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RESEARCH PAPER

Utricularia carnivory revisited: plants supply photosynthetic carbon to traps

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Abstract

The rootless, aquatic *Utricularia* species belong to the largest and most cosmopolitan carnivorous plant genus. Populations of *Utricularia* plants are an important component of many standing, nutrient-poor, and humic waters. Carbon (C) allocation is an aspect of *Utricularia*'s ecophysiology that has not been studied previously and there is considerable uncertainty about the functional and ecological benefit of the trap-associated microbial community and the potential role played by C exudation in enhancing plant–microbe interactions. A ^{13}C-labelling experiment was conducted in greenhouse conditions to determine the C allocation between plant tissues of increasing age and trap fluid in two *Utricularia* species. Both species allocated a majority of the newly fixed C into the fast growing shoot apex (46.1±8.6% in *U. vulgaris* and 56.1% in *U. australis*). Carbon allocation rapidly decreased with increasing age of the shoot, constituting only 8.0±4.0% and 6.7% of the total newly fixed C in the oldest analysed segments in *U. vulgaris* and *U. australis*, respectively. In the trap-bearing shoot segments, the ratio of C exuded into the trap fluid to that in plant tissues increased markedly with age—in the oldest analysed segments twice as much newly fixed C was allocated into the trap fluid than the plant tissue. Overall, a significant amount of the newly fixed C, approximately 25% (*U. vulgaris*) and 20% (*U. australis*), was allocated to the trap fluid. The importance of C exudation for the development of the microbial community associated with the traps as well as for the growth and ecology of aquatic *Utricularia* is discussed.

Key words: Aquatic carnivorous plants, ^{13}C labelling, carbon exudation, microbial community, *Utricularia australis*, *Utricularia vulgaris*.

Introduction

The rootless genus *Utricularia* (Lentibulariaceae) is the largest and most cosmopolitan genus among plants classified as carnivorous. The traps or utricles, characteristic for the genus, are among the most intricate structures in the plant kingdom (Juniper *et al.*, 1989) and have fascinated scientists since the times of Darwin (1875). These hollow utricles, usually 1–4 mm long and filled with trap fluid, are thought to be designed mainly to attract, capture, and digest live microzooplankton (Harms, 1999; Richards, 2001). Any mechanical irritation can trigger the trap, hence various detritus and organic particles of suitable size, including algae and bacteria, are frequently trapped as well as the traditionally considered microzooplankton (Harms, 1999; Richards, 2001; Peroutka *et al.*, 2008; Sirová *et al.*, 2009). A single trap is capable of firing several times during its lifetime, at least every 30 min or longer (Sydenham and Findlay, 1975). Despite the potential to re-fire, the traps contain particles and organic solutes sealed inside them. Once inside the trap, material other than water and inorganic ions cannot escape into the surrounding

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environment unless the trap walls are damaged or the trap is no longer functional (for details, see Sydenham and Findlay, 1975; Juniper *et al.*, 1989).

Linear *Utricularia* shoots exhibit a marked growth polarity: they grow rapidly at the apex but the shoots decay at their bases at the same rate (Adamec, 2009). Previous studies confirmed nutrient uptake from artificially fed prey in *Utricularia* (Lollar *et al.*, 1971; Friday and Quarmby, 1994) and digestive extracellular enzymes have been detected on the various trap glands and in the trap fluid (Sirová *et al.*, 2003, 2009; Płachno *et al.*, 2006). Some authors suggested that trap formation and biomass (investment in carnivory) was directly regulated by prey availability or tissue nutrient content (Bern, 1997; Englund and Harms, 2003; Kibriya and Jones, 2007; Adamec, 2008*a*). However, the nutritional benefit of capturing live animal prey has been debated (Bern, 1997; Richards, 2001; Sirová *et al.*, 2003, 2009; Peroutka *et al.*, 2008), especially in environments with low microzooplankton abundances due to nutritional constraints. Recently, the microbial community inside *Utricularia* traps was evaluated and viable components of a complete microbial food web were found in the trap fluid, including a large biomass of bacteria and fungi (Sirová *et al.*, 2009). The micro-organisms, especially bacteria, were found to produce extracellular phosphatases. Considering their large biomass and high metabolic potential, they are likely to play an important role in nutrient transformation inside the traps. Furthermore, high concentrations of nutrients were detected in traps without microzooplankton prey, specifically a large amount of dissolved nitrogen (N), phosphorus (P), and particularly C (Sirová *et al.*, 2009).

