Effects of aluminium toxicity and low pH on the early development of *Isoëtes echinospora*

Vliv toxicity hliníku a nízkého pH na raný vývoj *Isoëtes echinospora*

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A relict population of *Isoëtes echinospora* Durieu survived a thirty-year period of severe acidification and high concentrations of phytotoxic aluminium (Al) in Plešné Lake (Bohemian Forest, Czech Republic). The population consisted of only adult plants. Sporeling survival and age structure were examined during the population recovery in 2004–2008. Laboratory experiments were conducted to assess the effect of various pH values (4–8) and Al concentrations (0–1000 $\mu g \cdot l^{-1}$) on sporeling development. The responses of the sporelings to the experimental treatments were evaluated and compared with those observed in the lake. The experiments showed that an Al concentration higher than 300 $\mu g \cdot l^{-1}$, and high acidity (pH 4), inhibit sporeling growth, in particular resulted in a pronounced reduction in absorptive organs (macrogametophyte rhizoids, roots and root hairs). With increasing concentrations of Al and at pH 4, the ratio of the below-ground to above-ground sporeling biomass decreased to less than 1. The responses of the lake sporelings, rooting in the upper sediment layer, were similar to those exposed to 100–300 $\mu g \cdot l^{-1}$ of Al in the laboratory, and reflected the Al toxicity of the lake water. The quillworts at Plešné Lake survived because adult plants can tolerate these adverse conditions and are very long-lived. The population recovered when the pH of the water increased to over 5 and the Al concentration decreased to below 300 $\mu g \cdot l^{-1}$.

Keywords: acidification, demography, *Isoëtes echinospora*, quillwort, recovery, reproduction, sporeling

Introduction

The quillwort *Isoëtes echinospora* Durieu is a perennial submerged aquatic lycopsid and a characteristic macrophyte of oligotrophic softwater lakes in N and W Europe (Arts 2002, Roelofs et al. 2002, Smolders et al. 2002). In the Czech Republic, it is a critically endangered glacial relict found only in Plešné Lake (Bohemian Forest, Šumava Mts, Böhmerwald). The population has survived severe acidification caused by industrial air pollution for approximately thirty years. During this time, the pH of the lake water decreased to below 5 and the concentration of aluminium (Al) increased to as high as ~1000 $\mu g \cdot l^{-1}$ in the 1980s (Veselý et al. 1998, Majer et al. 2003). Both the high acidity and Al toxicity caused the extinction of the fish, a substantial change in the composition of the plankton in the lake (Veselý 1994, Vrba et al. 2003) and a decline in the abundance of *I. echinospora*.

In the 1990s, repeated inspections revealed that the *I. echinospora* population consisted entirely of adult plants (Husák et al. 2000). Other reports indicate that the plant cover declined markedly when re-colonization by sporelings did not occur after adult plants were damaged by human activities. Elsewhere throughout Europe, populations of *I. echinospora* declined or disappeared from many acidified oligotrophic lakes (Roelofs 1983, 1996, Arts et al. 1990, Bobbink et al. 1998, Arts 2002, Roelofs et al. 2002, Smolders et al. 2002, Szmeja & Bociag 2004).

Although the effects of acidification on softwater macrophyte communities are described by others (Arts 2002, Szmeja & Bociag 2004) the causal factors and their interaction are less well understood. *Isoëtes echinospora* is considered to be sensitive to low pH (Arts 2002, Brouwer et al. 2002). In Scandinavia and the Southern Baltic lowlands this species has persisted, at least temporarily, in conditions of pH 4.5–5.0 (Rørslett & Brettum 1989, Szmeja et al. 1997, Vöge 1997, Roelofs et al. 2002) without showing any symptoms of stress (Szmeja 1994a, Vöge 1997).

Quillworts, however, become extinct in lakes where the pH of the water is permanently below 4.5 (Szmeja et al. 1997). In acidified lakes, small slow-growing quillworts are outcompeted by robust, fast-growing species, e.g. *Juncus bulbosus*, *Sphagnum* sp., or by nymphaeids, filamentous algae etc., especially in eutrophic lakes (Roelofs 1983, Bobbink et al. 1998, Arts et al. 1990, Sand-Jensen et al. 2000, Roelofs et al. 2002, Smolders et al. 2002). Nevertheless, it is unknown whether the disappearance of quillwort is due to interspecific competition (Schuurkes et al. 1988, Farmer 1990, Arts 2002) as they coexist with these species in acid-tolerant communities (Arts 2002). Interspecific competition was virtually eliminated during the acidification of Plešné Lake as *I. echinospora* is now the only plant in the lake (Husák et al. 2000).

Elevated Al levels are reported to have little or no effect on quillworts in N European lakes (Maessen et al. 1992, Roelofs et al. 2002), but their reproduction and demography was not studied. Phytotoxicity of Al is caused by ionic forms at low pH and characterized by a rapid inhibition of root growth, decrease in nutrient uptake and poor growth of the plants (Kochian 1995, Matsumoto 2000, Mossor-Pietraszewska 2001, Rout et al. 2001). The most noticeable effect of Al toxicity is a severe inhibition of the growth of both the main and lateral roots. They are usually stubby and brittle, and the root tips and lateral roots are broad and may turn brown (Mossor-Pietraszewska 2001, Rout et al. 2001). Aluminium is reported not to inhibit germination but to impair the growth of new roots and establishment of plantlets (Nosko et al. 1988).

Quillwort reproduction relies exclusively on spore production. This study determined the effect of Al toxicity and acidity on sporeling (i.e., plantlet) establishment and survival, which is a fundamental prerequisite for population renewal. The aims were to (i) asses experimentally the effects of Al toxicity (at pH 5) and various pH values on *I. echinospora* sporeling establishment, (ii) examine sporelings in the lake for the symptoms of Al toxicity recorded in the experiments, and (iii) determine the age structure of the population in Plešné Lake.

