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Micronutrient content does not reflect the status of health in the aquatic carnivorous plant *Aldrovanda vesiculosa*

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Abstract: Tissue micronutrient content (B, Fe, Mn, Zn, Cu, Mo, Co) was compared in healthy and ill shoots of a rootless aquatic carnivorous plant *Aldrovanda vesiculosa* growing in an outdoor culture, in plants in the field, in different accessions from around the world, and in shoots and turions. On the basis of microelemental analyses, the observed disorder of shoot apices cannot be explained as deficiency of any of the above micronutrients.

Key words: rootless aquatic plant; disorder of shoot apices; tissue B, Fe, Mn, Zn, Cu, Mo, Co content

Abbreviations: DM – dry mass, EP – plants from E Poland, NA – plants from N Australia, NWA – plants from NW Australia.

Introduction

Aldrovanda vesiculosa L. (*Droseraceae*) is a critically endangered and rare aquatic carnivorous plant of the Old World. It is 8–15 cm long, rootless, free-floating, and grows just below the surface in shallow standing dystrophic waters (Adamec 1995, 2000). In the last 10 years, it has been the subject of extensive ecophysiological research on photosynthesis, mineral nutrition, physiological polarity, growth properties, importance of carnivory, winter bud (turion) overwintering (Adamec 1997, 2000, 2003a,b) as well as molecular genetic study (Maldonado San Martín et al. 2003). To support its very rapid apical shoot growth (over one new leaf whorl a day; e.g., Adamec 2000), it has to take up all necessary mineral nutrients including microelements only from nutrient-poor water or animal prey, besides having very effective re-utilization of N and P from senescent shoot segments (Adamec 2000). In cultures of *Aldrovanda,* a disorder of shoot apices called "Aldrovanda disease" has been described (Adamec 2003a). The disorder is manifested as damage (malformation), yellowing to blackening, and finally dying of shoot apices. As the disorder does not exhibit typical features of infection disease and boron deficiency in terrestrial plants, which causes well-known similar damages to shoot apices (e.g. Loomis & Durst 1992; Brown et al. 2002), deficiency of B and/or other microelements has been suspected to cause the disorder. The suspicion has been supported by a partial relief of the disorder after adding boric acid (0.5 mg L^{-1}) alone, or in a mixture with other microelements, to culture water in many cases (Adamec 2003a). Moreover, very rapid shoot turnover in *Aldrovanda* leads to a loss of a substantial amount of some macro- or microelements with senescent shoots (proved for K, Ca, Mg; Adamec 2000), which could cause growth limitation or, theoretically, even deficiency symptoms. However, nothing is known of the morphological symptoms of microelement deficiency in aquatic plants. As follows from the literature, data on tissue microelement content (B, Fe, Mn, Zn, Cu, Mo, Co) in aquatic plants are very variable among species and sampling sites, by up to two orders of magnitude (Boyd & Walley 1972; Glandon & McNabb 1978; Dykyjová 1979). As B content in rapidly growing species of duckweeds (Lemnaceae) is extremely high (Glandon & McNabb 1978) it is possible to assume that most unrooted, free-floating aquatic plants with rapid growth like *Aldrovanda* will have higher tissue B content.

The aim of this study was to compare tissue micronutrient content (B, Fe, Mn, Zn, Cu, Mo, Co) in healthy and ill shoots of *Aldrovanda* growing in an outdoor culture, in plants in the field, in different world accessions, and in shoots and turions. In this way, to confirm or deny that the "Aldrovanda disease" is caused by micronutrient deficiency.

Material and methods

Fourty healthy, adult *Aldrovanda* plants 6–12 cm long were collected for B analyses from each of the artificial *Aldrovanda* sites in the Třeboň basin, S Bohemia, Czech Republic (Adamec 2005): fen lake Karštejn, fen pool No. 1 at Ptačí blato fishpond, dystrophic pool in the sand-pit Branná (28 June 2002), and fen pool at Výtopa fishpond (12 July 2003). Seventy-two healthy, non-germinating turions were collected from each of three sites (Karštejn, P. blato, Branná) on 23 March 2003. All these plants originated from E Poland (EP; Maldonado San Martín et al.

