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Ecophysiological characterization of carnivorous plant roots: oxygen fluxes, respiration, and water exudation

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Abstract

Various ecophysiological investigations on carnivorous plants in wet soils are presented. Radial oxygen loss from roots of *Droseraceae* to an anoxic medium was relatively low 0.02 - 0.07 μmol(O_2) m^{-2} s^{-1} in the apical zone, while values of about one order of magnitude greater were found in both *Sarracenia rubra* roots and *Genlisea violacea* traps. Aerobic respiration rates were in the range of 1.6 - 5.6 μmol kg^{-1}(f.m.) s^{-1} for apical root segments of seven carnivorous plant species and 0.4 - 1.1 μmol kg^{-1}(f.m.) s^{-1} for *Genlisea* traps. The rate of anaerobic fermentation in roots of two *Drosera* species was only 5 - 14 % of the aerobic respiration. Neither 0.2 mM NaN_3 nor 0.5 mM KCN influenced respiration rate of roots and traps. In all species, the proportion of cyanide-resistant respiration was high and amounted to 65 - 89 % of the total value. Mean rates of water exudation from excised roots of 12 species ranged between 0.4 - 336 mm^3 kg^{-1}(f.m.) s^{-1} with the highest values being found in the *Droseraceae.* Exudation from roots was insensitive to respiration inhibitors. No significant difference was found between exudation rates from roots growing *in situ* in anoxic soil and those kept in an aerated aquatic medium. Carnivorous plant roots appear to be physiologically very active and well adapted to endure permanent soil anoxia.

Additional key words: aerobic respiration, anaerobic CO_2 release, CN^--resistant respiration, *Dionea*, *Drosera*, *Genlisea*, *Sarracenia*, soil anoxia.

Introduction

The majority of terrestrial carnivorous plants grow in bog and fen soils, where they endure persistent unfavourable conditions. The soils are usually wet or waterlogged, mostly acidic, and usually poor in available mineral nutrients (N, P, K, Ca, Mg; Juniper *et al.* 1989, Adamec 1997a). Thus, carnivory in most terrestrial plants may be considered as an adaptation to all these stress factors. However, the extent of adaptation of carnivorous plant roots to anoxic soil conditions has never been studied. It is possible that terrestrial carnivorous plants have adapted to these unfavourable factors by growing slowly. As summarized by Adamec (1997a) a weakly developed root system is a common characteristic of most carnivorous plants. The root/total biomass ratio in carnivorous plants species lies within the range 3.4 to 23 % (usually 10 to 20 %) in various species, which is less than in other plants. Roots are usually short, weakly branched, or unbranched, and are able to regenerate easily. Although roots have relatively low development of air spaces (Kohout 2002) they are able to tolerate, in an unknown way, anoxia and related phenomena (*e.g.* H_2S) in wet soils. This is in marked contrast with the development

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Abbreviations: CN^--R - cyanide-resistant respiration, d.m. - dry mass, f.m. - fresh mass, $J_{f.m.}$ - fresh-mass based water exudation, J_L - root-length based water exudation, R_D - dark respiration rate, RH - relative humidity, ROL - radial oxygen loss, SHAM - salicylhydroxamic acid.

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of large air spaces in roots of wetland graminoids (*e.g, Phragmites*) and cyperoids (*e.g, Carex*) where the main adaptation is the facilitation of oxygen diffusion from the shoots to the roots (*e.g*. Armstrong 1979).

Despite the lack of knowledge of the ecophysiology of carnivorous plant roots, it is reasonable to assume that the principal function of their roots is the uptake of mineral nutrients. Reliable evidence based on growth experiments in the last 20 years has shown that foliar mineral nutrient uptake stimulates considerably the mineral nutrient uptake from nutrient-poor soil by roots (Hanslin and Karlsson 1996, Adamec 2002). This inter-organ correlation between leaves and roots of carnivorous plants appears to be the basis of the carnivorous habit and represents the main physiological effect of foliar nutrient uptake from prey even though it has not yet been explained. Owing to the relatively low K, Ca, and Mg content of insects as a prey, it is possible to assume a much greater stimulation of K, Ca, and Mg root uptake as compared to that of N and P. Thus, it can be argued that it is the roots that are the first organs to benefit from carnivory. However, no increase in root tissue mineral content per unit biomass, respiration activity, or root length occurred in stimulated roots. There is, however, observed a marked increase in root biomass and diameter as a result of the foliar nutrient uptake (Adamec 2002).

This paper presents the findings of some ecophysiological studies on carnivorous plant roots including measurements of radial oxygen loss, respiration characteristics (aerobic respiration and anaerobic fermentation rates, cyanide-resistant respiration), and water exudation from excised roots. Traps of foliar origin of the carnivorous rootless genus *Genlisea* (*Lentibulariaceae*), which are functionally and ecologically analogous to carnivorous plant roots (Juniper *et al.* 1989), were also used for this study. A further experiments were carried out on the ability of carnivorous plant roots to survive under anoxia. Ecophysiological characteristics of carnivorous plant roots are discussed from the point of view of root adaptation to living in anoxic soils and their characteristics are compared with roots of non-carnivorous plants.

