Available online at www.sciencedirect.com
ScienceDirect
Aquatic Botany 89 (2008) 66–70
Aquatic botany
www.elsevier.com/locate/aquabot

Short communication

The influence of prey capture on photosynthetic rate in two aquatic carnivorous plant species

Lubomír Adamec *

Institute of Botany of the Academy of Sciences of the Czech Republic, Section of Plant Ecology, Dukelská 135, CZ-379 82 Třeboň, Czech Republic

Received 3 October 2007; received in revised form 15 January 2008; accepted 18 January 2008
Available online 2 February 2008

Abstract

Photosynthetic (P_N) and dark respiration rate (RD) were measured in two species of aquatic carnivorous plants, *Aldrovanda vesiculosa* and *Utricularia australis*, growing with or without prey in an outdoor growth experiment. After 7–14 days, the positive growth effect of feeding on prey (apical growth rate, plant size, branching) was evident in both species. Tissue N content in young leaf whorls of both species fed on prey was significantly lower (by 54% in *Aldrovanda*, by 86% in *Utricularia*) than that in unfed variants, while tissue P content was about the same. In both species, chlorophyll *a* content was consistent with tissue N content and RD in fed and unfed variants was very similar. However, P_N was significantly greater in fed plants of *Aldrovanda*, while lower in *Utricularia*. It is possible to conclude that feeding on prey in aquatic carnivorous plants leads to neither an increase of shoot tissue N content nor P_N per unit biomass. Thus, the main physiological effect of catching prey is not enhancement of P_N, but to provide N and P for essential growth processes in juvenile tissues.

Keywords: *Aldrovanda vesiculosa*; *Utricularia australis*; Feeding on prey; Apical shoot growth rate; Tissue N and P content; Net photosynthetic rate; Dark respiration

1. Introduction

Aquatic carnivorous plants comprise the species *Aldrovanda vesiculosa* L. (Droseraceae) and about 50 species of the genus *Utricularia* L. (Lentibulariaceae; Juniper et al., 1989; Taylor, 1989). Generally, rootless aquatic carnivorous plants grow in shallow standing dystrophic waters and their growth can often be limited by a shortage of N and P, and also of K in these waters (for the review see Adamec, 1997a; Ellison, 2006). Contrary to terrestrial carnivorous plants, most species of aquatic carnivorous plants exhibit very rapid apical shoot growth (1–3 leaf whorls/day) and high relative growth rate (Friday, 1989; Adamec, 2000, 2002; Adamec and Kovářová, 2006). Considering the ecological cost–benefit relationships, Givnish et al. (1984) hypothesized that carnivory for terrestrial carnivorous plants provides these plants with a greater mineral nutrient availability and may lead to increasing a plant's total rate of photosynthesis (P_N) as a principal effect of its carnivory. This effect may either be due to an increased P_N per unit leaf biomass or an increased total leaf biomass that it can support. Although the hypothesis was verified in some terrestrial species, the results remain very ambiguous and, generally, P_N increase per unit leaf biomass has not been demonstrated so far (cf. Méndez and Karlsson, 1999; Ellison and Farnsworth, 2005; Wakefield et al., 2005).

This hypothesis has never been tested in aquatic carnivorous plants. To ensure their very rapid growth, aquatic carnivorous plants exhibit high P_N (per unit fresh weight; FW). This high rate includes the maximum values known within submerged plants (Adamec, 1997b, 2006). In aquatic carnivorous plants, prey capture is very important to attain a high growth rate (for the review see Adamec, 1997a, 2000; Englund and Harms, 2003). However, prey capture in *A. vesiculosa* led to a slightly lower tissue N and P content in shoot apices and young shoot segments, when compared to the control without prey (Adamec, 2000).

A. vesiculosa and *Utricularia australis* R.Br. are free-floating submerged carnivorous plants growing commonly in the same shallow dystrophic waters (e.g., Kamiński, 1987). While *Aldrovanda* is a very rare and critically endangered plant species throughout its European range (Adamec, 1999), *U. australis* represents a very common submerged plant species

* Tel.: +420 384 721156; fax: +420 384 721136.
E-mail address: adamec@butbn.cas.cz.

0304-3770/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquabot.2008.01.008

(Taylor, 1989). Unlike *Aldrovanda*, which is considered to be rather stenotopic (Adamec, 1999), *U. australis* behaves as a eurytopic species (Adamec and Kovářová, 2006). In the present paper, P_N and dark respiration rate (RD) were measured in *A. vesiculosa* and *U. australis* plants growing with or without prey in an outdoor culture. Apical growth rate, shoot N and P content, chlorophyll content, and photosynthetic CO_2 compensation point were also estimated. The aim was to assess whether photosynthesis and related processes are influenced by prey capture.

