Germination rate and longevity of seeds of *Aldrovanda vesiculosa* and *Utricularia vulgaris*

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

The ecological group of aquatic carnivorous plants includes monotypic *Aldrovanda vesiculosa* L. (Droseraceae) and around 60 species of *Utricularia* L. (Lentibulariaceae). Although the majority of aquatic carnivorous species are perennials and propagate mainly vegetatively by branching, shoot fragmentation and turions, the majority of them also set seeds (Taylor 1989; Cross 2012; Cross *et al*. 2016, 2018). While these modes of vegetative propagation in temperate species ensure the production of high plant biomass at sites during each season and turions also serve population overwintering (Adamec 1999a, 2011), functional seeds form a viable seed bank as a safety measure to overcome crucial habitat disturbances (mainly drought) leading to biomass extinction and are usually spread by water birds to colonize new potential distant sites (Santamaria 2002; Cross 2012; Cross *et al*. 2016, 2018).

Temperate native European and introduced North American populations of *Aldrovanda vesiculosa* flower relatively commonly under optimum habitat conditions (Front Cover) but their seed set is rare and limited (Adamec & Tichý 1997; Adamec 1999b; Cross 2012; Cross *et al*. 2015, 2016). *Aldrovanda* produces ovoid, rigid seeds 1.2-1.5 mm long with highly developed honeycomb-like exotesta and it is assumed that they are spread via water birds (Cross 2012; Cross *et al*. 2016, 2018). Older fragmentary pieces of knowledge on *Aldrovanda*'s seed set and germination features (see Cross 2012 and the references therein) have recently been complemented by Cross *et al*. (2016). For freshly collected seeds from SW Australia, the optimum temperature for germination was 25°C in light, while no germination was observed in darkness. Germination was also the best with ethylene and after 8 weeks of cold stratification (5°C) interrupted by an 8-week warm period at 25°C; control seeds were kept at 5°C in darkness. To estimate seed longevity, when fresh seeds in bags were buried in sediment 1 cm deep at a natural site for 6 months, only 20% of the seeds survived and after burial for 1 year, all seeds died. Thus, this result can be interpreted as rather limited seed longevity under natural conditions of bottom sediment which would prevent the existence of a seed bank. On the other hand, at a natural Hungarian site, population recruitment after a several-year break and lake drying out occurred (Adamec, unpubl. data), which confirms a functioning of seed bank for several (ca. 10) years. Moreover, seeds stored in a dry state reduced partly their germination rate after 1 month, but completely after 1 year. Similarly, freezing fresh seeds to -18°C for only 24 h reduced the germination rate by 10-45%, while freezing for 3 months completely inhibited germination (Cross *et al*. 2016). Physiological dormancy is stated for *Aldrovanda* seeds (Cross *et al*. 2018).

European populations of *Utricularia vulgaris* flower and set seeds prolifically (Taylor 1989). The seeds usually ripen in capsules above the surface but fall soon to water and sink to the bottom. The seeds ca. 0.6 mm large have a morpho-physiological dormancy (Holzbauerová 2015; Cross *et al*. 2018). Similar to *Aldrovanda*, *U. vulgaris* seeds germinate better at a higher temperature of 25°C and in light and break their dormancy after weeks of cold stratification, with ethylene, or after a frost treatment (Holzbauerová 2015). Unlike *Aldrovanda*, seeds stored both under water or in dry state for 1-2 years germinated equally well. In *U. vulgaris*, a persistent seed bank exists. Gálová & Hájková (2014) found native seedlings of this species at a former fishpond site in S Moravia, Czech Republic, where the species was considered extinct for ca. 23 years. From organic bottom sediments dated between 70-100 years old, released *U. vulgaris* seeds germinated in light as soon as 6 days. Pieces of evidence thus indicate that the longevity of the *Aldrovanda* seed bank is very variable and much shorter than that of *U. vulgaris*. The aim of this study is to compare the survival and germination rate of *Aldrovanda* and *U. vulgaris* seeds exposed in nylon bags on top of a bottom sediment or slightly buried in it in a sand-pit pool in the Czech Republic for 11 months (cf. Cross *et al*. 2016).