Materials and methods

Cultivation of plants: Nearly all plant material was provided from the indoor carnivorous plant collection in the Institute of Botany at Třeboň, Czech Republic. The plants were grown in a glasshouse under natural light in plastic 0.1 × 0.1 × 0.1 m pots in acid organic peaty soils with *ca*. 10 % (v/v) of *Perlite*. The only exceptions were *Drosera capensis* L. and *Dionaea muscipula* Ell. which were grown in conifer needle mould with *ca*. 10 % (v/v) of *Vermiculite* (for details see Adamec 2002). The pots with the plants were placed in a 0.84 m^2 white polypropylene container 0.4 m high, filled with water (tap or rain water) to a depth of 2 - 3 cm. The container was covered with a neutral-density plastic foil to reduce irradiance and increase relative air humidity (RH). In summer, the irradiance at plant level was 12 - 15 % of full sunlight within the period 16 May - 20 September, when daily temperatures at plant level fluctuated between 20 to 36 °C and RH between 60 - 90 % during the day, and between 16 to 22 °C and 80 - 96 % RH at night. This manner of cultivation and the summer microclimatic conditions were the same for all glasshouse-raised plants used throughout this study. *Sarracenia purpurea* L. was grown in a 5-dm^3 plastic container in a wet fen soil outdoors, while *Drosera rotundifolia* L. was kept in a *Sphagnum*-dominated artificial peat-bog in a 2.5-m^2 plastic container also kept outdoors. As all experimental plants of all species were grown permanently in wet anoxic soils it is possible to assume that their roots were acclimated to these conditions.

Radial oxygen loss: To estimate the permeability of the surface of carnivorous plant roots and *Genlisea* traps to oxygen, radial oxygen loss (ROL) from these organs was measured polarographically (for details see Armstrong 1979). The ROL measurements are very sensitive and mimick anoxic conditions in wet soils. ROL from carnivorous plant roots was measured in a 30 cm^3 thermostatted glass chamber closed by a rubber plug. As an electrolytic solution, 8 mM KCl + 0.1 mM $CaCl_2$ with 0.5 % (m/v) carboxy-methyl-cellulose was used. The latter inert substance increased the solution viscosity without having any observable effect on both plant material and ROL measurement in comparison with 0.05 % (m/v) agar used by Armstrong and Armstrong (2001). The pH of the solution was 6.9. The 5.0 mm high cylindrical Pt electrode had an inner diameter of 2.25 mm. A saturated Ag/AgCl electrode was used as reference electrode. Before the insertion of plant material, the electrolytic solution was thoroughly bubbled by N_2 for 1.5 - 2 h to expel O_2 the concentration of which was always < 0.06 mg dm^{-3}. Cleaned, intact and excised roots of *Drosera capillaris* Poir., intact roots of *Drosera rotundifolia*, excised roots of *Dionaea muscipula* and *Sarracenia rubra* Walt., and excised traps of *Genlisea violacea* St. Hil. were carefully inserted into the chamber through a fine hole in the rubber plug. Measurements on intact roots were preferred but they could be performed in small species only, for comparison with excised roots. In different species, the length of roots or traps measured was 20 - 81 mm. After insertion, the cut end of the excised root or trap above the rubber plug was dried with a piece of soft paper tissue and exposed to a constant fine

stream of air, in order to prevent plugging of the air spaces of the cut end by xylem sap. The temperature in the chamber was kept at 25 ± 2 °C in one set of measurements, at 24.0 ± 0.1 in a second set, and 3.0 ± 0.1 °C in another.

Using a polarograph (*PA4*, *Laboratory Equipments*, Prague, Czech Republic) and a double-channel pen recorder, a polarization curve was recorded first for each organ. The optimum polarization voltage was between -560 to -650 mV. In each organ, a stable polarization current was measured in the root or trap tip (0 - 5 mm) and in 5-mm steps (measured from lateral side by a plastic ruler to the nearest 0.5 mm) towards the root or trap base, covering a total distance from the tip of about 20 - 35 mm. A stable reading was usually reached within 8 - 120 min. Residual polarization current was measured in the empty Pt electrode without a plant organ after ROL measurements in each organ. Its value within 0.07 - 0.17 μA was subtracted from all measured values of the stable polarization current and ROL values in μmol(O_2) cm^{-2}(root surface area) s^{-1} were calculated from this difference, which is related to the O_2 leakage from only the 5-mm root or trap zone inserted into the Pt eletrode. To calculate this root or trap surface area root or trap diameter in the 5-mm successive zone was measured by a *CD-15* electronic caliper (*Mitutoyo Corp*., Kawasaki, Japan) to the nearest 0.01 mm. It was checked microscopically that the maximum difference of the both methods was only 0.02 mm. The organs used had a diameter of 0.19 - 0.55 mm. In each species at a given temperature, ROL was measured in 5 - 14 different organs and totally 23 - 49 single measurements were performed. The results have been subjected to a linear regression dependent on the distance from the organ tip.

Measurement of respiration and fermentation rates: Aerobic (oxygen-based) respiration rate (R_D) with or without respiration inhibitors was measured in 3-cm long apical segments with healthy root tips from functional roots of *Drosera capillaris* and *D. capensis* and in basal root segments (3 - 6 cm from root tips) from the latter species. Respiration rate of root segments (2 - 5 roots, f.m. 9 - 120 mg) was measured in a 50 times diluted mineral nutrient solution (pH *ca.* 4.7; Adamec 2002) in a 8-cm^3 stirred thermostatted chamber at 22.0 ± 0.1 °C darkness, using a Clark-type oxygen sensor (*Labio*, Prague, Czech Republic) and a pen recorder (Adamec 1997b, 2003). Oxygen concentration during the measurements was 80 - 90 % of saturation. After the R_D had been measured under control conditions (15 min) the medium was exchanged and R_D of the same root was measured in 0.2 mM NaN_3 or 0.5 mM KCN within 10 - 20 min. Another experiments were performed with 3-cm long apical root segments of *Sarracenia rubra*, *Dionaea muscipula*, *Drosera adelae* F.Muell. cv. Giant, *Cephalotus follicularis* Labill., and with traps (1.5 - 5 cm long, shortened to 2 - 2.5 cm segments) of *Genlisea violacea, G. violacea* cv. Broad Leaf, and *G. hispidula* Stapf.