2. Materials and methods

2.1. Experimental plants

Plants used for laboratory experiments and analyses were taken from a 14-day outdoor growth experiment (from 26 July to 9 August 2006). *A. vesiculosa* (collected originally from Lake Długie, E Poland) used for the growth experiment was grown outdoors in a 1 m^2 plastic container with a substrate of *Carex* sp. litter (see Adamec, 1997b). *U. australis* (collected originally from Třeboň region, Czech Rep.) was grown outdoors in a 2 m^2 plastic container with a culture of *Nymphoides peltata*. On 26 July, 14 unbranched plants of *Aldrovanda* from the stock culture were shortened to 10 adult leaf whorls (shoot length 6.3–8.3 cm). The internode between the second and third adult whorls was tagged carefully by a short piece of fine thread for measuring the apical shoot growth rate. The plants were transferred to floating plastic frames (see Adamec, 2000; Adamec and Kovářová, 2006). Simultaneously, 14 unbranched plants of *Utricularia* from the stock culture were shortened to 20 adult leaf whorls (shoot length 10.3–16.9 cm), tagged in an analogous way between the second and third adult leaf whorls (Adamec and Kovářová, 2006), and placed within different floating plastic frames. As both species have quite different number of adult whorls and shoot length (Adamec and Kovářová, 2006) the different original number of whorls in both species was selected. In this way, the physiological age of shoots of both species was similar. The floating frames were 0.3 m × 0.3 m by about 6 cm depth and had solid walls to retain plants within each frame. To some frames mesh with a pore size of 150 μm was placed on the bottom; to exclude zooplankton but maintain unimpeded water exchange with the ambient water. The frames with nets represented the experiment without prey. Fourteen parallel-tagged plants of each species of the same size as above were placed in identical floating bottomless frames that lacked a bottom of fine plastic mesh. These frames allowed the plants to fully capture zooplankton. These frames kept the plants together and served as controls for the prey. All frames, with and without nets, with the plants inside floated on the surface of the same 2.5 m^2 plastic cultivation container. A substrate of *Carex* litter covered its bottom.

The container mimicked a shallow dystrophic pool in which both species occur naturally. Thus, all plants grew in uniform conditions but those within nets were deprived of prey. The position of the nets and frames varied randomly within the container. Every other 2 days, the mesh on the floating frames with nets was cleaned and washed by tap water. Fine zooplankton (copepods, ostracods, size 0.6–2 mm) was added repeatedly to the container to feed the control plants. During the growth experiment, the minimum water temperature at plant level in the nets and frames varied between 14.1 and 22.1 °C. The maximum water temperature at plant level varied between 16.6 and 30.9 °C. The difference between temperatures of the nets and frames never exceeded 0.6 °C. The irradiance at plant level was 70–80% of the incident light in the adjacent open area. Basic water chemistry parameters were estimated in the cultivation water at the beginning and at the end of the growth experiment (for the methods see Adamec, 2000). The water was rather poor in mineral nutrients (14–15 μg L^{-1} NO_3^--N; 15–87 μg L^{-1} NH_4^+-N; 10–17 μg L^{-1} PO_4-P). Over the course of the experiment, pH was 6.90–7.27, [O_2] 0.15–0.25 mM, total alkalinity 0.60–0.92 mequiv. L^{-1}, and [CO_2] 0.1–0.2 mM. No difference in pH and [CO_2] was measured between the frames with and without nets.

2.2. Estimation of apical shoot growth rate

The number of adult leaf whorls, position of the tag, and branching of shoots were estimated in all plants at 3–4-day intervals (for all details see Adamec, 2000; Adamec and Kovářová, 2006). Main shoot length was measured at the end of the growth experiment. Since the stock plants of both species captured prey before the start of the growth experiment, this effect could influence an initial period of the experiment. Therefore, the apical shoot growth rate (as new leaf whorls/day) was taken into account only between the 7th and 14th day.