Material and methods

Sampling site

The only population of *I. echinospora* in the Czech Republic is in Plešné Lake (48°47' N, 13°52' E, 1087 m a.s.l.). This mesotrophic lake is situated in the Šumava National Park, in a rather small, geologically sensitive catchment area on granitic bedrock, which is forested by Norway spruce (Kopáček et al. 2004). The effect that the acidification of the lake by athmospheric pollution had on water chemistry and biota is extensively reported by e.g., Majer et al. (2003) and Vrba et al. (2003); surface pH and Al concentrations recorded over the past decade are shown in Fig. 1 (Kopáček et al. 2006 and unpublished data).The only macrophyte growing in the lake is quillwort, where it covers the inshore area of ~300 m^2 at depths ranging from 0.3 to 1.0 m.

Quillwort morphology and ontogenesis

Rhizoids are fibrous structures that grow from the surface of macrogametophytes after fertilization (Eames 1936, Foster & Gifford 1959, Bennert et al. 1999; Fig. 2A). They rise from the macrogametophyte in the spore surrounding. Later, sporeling sprouts begin to mature, first producing a leaf then a root, and then a second leaf and root etc. (Eames 1936, M. Čtvrtlíková unpublished results; Fig. 2A). Quillwort sporelings remain attached for weeks or even months to the rich storage tissue of the macrogametophyte, until they reach the 2–4 leaf stage (Eames 1936, Foster & Gifford, 1959). In this study the term "sporeling" denotes the stage in the development of a sporophyte when there are at most three leaves. A juvenile sporophyte has four or more leaves and a rosette of leaves, which unlike in adult plants is not radially symmetrical.

In situ observations

Age structure of the quillwort population in Plešné Lake was studied to determine sporeling survival and population renewal. This was done by snorkelling during 2004–2008. Adult and juvenile plants were counted every May–July and the age structure of the whole population evaluated. All the plants in the population were counted in strip transects (0.5 × 10–30 m).

In September 2007, 38 rooted sporelings of *I. echinospora* were taken from the lake using a syringe sampler (200 ml). These sporelings were examined microscopically and compared with those that developed in the experiments (see below).

In vitro experiments: plant material and growth conditions

Mature spores of *I. echinospora* were obtained from intact and open sporangia of 60 macro-trophosporophylls and 60 micro-trophosporophylls harvested from the lake in October 2006. In the laboratory, the spores were released and cleaned of debris by rinsing them with distilled water. The spores were then kept in the dark in distilled water for five months at 5°C.

Five ml of a suspension of microspores and macrospores (92–286 spores) were added to Petri dishes (55 mm in diameter; 5–8 dishes as replicates) containing 12 ml of a solution similar in chemical composition to the lake water (in mg·l^{-1}: 5.57 SO_4^{2-}, 0.49 Cl^-, 1.01 Na^+, 0.47 K^+, 1.08 Ca^{2+}, 0.24 Mg^{2+}; according to Bittl et al. 2001). The solution was enriched

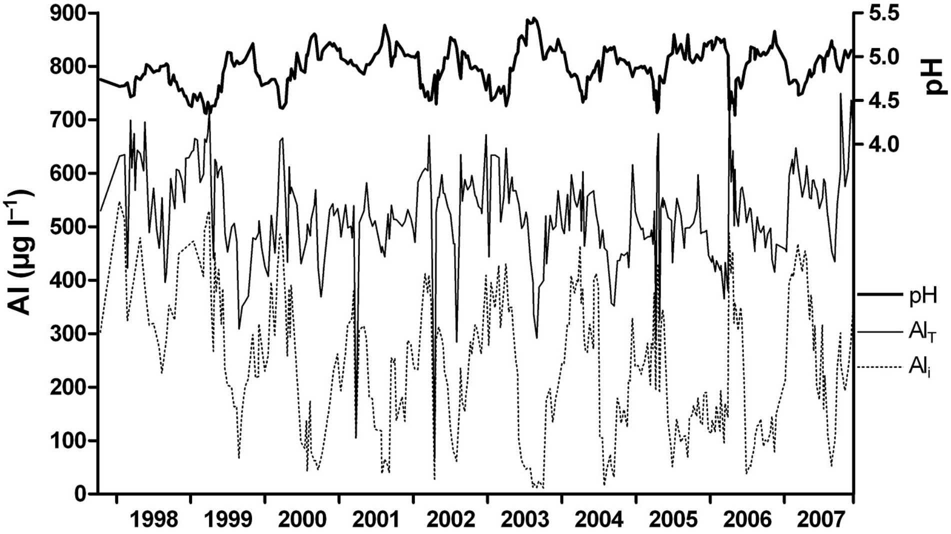

Fig. 1. – Annual variations in pH and aluminium concentration in the surface water of Plešné Lake. Al_i – ionic Al, Al_T – total Al. Data from Kopáček et al. (2006) and unpublished sources.

with macronutrients (in mg·l^{-1}: 0.48 P and 0.05 N as $NaH_2PO_4{\cdot}2H_2O$ and NH_4NO_3, respectively). As the toxic effect of Al only becomes pronounced at a low pH (e.g., Stanley 1974, Arts et al. 1990, Maessen et al. 1992, Rai et al. 1998) the experiment on Al toxicity was done at pH 5, which is similar to the pH of the lake water and avoids the negative effect of H^+ at lower values of pH. The concentration of Al (c_{Al}) were adjusted by the addition of $AlCl_3{\cdot}6H_2O$ giving: 0 (Al control), 100, 300, 500 and 1000 µg·l^{-1}. According to Stumm & Morgan (1981), ionic forms of Al (Al^{3+}, $Al(OH)^{2+}$, $Al(OH)_2^+$), i.e. the Al_i present in lake water (Fig. 1), account for over 85% of total Al added at pH 5. The pH experiments were conducted at pH of 4, 5, 6, 7 and 8 obtained by the addition of HCl and $NaHCO_3$. The culture medium was changed every four days. These experiments were carried out at 16°C and a 14:10 light:dark period provided by fluorescent lights (PAR = 100 µM·m^2·s^{-1}) over a period of three and half months (March–July 2007).