The proportion of cyanide-resistant respiration (CN^--R) was measured in 3-cm long apical root segments of *Sarracenia rubra*, *Drosera capillaris*, *D. capensis*, *D. rotundifolia* L., *Dionaea muscipula*, and in traps of *Genlisea violacea* and *G. hispidula* in a 5-cm^3 chamber at 22.0 ± 0.1 °C. R_D was measured both in freshly harvested organs in the diluted nutrient solution (controls) and in the organs after a 3-h exposure to either 0.5 mM KCN, or 5 mM salicylhydroxamic acid (SHAM; *Sigma-Aldrich Chemie*, Steinheim, Germany), or 0.5 mM KCN + 5 mM SHAM. The latter solution is known to inhibit cyanide-resistant respiration (*i.e.*, alternative oxidase pathway; *e.g.*, Kapulnik *et al*. 1992). CN^--R was expressed in % of values for 0.5 mM KCN alone. Effective KCN and SHAM concentrations and exposure period used were chosen according to Webb and Armstrong's (1983) study on pea roots.

To compare oxygen-based respiration rate of roots with anaerobic fermentation rate, anaerobic release of CO_2 was measured in 3-cm long apical root segments of *Drosera capillaris* and *D. capensis.* Freshly harvested, cleaned root segments were rinsed with the diluted nutrient solution, partly blotted dry, and put in Warburg manometer flasks (Schlüter and Crawford 2001, Adamec 2003/4). The manometers were flushed with N_2 for five min and then closed. They were equilibrated at 22.0 ± 0.02 °C. The fresh mass of the roots was *ca*. 0.35 g in *D. capillaris*, and 0.8 - 1.2 g in *D. capensis.* The measurement of CO_2 release in humid N_2 usually lasted between 30 - 150 min after the manometers were closed.

Aerobic R_D of root segments of *Drosera capillaris* and *D. capensis* was also used as a criterion for their survival under anoxia (Schlüter and Crawford 2001). Three-cm long apical root segments of the both species were put in 50-cm^3 glass flasks in the 50 times diluted nutrient solution. The aerobic segments were immersed in an air-saturated solution, while the anaerobic treatment used a nitrogen-saturated solution with an O_2 concentration below 0.1 mg dm^3. The flasks with the root segments were tightly closed by glass stoppers and maintained at 22 ± 1 °C in darkness for 48 h. After this treatment, root R_D was measured as above. R_D of freshly harvested root segments of the both species was measured as controls. All respiration and fermentation measurements were usually repeated 6 times. Dry mass of the plant material was also estimated.

Root exudation of water: To obtain data on the functioning of carnivorous plant roots, water exudation rate from excised roots was measured in 12 carnivorous plant species (*Drosera rotundifolia*, *D. capillaris, D. capensis, D. aliciae, D. spathulata* Labill.*, D. adelae*, *D. prolifera* C.T.White*, Dionaea muscipula, Sarracenia*

purpurea, *S. rubra*, *Cephalotus follicularis*, and *Pinguicula agnata* Casper). Water exudation was chosen as a criterion for active water flow through roots since this process, as well as radial ion fluxes through roots, are known to be dependent on metabolism (Clarkson 1974, Anderson 1976, Marschner 1995).

A capillary-based glass microvolumeter after Dainty and Ginzburg (1964) was used for these measurements. Its resolution was *ca*. 0.05 mm^3 and its stability in time better than 0.00004 mm^3 s^{-1}. Freshly harvested roots of the (sub)adult plants were thoroughly deprived of all soil particles by washing by tap water, their basal parts blotted dry by a soft paper tissue, and one root fixed in the microvolumeter just below the excised basal part. An adhesive silicon cement was used for water-tight fixation of the roots. The measured functional, non-senescent roots were 3 - 13 cm long and their f.m. was 4 - 120 mg. Both compartments of the equipment were filled with the 50 times diluted mineral nutrient solution as above. The equipment with the root was in a water bath at temperature of 22.0 ± 0.02 °C under shade. Water level in the capillary was read out first after 6 - 8-min stabilization in the water bath and then in 10 - 20-min intervals, totally for 20 - 60 min. It was verified that water flow rates were constant (± 5 %) for at least 2 h. After measurement, the root was excised just below the plug, weighed (f.m.), and the length of the main root measured. The measured water flows were expressed on both root f.m. ($J_{f.m.}$; mm^3 kg^{-1} s^{-1}) and root length basis (J_L; mm^3 m^{-1} s^{-1}). It was found in all species, however, that water flows correlated much better with root f.m. than root length. In each species, 6 - 18 parallel measurements were performed on roots from 4 - 8 plants. In some plant species, the parallel roots used differed considerably in their length, age, and branching from each other. These facts could account for a relatively high variation of the results. The effect of respiration inhibitors (0.2 mM NaN_3, 0.5 mM KCN) on root exudation rate was tested on roots of *Drosera capillaris* and *D. capensis*. After the exudation rate had been measured on a root under control conditions NaN_3 or KCN were added to the root compartment of the microvolumeter. Water flows were measured within 10 - 40 min after the inhibitor addition.

