Ecophysiological characterization of dormancy states in turions of the aquatic carnivorous plant *Aldrovanda vesiculosa*

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Abstract

Two main dormancy states, innate and imposed dormancy, were characterized in turions (winter buds) of the aquatic carnivorous plant *Aldrovanda vesiculosa* L. (*Droseraceae*) kept at 3 ± 1 °C in a refrigerator over the winter. As a result of the breaking of imposed dormancy by a temperature increase (at 15 - 20 °C), some of the turions rose to the water surface within 1 - 3 d and germinated. Turion leaves contained large lacunae with a slimy reticulum and were filled by water over winter. As a result of breaking imposed dormancy, the proportion of gas volume in inner turion leaves rose from 10 - 20 % to 100 % of leaf lacunae volume. The aerobic dark respiration rate of the turions [0.74 - 1.5 μmol (O_2) kg^{-1}(FM) s^{-1}] slightly increased during innate dormancy after 1 - 2 d at 20 °C, while it was almost constant during the breaking of imposed dormancy. The anaerobic fermentation rate of the turions was only 1.5 - 7 % of the oxygen respiration rate and also was constant during the breaking of imposed dormancy. In turions, the content of glucose, fructose, and sucrose was the same for the two states of dormancy, but starch content was greatly reduced for the imposed dormancy (10 - 11 *vs.* 32 % DM). It may be suggested that a temperature increase causes an increase of fermentation or respiration which is responsible for the evolution of gas in turion lacunae and, thus, for turion rising.

Additional key words: anatomy, gas volume, turion rising and germination, breaking imposed dormancy, dark respiration, fermentation, saccharide content.

Introduction

Turions (overwintering buds) are vegetative dormant organs produced by perennial aquatic plants. Turions are formed in subtropical to polar zones as a response to unfavourable ecological conditions, and protect fragile plant shoots from freezing and decaying. Turions are modified shoot apices. Turions of free-floating aquatic plants (*e.g., Spirodela polyrhiza, Hydrocharis morsus-ranae, Utricularia* spp., *Aldrovanda vesiculosa*) break their dormancy at the bottom of an aquatic habitat, but usually germinate at the water surface, in warmer water and at higher irradiance (Bartley and Spence 1987).

Turions of aquatic rootless carnivorous plants are

spherical (*Utricularia*) or rhomboid (*Aldrovanda*; see Fig. 1), tough and sturdy organs, formed by extreme condensation of very short modified leaves in the shoot apex at the end of the growing season. The turions are frost resistant (Winston and Gorham 1979a, Adamec 1995, 1999a), but their main ecological function is to sink to warmer water at the bottom in autumn and to rise to the water surface after winter again. *Aldrovanda* turions break off the dying mother shoots, actively sink in autumn and rise in the spring. Turions of aquatic carnivorous plants are storage organs. In autumn, they accumulate starch (25 - 32 % DM) and free sugars

(in total 7 - 14 % DM; Adamec 1995, 1999b). At the bottom, they overwinter under hypoxic or anoxic conditions, lightly covered by organic sediments.

In Canadian *Utricularia vulgaris* two dormancy states of formed turions were distinguished (Winston and Gorham 1979a). The states – innate and imposed dormancy – are controlled by endogenous contents of stimulating and inhibiting phytohormones (Winston and Gorham 1979b). In the state of innate dormancy, the turion is held dormant endogenously by inhibiting phytohormones, while imposed dormancy is maintained exogenously by low temperatures. The states of innate and imposed dormancy have also been found preliminarily in *Aldrovanda* turions (Adamec 1999b). *U. vulgaris* and *Aldrovanda* turions break imposed dormancy by a temperature increase; light may not play a ɪ role (Winston and Gorham 1979a, Adamec 1999b). Breaking imposed dormancy of *Aldrovanda* turions results in reduction of turion density within 1 - 2 d so that turions rise to the water surface where they can germinate in warmer water and in the light (Adamec 1999b). The mechanism of rising for *Aldrovanda* turions is still unknown. So far, greater attention has been paid to processes concerned with turion germination *sensu stricto*

(sprouting; *e.g.*, Winston and Gorham 1979a, Villanueva *et al.* 1985, Bartley and Spence 1987, Appenroth *et al.* 1990, Xyländer *et al.* 1992) than with breaking imposed dormancy and/or turion rising (Newton *et al.* 1978, Beer 1985, Kamiński 1987, Adamec 1999b). However, in the flat waxy turions of *Spirodela polyrhiza* (greater duckweed), a small gas bubble which is produced externally pulls the turions to the water's surface (Newton *et al.* 1978). Similarly, rising of *Aldrovanda* turions might be caused, besides reduction of starch content, by the production of a gas into the voluminous gas spaces in turion leaves, expelling water from them (Adamec 1999b).

