Is long-term survival of dried turions of aquatic carnivorous plants possible?

Lubomír Adamec • Institute of Botany of the Czech Academy of Sciences • Dukelská 135 • CZ-379 82 Třeboň • Czech Republic • lubomir.adamec@ibot.cas.cz

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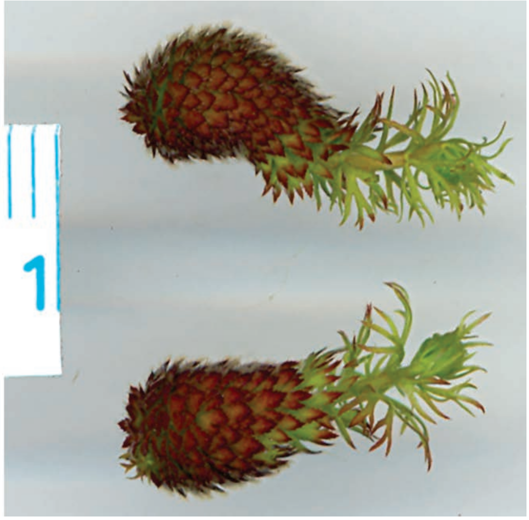
Introduction

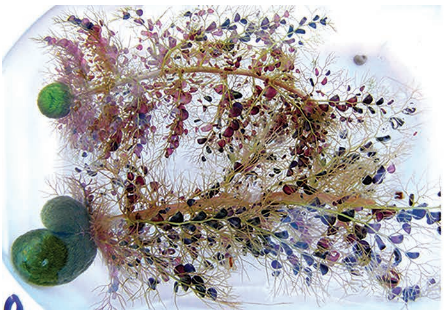
Turions of perennial aquatic carnivorous plants of the genera *Aldrovanda* and *Utricularia* are dormant, overwintering buds and are formed by extreme condensation of short, modified leaves in the shoot apex at the end of the growing season (Fig. 1) (Sculthorpe 1967; Bartley & Spence 1987; Adamec 1999). These tough organs represent also storage organs both for carbohydrates and some mineral nutrients (N, P; Winston & Gorham 1979a; Adamec 1999, 2003, 2010, 2011; Płachno *et al.* 2014) and their respiration rate is greatly reduced as compared to that of summer shoots (Adamec 2003, 2008a, 2011). Turions are partly frost-resistant organs which usually overwinter in shallow waters close to the bottom and escape from being included in ice (Adamec 1999). In some species and very shallow habitats, however, turions overwinter also above the water surface in the terrestrial ecophase, on wet organic substrate. Thus, these turions can face both freezing and drying in these microhabitats. Adamec & Kučerová (2013) investigated the characteristics of frost-resistance of turions of 8 aquatic carnivorous plant species and measured the freezing temperature of autumnal, dormant, non-hardened turions (interpreted as freezing of extracellular water) within a range of -7.0 to -10.2°C, whereas the freezing temperature of outdoor hardened turions of 6 species was within a very narrow range of -2.8 to -3.3°C. These characteristics suggest that turions can be hardened by weak frosts and that their hardiness is based on the shift from frost avoidance in non-hardened autumnal turions to frost tolerance in the spring.

Data are available that turions of several *Utricularia* species are able to survive drying and sprout. Maier (1973a) found that *U. vulgaris* turions dried for 1-123 days were able to sprout and grow. However, the older the turions were, the less tolerant of drying they were. Turions refrigerated for five months before being dried out survived very poorly. 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: from 12-48 days in control turions to only 5-7 days in dried ones (Maier 1973b). In another study, Adamec (2008b) found that the survival of spring dried turions was distinctly species-specific: *U. australis* and *U. ochroleuca* s. str. completely survived drying at 3°C for 5 days, but *U. bremii* and *Aldrovanda vesiculosa* did not. *U. australis* showed to be very resistant to drying and survived at 89-100% even a long dry period of 375 days, but *U. ochroleuca* did not at all. Moreover, dried *U. australis* turions even survived (at 100%) freezing at -11°C for 5 days indicating a possibility for a long-term turion survival in dry state. As turions lose major part of their storage carbohydrates over winter (Winston & Gorham 1979a; Adamec 1999, 2003, 2008a), under natural conditions (and also in a refrigerator), they can survive only from one season to another (Adamec 1999, 2003). On the physiological level, the