To estimate the activity of roots of carnivorous plants growing directly under *in-situ* anoxic soil conditions, water exudation rate was measured first *in situ* in excised roots of *Drosera capensis, D. aliciae*, and *D. capillaris* growing in pots in anoxic soil and then, in the same roots in the aerated nutrient solution. A calibrated glass capillary (inner diameter 0.60 - 0.76 mm, length *ca*. 4 cm) was fitted using a short piece of fine flexible silicon tubing (inner diameter 0.8 - 1.2 mm) to a freshly cut root stump the uppermost part of which (*ca*. 5 mm) was partly isolated from the soil. The pots with capillaries fitted to roots in peaty soil were placed on Petri dish in distilled water and exposed immediately in a growth chamber (Adamec 2003/4) at 22.0 ± 0.5 °C in light. During 0.5 - 3 h, exudation rate was measured by a plastic ruler to the nearest 0.2 mm. The root was then carefully pulled out from the soil, washed, and the exudation rate was measured in the aerated nutrient solution at 22.0 ± 0.02 °C in the microvolumeter as above. Results obtained with senescent roots were discarded.

To obtain orientation values, electrical redox potential was measured using a Pt electrode and a saturated Ag/AgCl reference electrode in the pots with experimental plants usually 5 cm below the top soil. Where possible all paired data were statistically evaluated by a two-tailed paired *t*-test. Other data were processed by one-way *ANOVA* (Tukey HSD test).

Results

ROL in roots of *Droseraceae* (*Drosera capillaris, D. rotundifolia, Dionaea muscipula*) reached relatively low values of 0.020 - 0.070 μmol(O_2) m^{-2} s^{-1}, while values of about one order of magnitude greater were

Table 1. Radial oxygen loss (ROL) from carnivorous plant roots and *Genlisea* traps to an anoxic medium, in intact plants or excised roots or traps, at 25 ± 2 °C. Linear regression between ROL and mean distance from root or trap tip (D; mm) is shown. SD interval is shown for the slope of ROL. *r* - correlation coefficient; statistically significant linear correlation, ** - $P < 0.01$; * - $P < 0.05$; *ns* - non-significant; $n = 23$ - 49.

Species	Organ type	Linear regression for ROL [μmol(O_2) m^{-2} s^{-1}]	*r*
Drosera capillaris	intact roots	(0.00650 ± 0.00220) D + 0.070	0.403^{**}
Drosera capillaris	excised roots	(0.00860 ± 0.00200) D + 0.036	0.691^{**}
Drosera rotundifolia	intact roots	(0.00036 ± 0.00057) D + 0.020	0.111^{ns}
Dionaea muscipula	excised roots	(-0.00035 ± 0.00037) D + 0.030	0.167^{ns}
Sarracenia rubra	excised roots	(-0.02800 ± 0.00500) D + 0.660	0.715^{**}
Genlisea violacea	excised traps	(0.00100 ± 0.00210) D + 0.440	0.074^{ns}

Table 2. Radial oxygen loss (ROL) from carnivorous plant roots to an anoxic medium, in intact *Drosera capillaris* or excised *Sarracenia rubra* roots at 24 or 3 °C. Linear regression between ROL and mean distance from root tip (D; mm) is shown. SD interval is shown for the slope of ROL. *r* - correlation coefficient; statistically significant linear correlation, ** - $P < 0.01$; * - $P < 0.05$; *ns* - non-significant; $n = 32$ - 35.

Species	Temperature [°C]	Linear regression for ROL [μmol(O_2) m^{-2} s^{-1}]	*r*
Drosera capillaris	24	(0.0041 ± 0.0029) D + 0.044	0.244^{ns}
Drosera capillaris	3	(0.0041 ± 0.0046) D + 0.099	0.153^{ns}
Sarracenia rubra	24	(-0.019 ± 0.0030) D + 0.45	0.728^{**}
Sarracenia rubra	3	(-0.031 ± 0.0040) D + 0.61	0.837^{**}

found in both *Sarracenia rubra* roots and *Genlisea violacea* traps (Tables 1, 2). In *Drosera capillaris,* ROL from intact roots was about twice greater than that from excised roots. In this species, ROL values increased markedly and statistically significantly ($P < 0.01$) towards root bases, as opposed to the decline in *S. rubra* roots. The trend of ROL along the organ was ambiguous in other species. ROL values in root tip were by 37 - 125 % greater at 3 than 24 °C (Table 2).

Aerobic respiration rates of *Drosera capillaris* and *D. capensis* roots were within 1.6 - 3.5 μmol kg^{-1}(f.m.) s^{-1} (Table 3). Addition of 0.2 mM NaN_3 increased the R_D in both apical and basal *D. capensis* root segments but was without any effect in *D. capillaris* (Table 3) and no effect was found for short-term addition of 0.5 mM KCN in the both species (data not shown). The rate of anaerobic fermentation (CO_2 release) in apical root segments was low compared with R_D and amounted to only 5 % in *D. capillaris* and 14 % in *D. capensis* (Table 3). R_D of roots of other four species ranged within 2.2 - 3.5 μmol kg^{-1}(f.m.) s^{-1} (Table 4). Short-term addition of 0.5 mM KCN to these species led to no statistically significant difference and that of 0.2 mM NaN_3 usually slightly increased R_D; this difference was statistically significant in *Dionaea* roots only. However, the R_D of *Genlisea* traps was only within 0.46 - 1.1 μmol kg^{-1}(f.m.) s^{-1} and the effect of both 0.5 mM KCN and 0.2 mM NaN_3 was statistically non-significant. The relatively low R_D per unit f.m. in the *Genlisea* traps reflects their 2 - 3 times lower d.m. as compared to the roots (Table 4). The short-

Table 3. Aerobic O_2 consumption, R_D, of *Drosera capillaris* and *D. capensis* roots as dependent on 0.2 mM NaN_3 and the rate of anaerobic CO_2 release of the roots in nitrogen. In *D. capensis*, the apical root segments reached 3 cm from root tips, while the basal ones did 3 - 6 cm from root tips. Within each row for aerobic O_2 consumption, the means (± SE) labelled by the same letter are not significantly different at $P < 0.05$. $n = 6$ - 8.