Methods

The experiment was conducted in a ca. 250 m^2 shallow sand-pit pool within the complex of excavated sand-pit Cep I near Suchdol nad Lužnicí in S Bohemia, Czech Republic (48°55'08.5"N, 14°53'3.1"E, 450 m a.s.l., see Fig. 1). The pool is about 15 years old, about 50-60 cm deep and the bottom sediment around 5-10 cm deep consists of fine clayish acidic sand mixed with organic substrate from decomposed plant litter. The pool is supplied with rainwater coming from a near terrace and its quite transparent water is extremely soft (electrical conductivity between 22-187 $\mu S\ cm^{-1}$,

Figure 1: Shallow pool in a sand-pit Cep I near Suchdol nad Lužnicí in S Bohemia, Czech Republic, is inhabited by introduced *Aldrovanda vesiculosa* and *Utricularia bremii*. The seed exposure experiment was conducted near the left shore.

usually only 30-50 µS cm^{-1}), slightly humic (dystrophic) and oligotrophic; pH ca. 6-7 (for details, see Adamec 2009; Cross *et al*. 2016). Native plant dominants in the pool were *Eleocharis palustris*, *E. acicularis*, *Potamogeton natans*, *Juncus bulbosus*, and *J. effusus*. Introduced dominants were *Utricularia bremii*, *Luronium natans*, and *Potamogeton alpinus* (Adamec & Kučerová 2013). Since 2009, also Hungarian red plants of *Aldrovanda* have been growing in the pool. In 2013 and 2014, its population contained at least 5000 plants and in both summer seasons each, at least 1000 plants flowered here and hundreds of ripe capsules were collected (Cross *et al*. 2016). In the 2015 season, its population was drastically decimated by the unknown (perhaps fungal) '*Aldrovanda* disease' (see Adamec 2007) and only dozens of plants have survived up to now.

Ripe seeds of *Aldrovanda* were collected from this pool in summer 2014 and the clean seeds were stored in humic water in a refrigerator for about one year before the experiment. Ripe seeds of *U. vulgaris* were freshly collected from an outdoor culture in the Institute of Botany at Třeboň in summer 2015 and stored in dry state in a refrigerator. Twenty *Aldrovanda* seeds were checked for mechanical stiffness and 40 *U. vulgaris* seeds were put into each of 10 fine nylon bags of 3.5 × 3.5 cm (mesh size 150 µm) and the tagged bags with seeds were closed with a flame. Five bags with the seeds were connected with each other using a nylon line to form a star (Fig. 2). On 1 Sep. 2015, five of the bags were placed on top soil in the pool ca. 40 cm deep near the shore, while the other five were buried 1-2 cm in the soft sediment about 25 cm nearby. Both sets of bags were fixed by a wooden stick. The seeds in the bags were exposed in the pool over winter for 11 months until 30 Jul. 2016. To estimate oxidation-reduction conditions in the exact place of seed exposure of both

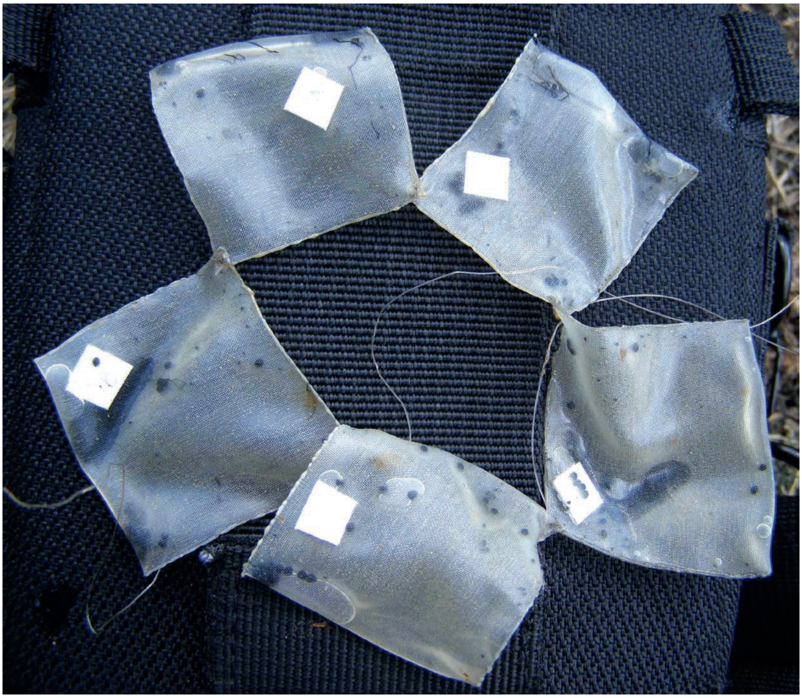

Figure 2: Fine nylon bags with *Aldrovanda* and *U. vulgaris* seeds after they were taken out of the shallow sediment after the 11-month exposure.