The appearance of the first archegonium, leaf and root were recorded. At the end of the experiments, the sporelings with leaves and roots were examined for symptoms of Al toxicity and/or acidity, and the length of the sprouts measured.

Plants were examined using a stereomicroscope (Olympus SZ61; magnification 5–45×) and an inverted microscope (Olympus IMT 1; magnification 40–200×). Due to the curved shape of sprout, the length is expressed in terms of the diameter of a macrospore (MD); one MD equals ~0.46 mm (Fig. 2A). The cumulative length of either the leaves or the roots is a sum of the lengths of all the sprouts, expressed in MD units. The total length of the sporelings was the sum of cumulative lengths of all the sprouts. Furthermore, the total surface area of absorptive sprouts (absorptive area, AB) was calculated and related to the total surface area of leaves (assimilative area; AS), using the following formula:

$$AB{:}AS=(\pi d_G \Sigma\, l_G + \pi d_R \Sigma\, l_R + \pi d_H \Sigma\, l_H) / \pi d_L \Sigma l_L.$$

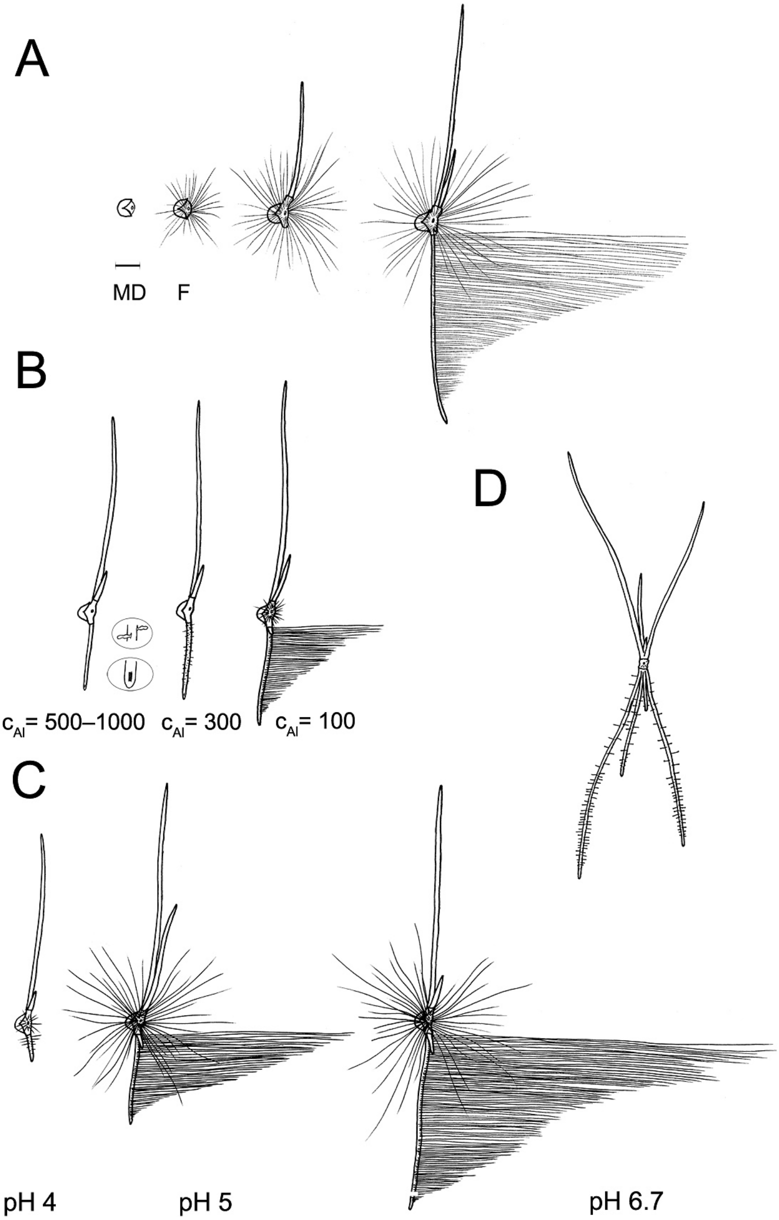

Fig. 2. – Illustrations of the early stages of development of quillworts (A) that developed in the different experimental treatments (B, C) and of sporelings collected from the lake (D). Sporeling dimensions are proportional. MD: macrospore diameter (~0.46 mm) is used as an arbitrary unit of length throughout this paper. Early development (A): After fertilization (F) rhizoids develop on the macrogametophyte in the area surrounding the spore, emergence of the first leaf is followed by other sporofyte sprouts. Treatments involving the addition of Al (B): elevated Al concentrations (c_{Al} in $\mu g \cdot l^{-1}$) inhibited growth of rhizoids, roots, root hairs and leaves. Details of the stubby root hairs and root-tip necrosis are shown in the small insets and a drawing of long root hairs on the right of the figure. Treatments in which the pH is varied (C): pH 4 resulted in inhibition of the growth of sporelings, pH5.0 stimulated the growth of leaves but inhibited root growth, pH 6 and 7 are optimum conditions for quillwort growth. Morphology of the lake sporelings (D) corresponds to that of sporelings that developed in the c_A 100–300 $\mu g \cdot l^{-1}$ treatments.