The aim of this paper is to present basic data on the overwintering ecophysiology of turions of *A. vesiculosa* L.: turion anatomy, breaking of dormancy, saccharide content and respiration characteristics. In turions of aquatic carnivorous plants, such complex data have not been published so far. Attention is mainly focused on the processes associated with breaking imposed dormancy of the turions and their rising to the water surface. This paper follows up on recent ecophysiological study on *Aldrovanda* (Adamec 2000).

Materials and methods

Plants: *Aldrovanda vesiculosa* L. plants, 2 - 3 cm long and with unripe turions, were collected from fen pools near Ptačí blato fishpond in the Třeboňsko Biosphere Reserve, S. Bohemia, Czech Republic, from late September to early October. The plants from E. Poland were introduced to the pools in 1995 (Adamec and Lev 1999). The turions were placed in a nylon net and allowed to fully ripen outdoors in dystrophic water in a plastic container in which *Aldrovanda* were grown (Adamec 2000). These conditions of turion ripening mimicked partly those which the turions receive under natural conditions (Adamec 1999b). Water temperature in

Fig. 1. Standard turions of *Aldrovanda* after overwintering in a refrigerator at the end of April. *Bar* represents 1 mm.

the container was 2 - 12 °C and natural irradiance was markedly reduced due to self-shading. In the first half of November, when water temperature fell to zero, ripe turions of standard size (length 5 - 6 mm, width 3 - 4 mm; Fig. 1) were devoid of dead shoots and thoroughly

washed using tap water. Clean turions were stored in darkness in filtered cultivation medium (for the composition see Adamec 2000) in a refrigerator at 3 ± 1 °C over the winter. Every 1 - 2 months, the turions were checked in dim white light and washed thoroughly with cold tap water, and the medium was renewed.

Characterization of dormancy states: To characterize the main states of *Aldrovanda* turion dormancy, *i.e.*, innate and imposed, sets of 20 sunken (lying at the bottom) turions from the same batch from the refrigerator were washed by cold tap water, put in 25 cm³ of filtered cultivation medium in 30-cm³ glass vials, and exposed immediately in a low-volume (80 dm³) self-made refrigerated growth chamber equipped with white fluorescent tubes (irradiance of 280 ± 30 μmol m⁻² s⁻¹ at the level of the turions). Turions were exposed at the temperatures of 5, 10, 15, or 20 (± 0.5) °C in continuous light (CL), or light pulse during an initial 10 min followed by darkness (LP), or darkness (DD) for 8 - 9 d. The dark variants were covered with a black cloth. Every 10 - 24 h, rising of the turions to the water surface (buoyancy) and symptoms of germination were investigated in dim white light. Overall, due to this manipulation, the dark variants received a low white-light dose which could be approximately two orders of magnitude lower than that of the LP variants. Turions were classified as germinating if they distinctly reflexed

their basal leaf whorls and opened themselves at least slightly at the apex of the turion (see Weber and Noodén 1976, Adamec 1999b). Oxygen concentration in the medium was always > 4 mg dm^{-3}. In other experiments, turion rising and germination were investigated at 20 °C in cultivation medium supplemented with 0.1 mM NaN_3 or in a closed glass vial in which the medium had been bubbled with N_2 and the initial $[O_2]$ was < 0.08 mg dm^{-3} (< 1 % of saturation). All experiments on *Aldrovanda* turion rising and germination in the state of imposed dormancy were performed in February - March, when innate dormancy was completely broken, while those performed on plants in the state of innate dormancy took place in mid-November. Results of 1 - 5 repetitions were pooled together. The range of times pertinent to the appearance of the first and 50 % turion rising and germination is shown. When turion rising and germination exceeded 50 % the time of reaching this level was interpolated.