Material	Aerobic O_2 consumption [μmol kg^{-1}(f.m.) s^{-1}]		Anaerobic CO_2 release [μmol kg^{-1}(f.m.) s^{-1}]
	controls	0.2 mM NaN_3	
D. capillaris - apical root segments	3.52 ± 0.29^a	3.53 ± 0.19^a	0.18 ± 0.04
D. capensis - apical root segments	2.06 ± 0.16^a	2.72 ± 0.28^a	0.28 ± 0.01
D. capensis - basal root segments	1.56 ± 0.08^a	2.13 ± 0.23^b	-

Table 4. Aerobic respiration of 3-cm long apical root segments of carnivorous plants and *Genlisea* traps (roots and traps are separated by dotted line) and the short-term effect of respiration inhibitors. d.m in % of f.m. is shown. Means ± SE. Different letters within the pairs of results (controls *vs*. inhibitor) denote statistically significant difference ($P < 0.05$); $n = 2$ - 6.

Species	d.m. [% f.m.]	Respiration rate [μmol kg^{-1}(f.m.) s^{-1}]			
		controls	0.5 mM KCN	controls	0.2 mM NaN_3
Sarracenia rubra	15.1	2.58 ± 0.53^a	2.58 ± 0.47^a	2.82 ± 0.38^a	3.21 ± 0.34^a
Dionaea muscipula	13.6	3.48 ± 0.21^a	2.96 ± 0.16^a	3.41 ± 0.16^a	3.90 ± 0.13^b
Drosera adelae cv. Giant	12.1	3.09	2.71	3.59	3.53
Cephalotus follicularis	-	2.23	2.95	1.86	2.18
Genlisea violacea	5.5	1.14 ± 0.14^a	1.22 ± 0.08^a	0.97 ± 0.15^a	1.11 ± 0.12^a
G. violacea cv. Broad Leaf	4.9	0.68 ± 0.06^a	0.80 ± 0.06^a	0.68 ± 0.06^a	0.83 ± 0.08^a
G. hispidula	6.8	0.46 ± 0.04^a	0.54 ± 0.04^a	0.45 ± 0.05^a	0.56 ± 0.05^a

Table 5. R_D of 3-cm long apical root segments of carnivorous plants and *Genlisea* traps as dependent on respiration inhibitors after 3-h treatment. d.m in % of f.m. is shown. CN^--R, proportion of the cyanide-resistant respiration (*i.e.*, the effect of 0.5 mM KCN + 5 mM SHAM) in % of values for 0.5 mM KCN. Means ± SE, $n = 6$.

Species	Respiration rate [μmol kg^{-1}(f.m.) s^{-1}]					CN^--R
	d.m. [% f.m.]	controls	0.5 mM KCN	5 mM SHAM	KCN + SHAM	[%]
Sarracenia rubra	24.0	2.42 ± 0.10[a]	2.47 ± 0.14[a]	1.60 ± 0.22[b]	0.84 ± 0.11[c]	64.7 ± 5.9
Drosera capillaris	15.4	2.51 ± 0.28[a]	2.51 ± 0.21[a]	1.82 ± 0.25[a]	0.39 ± 0.03[b]	84.0 ± 1.7
Drosera capensis	10.5	2.15 ± 0.11[a]	1.88 ± 0.10[a]	1.83 ± 0.12[a]	0.46 ± 0.04[b]	76.0 ± 1.6
Drosera rotundifolia	15.8	5.61 ± 0.61[a]	5.97 ± 0.31[a]	1.45 ± 0.21[b]	0.64 ± 0.10[b]	89.2 ± 1.8
Dionaea muscipula	17.3	4.72 ± 0.50[a]	4.36 ± 0.31[a]	3.36 ± 0.42[a]	1.11 ± 0.12[b]	73.7 ± 4.0
Genlisea violacea	6.4	0.38 ± 0.03[a]	0.49 ± 0.06[a]	0.38 ± 0.03[a]	0.07 ± 0.01[b]	85.3 ± 2.5
Genlisea hispidula	5.7	0.53 ± 0.07[a]	0.48 ± 0.10[a]	0.37 ± 0.02[a]	0.05 ± 0.00[b]	86.9 ± 2.6

Table 6. R_D of 3-cm-long apical root segments of *Drosera* measured in freshly collected roots (controls) and after two-day exposure by water with air ($+O_2$) or nitrogen ($-O_2$). Means ± SE, $n = 6$. The same letter within single rows denotes no statistically significant difference at $P < 0.05$.