The surface area was calculated assuming a cylindrical shape, without top and bottom, where d is the diameter, l the length and πd the circumference of the sprout. Subscript letters indicate gametophyte rhizoids (G), roots (R), root hairs (H) and leaves (L). The AB:AS ratio was calculated for quillwort sporelings that developed in the experiments and were collected from Plešné Lake. The sprout surface area was used to determine the proportion of the total biomass below-ground as it was not possible to measure the dry weight because of the small size of the sporelings.

Statistical analysis

The effects of Al and pH on sporeling growth were analysed using a hierarchical ANOVA (Statistica, StatSoft, Inc., Tulsa, OK, USA), where replicated samples (Petri dishes) were a nested factor in the c_{Al} or pH experiments. To determine differences between treatments a Tukey's HSD test was performed on logarithmically transformed data and differences accepted as significant if $P < 0.05$.

Results

Experiments

As quillwort in the field grows at pHs that are circumneutral (6–7; Roelofs 1983, Rørslett & Brettum 1989, Arts et al. 1990, Szmeja & Bociag 2004) and there were no differences in the growth recorded at pH 6 and 7 in the laboratory, these henceforth are referred to as the optimum conditions for growth. The onset of growth was not influenced by the concentration of Al or pH. In all treatments, the first archegonium appeared before day 20, the first leaf of the sporelings before day 28, the first root before day 36 and second leaf before day 40. Nonetheless, some pH and c_{Al} treatments markedly affected the growth of macrogametophyte rhizoids (Table 1, Fig. 2B,C). Rhizoid number and length were reduced at low pH (pH 4) and c_{Al} of 100 $\mu g \cdot l^{-1}$. At c_{Al} of 300 $\mu g \cdot l^{-1}$ and higher, no rhizoids developed (Fig. 2B).

Low pH values and elevated c_{Al} had a pronounced negative effect on the growth of the roots, root hairs and leaves of sporelings (Table 1, Figs 2B,C, 3A–D). The total length of sporeling in Al control ($c_{Al} = 0$ $\mu g \cdot l^{-1}$) corresponded to that obtained at optimum pH, however, in c_{Al} control leaf growth was noticeably stimulated while root growth was inhibited (Fig. 3C). Therefore, the effects of the Al treatments were related to growth at the optimum pH (6–7) rather than that of the c_{Al} control (pH 5).

The addition of Al inhibited root growth at all the concentrations tested (Fig. 3B,D). While leaf growth at c_{Al} of 100 $\mu g \cdot l^{-1}$was similar to that recorded at the pH optimum it was inhibited at a higher c_{Al} (>300 $\mu g \cdot l^{-1}$). Similarly, c_{Al} of 100 $\mu g \cdot l^{-1}$ had a moderate effect on the growth of root hairs. While root hair density did not vary much, their length was reduced by 30% at c_{Al} of 100 $\mu g \cdot l^{-1}$. At higher c_{Al} the hairs were shorter, which resulted in the hairs being short and stubby in form (Table 1, Fig. 2B). In addition, the growth of root hairs and most other parts of the plant decreased, notably at pH 4 (Table 1, Figs 2C, 3A,C).

Despite the changes in sporeling growth induced by low pH or high concentrations of Al, the first leaf grew well in all treatments (Table 1, Fig. 3A,B). When the first root appeared, growth of the first leaf was almost complete. Results of the statistical analysis are presented in Table 2.

Table 1. – Overview of the symptoms shown by *Isoëtes echinospora* sporelings reared at various pH and Al concentrations in the laboratory. The sporelings were at the two-leaf-two-root stage and still attached to a macrogametophyte. All the sporelings reared in a particular treatment showed the same symptoms. MD: an arbitrary unit of length corresponding to a macrospore diameter of ~0.46 mm; the number of root hairs are expressed per root. For parameters sprout growth see Fig. 3.

