO_2 (CP CO_2) of aquatic carnivorous plants grown under nearly-natural conditions in containers or aquaria, with those for plants growing in *in vitro* in a mineral medium with 2.5% sucrose for 3-5 weeks. pH and TA in the water are shown for the plants from containers or aquaria; the pH range of used *in vitro* media is shown (1-3 flasks). Means±SE are shown; n=5. Statistically significant differences between the same species or strains, *, $p<0.01$; NS, non-significant, $p>0.05$.

No.	Linear regression model	r^2	p
1	CP CO_2 (w) = 25.1 – 2.61 pH	0.47	0.003
2	CP CO_2 (w) = 3.74 + 0.015 [CO_2]	0.52	0.002
3	CP CO_2 (w) = 11.2 – 1.09 CP CO_2 (iv)	0.42	0.044

Table 2: Linear regression models between variables showing all statistically significant ($p<0.05$) meaningful correlations; (w), for plants from dystrophic waters in containers and aquaria; (iv), for *in vitro* plants; n=10-16; r^2, coefficient of determination; p, probability level.

Results and Discussion

ACPs used for this study grew in containers and aquaria under very different pH and CO_2 conditions, while TA values were relatively stable (Table 1). Overall, pH ranged between 6.6 and 8.7 and [CO_2] between only 3.4 and 450 µM. Co-occurring filamentous algae could have a certain influence on higher pH and lower [CO_2] but their influence was not tested. Though pH and [CO_2] presumably mildly oscillated in the containers and aquaria during day and night time, only one estimation of these parameters was performed before plant sampling but it gave reliable information on growth conditions. Values of CP CO_2 in 17 plant strains or species from nearly-natural cultures ranged between 1.9-13.6 µM (mean 5.3±0.7 µM) and about the same values between 2.5-8.8 µM (mean 5.2±0.6 µM) were found in all 11 *in vitro* strains or species but *U. hydrocarpa*. The *in vitro* culture of the latter species was more aged than in all other species and its values of CP CO_2 were as great as 147±35 µM. pH values of used *in vitro* media ranged between 3.08 and 6.13 but they were usually within only 3.2-4.0 (Table 1); the difference between parallel flasks for each species was usually only 0.1-0.3 pH due to a relatively higher buffering capacity of the medium. Linear regression models (*U. floridana* as an outlier was excluded) revealed a highly significant correlation between the values of CP CO_2 of APCs grown in containers or aquaria and between pH in these waters (Table 2, No. 1). As these pH values correlated very strongly $r^2=0.64$; $p=0.00011$; data not shown) and negatively with [CO_2] in the waters, CP CO_2 of APCs correlated also highly significantly with [CO_2] (No. 2). Except for *U. aurea*, the values of CP CO_2 within each of all other 9 ACP strains or species growing under *in vitro* and nearly-natural conditions were highly significantly different (Table 1). Yet, a significant correlation was found between these values of CP CO_2 for all 10 strains or species (Table 2, No. 3).

It follows clearly from the results (Table 1) that different *Aldrovanda* strains and 11 *Utricularia* species, regardless of their geographic origin, behave photosynthetically as typical, strict CO_2 users (*cf.* Maberly & Spence 1983). Evidently, there was no difference between *Aldrovanda* strains and *Utricularia* species on one hand and between *Utricularia* species with monomorphic and dimorphic shoots on the other. Based also on all literature data available (Moeller 1978; Adamec 1995; Adamec & Kovářová 2006), it may be concluded that all ACP species are strict CO_2 users. The recent finding of a weak HCO_3^- use in *U. australis* growing at a very high pH (Adamec 2008) may be a very rare exception from this rule and does not contest much this generalization. Within the ecological group of ACPs, the ecological strategy of a strict CO_2 use is consistent with relatively high [CO_2] which commonly occurs at most of natural sites of ACPs (Adamec 1997a, 1997b). In aquatic non-carnivorous plants, values of CP CO_2 depend markedly on concentration of inorganic carbon, mainly of CO_2, in the ambient water and this relationship also holds for strict CO_2 users (Madsen *et al.* 1996); low [CO_2] leads to low values of CP CO_2 and vice versa. The same adaptational relationship has also been confirmed for ACPs (Table 2, No. 2). Therefore, the differences in CP CO_2 found between the species can be attributed to differences in [CO_2] during the growth of the plants rather than to interspecific differences alone. Thus, a more efficient CO_2 uptake is induced by a shortage of CO_2 or a higher pH value. Nevertheless, when ACPs grow under the conditions of a marked CO_2 limitation this ecologically unfavorable factor can also be alleviated by prolifically catching prey and absorption of organic carbon from prey (Adamec 1997a, 1999). Anyway, shortage of CO_2 leads to a significant decrease in apical shoot growth

rate as found in *Aldrovanda* (Adamec, unpubl.).