It has been hypothesized that C, supplied into the traps in the form of plant exudate, benefits the associated microbial community, while nutrients (N and P) derived from this community become available for plant uptake in a manner similar to the rhizosphere interactions of terrestrial plants (Sirová *et al.*, 2009). Knight (1992) measured photosynthetic and respiration rates along shoot segments of different age in *U. macrorhiza* and demonstrated the greatest C uptake rate (biomass-based) in mature older segments. However, there has been no direct study on C allocation in *Utricularia* so far. To address the above hypothesis, a simple ^{13}C labelling experiment has been carried out to evaluate the pattern of C distribution between trap plant tissue and trap fluid of increasing age in *U. vulgaris* L. and *U. australis* R. Br.

Materials and methods

Experimental plants

Adult plants of *U. vulgaris* collected from the Czech Republic were precultivated outdoors in a 2.5 m^2 plastic container which approximately simulated natural conditions (for details see Adamec, 1997, 2008*b*; Sirová *et al.*, 2003). The pH of the cultivation medium was 7.7 and total alkalinity 838 μeq l^{-1} at the time of material collection. The length of plants selected for the ^{13}C-labelling experiment ranged from 48–70 cm. Based on the concentrations of nutrients, the water was considered oligotrophic. Addition of fine zooplankton prey to the cultivation container was interrupted one week before plant collection.

Adult plants of *U. australis* ranging from 30–52 cm in length, were collected from Ruda fishpond at Branná (Třeboňsko Biosphere Reserve, Czech Republic) one day before the start of the experiment and were placed into plastic tubs containing filtered water (40 μm mesh size; pH 5.7; total alkalinity 110 μeq l^{-1}) from the site.

^{13}C-labelling experiment

Plants of both species were placed into experimental plastic containers in a manner schematically represented in Fig. 1. Two containers, each containing 6.8 l of filtered water (mesh size 40 μm) from their respective growth sites, were aligned next to each other. The apical section of a main shoot, representing approximately one-third of the entire length of the plant, was placed into one plastic container while the remaining basal part with two-thirds of the plant was placed in the other. This resulted in an air-exposed shoot segment approximately 4 cm in length that was outside the tubs, which was immediately covered by a plastic foil to prevent desiccation or tissue damage. The 'basal' compartment received labelled $Na^{13}CO_3$ (^{13}C 98%, ISOTEC, Miamisburg, OH, USA). A solution of $Na^{13}CO_3$ provided 1.24 and 1.59 mg l^{-1} $Na^{13}CO_3$ for *U. vulgaris* and *U. australis*, respectively, in two successive additions 24 h apart. The resulting proportion of ^{13}C to the total inorganic carbon (TIC) was 3.85% and 2.20% for *U. vulgaris* and *U. australis,* respectively. The ^{13}C amounts added were based upon previously published photosynthetic and growth rate estimates for the two species (Adamec and Kovářová, 2006; Adamec, 2006,

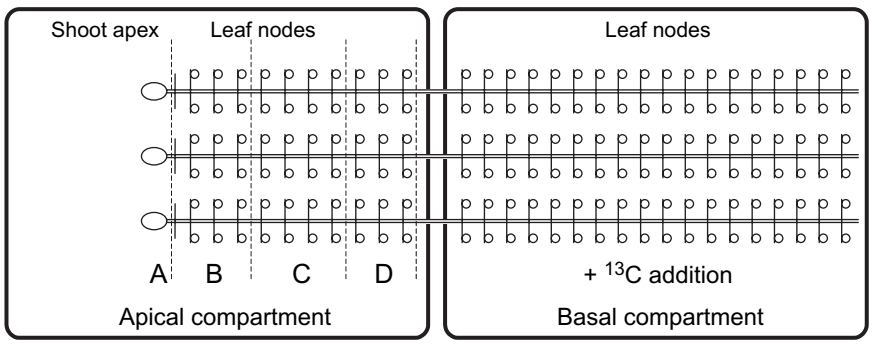

Fig. 1. Schematic representation of ^{13}C-labelling experiment setup. (A–D) Plant segments of increasing age. The basal compartment received ^{13}C addition. For measuring ^{13}C enrichment in plant tissues and trap fluid, the plant material in the apical compartment was used.

2008*b*). Three replicates and an unlabelled control each containing three *U. vulgaris* plants and one replicate and one unlabelled control each containing ten *U. australis* plants, were incubated in a naturally lit greenhouse for 48 h. More than one replicate of the *U. australis* treatment was not possible due to the smaller size of traps in this species, and the relatively large volumes of trap fluid needed for analysis. Water temperature ranged between 24–26 °C during the experiment.