<table>
<tr><th rowspan="2">Ontogenetic stage</th><th rowspan="2">Trait</th><th colspan="9">Experimental treatment (Al µg l^{-1})</th></tr>
<tr><th>pH 4
0Al</th><th>pH 5
1000Al</th><th>pH 5
500Al</th><th>pH 5
300Al</th><th>pH 5
100 Al</th><th>pH 5
0Al</th><th>pH 6
0Al</th><th>pH 7
0Al</th><th>pH 8
0Al</th></tr>
<tr><td rowspan="4">Macro-gametophyte</td><td>Colour of macrogametophyte surface</td><td>reddish</td><td colspan="8">colourless (no change)</td></tr>
<tr><td>Rhizoid number (items)</td><td>rare (<25)</td><td colspan="3">absent</td><td>reduced (<50)</td><td colspan="4">numerous (50-100)</td></tr>
<tr><td>Rhizoid length (MD)</td><td>short (<1)</td><td colspan="3">0</td><td>short (<1)</td><td colspan="4">long (>7)</td></tr>
<tr><td>Turgor</td><td>tense</td><td colspan="4">flaccid, adhered to the leaf basis</td><td colspan="4">tense</td></tr>
<tr><td rowspan="9">Sporeling</td><td>Root colour</td><td>brown</td><td colspan="8">colourless (brownish cell walls)</td></tr>
<tr><td>Root hair size (MD)</td><td>short (<1) not stubby</td><td colspan="2">absent</td><td>stubby (<1/4)</td><td>short (<7)</td><td colspan="4">long (>25)</td></tr>
<tr><td>Root tip necrosis</td><td>no</td><td colspan="3">present regularly</td><td colspan="5">no</td></tr>
<tr><td>Root hair density (items per 1 mm root-basis-length)</td><td>rare (<10)</td><td colspan="2">absent</td><td>rare (<10)</td><td colspan="5">numerous (>25-50)</td></tr>
<tr><td>Leaf colour</td><td colspan="9">green (no difference)</td></tr>
<tr><td>Growth of roots</td><td>minimum</td><td colspan="5">inhibited</td><td colspan="2">optimum</td><td>maximum</td></tr>
<tr><td>Growth of the first leaf</td><td colspan="4">inhibited</td><td colspan="5">optimum</td></tr>
<tr><td>Growth of the second leaf</td><td>minimum</td><td colspan="4">inhibited</td><td>maximum</td><td colspan="3">optimum</td></tr>
<tr><td>Total growth</td><td>minimum</td><td colspan="4">inhibited</td><td colspan="3">optimum</td><td>maximum</td></tr>
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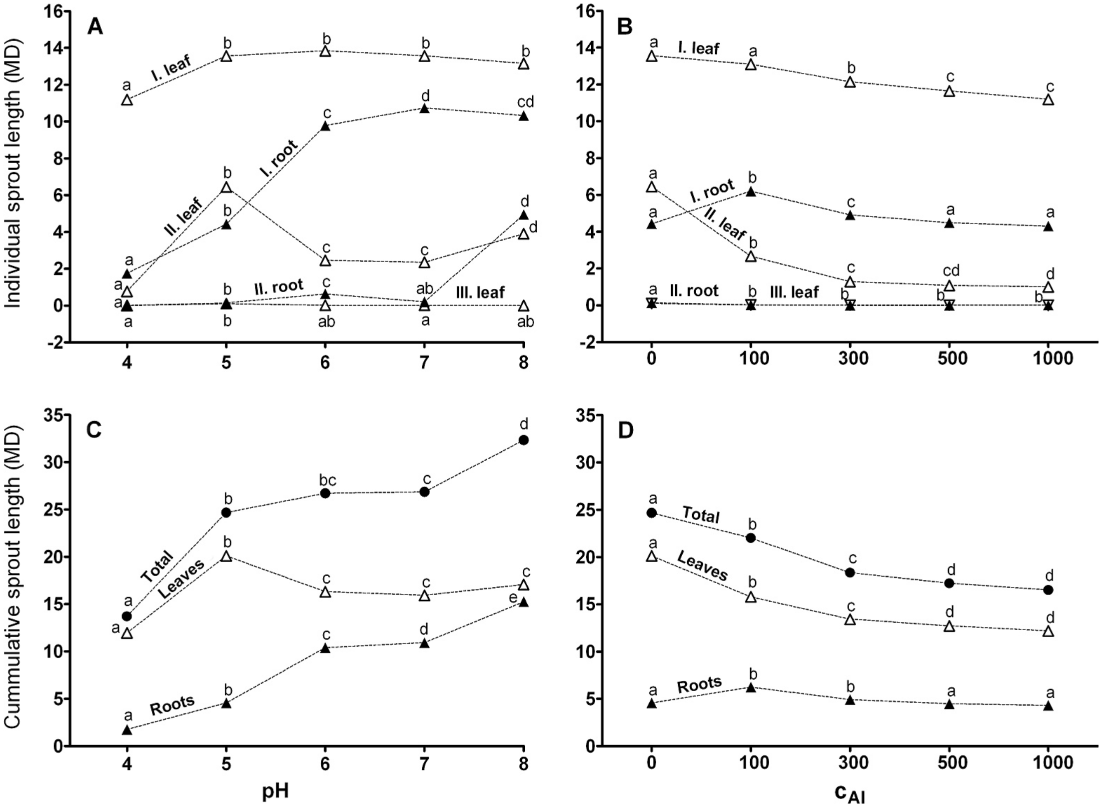

Fig. 3. – Summary of the effects of various pHs (A, C) and Al concentrations (c_{Al}, $\mu g \cdot l^{-1}$; B, D) on the lengths of the leaves and roots of *Isoëtes echinospora*. Panels A and B show lengths of individual sprouts, panels C and D cumulative lengths of sprouts and total length of sporelings. Differences among treatments were tested using hierarchical ANOVA and a post hoc Tuckey's HSD test. All symbols in the panels are means, SDs are not shown, as they are smaller than the symbols. Lower case letters above the symbols (a–e) indicate significant differences among treatments. Open triangles – length of leaves; solid triangles – length of roots; solid circles – total length of sporeling.

Lake survey

The population of *I. echinospora* in Plešné Lake recovered during the survey period, 2004 to 2008. The unbalanced age structure of few juveniles and mainly adults recorded in 2004 improved in 2005 (Fig. 4), when the percentage of juveniles increased considerably, indicating an increase in sporeling survival. In addition, the number of adult plants increased from ~2300 to ~6700 between 2004 and 2008. The number of plants present in spring (adults and juveniles) increased from ~2350 in 2004 to ~9200 in 2008. The percentage of juvenile plants rose from ~2% in 2004 to ~67% in 2005 and ~20–33% in 2006–2008.

Table 2. – Results of the statistical analysis of the measurements of the sporelings that developed in the different experimental treatments and are presented in Fig. 3.

Variable	pH		c_{Al}	
	F-ratio	P level	F-ratio	P level
First leaf	44.8	<< 0.001	47.8	<< 0.001
Second leaf	161.1	<< 0.001	397.6	<< 0.001
Third leaf	4.1	0.0026	9.0	<< 0.001
First root	464.2	<< 0.001	41.6	<< 0.001
Second root	151.7	<< 0.001	38.4	<< 0.001
Leaves	103.8	<< 0.001	217.9	<< 0.001
Roots	479.9	<< 0.001	40.2	<< 0.001
Total	182.6	<< 0.001	126.1	<< 0.001
AB:AS ratio	472.0	<< 0.001	669.6	<< 0.001

Each year of the study, sporelings developed in June–July and their abundance in the lake exceeded that of adults by an order of magnitude. Since 2005, ~10% of all sporelings survived their first winter and reached the juvenile stage. Sporelings were repeatedly observed being dislodged by waves or becoming detached from the sediment surface.