Species	Respiration rate [μmol kg^{-1}(f.m.) s^{-1}]		
	controls	$+O_2$	$-O_2$
D. capillaris	2.52 ± 0.25[a]	2.08 ± 0.12[ab]	1.69 ± 0.18[b]
D. capensis	1.85 ± 0.17[a]	1.00 ± 0.08[b]	1.71 ± 0.11[a]

Table 7. Water exudation rates from excised roots of carnivorous plants at 22 °C. The exudation rates are expressed per both root fresh mass ($J_{f.m.}$) and root length (J_L). Within each column, the means (± SE) labelled by the same letter are not significantly different at $P < 0.05$; $n = 6$ - 18.

Species	$J_{f.m.}$ [mm^3 kg^{-1}(f.m.) s^{-1}]	J_L [10^3 mm^3 m^{-1} s^{-1}]
Drosera rotundifolia	336.0 ± 34.0[e]	26.4 ± 1.9[c]
Drosera capillaris	66.9 ± 6.4[c]	20.6 ± 2.2[ac]
Drosera capensis	61.9 ± 5.6[c]	82.2 ± 15.0[b]
Drosera aliciae	36.7 ± 6.9 [acd]	17.8 ± 5.3[ac]
Drosera spathulata	56.1 ± 14.0[ac]	6.9 ± 1.4[ac]
Drosera adelae	51.7 ± 8.3[ac]	20.3 ± 5.0[ac]
Drosera prolifera	8.9 ± 1.5[bd]	3.3 ± 0.47[ac]
Dionaea muscipula	59.7 ± 13.0[c]	13.6 ± 2.8[ac]
Sarracenia purpurea	17.2 ± 3.1[ad]	25.8 ± 5.0[ac]
Sarracenia rubra	10.0 ± 2.8[bd]	6.9 ± 1.9[ac]
Cephalotus follicularis	0.4 ± 0.4[bd]	0.4 ± 0.4[a]
Pinguicula agnata	8.9 ± 5.8[bd]	1.5 ± 0.9[a]

term effect of 0.5 mM KCN on aerobic R_D of roots and traps was the same as that after 3-h exposure (Table 5). SHAM (5 mM) usually mildly decreased R_D of roots and traps but this decrease was statistically significant at $P < 0.05$ in only *Sarracenia rubra* and *Drosera rotundifolia* roots. However, the combination of KCN and SHAM led to a marked, statistically significant decrease in R_D in all species used. In all species, the proportion of cyanide-resistant R_D was high and amounted to 65 - 89 % of the value in KCN alone (Table 5). R_D of *D. capillaris* apical root segments were slightly decreased after 2-d exposure to aerobic medium but decreased significantly by 33 % after 2 d in nitrogen (Table 6). In *D. capensis* root segments, however, R_D was significantly decreased (by 46 %) after 2 d in aerobic medium.

Table 8. Water exudation rate in excised *Drosera* roots as dependent on 0.2 mM NaN_3 or 0.5 mM KCN. Within each row for each single treatment, the means (± SE) labelled by the same letter are not significantly different from the controls at $P < 0.05$; $n = 8$.

Species	Exudation rate [mm^3 kg^{-1}(f.m.) s^{-1}]			
	controls	NaN_3	controls	KCN
D. capillaris	66.9 ± 4.2[a]	55.8 ± 6.1[a]	47.8 ± 3.9[a]	40.0 ± 5.6[a]
D. capensis	61.7 ± 8.9[a]	60.3 ± 10[a]	-	-

Table 9. The comparison of water exudation rate in excised *Drosera* roots at 22 °C, either *in situ* in an anoxic peaty soil (controls) or in an aerated medium in a microvolumeter. For single species, the means (± SE) labelled by the same letter are not significantly different from the controls at $P < 0.05$; $n = 5$ - 10.

Species	Exudation rate [mm^3 kg^{-1}(f.m.) s^{-1}]	
	soil *in situ*	medium
D. capensis	13.5 ± 2.5[a]	20.3 ± 4.6[a]
D. aliciae	12.2 ± 2.1[a]	12.4 ± 4.7[a]
D. capillaris	48.1 ± 6.6[a]	55.0 ± 5.6[a]

Mean rates of water exudation from excised roots of the 12 carnivorous plant species examined differed by up

to three orders of magnitude from each other (Table 7). However, in all the eight species of *Droseraceae* (genera *Drosera* and *Dionaea*), mean rates ranged within 9 - 336 mm^3 kg^{-1}(f.m.) s^{-1} and except for *Drosera prolifera,* were significantly greater than those in other carnivorous plant species. *Drosera rotundifolia* was found to have an activity on a f.m. basis about 5 - 9 times greater than other similar *Drosera* species. Except for one value of 3.1 mm^3 kg^{-1}(f.m.) s^{-1}, zero rates were measured in *Cephalotus follicularis.* When exudation rates were expressed per length of main root variation was greater. On the basis of root length, mean rates in *D. capensis* (with very thick roots) of 0.082 mm^3 m^{-1} s^{-1} differed significantly from all other species (Table 7). Exudation rates from *Drosera capillaris* and *D. capensis* roots were found to be insensitive to either 0.2 mM NaN_3 or 0.5 mM KCN (Table 8). Water exudation rates from roots in three *Drosera* species measured *in situ* in anoxic soils were by 2 - 33 % lower than those in an aerated medium in a microvolumeter but these differences were statistically non-significant at $P < 0.05$ (Table 9).

All experimental plants used grew in soils with zero oxygen concentration and relatively low redox potential (range of means for different species in pots or outdoors +74 to -161 mV, total mean -26 mV, SD = 58 mV, $n = 15$).