Measurement of respiration and fermentation rates: The aerobic respiration rate of *Aldrovanda* turions was measured in mid-November (innate dormancy) and at the end of March (imposed dormancy). Three turions (FM *ca.* 100 mg) having been stored in a refrigerator at 3 ± 1 °C were used for single measurements. The turions were thoroughly washed with tap water and immediately used for measurements. The dark respiration rate (R_D) was measured in 2 mM $NaHCO_3$ (pH 8.1), in a 8-cm^3 stirred thermostatted chamber at 20.0 ± 0.1 °C, using a Clark-type oxygen sensor (*Labio*, Prague, Czech Republic) and a pen recorder (Adamec 1997a). The oxygen concentration during the measurements was 80 - 90 % of the saturation. R_D was measured with the same turions repeatedly after 0, 4, 8, 24, and 48 h exposure in darkness at 20.0 ± 1 °C, thus under conditions of breaking imposed dormancy. After the last measurement, FM of blotted turions was estimated. For estimation of respiration Q_{10}, R_D was measured first at 20.0 °C in the turions freshly taken out from a refrigerator and then, after 2 - 3 h at 4.0 °C in darkness. To estimate a net photosynthetic rate (P_N) of the turions, O_2 exchange was measured (see Adamec 1997a) at 20.0 ± 0.1 °C, CO_2 concentration 0.25 mmol dm^{-3}, and irradiance (PAR) of 70 W m^{-2} from a halogen lamp.

To compare aerobic R_D of turions with their anaerobic fermentation rate, anaerobic release of CO_2 was measured

in sets of 20 *Aldrovanda* turions (FM 0.6 - 0.9 g) stored in a refrigerator, both in mid-November and in mid-April. The turions were thoroughly washed with tap water, rinsed with distilled water, partly blotted dry, and put in the flasks of Warburg manometers. The manometers were flushed with N_2 for 5 min and then closed and maintained at 20.0 ± 0.02 °C. The measurement of CO_2 release in humid N_2 in darkness usually lasted from 30 - 150 min after the manometers were closed using the same turions within 1 - 3 and 4 - 16 h at innate dormancy or 3 - 6 and 48 - 53 h at imposed dormancy. Results of all respiration measurements are expressed in $\mu mol\ kg^{-1}\ s^{-1}$ on the FM basis.

Analyses of saccharide content: To investigate saccharide content in *Aldrovanda* turions during breaking imposed dormancy, starch and sugar content were analysed in sets of 7 - 8 turions (FM 140 - 240 mg; DM 40 - 55 mg) in early or mid-November or at the end of March. The turions were analysed for saccharides just after taking them out of a refrigerator and after 8, 24, and 48 h exposure in the filtered cultivation medium at 20.0 ± 0.5 °C in darkness. Soluble sugars were extracted with 80 % ethanol at 70 °C and sucrose, glucose, and fructose concentrations in lyophilised and redissolved samples were estimated using HPLC. In the extracted DM, starch was hydrolysed with 32 % $HClO_4$ and glucose was estimated by the anthrone method (for all analytical details see Čížková *et al.* 1996). All saccharide analyses were performed in triplicates.

Anatomical investigations: To obtain basic information on the anatomy of turions, *Aldrovanda* turions at the state of innate or imposed dormancy were fixed with 70 % FAA solution and embedded in paraffin. Cross and longitudinal sections 8 - 10 μm thick were stained by 0.1 % Alcian blue in 3 % acetic acid for 3 - 4.5 h. Preparations were photographed using a computer-controlled microscope camera *Hitachi HV-20C* (*Hitachi Denshi Ltd.*, Tokyo, Japan; software *Lucia*; *Imaging Industry*, Prague, Czech Republic). Isolated intact turion leaves were photographed in the same way.

Statistics: Where possible all paired data were statistically evaluated by a two-tailed paired *t*-test. Other data were processed by one-way *ANOVA* (Tukey HSD test).

Results

Leaves of *Aldrovanda* turions contain septae which subdivide leaf lamina into large regular lacunae (Fig. 2) which are homologous to those in adult leaves. In inner leaves of turions, the lacunae usually range from 310 - 460 μm (length) × 150 - 190 μm (width) × 40 - 86 μm

(depth). Turion leaves contained different volume of gas in their lacunae as dependent on their leaf position and dormancy state. Generally in all turions, the proportion of gas volume in leaf lacunae increased from the outermost turion leaves at the turion base toward the inner ones.

Turions at the state of innate (data not shown) or imposed dormancy (Fig. 2) contained gas in less than ca 10 % of the volume of leaf lacunae in their 6th leaves. While after breaking imposed dormancy at 20 °C in darkness after 2 d, the proportion of gas volume in the total leaf lacunae volume usually ranged from 20 - 100 % in 6th turion leaves (Fig. 3). Outer turion leaves usually contained no gas bubbles during the both dormancy states, and the proportion of gas volume was only approximately 20 % or less after breaking of imposed dormancy (data not shown). Cross-sections through Aldrovanda turions confirmed the occurrence of voluminous leaf lacunae which could amount approximately a half of a leaf cross-section area (Fig. 4). Leaf thickness and size of lacunae were usually smaller in inner leaves than in outer ones. Leaf lacunae were surrounded by one-layered-celled septae the cells of which were 25 × 25 to 40 × 40 μm large. Turions consisted of 8 - 9 fully developed leaf whorls and 6 - 7 leaf whorl primordia at the turion apex (Fig. 5). As follows from detailed cross-sections (Figs. 6, 7) some leaf lacunae were filled with slimy reticulum which was stained by Alcian blue. Fragments of the reticulum often fringed the inner side of leaf lacunae.

