

## SURVIVAL OF DRIED TURIONS OF AQUATIC CARNIVOROUS PLANTS

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### Introduction

Aquatic carnivorous plants in the genera *Aldrovanda* and *Utricularia* produce turions: dormant, overwintering buds modified into storage organs (Sculthorpe 1967; Bartley & Spence 1987; Adamec 1999, 2003). Turions are durable, partly frost-tolerant organs which usually overwinter in shallow water close to the bottom, below ice cover. However, in some species turions overwinter also above the water surface in the terrestrial ecophase, on wet organic soil. Thus, these turions can face drying *in situ*. Maier (1973a) found that *U. vulgaris* turions, dried at 33% relative humidity (RH) and 23°C for 1-123 days, were able to sprout and grow. These turions contained water equivalent to only 5-6% of their dry weight (DW). However, the longer the turions were stored in a refrigerator before drying, the less tolerant of drying they were. Turions refrigerated for five months before being dried out survived very poorly. In another study (Maier 1973b), turions of *U. vulgaris*, *U. australis*, *U. intermedia*, and *U. minor* were able to withstand drying out (24±3°C, 33% RH) for 5-19 days and, in addition, the drying markedly shortened innate turion dormancy. Upon being returned to suitable growing conditions at 24°C, partially dried turions sprouted after only 5-7 days while control turions sprouted after 12-48 days. Although the fresh-weight based respiration rates of carnivorous plant turions are relatively low when compared to those of adult shoots of the same species, turions lose major part of their storage carbohydrates over winter (Winston & Gorham 1979a; Adamec 1999, 2003, 2008). Therefore, under natural conditions (and usually also in a refrigerator), they can survive only from one season to another (Adamec 1999, 2003). It was demonstrated in *U. vulgaris* turions that their overwintering and stages of dormancy were regulated by native phytohormones, mainly by abscisic and gibberellic acid (Winston & Gorham 1979b; see also Minorski 2003).

The life-spans of intact wet turions are relatively short (Adamec 2003). If dried turions have a longer life span, storing turions after a drying period might improve their long-term survival. The aim of this paper was to investigate sprouting of turions of four aquatic carnivorous plant species after various periods of drought in a combination with frost.

### Materials and Methods

Experiment #1 began on 9 March 2003 and tested the effects of cold desiccation on dormant turions. Thirty turions of *U. ochroleuca*<sup>1</sup> collected from a wet sediment in an outdoor culture (which had been dormant for about five months and had withstood a normal Czech winter (temperatures ranging from about -20°C to 6°C), and twenty turions of *U. australis* overwintered in water in a refrigerator at 3±1°C, were thoroughly blotted dry. As a drying treatment, they were put on an open Petri dish in a refrigerator at 3±0.5°C and 63-65% RH for 5 days. As a control sample, wet turions of both species were kept on a wet paper tissue in the same refrigerator for the same time. After 5 days, dry and wet turions were allowed to sprout in tap water in natural light (day-length approximately 11.5 h) at 20±1°C. The time of the first sprouting symptoms and percentage of turion sprouting were estimated for 7 days as this period markedly extended that necessary for full sprouting of the controls. Turions were scored as sprouting if they distinctly reflexed their basal leaf whorls and partly opened themselves (see Adamec, 2003).

Experiment #2 was started on 18 March 2003, and explored the effects of desiccation and freezing on dormant turions. We used 5-10 turions of each of *Aldrovanda vesiculosa* (E Poland), *U. aus-*

<sup>1</sup>All references to *U. ochroleuca* in this paper involve plants that, on the basis of microscopic characteristics, could be considered *U. stygia*.



Figure 1: *Utricularia* turions. Clockwise from top left: *Utricularia vulgaris*, *U. stellaris*, *U. macrorhiza* (Massachusetts, USA), *U. intermedia* (Massachusetts, USA), *U. ochroleuca* s. lat. (*U. stygia*). All images by author, except *U. macrorhiza* and *U. intermedia* by Barry Rice.