Discussion

All the carnivorous plants used in this study are wetland plants and take up only a variable part of their mineral nutrients by roots from peaty soils (Adamec 1997a). Therefore, it is necessary to discuss whether or not the results presented here are comparable with literature data on roots of other, non-carnivorous wetland plants or whether the differences found may be attributed to carnivory itself. It is accepted that ROL in roots depends generally on root porosity (*i.e.*, oxygen availability in root tissues), root respiration rate, diffusion barriers in root exodermis, and root length (Armstrong 1979, Webb and Armstrong 1983, Armstrong and Armstrong 2001). The ROL values measured in *Sarracenia rubra* roots and *Genlisea violacea* traps at room temperature and related to root tip (0.44 - 0.66 μmol m^{-2} s^{-1}; Tables 1, 2) are comparable with the values for apical regions of *Phragmites* and rice adventitious roots (0.42 and 0.68 μmol m^{-2} s^{-1}, respectively; Armstrong and Armstrong 2001). The ROL values in two *Drosera* species and *Dionaea* (0.02 - 0.07 μmol m^{-2} s^{-1}) are similar to those in apical regions of adventitious roots in 12 rice cultivars grown in stagnant anoxic solution (usually 0.06 - 0.15 μmol m^{-2} s^{-1}; Colmer 2003). However, in contrast with a sharp ROL decrease towards root bases found in the latter two studies and caused by exodermal diffusion barriers, ROL values in *D. capillaris* roots rose markedly towards bases (0.21 - 0.38 μmol m^{-2} s^{-1} 40 mm from the tip; Tables 1, 2).

It is difficult to interpret clearly the significance of the increased ROL at 3 °C (Table 2), due to twice reduced oxygen diffusivity in gaseous phase at 3 °C (Armstrong 1979). Since ROL increased markedly in both *S. rubra* (no root hairs) and *D. capillaris* (dense root hairs) this effect alone could not be due to the respiration of root hairs inside the Pt electrode at room temperatures but rather to a greatly reduced R_D of root tissues, hence leading to a higher internal oxygen concentration in root air spaces. Combining the ROL results with the knowledge of root anatomy in some carnivorous plants (Kohout 2002) two strategies of oxygen economy might be suggested in carnivorous plants. In *Droseraceae* roots (except *D. capillaris*) with a relatively low proportion of air space (5 - 10 %), oxygen diffusion towards root tips is limited but roots reduce thoroughly ROL by formation of exodermal barriers and are able to conduct oxygen down to the root tips. Whereas in *Sarracenia* roots without an exodermal barrier, with a greater proportion of air spaces (20 - 25 %, Kohout 2002) and also in *Genlisea* traps (20 - 25 %, Adamec 2003), longitudinal oxygen fluxes are much greater but a considerable part of oxygen leaks as ROL. The proportion of air spaces in roots of wetland plants is usually much greater in non-carnivorous than carnivorous species (10 - 45 %, Justin and Armstrong 1987).

R_D of roots of seven carnivorous plant species (1.6 - 5.6 μmol kg^{-1}(f.m.) s^{-1}; Table 3 - 6) are comparable with values reported for roots of wetland cyperoids (*Carex diandra,* 1.2 - 2.9 μmol kg^{-1}(f.m.) s^{-1}; *C. acutiformis*, 0.8 - 2.2 μmol kg^{-1}(f.m.) s^{-1}, Van der Werf *et al.* 1988), aquatic plants (0.7 - 1.6 μmol kg^{-1}(f.m.) s^{-1}, Smits *et al.* 1990), some crop plants (1.1 - 1.8 μmol kg^{-1}(f.m.) s^{-1}, Veen 1989), 16 herb or tree species (range 1.7 - 10.7, usually 4.3 - 7.1 μmol kg^{-1}(f.m.) s^{-1}, Loveys *et al.* 2003), or 8 meadow species (2.5 - 3.4 μmol kg^{-1}(f.m.) s^{-1}, Atkin *et al.* 1995). Relatively high RRs in *Drosera rotundifolia* and *Dionaea muscipula* correspond to high rates of water exudation in these species (*cf.* Table 7). In the same species, different batches of roots could differ considerably in their respiration and exudation rates (*cf.* Tables 3 - 9). This variability could be due to a different age of each root batch. R_D of *Genlisea* traps (Tables 4, 5) expressed per unit d.m. are comparable with those in the roots of carnivorous plants. However, the rate of anaerobic fermentation (CO_2 release) found in the *Drosera* roots was only 5 - 14 % of the aerobic rate (Table 3). Such a low ratio can suggest that the *Drosera* roots are not adapted to internal anaerobiosis, which can lead to shortage of needed metabolic energy, and they prevent it by an aeration mechanism, as in some aquatic plants (*see* Smits *et al.* 1990). In *D. capensis* roots in air,

the respiration quotient (CO_2/O_2) of 0.97 was found (data not shown). R_D was used as a measure of viability of *Drosera* roots after 2-d exposure to either an aerobic or anoxic medium (Table 6), instead of tetrazolium staining which appeared to be inapplicable in this case due to low sensitivity (*cf.* Smits *et al.* 1990). In *D. capillaris* roots, which may be less adapted to anoxia than *D. capensis* roots (see also Table 3), the aerobic respiration rate significantly decreased after anoxia, while in *D. capensis,* after the aerobic treatment. It is not possible to explain these differences between the species on the basis of sugar content in their roots as a similar free-sugar content (1.3 - 2.1 % d.m.) and a starch content (30 - 47 % d.m.) was found in freshly cut 3-cm apical root segments in *D. capillaris* and *D. capensis* (Adamec, unpublished).