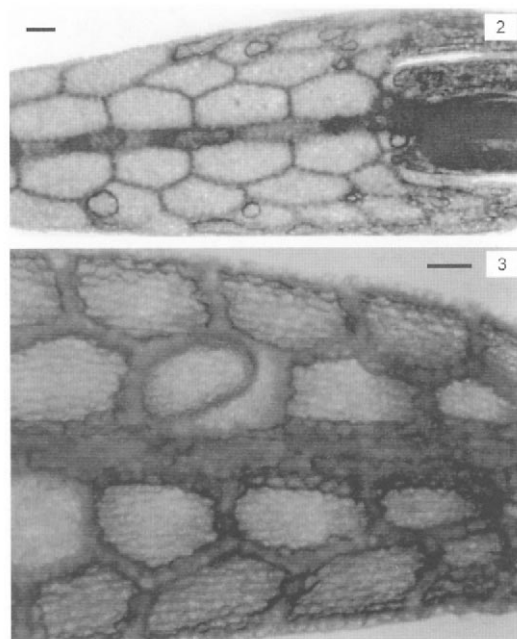Ripe Aldrovanda turions neither rose nor germinated under all light regimes at 20 °C in November, after they

Fig. 2. Intact leaf of the 6th leaf whorl (from the turion base) of an Aldrovanda turion at the end of February. The turion was kept in a refrigerator. Small volume of gas bubbles occurs in leaf lacunae. Bar represents 100 μm.

Fig. 3. Intact leaf of the 6th leaf whorl (from the turion base) of an Aldrovanda turion in mid-May. Basal leaf part is shown. Before microscopic examination, the turion was exposed to 20 °C in darkness for 2 d. Almost the whole volume of leaf lacunae is filled with gas. Bar represents 100 μm.

had been transferred from the outdoor container to a refrigerator (data not shown). They could partly rise and germinate at 20 °C in early January, while their full breaking of innate dormancy occurred at the end of January (data not shown). However, even after this date, the characteristics of turion rising and germination were very variable (Table 1). The turions were totally unable to rise and germinate at 5 °C and germinated at 10 °C only in continuous light after 7 d, without any rising. Turion rising was approximately the same at 15 and 20 °C, with the first rising after 24 - 104 h, but germination was mildly accelerated at 20 °C. The light regime did not influence turion rising, while turion germination was enhanced by light. At 20 °C, a 10 min light pulse usually accelerated both the start of germination and reaching of the 50 % level by about 1 d and CL by about 2 d, as compared to the effect of darkness. Both azide and nitrogen (i.e., anoxia) totally blocked turion rising and germination in DD, while these processes were not influenced in CL (Table 1). After the turions in DD with nitrogen had been returned to CL and oxygenated water, they normally rose and germinated, while light alone was without any effect (data not shown).

Table 1. Rising and germination of Aldrovanda turions in the state of imposed dormancy (February - March). Range of times of the first symptoms and reaching 50 % is shown. The data in parentheses indicate that the phenomenon did not occur within the time period given. ∞, the 50 % rising or germination was not reached within the experiment. DD, darkness; LP, initial 10-min light pulse; CL, continuous light.

Temp. [°C]	Conditions	Turion rising [h] 1st symptom	50 %	Germination [h] 1st symptom	50 %
5	DD	(>216)	∞	(>216)	∞
5	LP	(>216)	∞	(>216)	∞
5	CL	(>216)	∞	(>216)	∞
10	DD	(>209)	∞	(>209)	∞
10	LP	(>209)	∞	(>209)	∞
10	CL	(>209)	∞	167	177
15	DD	24	(>129)	108	119
15	LP	48	(>129)	86	102
15	CL	34	58	84	100
20	DD	96-∞	∞	72-98	72-148
20	LP	25-104 (∞)	∞	50-80	72-168
20	CL	24-91	63-90 (∞)	24-53	33-110
20	DD + NaN3	(>245)	∞	(>245)	∞
20	CL + NaN3	47	73	75	82
20	DD + N2	(>134)	∞	(>134)	∞
20	CL + N2	23	58	45	56