*tralis*, *U. breinii* (Lake Oniega in N. Russia), and *U. ochroleuca*). These turions had been previously kept in water in a refrigerator at  $3\pm 1^\circ\text{C}$  for four months, and were then dried at  $3\pm 0.5^\circ\text{C}$  and 63-65% RH for 5 days. Once dried, they were kept frozen at  $-11\pm 1^\circ\text{C}$  for 5 days.

Experiment #3 (also begun on 18 March 2003) tested the hypothesis that dried *Utricularia* turions (*U. australis* turions freshly collected from a dystrophic pool nearby, the other species from a refrigerator) are much more tolerant of freezing at  $-11\pm 1^\circ\text{C}$  for 5 days than wet turions are. To investigate the effect of long-term drying of turions on their sprouting, 10-47 turions of the above 4 species (*U. australis* turions both from the culture and the field) were kept dry at  $3\pm 1^\circ\text{C}$  and 63-65% RH for 370-375 days. Turion sprouting in tap water was tested in a miniphytotron at  $20\pm 1^\circ\text{C}$  in white fluorescent light (irradiance of  $280\pm 30 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; 12/12 h L/D regime).

## Results and Discussion

Turions of *U. ochroleuca* and *U. australis* were able to fully sprout after a 5-day desiccation period and the first sprouting symptoms were visible after 2 days (Table 1). Sprouting turions grew and sprouted normally. Moreover, the dried turions of both species sprouted slightly faster than the control ones. Thus, drying out reduces partly not only the innate dormancy (Maier 1973b) but also the imposed dormancy of turions. The protective effect of turion drying upon subsequent freezing was clearly demonstrated in *U. australis* (Table 2); in this species, control wet turions were totally killed by freezing at  $-11\pm 1^\circ\text{C}$  for 5 days, while dried ones sprouted fully. However, while *U. australis* turions were able to fully sprout after this combined treatment, only 20% of *U. breinii* turions survived and *U. ochroleuca* turions did not survive at all (Table 2). *Aldrovanda* turions proved to be very susceptible to drying out and a 5-day desiccation period killed them totally, regardless of freezing treatment. During rehydration, the *Aldrovanda* turions released a yellow naphthoquinone plumbagin after 30-40 min which indicated their dead status. After one-year desiccation period, only *U. australis* turions were viable (Table 2). Minor differences in germination were found between the batches of *U. australis* turions; those overwintered in a refrigerator germinated at 100%, while those collected from the field at only 89%.

In this study, great interspecific differences were found in the tolerance of short-term (5 days) drying of turions of aquatic carnivorous plants. Turions of the *Utricularia* species tested were tolerant of drying while *Aldrovanda* turions were intolerant of. It is not clear what physiological differences cause these disparate responses. The temperate *Utricularia* and *Aldrovanda* all grow in ecologically similar shallow dystrophic waters. During the summer season, the probabilities of their growing shoots becoming stranded and drying out should be the same. On the other hand, considerable ecological differences between both genera can occur during turion overwintering. As *Aldrovanda* turions detach from mother shoots and sink to the bottom in autumn, the turions of these *Utricularia* do not. So while only a negligible portion of *Aldrovanda* turions are at risk of drying and freezing over winter (Adamec 1999), a relatively large fraction of *Utricularia* turions are at risk of drying out or freezing over winter. So while the origins of the differences in the tolerance of drying out and freezing between both genera are not well understood, their value of these adaptations to the environment are clear and reflect differences in turion ecology.

The tolerance of *Utricularia* turions of desiccation and freezing apparently varies within the genus (Table 2). In some *Utricularia* species, great resistance of turions to drying out and freezing could be of a great ecological importance. However, it is not clear which physiological characteristics result in the different tolerances turions have of drying and freezing (Table 2). Since the tolerance of *U. vulgaris*

Species	Number of turions	Drought period (d) at $3\pm 1^\circ\text{C}$	First sprouting symptoms (d)	% of sprouting (by the time)
<i>Utricularia ochroleuca</i>	20	0 (control)	2	100 (3 d)
-''-	30	5	2	100 (3 d)
<i>Utricularia australis</i>	20	0 (control)	2	100 (3 d)
-''-	20	5	2	100 (3 d)