variants, the electrical redox potential was measured on the top soil and 1-2 cm deep in the sediment near the exposed bags on 9 Jul. 2016. Platinum and Ag/AgCl reference electrode and a portable digital mV meter were used for the measurements. Ten parallel redox potential measurements were conducted for each variant and the potential of the reference electrode (218 mV) was subtracted from the measured value.

On 30 July 2016, the seed exposure was terminated, the bags were washed by tap water, and the seeds taken out from the bags. Within each bag, just germinating and non-germinating seeds were counted; the remaining seeds were considered damaged as no intact seed could escape from the bags. The remaining clean seeds from each bag were put in a small translucent plastic box in 20 ml of filtered humic water to germinate. All 10 plastic boxes were put into a 3.5-l miniaquarium filled with tap water which floated in an outdoor 2 m^2 plastic container. The seeds in humic water were thus naturally lit and exposed near the water surface at temperatures similar to those in natural habitats. The boxes with the seeds were alternatively exposed outdoors or at ca. 3.5°C in darkness in a refrigerator to break seed dormancy (*sensu* Holzbauerová 2015; Cross *et al*. 2016). After 4 days of outdoor exposure, they were put into a refrigerator on 3 Aug. 2016 for 3 days until 6 Aug. 2016. Then, after 6 days of outdoor exposure on 12 Aug. 2016, they were put into a refrigerator for 10 days until 22 Aug. 2016. After 9 days outdoors, they were put into a refrigerator on 31 Aug. 2016 for 3 days. On 3 Sep. 2016, they were exposed outdoors for 11 days until 14 Sep. 2016. After 5 days in a refrigerator, on 19 Sep. 2016, they were exposed outdoors for 29 days until 18 Oct. 2016. The remaining seeds then overwintered in a refrigerator for 9 months until 24 Jul. 2017. After another 13-day outdoor exposure, the boxes were put in a refrigerator on 6 Aug. 2017 for 8 days until 14 Aug. 2017. They were then exposed outdoors until 4 Sep. 2017 for 21 days and then put into a refrigerator until 14 Sep. 2017 for 10 days. They were then exposed outdoors until 2 Oct. 2017 for 18 days. At the end of each outdoor exposure, germination of seeds was scored and germinated seeds were removed from the boxes. The cumulative germination rate within each box (bag) is expressed in % of the original seed count. Means ± 1 SE intervals are shown for 5 bags within each variant but the data clearly followed a non-normal distribution.

Results and Discussion

The redox potential on the top soil was -209.8 ± 8.7 mV, while that 1-2 cm deep in the bottom was -376.4 ± 6.6 mV, indicating an anoxic zone in the sediment. Between 3-7% of seeds were damaged and their testa was ruptured at the conclusion of 11 months of storage in sediment or top soil (Table 1). The difference between both treatments is not marked. The first seeds started rarely germinating only after two short cold/warm stratification treatments. However, the germination was markedly stimulated after the next 11-month cold stratification in a refrigerator as a result of which about 19% of the seeds germinated in light outdoors (Fig. 3). A further germination was very low. Unlike these results, fresh *Aldrovanda* seeds did not survive a one-year period of shallow burial in a bottom sediment at an Australian site (Cross *et al*. 2016).

Only 3-5% of *U. vulgaris* seeds were damaged after the field exposure in both variants (Table 1). Generally, the germination pattern was very similar to that in *Aldrovanda*. First *U. vulgaris* seeds germinated as early as at the end of the field exposure in the bags in the top soil variant. Germination was very low even after two short cold/warm stratification treatments. Seed dormancy was markedly broken after the next 11-month cold stratification and about 37% seeds germinated in light outdoors afterwards (Fig. 3). Only the next 2-3% of the seeds germinated by the end of the summer season.