In Aldrovanda turions in innate dormancy, the aerobic dark respiration rate increased by 38 - 45 % after 1 - 2 d at 20 °C, while R_D was almost constant during the 2-d period of breaking imposed dormancy (Table 2). R_D of Aldrovanda turions in the imposed dormancy was by

Table 2. Aerobic dark respiration rate [μmol (O_2) kg^{-1} (FM) s^{-1}] of turions of *A. vesiculosa* measured at 20 °C at the state of innate (mid-November) or imposed dormancy (late March) within 0 - 48 h after their transfer to 20 °C in darkness (* - another experiment with different batch of turions). Means ± SE, n = 4. Different letters in rows within different dormancy states denote significant differences at P = 0.05.

Species	Innate dormancy			Imposed dormancy				
	0 h	24 h	48 h	0 h	4 h	8 h	24 h	48 h
AV	0.74 ± 0.04[a]	1.02 ± 0.02[b]	1.07 ± 0.06[b]	1.12 ± 0.12[a]	-	1.29 ± 0.05[a]	1.24 ± 0.05[a]	1.20 ± 0.09[a]
AV*	-	-	-	1.39 ± 0.09[a]	1.54 ± 0.19[a]	1.27 ± 0.26[a]	1.16 ± 0.16[a]	1.37 ± 0.12[a]

51 - 88 % higher (P < 0.05) than that in innate dormancy. Q_{10} of R_D was 2.78 in *Aldrovanda*. At the imposed dormancy, P_N was negative in *Aldrovanda* turions [-0.78 ± 0.08 μmol kg^{-1}(FM) s^{-1}]. Anaerobic fermentation rate of *Aldrovanda* turions at the innate dormancy was only 0.05 - 0.07 μmol kg^{-1}(FM) s^{-1} (Table 3), *i.e.*, about

Table 3. Anaerobic fermentation rate [μmol (O_2) kg^{-1} (FM) s^{-1}] of *Aldrovanda* turions measured in N_2 at 20 °C in darkness at the state of innate (mid-November) or imposed dormancy (mid-April) within 1 - 53 h after their transfer to 20 °C in darkness. Means ± SE, n = 4. The same letters for the same dormancy state denote no significant differences at P = 0.05.

Innate dormancy		Imposed dormancy	
1 - 3 h	4 - 16 h	3 - 6 h	48 - 53 h
0.050 ± 0.009[a]	0.069 ± 0.016[a]	0.021 ± 0.006[a]	0.032 ± 0.004[a]

15 times lower than the aerobic R_D, and was only 0.02 - 0.03 μmol kg^{-1}(FM) s^{-1} in the course of breaking imposed dormancy, *i.e.*, about 40 - 70 times lower than the corresponding aerobic R_D.

In *Aldrovanda* turions, no statistically significant changes of saccharide content occurred both at the innate and imposed dormancy after the transfer to 20 °C (Table 4). In innate dormancy, starch content in turions varied from 21 to 32 %, glucose 5.3 - 6.7 %, fructose 0.9 - 1.3 %, sucrose 6.4 - 7.3 %, and total saccharide content 35 - 46 % of total DM. However, within the same batch of turions, those in imposed dormancy had statistically a significantly lower proportion of DM (P < 0.05) and content of starch and total saccharides (P < 0.001) compared to turions in innate dormancy. In the course of breaking imposed dormancy, the amount of all saccharide types was constant. The amount of glucose, fructose, and sucrose was the same at the both states of dormancy.

Table 4. Saccharide content in *Aldrovanda* turions in the state of innate (early to mid-November) and imposed (end of March) dormancy within 0 - 48 h after their transfer to 20 °C in darkness (* - another experiment with different batch of turions). Saccharide content is given in % of DM; DM of the turions in % of FM. Means ± SE, n = 3. Different letters in individual columns for each batch of turions denote significant difference at P = 0.05.