Although the physiological age of the *in vitro* cultures of ACPs used was partly different due to different depletion of the media, the pH values in used media in most species (except for *U. floridana* and *U. striata*) were well below 5.0 and usually only 3.2-4.0 (Table 1). Such low pH values in a half-strength Gamborg B5 media were also reported by Adamec & Pásek (2000) and Adamec & Kondo (2002) for *Aldrovanda* cultures. As follows from the cited studies the very low pH in used Gamborg B5 media reflects the availability of NH_4^+ and NO_3^- in the fresh media as well as a great uptake preference for NH_4^+ over NO_3^- (Adamec 2000; Adamec & Pásek 2000). Thus, it is possible to suggest that *U. floridana* and *U. striata* take up also a considerable proportion of NO_3^- which leads to medium alkalization.

The fact that CP CO_2 values in *in vitro* plant species are comparable with those in the plants growing under nearly-natural conditions (Table 1) testifies that the former group of plants is able to normally photosynthetically use CO_2, with a CO_2 affinity common for the latter group. Yet, these results do not show how great net photosynthetic rate of these *in vitro* raised plants can be as compared to nearly-natural cultures. To our knowledge, no similar measurement of CP CO_2 has been conducted in aquatic non-carnivorous plants *in vitro* so far and, thus, our data cannot be compared. However, *U. hydrocarpa* grown *in vitro* exhibited weak symptoms of senescence and its CP CO_2 was enormously high (147±35 μM). It is therefore possible to assume that the physiologically older a culture was, the higher was its CP CO_2. In terrestrial non-carnivorous plants growing *in vitro* with sucrose, net photosynthetic rate per unit leaf area is only about a half of that measured in the same species under *ex vitro* conditions (Fila *et al.* 2006) and sucrose added to the medium can reduce net photosynthetic rate by 30-45% (Lucchesini *et al.* 2006). Although the values of CP CO_2 in nearly-naturally grown ACP species depended strongly on the ambient [CO_2] they also correlated significantly with those *in vitro* (Table 2, No. 3). This might indicate that certain interspecific differences in CP CO_2 among the species or strains do exist.

It should be noted that the *in vitro Utricularia* species looked quite different than the plants from the nearly-natural cultures. The former group of plants had relatively abundant but small traps the maximum size of which did not extend 1.5 mm (or 2.5 mm in *U. reflexa*; see Figure 1), while the latter plant group had much larger traps (usually 2-4 mm, but up to 7 mm in *U. reflexa*; see Figure 2). It is possible to assume that the proportion of trap biomass to the total plant biomass (*i.e.*, investment in carnivory) is much lower in grown aquatic species than in the same

Figure 1: *Utricularia reflexa* (a clone noted as having relatively large traps) from Zambia grown under nearly-natural conditions in outdoor aquaria. Scale bar indicates 10 mm; the largest trap size is 7 mm; September 2007. Photograph by Lubomír Adamec.

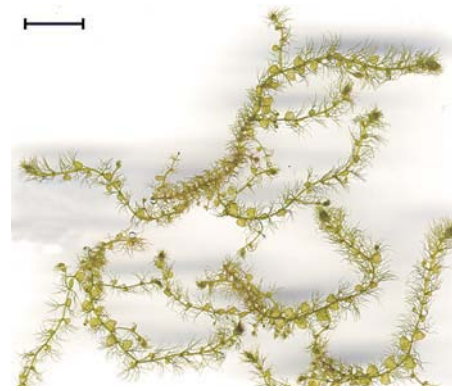

Figure 2: The same Zambian clone of *U. reflexa* as shown in Figure 1 grown *in vitro*. Scale bar indicates 10 mm; the largest trap size is only 2.5 mm. Photograph by Lubomír Adamec.

species grown in nearly-natural cultures. Thus growing ACPs in concentrated mineral media with sucrose presumably causes great changes (decrease) of investment in carnivory.

Acknowledgements: This paper has been dedicated to our friend Miloslav Studnička, Liberec, Czech Republic, on the occasion of his 60th birthday and for his great long-term merits on studying carnivorous plants and their popularization. This study was supported partly to L.A. by the Research Programme of the Academy of Sciences of the Czech Republic (No. AV0Z60050516).

References

Adamec, L. 1995. Photosynthetic inorganic carbon use by aquatic carnivorous plants. Carniv. Pl. Newslett. 24: 50-53.

Adamec, L. 1997a. Mineral nutrition of carnivorous plants: A review. Bot. Rev. 63: 273-299.

Adamec, L. 1997b. Photosynthetic characteristics of the aquatic carnivorous plant *Aldrovanda vesiculosa*. Aquat. Bot. 59: 297-306.