2.3. Gasometric measurements

One to two days after the 14-day growth experiment, eight experimental plants of each species and variant were used for P_N and RD measurements as well as for all other analyses. P_N and RD were measured from the 5th to 6th adult leaf whorls of *Aldrovanda* with traps deprived of prey (FW 16–26 mg) and from *Utricularia* leaves from the 8th to 10th adult leaf whorls deprived of all traps (FW 8–24 mg). *Aldrovanda* traps were deprived of caught prey as the prey was usually large and could influence both P_N, RD, and biomass measurements. *Utricularia* leaves were deprived of traps as trap P_N is known to be 7–10 times lower per unit FW than that of trapless leaves (Adamec, 2006). RD and P_N were measured in a solution of 1 mM $NaHCO_3$ with 0.5 mM KCl and 0.1 mM $CaCl_2$ (80–90% O_2 saturation) in a 5-mL stirred chamber (kept at 22.0 ± 0.1 °C). A Clark-type oxygen sensor and a pen recorder (for details see Adamec, 1997b, 2006) was used. The initial pH of the solution was 7.07 and [CO_2] was 0.20 mM. After RD had been measured in darkness for 15 min, a light was switched on (halogen reflector, 400 μmol m^{-2} s^{-1} PAR) and P_N was measured for 15 min. FW was then determined for all measured leaves (the fluid was pressed out from *Aldrovanda* traps), while dry weight (DW; 80 °C) was estimated in pooled samples.

All measurements were repeated 8 times for the same conditions for a different plant material. RD and P_N are expressed in mmol kg_{FW}^{-1} h^{-1}. Chlorophyll *a* content was determined in the measured plant material (Pechar, 1987) in five replications. To assess the quantity of captured prey, the percentage of traps with any recognizable prey in the eight experimental plants was estimated using a binocular loupe in the adult 5th to 6th leaf whorls in *Aldrovanda*, and in the 10th whorls of *Utricularia*. To estimate CO_2 compensation point (CP) of photosynthesis as a measure of CO_2 affinity, an outdoor end-pH experiment was performed using parallel plant material from the growth experiment (apical shoot segments 5 cm long, with traps, in *Aldrovanda* and 7 cm long in *Utricularia*). CO_2 CPs were estimated in 10 mL test tubes in the above $NaHCO_3$ solution in natural light (550–900 μmol m^{-2} s^{-1}) at 21 ± 1 °C. The end-pH values were measured after a 5-h exposure in five replications (Maberly and Spence, 1983; Adamec, 1997b).

2.4. Tissue nutrient content and statistical treatment

N and P content was determined colorimetrically in adult 3rd to 4th leaf whorls with traps in *Aldrovanda* and in 6th to 7th trapless leaf whorls in *Utricularia* after acid mineralization (for all details see Adamec, 2002). Five replications were used for each variant. Throughout the paper, the mean with standard error was provided wherever possible. Significant differences were evaluated by a factorial two-way ANOVA (species and feeding as fixed effects, Tukey HSD test for multiple comparisons) and apical shoot growth rate by a two-tailed *t*-test.

3. Results

At the end of the growth experiment after 15 or 16 days, both *Aldrovanda* and *Utricularia* plants without prey had virtually no prey in their traps, while the control plants with prey had about 43 and 4.2% traps with prey, respectively (Table 1). Fed plants of both species were found to be longer (only in *Utricularia* significantly), and had significantly more adult leaf whorls on the main shoot than unfed plants. Effect of prey application interacted significantly with species only for shoot length but not for adult leaf whorls. As early as day 7 of the experiment, the fed and unfed plants showed a highly significant difference in the number of leaf whorls in *Aldrovanda* ($p < 0.01$). Yet the difference was only weakly significant in *Utricularia* ($p < 0.055$; data not shown). The apical shoot growth rate of fed control plants was by 33–53% higher than that of the unfed variants in both species between the 7th and 14th day of the growth experiment. However, these differences were not statistically significant at $p < 0.05$ (Table 1). A marked but non-significant difference in shoot branching was found between the fed and unfed *Aldrovanda* plants, while only a small and non-significant difference was found between *Utricularia* plants. Overall, the positive growth effect of feeding on prey was evident in both species.

Tissue N content in young leaf whorls of both species fed on prey was significantly lower (by 54% in *Aldrovanda*, by 86% in *Utricularia*) than that in unfed variants (Table 2). Tissue P content in these leaf whorls was significantly different between fed and unfed variants only in *Utricularia* and the species × prey interaction was highly significant. Feeding on prey had no significant influence on chlorophyll *a* content in measured leaf whorls of *Aldrovanda*, while the content from the unfed variant was highly significantly greater (by 65%) in *Utricularia* (Table 2). In both plant species, RD in fed and unfed variants was very similar. However, P_N was quite significantly greater in fed plants of *Aldrovanda*, while lower in *Utricularia* and the interaction between species and prey was highly significant. The photosynthetic CO_2 CP was the same in fed and unfed *Aldrovanda*, but it was highly significantly greater in the control plants of *Utricularia* fed with prey (9.2 μM vs. 5.2 μM; Table 2). In *Utricularia*, the photosynthetic CO_2 affinity was consistent with P_N and chlorophyll content.