Species	Dormancy	Exposure [h]	DM	Starch	Glucose	Fructose	Sucrose	Total
AV	innate	0	22.5 ± 0.3[a]	24.3 ± 0.8[a]	6.7 ± 0.1[a]	1.3 ± 0.1[a]	6.4 ± 0.3[a]	38.7 ± 1.0[a]
	innate	24	22.0 ± 0.2[a]	22.6 ± 0.7[a]	6.3 ± 0.2[a]	1.0 ± 0.1[a]	6.5 ± 0.1[a]	36.4 ± 1.0[a]
	innate	48	22.3 ± 0.2[a]	20.8 ± 1.9[a]	6.7 ± 0.1[a]	0.9 ± 0.1[a]	7.0 ± 0.1[a]	35.4 ± 1.9[a]
AV*	innate	0	26.1 ± 1.0[a]	32.1 ± 1.7[a]	5.3 ± 0.2[a]	1.1 ± 0.1[a]	7.3 ± 0.8[a]	45.7 ± 1.5[a]
	imposed	0	22.5 ± 0.1[b]	9.7 ± 0.3[b]	5.0 ± 0.4[a]	1.1 ± 0.2[a]	6.3 ± 0.6[a]	22.1 ± 0.9[b]
	imposed	8	22.8 ± 0.3[b]	9.7 ± 0.2[b]	5.0	0.7	6.2	21.6
	imposed	24	22.2 ± 0.2[b]	11.0 ± 0.3[b]	5.2 ± 0.6[a]	0.9 ± 0.2[a]	6.3 ± 0.8[a]	23.4 ± 1.8[b]
	imposed	48	22.2 ± 0.7[b]	10.0 ± 0.4[b]	4.7 ± 0.2[a]	0.9 ± 0.1[a]	5.8 ± 0.2[a]	21.4 ± 0.7[b]

Discussion

In spite of a different ecophysiological strategy of autumnal sinking and spring rising, two dormancy states of *Aldrovanda* turions were analogous to those described by Winston and Gorham (1979a) for *U. vulgaris* turions. After ripe *Aldrovanda* turions had been collected from an outdoor container and stored in a refrigerator (cold treatment) in early November, they were strictly in the state of innate dormancy for about 2 months, till early January. They could neither rise nor germinate at 20 °C in CL for at least 12 d (data not shown). A complete break of innate dormancy took place in late January. Afterwards, turions could fully (> 90 %) and reliably

germinate at the temperatures of 15 - 20 °C at any light regime or in darkness during 8 d or shorter times (Table 1). On the contrary, in the state of imposed dormancy during February - April, turion rising proved to be a very variable and unreliable phenomenon. Generally, the proportion of risen turions was 0 - 60 % (usually only 20 - 40 %) at the end of experiments (data not shown). Thus, a great number of turions could germinate without rising. *Aldrovanda* turions in imposed dormancy can rise after only 1 - 2 d at 15 - 20 °C (Table 1). As opposed to the turion germination rate, which clearly depended on both temperature and light, turion rising was about the same at 15 and 20 °C. The critical temperature for

Aldrovanda turion rising might be about 12 °C (Adamec 1999a,b). Though turion rising represents an important physiological marker of breaking imposed dormancy, this rise is not a strict prerequisite for germination. However, under natural conditions, *Aldrovanda* turions always rise before they germinate (Adamec 1999a,b). It was found that *Aldrovanda* turions could perform the initial stage of germination also in darkness but further stages (sprouting) strictly required light (Adamec 1999b). From an ecological point of view, turion rising is a crucial process since turions entangled in the bottom sediment could rot.
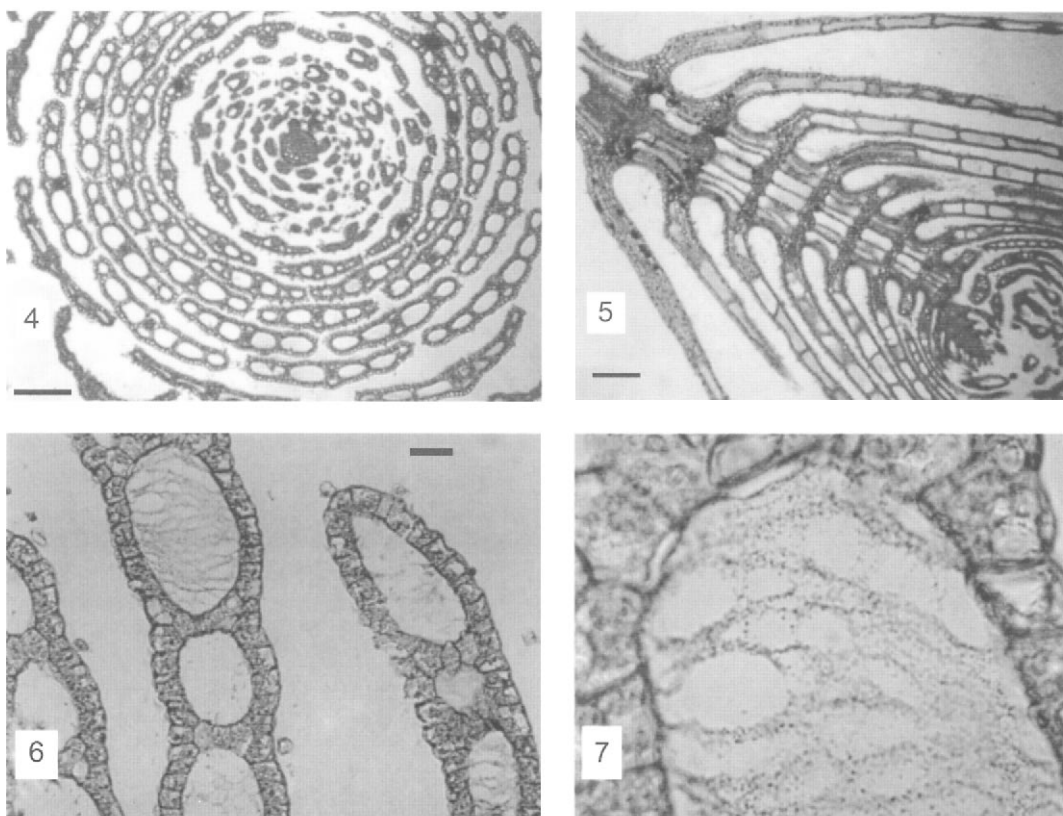
Fig. 4. Cross-section through an *Aldrovanda* turion, which overwintered in a refrigerator, at the end of March. The outermost turion leaves are artificially shrunken. *Bar* represents 400 μm.