Table 1. Boron content (in mg kg^{-1} DM) in *Aldrovanda vesiculosa* plants collected either from four artificial sites in the Třeboň basin, Czech Republic, from outdoor containers with or without an addition of boric acid, from *in-vitro* cultures, or in the form of turions from three sites. EP, plants from E Poland; NA, plants from N Australia; NWA, plants from NW Australia. Mean ± SE and range of values of four repetitions are always shown. Different runs of samplings or experiments are separated by dotted line. Statistically significant difference in B content between healthy and ill plants in the A outdoor culture and between shoots and turions was expressed for each site (*t*-test); NS – non-significant at $P > 0.05$; * – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$.

Origin of plants	Organ	Status	Treatment	B content (mg kg^{-1})
Fen lake Karštejn (EP)	shoot	healthy	–	16.2 ± 0.2 (15.7–16.6)
Ptačí blato fishpond (EP)	shoot	healthy	–	14.8 ± 0.3 (14.2–15.6)
Sand-pit Branná (EP)	shoot	healthy	–	15.3 ± 0.2 (14.2–15.7)
Výtopa fishpond (EP)	shoot	healthy	–	15.7 ± 1.1 (13.0–18.0)
Outdoor culture B (EP)	shoot	healthy	–	19.2 ± 0.1 (19.0–19.5)
Outdoor culture A (EP)	shoot	healthy	3 mg L^{-1} H_3BO_3	24.4 ± 0.6 (23.3–25.3)
Outdoor culture A (EP)	shoot	ill	3 mg L^{-1} H_3BO_3	23.5 ± 0.2^{NS} (23.0–23.7)
In-vitro culture (NA)	shoot	healthy	–	16.8 ± 1.3 (14.0–19.0)
In-vitro culture (NWA)	shoot	healthy	–	18.5 ± 1.8 (15.0–23.0)
Fen lake Karštejn (EP)	turion	healthy	–	$8.63 \pm 0.17^{***}$ (8.3–9.0)
Ptačí blato fishpond (EP)	turion	healthy	–	$9.83 \pm 0.33^{***}$ (9.0–10.6)
Sand-pit Branná (EP)	turion	healthy	–	$8.80 \pm 0.14^{***}$ (8.6–9.2)

2003). Other EP plants for B analyses were grown outdoors in a smaller (A; 1 m^2, 300 L) or larger plastic container (B; 2.5 m^2, 720 L) with *Carex* litter as the substrate (Adamec 1997, 2000). On 20 June 2002, a minor part of the plants in the smaller container exhibited first typical symptoms of the "Aldrovanda disease" and H_3BO_3 at the final concentration of 3 mg L^{-1} (i.e. 0.52 mg L^{-1} B) was added to the water. After 44 days, 40 adult healthy and 40 ill plants from the A culture and 40 healthy plants from the B culture (all plants 8–12 cm long) were collected for B analyses. Meristem *in vitro* cultures of *Aldrovanda* from N Australia (NA) and NW Australia (NWA; Maldonado San Martín et al. 2003) were also used for B analyses. The plants grew in a half-strength Gamborg's B5 liquid medium with only 500 mg L^{-1} KNO_3 [microelements also diluted twice, initial (H_3BO_3)1.5 mg L^{-1}], 100 mg L^{-1} casein hydrolysate, and 2% of sucrose (for the composition see Adamec & Kondo 2002) at 21–23 °C and 14/10 h L : D regime in fluorescent light for 41 days. Twenty healthy plants were collected from each of four Erlenmeyer flasks within NA and NWA plants for B analyses and other five parallel plants for other micronutrient analyses. For other micronutrient analyses, 8 healthy EP *Aldrovanda* plants were collected from each of three sites (Karštejn, P. blato, Branná) on 12 July 2003 and 12 healthy and 12 ill EP plants and 12 ill NA plants were collected from the A outdoor culture on 15 August 2003.