Aldrovanda vesiculosa



Utricularia stygia



Utricularia vulgaris

Utricularia macrorhiza

Utricularia bremii

Figure 1: Ripe or just sprouting turions of *Aldrovanda vesiculosa*, *Utricularia stygia*, *U. vulgaris*, *U. macrorhiza*, and *U. bremii*.

overwintering and stages of dormancy in *U. vulgaris* turions are regulated by native phytohormones, mainly by the ratio of activities of abscisic and gibberellic acid (Winston & Gorham 1979b).

The aim of this paper was to investigate survival of turions of 6 aquatic carnivorous plant species after long periods of drought in a combination with frost, to test a possibility to prolong the relatively short turion life-span when stored in water in a refrigerator. A protective effect of paraffin oil on turion storage was also tested.

Materials and Methods

Ripe dormant turions of *Utricularia australis* (collected from two different sites from Třeboň basin, S Bohemia, Czech Rep.: humic pool at Ptačí blato and at Pihulík), *U. intermedia* and *U. stygia* (formerly *U. ochroleuca* s. lato; both species from Třeboň basin), and *U. bremii* (from Lake Oniega, NW Russia; the latter three species freshly collected from the outdoor collection of the Institute of Botany at Třeboň) were thoroughly washed by tap water, blotted dry, put on an open Petri dish, and let dry out in a refrigerator at 3±1°C and 59±2% RH for 4 days. On 3 Nov. 2006, dozens of dried turions were put in plastic vials and kept either frozen at -12±1°C, refrigerated at 3±1°C or in darkness at room temperature of 20 to 26°C for 17 months (517 days). After this treatment, 15-20 turions of each variant were put in filtered humic culture water in a miniphytotron at 20±1°C in white fluorescent light (ca. 180 μmol m^{-2} s^{-1}; 12/12 h L/D) for 26 days to test their survival and sprouting ability. As the turion sprouting of some species was very low indicating that the innate dormancy might not be broken sufficiently by the drying (*sensu* Maier 1973b), turions in the culture water were then put to a refrigerator at 3±1°C for 2 months to break their innate dormancy. After this cold treatment, turions were allowed to sprout in the miniphytotron at 20±1°C in light for another 30 days. Non-sprouting but still living turions were then transferred outdoors and the vials with turions were let floating on the surface of an outdoor growth container in full sun at ca. 20-30°C for two days. Twenty parallel dried *U. australis* turions (from Pihulík pool) were kept frozen at -12±1°C for 29 months (882 days), then put in the culture water at 3±1°C for 31 days to break the innate dormancy, and were then allowed to sprout in the miniphytotron at 20±1°C in light. Another 20 parallel dried *U. australis* turions were kept in darkness at 20 to 26°C for 64 months (1936 days) and were then allowed to sprout at 20±1°C in light.

In another experiment on 2 Nov. 2011, 10-20 freshly collected dormant turions of each of *A. vesiculosa* (from SW Hungary, collected from Suchdol nad Lužnicí sand-pit), *U. australis* and *U. bremii* (both latter species taken from the collection) were thoroughly blotted dry, put in a small plastic vial with liquid paraffin oil, and kept refrigerated at 2±1°C for 68-81 days to learn whether this water-free medium can facilitate turion survival at low temperature. They were then blotted dry, put in the culture water and allowed to sprout in the miniphytotron at 20±1°C in light. In another experiment on 11 Dec. 2011, 10 dormant turions of each of *A. vesiculosa* (from E Poland), *U. australis* and *U. ochroleuca* s. str. (from Třeboň basin; all species taken from the collection) were thoroughly blotted dry, put in a small plastic vial with paraffin oil, and kept frozen at -14±1°C for 72 days. Then, turion sprouting was tested in the culture water at 20±1°C in light.