Adamec, L. 1997c. How to grow *Aldrovanda vesiculosa* outdoors. Carniv. Pl. Newslett. 26: 85-88.

Adamec, L. 1999. Seasonal growth dynamics and overwintering of the aquatic carnivorous plant *Aldrovanda vesiculosa* at experimental field sites. Folia Geobot. 34: 287-297.

Adamec, L. 2000. Rootless aquatic plant *Aldrovanda vesiculosa*: physiological polarity, mineral nutrition, and importance of carnivory. Biol. Plant. 43: 113-119.

Adamec, L. 2006. Respiration and photosynthesis of bladders and leaves of aquatic *Utricularia* species. Plant Biol. 8: 765-769.

Adamec, L. 2007. Investment in carnivory in *Utricularia stygia* and *U. intermedia* with dimorphic shoots. Preslia 79: 127-139.

Adamec, L. 2009. Photosynthetic CO_2 affinity of the aquatic carnivorous plant *Utricularia australis* (Lentibulariaceae) and its investment in carnivory. Ecol. Res. 24: 327-333.

Adamec, L., and Kondo, K. 2002. Optimization of medium for growing the aquatic carnivorous plant *Aldrovanda vesiculosa in vitro*. Plant. Biotechnol. 19: 283-286.

Adamec, L., and Kovářová, M. 2006. Field growth characteristics of two aquatic carnivorous plants, *Aldrovanda vesiculosa* and *Utricularia australis*. Folia Geobot. 41: 395-406.

Adamec, A., and Pásek, K. 2000. Medium optimization for growing *Aldrovanda vesiculosa in vitro*. Carniv. Pl. Newslett. 29: 122-124.

Englund, G., and Harms, S. 2003. Effects of light and microcrustacean prey on growth and investment in carnivory in *Utricularia vulgaris*. Freshwater Biol. 48: 786-794.

Fila, G., Badeck, F.-W., Meyers, S., Cerovic, Z., and Ghashghaie, J. 2006. Relationships between leaf conductance to CO_2 diffusion and photosynthesis in micropropagated grapevine plants, before and after *ex-vitro* acclimatization. J. Exp. Bot. 57: 2687-2695.

Friday, L.E. 1989. Rapid turnover of traps in *Utricularia vulgaris* L. Oecologia 80: 272-277.

Fuentes, G., Talavera, C., Oropeza, C., Desjardins, Y., and Santamaria, J.M. 2005. Exogenous sucrose can decrease *in vitro* photosynthesis but improve field survival and growth of coconut (*Cocos nucifera* L.) *in vitro* plantlets. In Vitro Cell. Dev. Biol.-Plant 41: 69-76.

Guisande, C., Granado-Lorencio, C., Andrade-Sossa, C., and Duque, S.R. 2007. Bladderworts. Funct. Plant Sci. Biotechnol. 1: 58-68.

Helder, R.J. 1988. A quantitative approach to the inorganic carbon system in aqueous media used in biological research: dilute solutions isolated from the atmosphere. Plant Cell Environ. 11: 211-230.

Hough, R.A., and Fornwall, M.D. 1988. Interactions of inorganic carbon and light availability as controlling factors in aquatic macrophyte distribution and productivity. Limnol. Oceanogr. 33: 1202-1208.

Juniper, B.R., Robins, R.J., and Joel, D.M. 1989. The Carnivorous Plants. Academic Press Ltd., London.

Kamiński, R. 1987. Studies on the ecology of *Aldrovanda vesiculosa* L. II. Organic substances, physical and biotic factors and the growth and development of *A. vesiculosa*. Ekol. Pol. 35: 591-609.

Lucchesini, M., Monteforti, G., Mensuali-Sodi, A., and Serra, G. 2006. Leaf ultrastructure, photosynthetic rate and growth of myrtle plantlets under different *in vitro* culture conditions. Biol. Plant. 50: 161-168.

Maberly, S.C., and Madsen, T.V. 2002. Freshwater angiosperm carbon concentrating mechanisms: processes and patterns. Funct. Plant Biol. 29: 393-405.

Maberly, S.C., and Spence, D.H.N. 1983. Photosynthetic inorganic carbon use by freshwater plants. J. Ecol. 71: 705-724.

Madsen, T.V., Maberly, S.C., and Bowes, G. 1996. Photosynthetic acclimation of submersed angiosperms to CO_2 and HCO_3^-. Aquat. Bot. 53: 15-30.

Moeller, R.E. 1978. Carbon-uptake by the submerged hydrophyte *Utricularia purpurea*. Aquat. Bot. 5: 209-216.

Taylor, P. 1989. The Genus *Utricularia*: A Taxonomic Monograph. Kew Bulletin, Additional Series, XIV.