The sporelings collected in September 2007 were approximately four months old and had three leaves and four roots. The mean lengths (MD units) and standard deviations of the first four leaves were: 48.6±2.2, 36.7±2.8, 19.3±2.4 and 2.3±1.3, and of the first four roots: 53.0±3.2, 40.9±3.2, 25.0±3.3 and 8.6±2.7. Thus, the mean total length of the lake sporelings was 234.4 ±14.8.

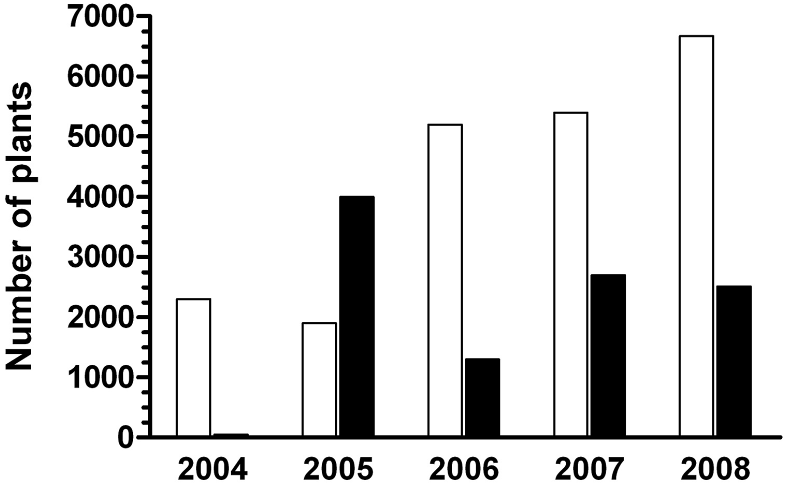

Fig. 4. – Abundance and age structure of the population of *Isoëtes echinospora* in Plešné Lake in the springs of 2004–2008. Counts of adult (white columns) and juvenile (black columns) plants were made in the lake.

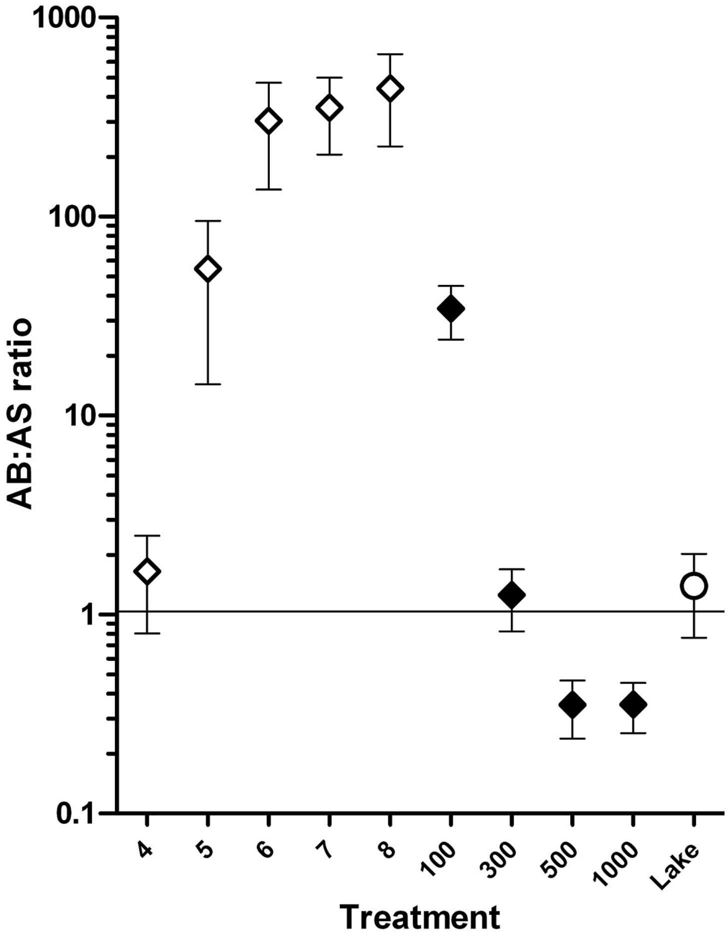

Fig. 5. – Mean AB:AS ratios of the sporelings that developed in experimental treatments and those collected from Plešné Lake. The labelling (from left) on the x axis correspond to various pH values (4–8) or Al concentrations (100–10,000 in $\mu g \cdot l^{-1}$) in the experimental treatments, and the lake (in situ). Bars indicate standard deviation. Solid line indicates an equal investment in absorptive and assimilative areas.

Macrogametophyte rhizoids were mostly absent or rare (<4 items) and never longer than 1 MD (Fig. 2D). Root hairs were stubby, shorter than 1 MD, but present in various numbers. Along the upper half of the sporeling roots, the number of root hairs ranged from 0 to13 and 36–62 on the lower part of the root. Necrosis of the tip of the second root was recorded for 11% of the lake sporelings.

The AB:AS ratio was highest at the optimum pH conditions and in the Al control (~400 and ~40–50, respectively, Fig. 5). The sporelings in the lake and those that developed at pH 4 and a c_{Al} of 300 $\mu g \cdot l^{-1}$ had similar AB:AS ratios (~1–2), whereas the ratio was less than 1 at higher $c_{Al,}$ which reflects the reduction in the quillwort root system at the higher concentrations of Al.