Judging from the insensitivity of root respiration of some carnivorous species to 0.2 mM NaN_3 and 0.5 mM KCN (Table 3 - 5), the capacity of the roots for cyanide-resistant respiration may be very high. Moreover, water exudation from roots of both *Drosera* species was insensitive to both NaN_3 and KCN (Table 8) although this process is generally considered to depend on ATP (Clarkson 1974, Marschner 1995). The great proportion of CN^--R from the total R_D (65 - 89 %) was confirmed in roots and traps of all carnivorous species tested (Table 5). However, it is difficult to interpret the effect of KCN or SHAM alone in the terms of the inhibition of cytochrome oxidative or cyanide-resistant pathway (Lambers 1980, Atkin *et al.* 1995). The effect of SHAM (5 mM) alone on R_D decrease in roots (by 15 - 74 %, mean 36 %) and traps (by 0 - 30 %, Table 5) of carnivorous plants was similar to the decrease found by Lambers (1980) in roots of three non-wetland plant species (by 25 - 74 %) and by Atkin *et al*. (1995) in roots of 8 meadow species (by 0 - 24 %, mean *ca*. 5 - 10 %). However, the proportion of CN^--R on the total R_D in roots in the latter two studies was only 2 - 55 % and 27 - 53 %, respectively; much less than in the present study. The data reported by Van der Werf *et al.* (1988) on a great proportion of CN^--R in roots of two wetland *Carex* species (65 - 80 %) and those in Table 5 support the view that great proportion of CN^--R is characteristic in roots of slowly growing wetland plant species. However, this characteristic does not imply a biochemical adaptation of these roots towards soil anoxia but it might rather reflect a relatively low growth rate and energy demand in these organs (see Atkin *et al.* 1995, Millar *et al.* 1998).

The results show that mean exudation rates in all *Drosera* species (except *D. prolifera*) and *Dionaea* (37 - 336 $mm^3\ kg^{-1}$(f.m.) s^{-1}) markedly exceeded those in other genera (0.4 - 17 $mm^3\ kg^{-1}$(f.m.) s^{-1}; *Sarracenia, Cephalotus, Pinguicula*, Table 7). With the exception of *Drosera rotundifolia* and *S. purpurea* growing outdoors, the other ten carnivorous species were grown under the same conditions. Therefore, the differences in exudation rates might be attributed to differences in growth forms of the individual species. Theoretically, the rosette-leaved species (*Drosera rotundifolia, D. capillaris, D. aliciae, D. spathulata*) with their leaves attached to wet soil should transpire less water and their exudation rates should be lower than those in erect (*D. capensis, D. adelae*) or erect-rosette species (*Drosera prolifera, Dionaea muscipula, Cephalotus follicularis, Pinguicula agnata*). However, this relationship does not follow from the results. Exceptionally high f.m.-based exudation rate in *Drosera rotundifolia* could partly be explained by a very low f.m. of its very thin roots (diam. 0.19 - 0.25 mm) which, however, keep fully all root functions. Low exudation rate in *Drosera prolifera* could reflect its adaptation to living in humid rain-forests. Low exudation rate in *P. agnata* could be caused by its partial adaptation to water uptake by lower leaves from the wet top soil (Juniper *et al.* 1989) and the almost zero rate in *Cephalotus* by its possible adaptation to uptake of rain water from pitchers; this could partly hold also for *Sarracenia*.

Exudation rates of the same order of magnitude or somewhat lower (range 0.1 - 31, mean *ca.* 5 - 14 $mm^3\ kg^{-1}$(f.m.) s^{-1}) were reported by Anderson (1976), Glinka and Abir (1989), and Quintero *et al*. (2001) for roots of several crop plants. Thus, in spite of living under unfavourable soil conditions (*i.e*., low mineral content, soil anoxia), roots of carnivorous plants actively pump water to shoots at comparable or even somewhat higher rates than non-carnivorous plants. Since longitudinal water flow in roots correlates closely with xylem ion fluxes (*e.g.*, Marschner 1995) and carnivorous plant roots are usually weak (Adamec 1997a) it is not clear how these roots can ensure such functions. In *Drosera capensis,* transpiration rates were measured from the adaxial leaf surface with tentacles of 7.0 $\pm$ 0.9 mmol $m^{-2}\ s^{-1}$ and from the abaxial leaf surface 3.4 $\pm$ 0.6 mmol $m^{-2}\ s^{-1}$ (n = 12; Adamec, unpublished). These values lie within the range of usual transpiration rates in herb leaves (*ca.* 1.5 - 8 mmol $m^{-2}\ s^{-1}$; Larcher 1984) and confirm the above assumption of considerably great water flows through roots of *Droseraceae*. Moreover, the comparison of exudation rates on *Drosera* roots measured under nearly-natural *in-situ* conditions in anoxic soils and those in an oxygenated medium in the microvolumeter did not show substantial differences (Table 9) which is in line with the insensitivity of the process to respiration inhibitors (Table 8). However, lower values measured *in situ* in soils could be due to some leaking of exudate from the fittings.

It may be concluded that roots of carnivorous plants are well adapted to living in anoxic soils. In spite of relatively weak proportion and development of the roots, they appear to be physiologically very active. Since the prevailing amount of mineral nutrients in carnivorous plants is gained by roots, how this activity is stimulated by leaf nutrient absorption from prey, is crucial for understanding mineral nutrition of carnivorous plants.

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