Table 1. Percentage of sprouting of turions at  $20\pm 1^\circ\text{C}$  and 11.5/12.5 h L/D regime on 9 March. Before the experiment, the turions overwintered in water at  $3\pm 1^\circ\text{C}$  in darkness (*U. australis*) or outdoors on a wet sediment (*U. ochroleuca*). Treated turions were dried at  $3\pm 0.5^\circ\text{C}$  and 63-65% RH in darkness.

turions of drying decreases markedly during the overwintering period (Maier 1973a) it seems likely that decreasing starch (and free sugar?) content in turions (Winston & Gorham 1979a; Adamec 1999, 2003) may be responsible for this decreasing tolerance. Another possibility might be the different phytohormone content turions have in different part of their overwintering (Winston & Gorham 1979b).

It is well-known that stored plant material is able to withstand freezing much better at a dried state than at a wet (hydrated) state. The results show that the effect of drying out on *Utricularia* turion sprouting is rather of a quantitative than qualitative character, depending on the length of the dried state (*cf.* Tables 1, 2). *Utricularia ochroleuca* and *U. bremii* turions were able to sprout after 5 days of drying out but one-year drying killed them totally. Therefore, it is possible to assume that certain, though very low, respiration rate persists even in dried turions kept at  $3\pm 1^\circ\text{C}$  in a refrigerator. Out of all four aquatic carnivorous plant species investigated, turions of *U. australis* showed clearly the greatest tolerance of drying and freezing. This great tolerance of *U. australis* turions might be associated both with the eurytopic character of this species and its cosmopolitan spread on all continents of the Old World. Out of all European temperate *Utricularia* species, only *U. australis* turions were able to survive in water in a refrigerator at  $3\pm 1^\circ\text{C}$  for 23 months and sprout (Adamec unpubl.). Nevertheless, if a long-term survival of autumnal, freshly collected *Utricularia* turions at a dry state in a refrigerator is much better than that in water, then storage of dry turions might be a convenient way of their long-term keeping in collections.

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Species	Number of turions	Drought period at $3\pm 1^\circ\text{C}$ (d)	Exposure to $-11^\circ\text{C}$ in dry state (d)	First sprouting symptoms (d)	% of sprouting (by the time)
<i>U. ochroleuca</i>	10	5	5	—	0 (7 d)
<i>U. australis</i> <sup>1</sup>	5	5	5	2	100 (2 d)
<i>U. bremii</i>	10	5	5	2	20 (7 d)
<i>Aldrovanda</i>	10	0 (control)	0	2	100 (3 d)
<i>Aldrovanda</i>	10	5	0	—	0 (7 d)
<i>Aldrovanda</i>	10	5	5	—	0 (7 d)
<i>U. australis</i> <sup>2</sup>	10	0 (control)	0	2	100 (2 d)
<i>U. australis</i> <sup>2</sup>	10	5	0	2	100 (4 d)
<i>U. australis</i> <sup>2</sup>	10	0	5 (wet turions)	—	0 (7 d)
<i>U. australis</i> <sup>2</sup>	10	5	5	2	100 (4 d)
<i>U. australis</i> <sup>1</sup>	15	375	0	1.5	100 (2.5 d)
<i>U. australis</i> <sup>2</sup>	47	370	0	1.5	89 (7 d)
<i>U. ochroleuca</i>	20	375	0	—	0 (4 d)
<i>U. bremii</i>	20	375	0	—	0 (4 d)
<i>Aldrovanda</i>	10	375	0	—	0 (4 d)

<sup>1</sup>Turions overwintered in a refrigerator.

<sup>2</sup>Turions collected fresh from the field before the experiment.

Table 2. Percentage of sprouting of turions at  $20\pm 1^\circ\text{C}$  and 12/12 h L/D regime on 18-23 March. Before the experiment, the turions overwintered in water at  $3\pm 1^\circ\text{C}$  in darkness. Treated turions were dried at  $3\pm 1^\circ\text{C}$  and 63-65% RH in darkness. Some variants of dry turions were exposed to the frost of  $-11\pm 1^\circ\text{C}$ . Different experimental runs are separated by dashed line.

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