4. Discussion

Although both species grew considerably faster as a result of feeding on prey (plant size, branching, apical shoot growth; Table 1), the relative quantity of feeding as a basis for ecophysiological responses was markedly different in both species. *Aldrovanda* displayed marked differences in feeding, while differences in *Utricularia* remained weak. However, it is possible that both species respond to prey and to nutrient availability in a different way as quantitatively shown in the

Table 1
Growth characteristics of *Aldrovanda vesiculosa* and *Utricularia australis* grown with (+) or without prey (−) in an outdoor culture for 14 days

Species or parameter of ANOVA	Prey	Traps with prey (%)	Shoot length (cm)	Adult leaf whorls of main shoot	Apical shoot growth rate (whorl/day)	Branches ($plant^{-1}$)
A. vesiculosa	+	43.4 ± 6.1	13.3 ± 0.4	17.6 ± 0.4	0.29 ± 0.06	0.42 ± 0.15
A. vesiculosa	−	0.0^{++}	10.6 ± 0.3^{ns}	$14.6 \pm 0.2^{++}$	0.19 ± 0.04^{ns}	0.00^{ns}
U. australis	+	4.22 ± 0.59	38.9 ± 2.2	49.4 ± 0.6	1.16 ± 0.16	0.64 ± 0.13
U. australis	−	0.0^{ns}	$28.0 \pm 1.4^{++}$	$45.8 \pm 0.7^{++}$	0.87 ± 0.18^{ns}	0.50 ± 0.15^{ns}
Species	–	***	***	***	–	**
Prey	–	***	***	***	–	*
Species × prey	–	***	**	ns	–	ns

Apical shoot growth rate was evaluated within the 7th to 14th day of the experiment. Mean ± S.E. are shown; $n = 14$. Significance (within each species): $^{++}p < 0.01$, $^{ns}p > 0.05$; significance (between species, prey application, and species × prey interaction) on the bottom of the table: $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, $^{ns}p > 0.05$.

Table 2
Physiological characteristics of *A. vesiculosa* and *U. australis* which had been grown with (+) or without prey (−) for 15–16 days

Species or parameter of ANOVA	Prey	DW (% FW)	Tissue content (% DW)		Chlorophyll *a* content ($mg\,kg_{DW}^{-1}$)	RD ($mmol\,kg_{FW}^{-1}\,h^{-1}$)	P_N ($mmol\,kg_{FW}^{-1}\,h^{-1}$)	Compensation point CO_2 (μM)
			N	P				
A. vesiculosa	+	10.9	1.48 ± 0.16	0.136 ± 0.004	2.39 ± 0.13	8.97 ± 0.72	34.1 ± 1.5	5.13 ± 0.44
A. vesiculosa	−	8.74	$2.28^{++} \pm 0.23$	$0.126^{ns} \pm 0.004$	$2.64^{ns} \pm 0.07$	$8.17^{ns} \pm 0.81$	$21.5^{++} \pm 2.2$	$4.95^{ns} \pm 0.16$
U. australis	+	11.5	0.81 ± 0.04	0.130 ± 0.008	1.84 ± 0.08	7.42 ± 0.93	66.7 ± 1.6	9.20 ± 0.89
U. australis	−	11.5	$1.50^{+} \pm 0.06$	$0.166^{+} \pm 0.013$	$3.03^{++} \pm 0.14$	$7.89^{ns} \pm 0.68$	$89.2^{++} \pm 3.7$	$5.20^{++} \pm 0.34$
Species	–	–	***	*	ns	ns	***	***
Prey	–	–	***	ns	***	ns	ns	**
Species × prey	–	–	ns	**	***	ns	***	**

RD and P_N were measured in 5th to 6th adult leaf whorls with traps in *A. vesiculosa*, while in excised trapless leaves from the 8th to 10th leaf whorls in *U. australis*; $n = 8$. Other parameters, $n = 5$. Mean ± S.E. are shown where possible. Significance (within each species): $^{++}p < 0.01$, $^{+}p < 0.05$, $^{ns}p > 0.05$; significance (between species, prey application, and species × prey interaction) on the bottom of the table: $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, $^{ns}p > 0.05$.

recent field-growth study (Adamec and Kovářová, 2006). Due to the very high-feeding rate of *Aldrovanda* in this present study, it is obvious that the plants could take up a large amount of organic substances from prey. Yet P_N of fed plants was not self-inhibited and its high value contrasted with relatively low tissue N and chlorophyll content (Table 2). In contrast, *Utricularia* captured only a small amount of fine prey, but P_N and CO_2 affinity of the fed plants were significantly lower. However, as follows from Table 2, chlorophyll-based P_N was higher in fed plants of both species. Yet photosynthetic effects of prey application were clearly species specific.