Trap fluid collection and ^{13}C analysis

At the end of the 48 h incubation period, all plants were split into apical parts (where ^{13}C enrichment was measured) and basal parts which were handled separately to avoid ^{13}C contamination of the samples. Basal plant parts from the same replicate were pooled, dried to a constant weight, and weighed. Apical plant parts were further divided as follows: shoot apex (included two immature, non-functional trap segments, Fig. 1A), 'B' segment (with youngest mature, functional traps; approximately six leaf nodes; Fig. 1 B), 'C' segment (medium-aged, approximately five leaf nodes with functional traps; Fig. 1C), 'D' segment (oldest analysed, approximately five leaf nodes with functional traps; Fig. 1D). Trap fluid from traps on each of the B–D segments (pooled from 300–400 traps within each replicate) was collected by a glass capillary (Sirová *et al.*, 2003) attached to a peristaltic pump. It was not possible to collect trap fluid from all the traps due to the small trap size or to trap damage; the efficiency of trap fluid collection has therefore been determined by estimating the ratio of traps used for collection to the total number of traps per segment. This estimate (70% on average) was taken into account in data evaluation—a 10% error in the estimate would cause an approximately 3% error in the results presented. Plant tissue including emptied, blotted-dry traps was dried to constant weight, ground, and weighed into tin cups. Unfiltered trap fluid samples were transferred into tin cups and dried.

Analyses of C and ^{13}C content in tissue and trap fluid samples were conducted on an NC Elemental analyser (ThermoQuest, Germany) connected to an isotope ratio mass spectrometer (IR-MS Delta X Plus, Finnigan, Germany). The ^{13}C atomic percentage of the sample was determined and ^{13}C enrichments expressed as atom per cent (atm%) in relation to Pee Dee Belemnite as the reference standard material. Atom per cent excess ^{13}C (APE; %) was calculated as the atm% ^{13}C difference between labelled (from the apical compartment, $^{13}C_{lab}$) and control plant part (unlabelled, $^{13}C_{con}$):

$$APE = atm\%^{13}C_{lab} - atm\%^{13}C_{con}$$

The total amount of ^{13}C taken up by a particular plant segment (C_{upt}) was calculated from APE, C content in 1 g of plant tissue (C_{tot}; μg C g^{-1}) and total dry weight of the plant segment (DW; g):

$$C_{upt} = APE \times C_{tot}/(DW \times 100)$$

The relative C uptake by a particular plant segment was expressed as % of the total C_{upt} fixed by the whole plant during the experiment, which is referred to as 'total newly fixed C' in the text. All results concerning *U. vulgaris* are presented as mean ±SD.

Results

Utricularia plants of both species allocated the majority of the total newly fixed C into the shoot apex (46.0±7.0% in *U. vulgaris* and 56.1% in *U. australis*; Fig. 2). Carbon allocation rapidly decreased with increasing age of the shoot segments, constituting only approximately 8.0±4.0% and 6.7% of the total newly fixed C in the oldest analysed segment ('D', Fig. 2) in *U. vulgaris* and *U. australis*, respectively. In the youngest two plant segments bearing traps, the largest proportion of the total newly fixed C was found in the plant tissue (Fig. 2). However, the ratio of C amount exuded into the trap fluid to the C allocated to plant tissues of the particular trap-bearing segment increased with age: from 0.9±0.4 and 0.6 (segment 'B'; Fig. 2) up to 2.0±0.3 and 2.0 (segment 'D', Fig. 2) in *U. vulgaris* and *U. australis*, respectively. The youngest traps (segment 'B', Fig. 2) of both species received the largest supply of C (16.3±2.7% and 11.7% of the total newly fixed C in *U. vulgaris* and *U. australis*, respectively).

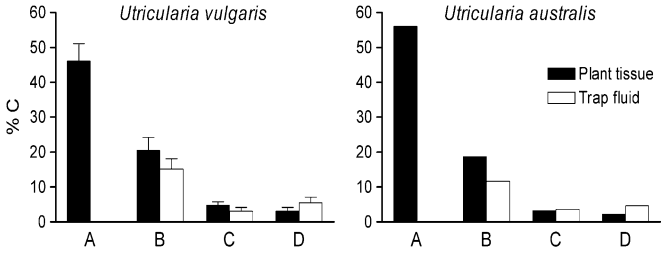

Fig. 2. Proportion of the total newly fixed C allocated to plant tissue and trap fluid in shoot segments of increasing age (A–D). Mean ±SD is shown for *U. vulgaris*.

On the whole, approximately 25% (*U. vulgaris*) and 20% (*U. australis*) of the total newly fixed C was allocated to the trap fluid in all the shoot segments analysed.

Discussion and conclusions

Most species of aquatic carnivorous plants exhibit very rapid apical shoot growth (1–4 leaf nodes d^{-1}) and high relative growth rates (Friday, 1989; Adamec, 2000, 2009; Adamec and Kovářová, 2006). To ensure their very rapid growth, aquatic carnivorous plants exhibit high net photosynthetic rates, which include the maximum values known within submerged plants (Adamec, 1997, 2006). Consistently with these results, our data show that approximately 50% of total newly fixed C in the two *Utricularia* species studied was supplied to the fast growing shoot apices. A similar pattern was observed in *Aldrovanda vesiculosa,* an aquatic carnivorous plant with ecology similar to *Utricularia*, fed on ^{14}C-labelled prey (Fabian-Galan and Salageanu, 1968).