Turions of all species were scored as sprouting if they distinctly reflexed their basal leaf whorls and partly opened themselves (see Adamec 2003, 2008), and as dead if they were blackened without any sign of sprouting. Although the sprouting of control turions, which were kept standardly in the culture water at 3±1°C in darkness, was not estimated within the above experiments, it is well-known from other experiments and tests (e.g., Adamec 2003, 2008b; Adamec & Kučerová 2013) that the percentage of sprouting at 20°C in light in each species was nearly 100%.

Results and Discussion

Similarly as in the previous study (Adamec 2008b), turions of *U. australis* showed to be the most resistant to long-term drying of all species investigated (Table 1). Dried dormant *U. australis* turions were able to survive both freezing at -12°C and keeping at 3°C for 17 months and fully sprouted afterwards only after additionally breaking the innate dormancy by a next 2-month cold treatment and after exposing to bright light. Thus, as opposed to results of Maier (1973b), who dried and stored turions of 4 *Utricularia* species at a relatively high temperature (24±3ºC, 33% RH) for 5-19 days, *U. australis* turions dried at 3±1°C in this study did not break their dormancy at all. However, a weak breaking of dormancy was found in turions of *U. bremii* and *U. stygia* frozen at -12°C. Dried *U. australis* turions could only survive when kept frozen or at 3°C for 17 months, but not at room temperature. In line, parallel dried *U. australis* turions kept at room temperature for 64 months died during rehydration (data not shown). Dried *U. intermedia* turions did not survive the rehydration after both treatments and, except for frozen *U. bremii* turions, the same also applied for turions of *U. bremii* and *U. stygia* (Table 1). Although dried *U. australis* turions were able to fully survive freezing at -12°C for 17 months, they completely died after 29 months of the same treatment during rehydration (data not shown). Thus, a marked species-specificity was found for long-term survival of dried turions at low temperatures.

Table 1. Sprouting of dried *Utricularia* turions originating from outdoor collection but *U. australis*[1], collected from Ptačí blato pool, and *U. australis*[2], collected from Pihulík pool. First, dormant autumnal turions were dried in a refrigerator at 3°C for 4 days. As a treatment, they were kept dry at -12°C, 3°C, or 20 to 26°C for 17 months. Afterwards, they were put in culture water and allowed to sprout in light at 20°C in a thermostat chamber for 26 d. Then, non-sprouting turions were kept in the water in a refrigerator at 3°C and darkness to break dormancy for next 2 months and allowed to sprout in light at 20°C for 30 d again (sprouting scored after 10 and 30 d). The remaining turions were then transferred outdoors where they could sprout in natural light. (dead), all remaining turions were blackened and dead.

Species	Number of turions	Treatment of dry turions for 17 months	Cumulative number of sprouting turions			
			At 20°C after 26 d	After next 2-month cold treatment at 3°C; in light at 20°C after next		Outdoors full sun (after 2 d)
				10 d	30 d	
U. australis[1]	20	-12°C	0	2	4	20
- " -	20	3°C	0	8	11	20
U. australis[2]	20	20 to 26°C	0 (dead)	--	--	--
U. intermedia	15	-12°C	0 (dead)	--	--	--
- " -	15	3°C	0 (dead)	--	--	--
U. bremii	20	-12°C	5	0	0	0 (dead)
- " -	20	3°C	0 (dead)	--	--	--
U. stygia	20	-12°C	3 (dead)	--	--	--
- " -	20	3°C	1 (dead)	--	--	--