Table 1. Results of the cumulative germination rate of *Aldrovanda vesiculosa* (AV) and *Utricularia vulgaris* (UV) seeds during the 2016 and 2017 seasons. On 1 Sep. 2015, five parallel nylon bags each with 20 seeds of *A. vesiculosa* and 40 seeds of *U. vulgaris* were placed on the top of a loose sediment (variant T) and another five bags were buried 1-2 cm deep in the sediment of a shallow sand-pit pool (variant B; see Fig. 1) for 11 months until 30 Jul. 2016. Damaged and germinated seeds were evaluated (in % of all seeds for each bag, means ± SE intervals shown, n=5). Seeds of both species were allowed to germinate in natural light in humic water in translucent vials. Light exposure was interrupted by periods of dark cold treatment at 3-4°C (see the text).

	30 Jul. 2016		12 Aug. 2016	31 Aug. 2016	31 Jul. 2017	4 Sep. 2017	2 Oct. 2017
Species	Damage	Cumulative germination rate (%)					
AV-T	7.0 ± 3.8	0	0	0.5 ± 0.5	19.0 ± 4.3	20.0 ± 4.7	20.0 ± 4.7
AV-B	3.0 ± 2.0	0	0	0.5 ± 0.5	20.0 ± 5.2	21.0 ± 5.6	21.0 ± 5.6
UV-T	5.0 ± 1.6	1.0 ± 1.0	1.5 ± 1.0	2.0 ± 0.9	39.5 ± 5.5	41.0 ± 5.7	41.5 ± 6.0
UV-B	3.0 ± 2.4	0	0.5 ± 0.5	1.0 ± 1.0	37.5 ± 5.3	39.5 ± 4.4	40.5 ± 5.3

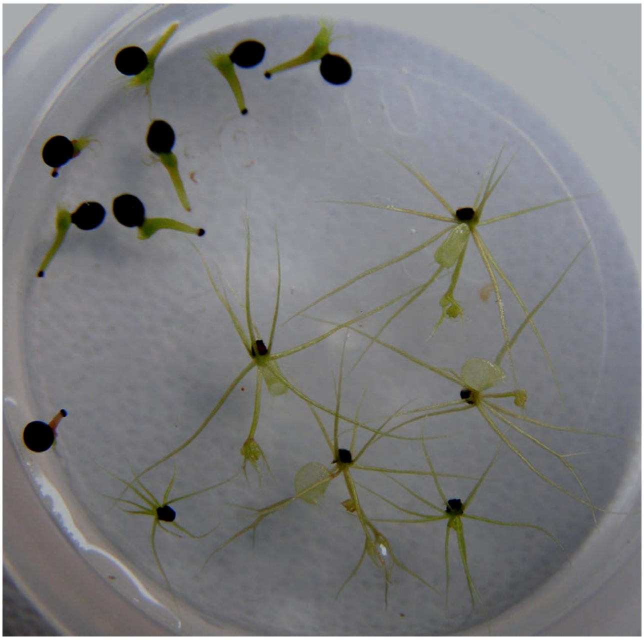

Figure 3: Germinating seeds of *Aldrovanda* (top) and *U. vulgaris* from the experiment. The diameter of the plastic lid is 25 mm. Photos by Lubomír Adamec.

Conclusions

The seasonal seed exposure on the top soil or 1-2 cm deep in the sediment had no influence on seed longevity and subsequent germination rate in *Aldrovanda* and *U. vulgaris* seeds. This indicates that a deeper anoxia is not harmful for these seeds. For both species, the germination rate in single bags was highly variable. The longevity of *Aldrovanda* seeds under natural conditions in water can be very variable and be shorter than one year but also longer than 2-3 years. The factors which decide seed longevity might be the population origin (temperate *vs*. subtropical), plant batch, or natural conditions on the bottom or in the sediment.