For all analyses, apical shoot segments 4–5 cm long were always used. Traps with captured prey were excised, shoot segments were thoroughly washed using tap water, rinsed with distilled water, and dried (80 °C). Ten-20 apical segments (dry mass, DM 60–110 mg) were used for single B analysis (or 18 turions; 150–200 mg DM) and 2–5 segments (5–8 mg DM) for other micronutrient analysis. Dry plant tissue was mineralized in teflon vials with highest analytical grade HNO_3 + H_2O_2 for B analyses and with analytical grade HNO_3 for other micronutrient analyses. Blank samples were used for both types of analyses. B was analysed by an ICP-OES spectrometer VISTA-PRO (Varian, Melbourne, Australia). Fe, Mn, Zn, Cu, Mo, and Co were analysed by atomic absorption flame spectrometry (SpectrAA 640, Varian). Four parallel plant samples were always analysed. Tissue micronutrient content is expressed in mg kg^{-1} (DM). Means ± SE are always stated. Where possible, pairs of data were evaluated by a two-tailed *t*-test.

Results and discussion

Mean B content in apical shoot segments of *Aldrovanda* was within a very narrow range from 15–19 mg kg^{-1} (DM) at different field sites or in an outdoor culture, while that in turions collected from the same sites was about a half (8.6–9.8 mg kg^{-1}; Table 1). The mean tissue B content in two accessions of Australian plants grown in *in-vitro* culture (16.8–18.5 mg kg^{-1}) was similar to that found in the field or outdoor culture. The addition of 3 mg L^{-1} boric acid to the A outdoor culture led to an increase of the tissue B content to about 24 mg kg^{-1} (Table 1). Similarly, in an orientation 40–day growth experiment in miniaquaria, addition of boric acid to EP *Aldrovanda* within 1–10 mg L^{-1} correlated linearly with B shoot content [B content in mg kg^{-1} = 2.69 (added boric acid in mg L^{-1}) + 21.1; $n = 5$; $r = 0.996$; $P < 0.01$; Adamec unpubl.]. No statistically significant difference in tissue B content was found between healthy and ill Polish plants in the A culture (Table 1).

As reviewed by Dykyjová (1979) B tissue content in many dozens of aquatic plant species ranged totally from 0.6–170 mg kg^{-1} (DM), but most commonly from 10–30 mg kg^{-1}. For single species at various sites, there were usually two- to threefold difference between minima and maxima. Thus, shoot B content in rapidly growing *Aldrovanda* is similar to that in many other aquatic plant species the growth rate of which is evidently lower. This is also the case of two ecologically very closely related, rootless aquatic *Utricularia* species with a comparable shoot B content of 8 and 37 mg kg^{-1} (Boyd & Walley 1972; Adams et al. 1973). However, extremely high tissue B content in two rapidly growing *Lemna* species (1 100–2 140 mg kg^{-1}) reflects enormous accumulation capacity for B within this plant

Table 2. Micronutrient content in 3–4-cm long apical segments of *Aldrovanda vesiculosa* plants with different status of health. Apices of ill plants exhibited evident features of a disorder. The plants were either collected from three artificial *Aldrovanda* sites in the Třeboň basin, Czech Republic, from an outdoor culture, or from an in-vitro culture. Boric acid was not added to the outdoor culture. EP, plants from E Poland; NA, plants from N Australia; NWA, plants from NW Australia. The EP and NA plants were grown in the same outdoor culture. Different runs of samplings are separated by dotted line. Mean ± SE ($n = 4$). Statistically significant difference between healthy and ill EP plants in the outdoor culture (t-test): NS, non-significant at $P > 0.05$; * – $P < 0.05$.

Origin of plants	Status	Fe	Mn	Zn	Cu	Mo	Co
		mg kg^{-1} (DM)					
Fen lake Karštejn (EP)	healthy	2081 ± 974	1077 ± 209	40.1 ± 9.1	2.50 ± 0.88	–	–
Sand-pit Branná (EP)	healthy	2783 ± 592	2047 ± 427	29.1 ± 2.5	3.60 ± 0.82	–	5.78 ± 1.39
Výtopa fishpond (EP)	healthy	4338 ± 1202	764 ± 62	72.4 ± 5.7	6.62 ± 2.37	–	2.77 ± 1.36
Outdoor culture (EP)	healthy	466 ± 84	249 ± 45	54.8 ± 13.5	7.22 ± 0.73	–	3.44 ± 0.60
Outdoor culture (EP)	ill	381^{NS} ± 56	358^{NS} ± 45	53.7^{NS} ± 5.8	4.47* ± 0.72	–	4.35^{NS} ± 0.43
Outdoor culture (NA)	ill	794 ± 75	628 ± 141	63.2 ± 3.4	6.55 ± 1.03	–	7.71 ± 1.35
In-vitro culture (NA)	healthy	73.3 ± 29.3	60.7 ± 6.1	24.3 ± 3.7	11.8 ± 4.7	3.9 ± 0.7	–
In-vitro culture (NWA)	healthy	53.8 ± 9.0	94.3 ± 8.4	32.2 ± 5.2	12.0 ± 6.1	3.00 ± 0.3	–