Discussion

This study provides additional empirical data that should help resolve how quillworts are able to tolerate acidification (Maessen et al. 1992, Arts 2002, Brouwer et al. 2002, Roelofs et al. 2002) and elevated levels of Al (Maessen et al. 1992, Vöge 1997, Roelofs et al. 2002). The results detail the external physical changes that quillwort sporelings (plantlets) undergo and are crucial to determining whether regeneration of quillwort populations occur in acidified lakes.

The growth of quillwort sporelings was inhibited at pH 4. This pH value is slightly below that recorded for the water in Plešné Lake during the period of acidification (Veselý et al. 1998, Majer et al. 2003, Kopáček et al. 2006). At pH 5, which is close to the current pH values recorded at Plešné Lake (Fig. 1), the growth of sporeling was less inhibited, but the growth was still abnormal, with the growth of the leaves stimulated but that of the roots inhibited (Fig. 2C). This response differs from the dominance of the root system in quillworts reported by Farmer & Spence (1986), Boston & Adams (1987), Szmeja (1994a,b), Madsen et al. (2002) and Smolders et al. (2002). Thus, it is assumed that the large root system and small rosettes of leaves of quillworts is an adaptation to living in infertile habitats (Farmer & Spence 1986, Boston & Adams 1987).

In general, the roots account for more than ~50% of the quillworts' biomass (Szmeja 1994a, Madsen et al. 2002, Szmeja & Bociag 2004) and their contribution is highest in the early juvenile stages (Szmeja 1994a). According to Szmeja (1994a), the weight ratio of below-ground (roots and bulbous stem) to above-ground (leaves) biomass in quillworts decreases from 1.8 in early juvenile to 1.2–0.7 in adult plants. In this paper the AB:AS ratio is used to quantify changes in biomass distribution in quillwort sporelings. Despite the stimulation of leaf and inhibition of root growth at pH 5 the AB:AS ratio was greater than 1 due to the presence of rhizoids and root hairs, therefore, the dominance of the root system was maintained. However, they risk being up-rooted due to their buoyancy if the plants are not well anchored by their roots (Raven 1988, Smolders et al. 2002).

As expected, elevated c_{Al} seriously damaged the root systems of sporelings and even the rhizoids of macrogametophytes. The growth of absorptive organs were negatively affected by c_{Al}>300µg·l^{-1}, resulting in a decrease in the AB:AS ratio to close to or below 1. Thus, these concentrations were harmful for quillwort sporeling survival. All absorptive organs of sporelings were negatively affected at a c_{Al} of 100 µg·l^{-1}, but AB was several times larger than AS. Although these sporelings were under stress results from Plešné Lake show that they can survive under these conditions in the field, as in 2005, when concentrations of ionic Al fell below 200 µg·l^{-1} (Kopáček et al. 2006, Fig. 1).

The morphology of lake sporelings (Fig. 2C), which experienced ionic Al (Al_i) concentrations of ~100–300 µg·l^{-1} (Fig. 1), correspond with that of sporelings that developed in the c_{Al} 100–300 µg·l^{-1} treatments (Fig. 2C). The mean AB:AS ratio of lake sporelings was similar to that measured at c_{Al} = 300 µg·l^{-1} (Fig. 5), which indicates that the quillwort life strategy of root system dominance was barely effective. This implies that a concentration of ~300 µg·l^{-1} of Al_i in lake water may limit the development and survival of quillwort sporelings. The recovery of the quillwort population might have started prior to our study as the ionic Al concentration was already less than ~300 µg·l^{-1} in 1999 (Fig. 1).

As noted above, roots are essential for quillworts as they are more dependent on the sediment than other submersed plants (Sand-Jensen 1982, Rørslett & Brettum 1989, Vöge

1997, Bennert et al. 1999, Roelofs et al. 2002, Smolders et al. 2002, Szmeja & Bociag 2004). Their extensive root system is important for obtaining nutrients (Sand-Jensen & Søndergaard 1979, Szmeja 1994a, Smolders et al. 2002), uptake of carbon dioxide (CO_2) (Roelofs et al. 1984, Farmer and Spence 1986, Boston et al. 1987, Madsen et al. 2002, Smolders et al. 2002) and anchorage (Sand-Jensen & Søndergaard 1979, Szmeja 1994a, Smolders et al. 2002). Since the quillwort leaf surface is thick, cutinized and, thus, hardly permeable, nutrient uptake is thought to take place exclusively via the roots (Boston & Adams 1987, Szmeja 1994a, Vöge 1997, Madsen et al. 2002, Smolders et al. 2002), which have vesicular-arbuscular mycorrhizae (Keeley 1998, Wigand 1998, Smolders et al. 2002). As a result, quillworts have developed morphological adaptations for obtaining CO_2 for photosynthesis (Keeley 1998). They use their roots to take up CO_2 from the sediment, where the CO_2 levels are 10–100 times higher than in the water (Boston et al. 1987, Sand-Jensen & Søndergaard 1979, Madsen et al. 1993, 2002, Smolders et al. 2002). In addition, the root system aerates sediment via radial oxygen loss, which stimulates mineralization and also decreases solubility of metal ions (Sand-Jensen & Søndergaard 1979, Sand-Jensen 1982, Arts et al. 1990, Roelofs 1996, Smolders et al. 2002).