Under favourable growth conditions, aquatic carnivorous plants with linear, modular shoot structure exhibit very fast apical shoot growth and high relative growth rate (Friday, 1989; Adamec, 2000; Adamec and Kovářová, 2006). Due to the relatively low water temperature during the 7th to 14th day of the growth experiment, the apical growth rate measured in fed plants of both species (Table 1) was only about 30–35% of that measured in warm water in the field (Adamec and Kovářová, 2006). Although both species of fed plants grew relatively slowly, low tissue N content in adult leaf whorls of fed plants suggests that tissue N was ''diluted'' by growth processes much more than in the unfed controls. The same relationship between tissue N content and growth rate, as dependent on feeding on prey, was reported in *Aldrovanda*, not only from a similar growth experiment (Adamec, 2000) but also ones done on several species of terrestrial carnivorous plants (Adamec, 1997a, 2002). It was calculated that for rapidly growing *Aldrovanda* its daily P_N was theoretically sufficient to cover about 180% of its daily need for carbohydrates, even when no re-utilization of carbohydrates would occur (Adamec, 1997b).

It is therefore possible to conclude that the growth rate in both faster growing aquatic and slower growing terrestrial carnivorous plant species is not limited by photosynthetic rate, but rather by a shortage of mineral nutrients necessary for growth processes. Feeding on prey in both ecological groups of carnivorous plant species clearly does not lead to any increase of shoot tissue N content (it usually decreases in aquatic ones) or P_N per unit biomass, either. However, it has not been tested whether the total plant P_N is increased in fed plants in both species. Therefore, the main physiological effect of catching prey and the associated uptake of mineral nutrients from this prey are not based on enhancement of P_N, as suggested by Givnish et al. (1984), but on providing N and P for essential growth processes such as cell division, DNA replication, and proteosynthesis in young tissues in shoot apices.

Acknowledgements

Sincere thanks are due to Dr. Robert Gibson and Mr. Kirk S. Hirsch, University of New England, Armidale, NSW, Australia, for correction of the language. This study was partly funded by the Research Project of the Academy of Sciences of the Czech Republic no. AV0Z60050516.

References

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., 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., 2002. Leaf absorption of mineral nutrients in carnivorous plants stimulates root nutrient uptake. New Phytol. 155, 89–100.
Adamec, L., 2006. Respiration and photosynthesis of bladders and leaves of aquatic *Utricularia* species. Plant Biol. 8, 765–769.
Adamec, L., Kovářová, M., 2006. Field growth characteristics of two aquatic carnivorous plants, *Aldrovanda vesiculosa* and *Utricularia australis*. Folia Geobot. 41, 395–406.
Ellison, A.M., 2006. Nutrient limitation and stoichiometry of carnivorous plants. Plant Biol. 8, 740–747.
Ellison, A.M., Farnsworth, E.J., 2005. The cost of carnivory for *Darlingtonia californica* (Sarraceniaceae): evidence from relationships among leaf traits. Am. J. Bot. 92, 1085–1093.
Englund, G., Harms, S., 2003. Effects of light and microcrustacean prey on growth and investment in carnivory in *Utricularia vulgaris*. Freshwater Biol. 48, 786–794.
Friday, L.E., 1989. Rapid turnover of traps in *Utricularia vulgaris* L. Oecologia 80, 272–277.

Givnish, T.J., Burkhardt, E.L., Happel, R.E., Weintraub, J.D., 1984. Carnivory in the bromeliad *Brocchinia reducta*, with a cost/benefit model for the general restriction of carnivorous plants to sunny, moist, nutrient-poor habitats. Am. Nat. 124, 479–497.

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

Kamiński, R., 1987. Studies on the ecology of *Aldrovanda vesiculosa* L. I. Ecological differentiation of *A. vesiculosa* population under the influence of chemical factors in the habitat. Ekol. Pol. 35, 559–590.

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

Méndez, M., Karlsson, P.S., 1999. Costs and benefits of carnivory in plants: insights from the photosynthetic performance of four carnivorous plants in a subarctic environment. Oikos 86, 105–112.

Pechar, L., 1987. Use of acetone-methanol mixture for extraction and spectrophotometric determination of chlorophyll *a* in phytoplankton. Arch. Hydrobiol. 78 (Suppl.), 99–117.

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

Wakefield, A.E., Gotelli, N.J., Wittman, S.E., Ellison, A.M., 2005. Prey addition alters nutrient stoichiometry of the carnivorous plant *Sarracenia purpurea*. Ecology 86, 1737–1743.