Dormant humid turions of three species (*A. vesiculosa*, *U. bremii*, *U. ochroleuca*) survived being immersed in paraffin oil at 2°C for 68-81 days and sprouted then at 83-100% – comparably with controls (data not shown). Similarly, non-dormant turions of the three species kept under these conditions in paraffin oil for 4 months sprouted at 90-100% (data not shown). However, after dormant humid turions of three species (*A. vesiculosa*, *U. australis*, *U. ochroleuca*) had been kept frozen at -14°C for 72 days, all turions died during rehydration (data not shown). Thus, keeping of blotted-dry turions in paraffin oil in a refrigerator at temperatures above zero or in a frozen state does not render any advantage for turion overwintering and cannot be recommended, though the effect of paraffin oil in itself is harmless.

As found recently (Adamec & Kučerová 2013), dormant, non-hardened wet turions of 8 aquatic carnivorous species froze within a range of -7.0 to -10.2°C and freezing was usually lethal for the turions. Out of all tested species in the present and previous study (Adamec 2008b), the turions of *U. australis* were clearly the most resistant both to drying and freezing of dried turions. Yet, the freezing temperature of both non-hardened (-9.0±0.3°C) and hardened turions (-3.1±0.1°C) of *U. australis* lay near the means for all species and did not indicate at all an exceptionally high frost resistance (Adamec & Kučerová 2013). In line, it is not clear which endogenous factor cause *U. australis* turions to be rather tolerant to long-term drying. Though this species grows also in very shallow habitats and can even survive longer periods of growing in the (semi)terrestrial ecophase on wet substrate (Adamec 2008b), another 4 relative species investigated (*U. bremii*, *U. intermedia*, *U. ochroleuca*, *U. stygia*) normally grow also in the terrestrial ecophase on wet substrate and their turions should be adapted better to occasional drying out and freezing over winter – but they are not.

In conclusion, turions of aquatic carnivorous plants have evolved to survive only a limited, ca. half-year period of unfavorable frosty or cold conditions, i.e., from one season to the next. Therefore, due to their metabolism, their life-span even under ecological, cold overwintering conditions is usually limited to ca. a half year (Adamec 2008a,b). Turions of some resistant species are able to withstand drying at low or room temperature and subsequent keeping in cold even for one year and normally sprout afterwards. Yet, drying of dormant, autumnal turions may not be followed by breaking the innate dormancy (Table 1), while drying of non-dormant, spring turions having been kept at above zero temperatures in a refrigerator may be harmless (Maier 1973b). As shown in this study, a long-term keeping of dormant dried turions either at a low temperature (ca. 2 to 3°C) or frozen at ca. -12°C is not possible (except for common *U. australis*). Thus, a long-term keeping of dried turions of aquatic carnivorous plants at any temperature is not possible and cannot be recommended. A storage using a paraffin oil cannot be recommended, either. The best way to overwinter the turions is to keep them at a very low, above zero temperature (1 to 2°C) under water or in a wet state.

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Literature review

By John Brittnacher

Bailey, T. 2015. *Drosera* × *eloisiana*, not *D.* × *belezeana*. Planta Carnivora 37(1): 42-47.

Camus (1891) described a plant he considered a hybrid between *Drosera rotundifolia* and *D. intermedia* and named it *D.* × *belezeana* after the collector, Marguerite Belèze. Jan Schlauer questioned whether the specimen in the Paris herbarium is in fact a hybrid. He suggested to a number of people that the specimen appears to be *D. rotundifolia* and a new type specimen be selected. Bailey (2015) collected a confirmed *D. rotundifolia* × *intermedia* hybrid in the UK and designated it as the type for the hybrid. He also proposed a new name, *Drosera eloisiana*. However in this situation, current rules of nomenclature (ICN Art. 57.1) demand the preservation of the current name rather than the creation of a new name. Stay tuned; this taxonomic drama is not yet finished. There is more likely to be said in the future.

Camus, E.G. 1891. Note sur les *Drosera*, observés dans les environs de Paris. Journal de Botanique 5: 196-199.