This study focused on the early development of quillwort sporelings. In general, plantlets are more susceptible to Al toxicity than older plants (Nosko et al. 1988, Mossor-Pietraszewska 2001). Even a small disruption in the development of the plantlet root system adversely affects its growth (McCully 1999). Quillwort plant establishment is reported to be more dependent on the quality of the sediment than of the water of lakes (Sand-Jensen 1982, Boston & Adams 1987, Szmeja 1994b, Vöge 1997). Sporeling roots develop in the upper sediment layer adjacent to the overlying acidic Al-rich lake water, which is harmful for the development of their root system. On the other hand, the extensive root systems of adult plants are buried deeper in the sediment, where the conditions differ markedly from those in lake water or at the surface of the sediment. For instance, pH values in the deeper sediment horizons (5–50 cm) at Plešné Lake are relatively high, ~5.7, despite the acidification of the lake (Kopáček et al. 2001). In acidified lakes with a depleted carbonate buffering system, a steep pH gradient between overlying water and interstitial water in the sediment commonly develops due to alkalinity generated by biogeochemical processes in the sediment (Herlihy & Mills 1986, Kopáček et al. 2001). At pH values higher than 5, the toxic ionic Al forms hydrolyze into colloidal form and its concentration in sediments decreases (Kopáček et al. 2001). Thus, strong acidification and Al toxicity of lake water does not necessarily adversely affect the root system of adult plants, their vitality or eventual production of viable spores.

Several adult plants of *I. echinospora* were removed from Plešné Lake and placed in pools during 2001–2006. These plants possessed spores, which germinated into viable sporelings immediately after removal (M. Čtvrtlíková, unpublished results). Viability of spores present on adult plants was the most likely cause of the boom in reproduction of *I. echinospora* that occurred at Plešné Lake (Fig. 4) when the ionic Al concentration decreased (Fig. 1). A viable spore bank in the sediment, however, may also play an important role in population recovery. In The Netherlands a population of *I. echinospora* was successfully re-established from spores surviving in the sediment of a softwater lake from which this species had disappeared more than 15 years previously (Roelofs 1996).

There were high Al_i concentrations ($>300\ \mu g \cdot l^{-1}$) in the strongly acidified Plešné Lake from the 1970s through to the end of the 1990s (Kopáček et al. 2006). The population of *I. echinospora* declined from ~3000–5000 plants in 1977 to 2000–2200 in 1997, when juvenile plants were completely absent (Husák et al. 2000). This decrease occurred when adult plants were damaged, and this population loss was not compensated for by the establishment of juveniles. For ~30 years of the strongest acidification there has been a reduction in or lack of sporeling survival at Plešné Lake.

Although the concentrations of Al used in this study are extremely low compared to those used in other studies on aquatic plants (e.g., Stanley 1974) the effects were clear and easily detected. The relatively high toxicity of the low concentrations of Al used in this study may be due to the low concentrations of calcium and magnesium in the water, which results in an increase in the Al:Ca and Al:Mg ratios that in many species counteract negative effects of aluminium (Maessen et al. 1992, Ryan et al. 1997).

This study shows that high acidity (pH 4) along with Al toxicity induced by c_{Al} higher than 300 $\mu g \cdot l^{-1}$ is responsible for the reduction or cessation of the growth of sporelings. Under such adverse conditions, the development of the first leaf was dependent on macrogametophyte storage tissue while the growth of the other sprouts was strongly inhibited. Under similar conditions at Plešné Lake sporelings were unable to complete their development and root in the sediment, which is essential for winter survival. As quillworts do not grow clonally, the survival of the population was entirely dependent on the longevity of adult plants. The lifespan of *I. echinospora* at Plešné Lake must be at least 20–30 years. A similar longevity for adult quillworts is suggested by Szmeja (1994b), who records a low mortality of *I. lacustris* adult plants at Lake Krasne, but a high juvenile mortality due to the acidity.

We document a progressive recovery of the *I. echinospora* population in Plešné Lake over a period of five years. The recent increase in plantlet survival has partly reversed the ageing of the population; however, acidity and Al toxicity still remain the underlying cause of the important bottleneck in quillwort reproduction. It is possible the population in Plešné Lake may become more vulnerable to persistent acid stress, e.g. due to reduced genetic variability. Further autecological studies on this lake are needed.

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Souhrn

Populace šídlatky ostnovýtrusné (*Isoetes echinospora* Durieu) přežila třicetileté období silné acidifikace Plešného jezera (Šumava, Česká Republika) provázené vysokými koncentracemi fytotoxického hliníku (Al). V letech 2004–2008 bylo sledováno přežívání klíčních rostlin a věková struktura zotavující se populace. Současně proběhly laboratorní pokusy s cílem zjistit vliv různých hodnot pH (4–8) a koncentrací Al (0–1000 $\mu g \cdot l^{-1}$) na ontogenezi klíčních rostlin šídlatky. Experimentální přídavek hliníku v množství vyšším než 300 $\mu g \cdot l^{-1}$ nebo snížení pH pod 5 výrazně zpomalily růst klíčních rostlin. To se projevilo zejména výraznou redukcí absorpčních orgánů (rhizoidů makrogametofytu, kořenů a kořenového vlášení) a poklesem poměru nadzemní a podzemní biomasy pod hodnotu 1, což je ve rozporu s životní strategií šídlatek založené na dominanci kořenového systému. Specifické příznaky na již přežívajících jezerních rostlinách odpovídaly působení koncentrací Al 100–300 $\mu g \cdot l^{-1}$ v experimentech, což jsou zároveň i koncentrace Al v jezeře. Při vyšších koncentracích Al během dlouhého období acidifikace jezerní

vody byl však vývoj a přežívání klíčních rostlin zastaven a populace mohla přežívat jen díky dlouhověkosti dospělých rostlin. Podle výsledků naší studie je obnova populace šídlatky ostnovýtrusné v Plešném jezeře spojena teprve se zvýšením pH nad 5 a poklesem koncentrací Al pod 300 $\mu g \cdot l^{-1}$.

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