Fig. 5. Longitudinal section through an *Aldrovanda* turion in mid-November. Some leaf lacunae are filled with a slime stainable with Alcian blue. *Bar* represents 400 μm.

Fig. 6. Detail of cross-section through an *Aldrovanda* turion in mid-November. Some leaf lacunae are filled with a slimy reticulum stainable with Alcian blue. *Bar* represents 50 μm.

Fig. 7. Detail of the slimy reticulum from the Fig. 6. *Bar* represents 10 μm.

The reason why *Aldrovanda* turions rise was highly variable among the experiments and why endogenous parameter(s) of the turions differed from those under natural conditions remains unclear. Experimental unripe turions were kept in an outdoor container in natural dim light for about one month after the field-grown turions had sunk to the bottom. The sunken turions are usually covered with a thin layer of sediment or litter and are in

darkness and overwinter under anoxic conditions (Adamec 1999a,b), while [O₂] may vary in the over-wintering medium for experimental turions. Presumably, turion rising in our experiments interferes in an unknown way with an ecophysiological property of the turions (*e.g.*, light exposure or temperatures during autumnal ripening, phytohormone content, starch or free-sugar content, degree of hypo- or anoxia in the medium, boron

tissue content; Adamec 1997b), which can be rather variable. Then, the resultant rate or capacity of turion rising could greatly be influenced. Under natural conditions, *Aldrovanda* turions break their imposed dormancy and rise reliably in darkness, anoxia, and high $[CO_2]$ of approximately 0.5 - 1.5 mmol dm^{-3} (Adamec 1999a). However, 1.5 mM CO_2 in the aerobic cultivation medium in darkness had no effect on rising (data not shown). In contrast, experimental turions were able neither to rise nor to germinate in darkness in N_2-bubbled medium, but only in light, and the same held also for azide treatment (Table 1). Similarly, *Spirodela polyrhiza* turions germinated in darkness only in oxygenated media, while the O_2 requirement could be compensated for by CL, allowing photosynthetic O_2 production (Appenroth *et al.* 1990). Overall, turion germination (sprouting) is very sensitive to hypoxia. As it follows from the insensitivity of *Aldrovanda* turion rising to light (Table 1) and turion natural overwintering in darkness, light itself has no effect on breaking imposed dormancy – only temperature increase has such an effect.

Inner turion leaves (6th whorl) could increase markedly their proportion of gas volume in lacunae after breaking imposed dormancy (Figs. 2, 3). It may therefore be concluded that a gas of an unknown composition is released into the lacunae of (mostly) inner turion leaves as a result of the breaking of imposed dormancy after the temperature has been increased. This mechanism of rising in turions of *Aldrovanda* is analogous to the production of external gas bubbles on flat turions of *Spirodela* (*cf.* Newton *et al.* 1978). Owing to the evolution of gas bubbles in turion leaf lacunae, the composition and function of the slimy reticulum (Figs. 6, 7) remain unknown. Judging from stainability by Alcian blue, it is probably an acidic polysaccharide. As the reticulum was more represented in longitudinal than in cross-sections (*cf.* Figs. 4, 5) it was more damaged in the latter sections. It might function as a catalyst for the conversion of dissolved gases to bubbles.