genus rather then a necessary requirement for their rapid growth (Glandon & McNabb 1978). As it follows from the latter study B tissue content in aquatic plants is not only dependent on B availability in the water, but inversely proportionally also on growth rate.

High mean shoot contents of Fe (2 080–4 340 mg kg^{-1}) and Mn (764–2 050 mg kg^{-1}) in field-grown *Aldrovanda* as compared to those in the outdoor culture (Fe, 466; Mn, 249 mg kg^{-1}; Table 2) indicate that a great deal of these elements could be adsorbed or precipitated on the plant surface. In field-grown *Aldrovanda* shoots, Kamiński (1987) found 2.5–13.0 g kg^{-1} Fe and similarly high values within 1.0–28 g kg^{-1} Fe (and 0.10–14.2 g kg^{-1} Mn) were also reviewed by Dykyjová (1979) for several ecologically related aquatic *Utricularia* species. In contrast, shoot Fe (54–73 mg kg^{-1}) and Mn content (61–94 mg kg^{-1}) in healthy Australian *Aldrovanda* plants grown in *in-vitro* culture was about by one order of magnitude lower than that in ill plants of the same accession in the outdoor culture (Table 2). Shoot Zn and Cu content in field-grown healthy *Aldrovanda* plants was similar to that found in healthy and ill plants in the outdoor culture (Table 2) and corresponded to mean values for aquatic *Utricularia* species (Dykyjová 1979). Shoot Mo content in Australian plants grown in *in-vitro* culture (3.0–3.9 mg kg^{-1}; Table 2) was about twice as high as that found in some non-repeated samples from the field or the outdoor culture (healthy and ill plants; 1.2–2.3 mg kg^{-1}; Adamec, unpubl.). Shoot Co content in field-grown plants was comparable with that in the outdoor culture in both healthy and ill plants (Table 2) but that in the oligotrophic fen lake Karštejn was below the detection limit of 0.90 mg kg^{-1}. In summary, except for Cu, no statistically significant difference was found in micronutrient content (Fe, Mn, Zn, Co) between the healthy and ill *Aldrovanda* plants growing in the same outdoor culture; shoot Cu content in the ill plants was the same as that in healthy field-grown plants. Thus, on the basis of microelemental analyses, the observed "Aldrovanda disease" cannot be explained as deficiency of any of the following micronutrients, B, Fe, Mn, Zn, Cu, Mo, and Co.

Recently, an unknown species of a mold of the genus *Fusarium* and of Deuteromycetes has been found in apices of ill *Aldrovanda* plants as potential agents of the "Aldrovanda disease" (Lebeda unpubl.). This finding is further supported by the fact that addition of fungicides of Topsin M or Amistar at the dose of 0.1 g L^{-1} to the aquarium water cures reliably the disease in Australian plants (Adamec unpubl.). Accepting that the "Aldrovanda disease" is caused by a fungal agent it is not clear why an application of boric acid at a weak concentration, which cannot be toxic to the fungal agent, can partly cure the disease. As B is known to act within cell walls and change their properties (incl. mechanical; Loomis & Durst 1992; Brown et al. 2002) it is possible to consider that B addition renders cell walls less susceptible to the agent of the disease. Another potential candidate for changing the properties of cell walls (and thus, susceptibility to fungal pathogens) might be calcium (e.g. Marschner 1995). However, as follows from a previous study (Adamec 2000) shoot Ca content in EP *Aldrovanda* grown outdoors under the same conditions was relatively high (0.17–0.49% of DM) and Ca could not limit the status of plant health.

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