It was also determined that a significant proportion (20–25%) of the newly fixed C in *Utricularia* was allocated to the trap fluid, mainly in the youngest functional traps. In aquatic *Utricularia*, traps may constitute 10–62% of total plant biomass (Englund and Harms, 2003; Adamec, 2008*a*). Although chlorophyll content and photosynthesis can be greatly reduced, traps are physiologically very active organs with high respiration rates (Knight, 1992; Adamec, 2006). Adamec (2006) also suggested that the main metabolic cost of carnivory in *Utricularia* species is the expenditure on organic C for trap production and on metabolic costs to maintain trap functions. The results presented here suggest

that carbon exudation into the trap lumen is an important additional maintenance cost.

The large proportion of ^{13}C allocated into the trap fluid of younger traps supports previous results where a change in trap function with age has been suggested (Sirová *et al.*, 2003, 2009; Vintéjoux and Shoar-Ghafari, 2005). New traps are most probably sterile and the increased C supply aids the rapid development of the microbial community which reaches the highest biomass values in the oldest traps (Sirová *et al.*, 2009). It is not yet known at what stage of trap development the uptake of nutrients begins, but this absorptive phase is likely to increase in importance as the trap ages (Friday, 1989; Friday and Quarmby, 1994) and large amounts of nutrients have accumulated (Sirová *et al.*, 2009).

The proportion of C allocated to trap exudation in *Utricularia* is very similar to root exudation measured in terrestrial plants, which ranges between 5% to 21% of all photosynthetically fixed C (Marschner, 1995). In addition to the classical roles of providing mechanical support and allowing water and nutrient uptake, roots also have the ability to synthesize, accumulate, and secrete a diverse array of compounds (Flores *et al.*, 1999). The ability to secrete such a variety of compounds into the rhizosphere is one of the most remarkable metabolic features of plant roots. Given the complexity and biodiversity of the soil ecosystem, roots are clearly not passive targets for soil organisms. Through the exudation of a wide variety of compounds, roots may regulate the soil microbial community in their immediate vicinity, encourage beneficial symbioses, and change the chemical and physical properties of the soil (Nardi *et al.*, 2000).

In a similar manner, rootless *Utricularia* plants most likely supply easily available organic C to the microbial community thriving within the trap environment while benefiting from its by-products. Compounds such as amino acids, organic acids, sugars, phenolics, and various other C-rich secondary metabolites are believed to comprise the majority of root exudates (Walker *et al.*, 2003). Our preliminary analyses of trap fluid composition in several *Utricularia* species revealed relatively high concentrations of simple sugars (1–120 mg l^{-1}; sucrose, glucose, fructose, galactose, ribose, xylose) and unidentified organic acids (L Adamec, unpublished results). Once inside the trap, nutrients accumulated in the traps from the surrounding water are no longer available for *Utricularia* competitors. The large absorptive surfaces of the trap lumen are thus permanently in contact with a medium where the concentrations of potentially utilizable nutrients, even in preyless traps, exceed those in the ambient water by 2–3 orders of magnitude (Sirová *et al.*, 2009). Supplying readily available C to the trap microbial community will increase turnover rates of the accumulated organic matter (including N and P) and will help to facilitate fast apical growth. Such an arrangement could enable the rootless 'pelagic' *Utricularia* to grow and utilize both inorganic and organic nutrients (either dissolved or particulate, i.e. from plankton as well as detritus) in the water column without the need for attachment to a substrate or sediment. Inhabiting this ecological niche provides advantages such as reduced competition with rooted aquatic macrophytes for nutrients and light and also increased diffusivity of available nutrients. This undoubtedly is an advantage that may have contributed to the success of *Utricularia* within the carnivorous plant group and to the ubiquitous distribution of the genus in a wide variety of nutrient-poor environments (Müller and Borsch, 2005).

Utricularia are plants with a terminal position in the phylogeny of the eudicots with some of the smallest angiosperm genomes found (Greilhuber *et al.*, 2006). One possible explanation for this phenomenon lies in selective constraints on a wide range of genomic regions that may have been lowered due to the use of an alternative mode of acquiring nutrients (Müller *et al.*, 2006). This 'alternative mode' does not necessarily need to be the capture of live microzooplankton prey, but can also include the exploitation of a close plant–microbe interaction analogous to the interactions in the rhizosphere of rooted plants. The nature of this relationship, however, including its stoichiometry, the composition of exudates and their bioavailability, or nutrient fluxes and turnover rates within the traps, remain to be assessed.

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