In *Aldrovanda* turions at both states of dormancy, R_D in aerobic medium at 20 °C [0.74 - 1.4 µmol kg^{-1}(FM) s^{-1}; Table 2] was about 2 - 3 times lower than that in summer shoots of *Aldrovanda* (Adamec 1997a). However, the values of R_D in turions are comparable with lower limits of those reported e.g. for roots of wetland sedges (*Carex* spp.; 0.75 - 2.9 µmol kg^{-1} s^{-1}; Van der Werf *et al.* 1988) or aquatic plants (0.72 - 1.6 µmol kg^{-1} s^{-1}; Smits *et al.* 1990), which also face hypo- or anoxia in their substrates. R_D of *U. australis* turions was 2 - 3 times lower (0.41 - 0.66 µmol kg^{-1} s^{-1}) than that of *Aldrovanda* turions and similar values of 0.43 and 0.51 µmol kg^{-1} s^{-1} were found in *U. vulgaris* and *Hydrocharis morsus-ranae* turions, respectively (Adamec, unpublished). P_N values measured even under optimum conditions show that the sign of the O_2 exchange depends on turion size (*i.e.*, surface-volume relationship). Only in small *U. australis* turions P_N was

positive (0.48 ± 0.09 µmol kg^{-1} s^{-1}), while in bigger *Aldrovanda*, *U. vulgaris*, and *Hydrocharis* turions, P_N values were negative (Adamec, unpublished). Generally, photosynthesis in turions of aquatic carnivorous plants has no importance under field conditions (darkness, low temperature) during the breaking of imposed dormancy. However, P_N was as great as 11 µmol kg^{-1}(FM) s^{-1} in tiny *Spirodela polyrhiza* turions (Beer 1985).

Values of oxygen-based R_D (Table 2) do not provide evidence of real respiratory activity of *Aldrovanda* turions overwintering under natural conditions of low temperatures (about 4 °C, darkness, and anoxia). R_D of *Aldrovanda* turions was only about 0.22 ± 0.02 µmol(O_2) kg^{-1} s^{-1} at 4 °C (Q_{10} 2.78; 2.10 in *U. australis*; Adamec, unpublished). However, as opposed to the roots of aquatic plants, in which anaerobic fermentation rates ranged from 10 - 100 % of aerobic R_D values (Smits *et al.* 1990), the values of anaerobic fermentation rates in *Aldrovanda* turions amounted to only 1.5 - 7 % of the oxygen-based ones (*cf.* Tables 2 and 3). Such a low ratio might reflect both low energy consumption in overwintering turions and their biochemical adaptation to anoxia, saving reserve sugars (Winston and Gorham 1979a). After turions rise to water with enough oxygen, their metabolism is switched from anaerobic to aerobic, and thus their R_D increases. In *Aldrovanda* turions during 48 h of breaking of imposed dormancy, no marked increase of R_D or anaerobic fermentation rate occurred (Tables 2, 3) which could be interpreted as a possible source of gas (CO_2) for bubble production in turion leaf lacunae. Moreover, orientation values of the respiratory quotient of *Aldrovanda* turions were close to unity and did not change during the breaking of imposed dormancy (Adamec, unpublished). In addition, the absence of any changes in saccharide content in *Aldrovanda* turions during 48 h of the breaking of imposed dormancy (Table 4; the same also in *U. vulgaris* turions; Adamec, unpublished) supports the view that no marked respiratory increase occurs during the 2-d period of breaking imposed dormancy, before turion germination (sprouting) starts. In addition, changes in saccharide content and metabolism preceding germination in *Spirodela* turions (Xyländer *et al.* 1992, Ley *et al.* 1997) are not understood by these authors as primary regulatory steps but as the securing of availability of soluble carbohydrates to support subsequent sprouting of the turion.

Taking into account all pieces of information on overwintering of turions of *Aldrovanda*, the mechanism of turion rising might be explained as follows. In the spring in cold water, the turions' rate of respiration or fermentation is very low. Their density is somewhat reduced as a result of starch degradation over the winter. Turion leaf lacunae are filled with water. As soon as water temperature is increased to a value of about 12 °C or higher, the fermentation rate is increased. As a result

of increased CO_2 production in turions in CO_2-rich bottom sediment, CO_2 in inner leaf lacunae appears in the form of bubbles and expels water from them. Thus, turions become lighter than water and gradually rise to the water surface where they germinate in warmer water, sufficient oxygen and light.

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