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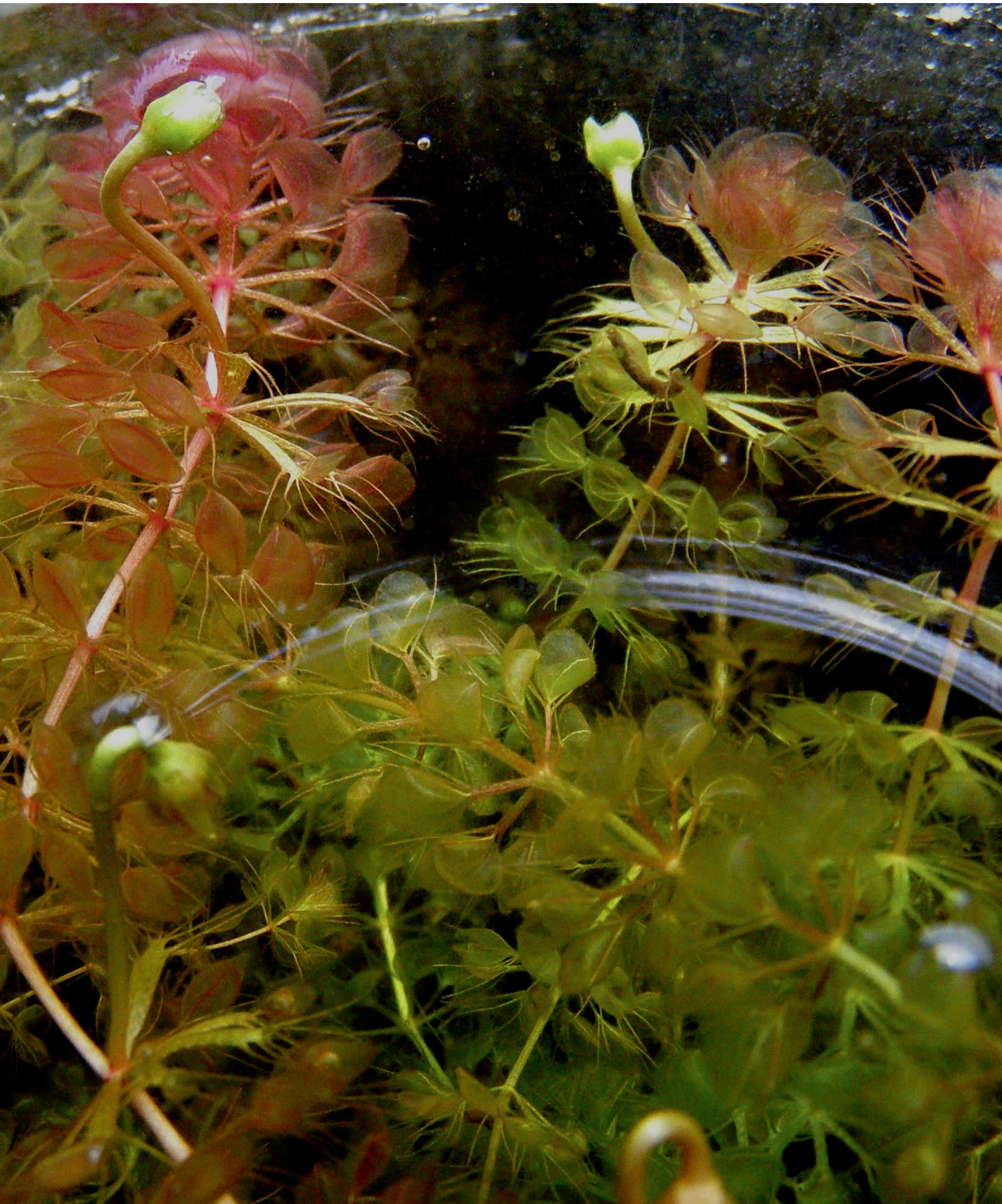

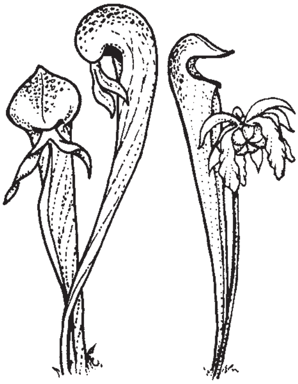

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Front Cover: Flowering African *Aldrovanda vesiculosa* from Botswana in an indoor culture. Photo by Lubomír Adamec. Article on page 64.

Back Cover: A cluster of *Nepenthes ampullaria* lower pitchers growing in Gunung Mulu National Park, Borneo. Photo taken by Richard Nunn. Article on page 47.

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International Carnivorous Plant Society, Inc.
2121 N. California Blvd., Suite 290
Walnut Creek, CA 94596-7351, USA
icps@carnivorousplants.org

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