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Feeding on prey increases photosynthetic efficiency in the carnivorous sundew *Drosera capensis*

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• *Backround and Aims* It has been suggested that the rate of net photosynthesis (A_N) of carnivorous plants increases in response to prey capture and nutrient uptake; however, data confirming the benefit from carnivory in terms of increased A_N are scarce and unclear. The principal aim of our study was to investigate the photosynthetic benefit from prey capture in the carnivorous sundew *Drosera capensis*.
• *Methods* Prey attraction experiments were performed, with measurements and visualization of enzyme activities, elemental analysis and pigment quantification together with simultaneous measurements of gas exchange and chlorophyll *a* fluorescence in *D. capensis* in response to feeding with fruit flies (*Drosophila melanogaster*).
• *Key Results* Red coloration of tentacles did not act as a signal to attract fruit flies onto the traps. Phosphatase, phophodiesterase and protease activities were induced 24 h after prey capture. These activities are consistent with the depletion of phosphorus and nitrogen from digested prey and a significant increase in their content in leaf tissue after 10 weeks. Mechanical stimulation of tentacle glands alone was not sufficient to induce proteolytic activity. Activities of β-D-glucosidases and N-acetyl-β-D-glucosaminidases in the tentacle mucilage were not detected. The uptake of phosphorus from prey was more efficient than that of nitrogen and caused the foliar N:P ratio to decrease; the contents of other elements (K, Ca, Mg) decreased slightly in fed plants. Increased foliar N and P contents resulted in a significant increase in the aboveground plant biomass, the number of leaves and chlorophyll content as well as A_N, maximum quantum yield (F_v/F_m) and effective photochemical quantum yield of photosystem II (Φ_{PSII}).
• *Conclusions* According to the stoichiometric relationships among different nutrients, the growth of unfed *D. capensis* plants was P-limited. This P-limitation was markedly alleviated by feeding on fruit flies and resulted in improved plant nutrient status and photosynthetic performance. This study supports the original cost/benefit model proposed by T. Givnish almost 30 years ago and underlines the importance of plant carnivory for increasing phosphorus, and thereby photosynthesis.

Key words: Carnivorous plant, cost/benefit, *Drosera capensis*, cape sundew, fruit flies, digestive enzymes, nitrogen, phosphorus, photosynthesis, sundew.

INTRODUCTION

Carnivorous plants have resulted from several independent evolutionary processes and are thus an example of convergent evolution (Givnish *et al.*, 1984; Ellison and Gotelli, 2001, 2009; Ellison, 2006; Peroutka *et al.*, 2008; Król *et al.*, 2012). In general, convergent evolution is the process in which organisms that are not closely related independently evolve similar traits as a result of adaptation to similar environments. The American naturalist Thomas Givnish was the first to realize that terrestrial carnivorous plants are mostly restricted to sunny, nutrient-poor and wet environments, where the marginal benefit derived from carnivory exceeds the cost. He thus introduced a cost/benefit model of carnivory, which soon became a framework for studying functional ecological relationships in carnivorous plants (Givnish *et al.*, 1984; Ellison and Gotelli, 2009; Ellison and Adamec, 2011). The cost of carnivory is mainly associated with carbon investment in the production of the lure, mucilage and digestive enzymes in photosynthetically inefficient traps (Thorén *et al.*, 2003; Adamec, 2006; Pavlovič *et al.*, 2007; Karagatzides and Ellison, 2009). Some authors also found that respiration rates of traps were greater during periods of high metabolic activity associated with their function, e.g. water pumping from *Utricularia* bladders (Adamec, 2006), rapid trap movement in *Dionaea* (Pavlovič *et al.*, 2010, 2011*a*) or tentacle movement in *Drosera* (Adamec, 2010). This confirms that modification of the cost/benefit model to include the respiratory cost, as proposed by Laakkonen *et al.* (2006), is correct. On the other hand, according to the original hypothesis by Givnish *et al.* (1984), benefits derived from prey capture associated with increased nutrient uptake include an increased photosynthetic rate (A_N), higher seed production and/or direct uptake of carbon from prey. Givnish *et al.* (1984) assumed that the primary benefit of carnivory is enhanced photosynthesis as carbon uptake alone from prey is, at least in terrestrial carnivorous plants, ecologically negligible (Chandler and Anderson, 1976*b*); however, it does occur (Dixon *et al.*, 1980; Rischer *et al.*, 2002).

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Since Darwin (1875), many studies have shown the significant positive effects that prey capture has on the growth and tissue mineral nutrient content (see reviews by Adamec, 1997, 2011*a*; Ellison, 2006) but experimental studies expressing these benefits in terms of increased photosynthetic rate are scarce and ambiguous (Ellison and Gotelli, 2009). Experimental results have shown no effect of prey addition on A_N in *Drosera rotundifolia*, in three *Pinguicula* species (Méndez and Karlsson, 1999), *Sarracenia purpurea* (Wakefield *et al.*, 2005) and the aquatic *Utricularia australis* (Adamec, 2008). Increased A_N in response to feeding has, however, been confirmed in pitcher plants, including ten *Sarracenia* species (Farnsworth and Ellison, 2008), three *Nepenthes* species (Pavlovič *et al.*, 2009, 2011*b*; He and Zain, 2012) and the aquatic *Aldrovanda vesiculosa*, which has snap traps (Adamec, 2008). Recently, Ellison and Adamec (2011) modified the cost/benefit model for aquatic carnivorous plants. Here, the primary benefit was growth stimulation of the shoot apex through the provision of more N and P, which are essential for growth processes such as cell division, DNA replication and proteosynthesis (see also Adamec, 2008; Adamec, 2011*b*).

As the carnivorous syndrome involves a complex set of traits, in this study we investigated the full hunting cycle of the carnivorous sundew *Drosera capensis*, including prey attraction, digestion and the benefit from nutrient uptake. Firstly, we tested the hypothesis that the red colour of the tentacles may lure insects onto the traps. Secondly, we measured the activities of different digestive enzymes in response to prey capture and mechanical stimulation of traps. We also investigated the nutrient uptake from prey and the subsequent benefit from prey capture by simultaneous measurements of gas exchange and chlorophyll *a* fluorescence. This study confirmed the original hypothesis of Givnish *et al.* (1984) that there must be a benefit in terms of increased A_N in response to feeding, prey digestion and nutrient uptake.

MATERIALS AND METHODS

Plant material and culture conditions

The carnivorous plant *Drosera capensis* (Cape sundew) is a small, erect perennial sundew native to the Cape region of South Africa. Experimental plants were grown from seed under standard greenhouse conditions within a collection of carnivorous plants in the Department of Plant Physiology in Bratislava. Well-drained peat moss in plastic pots (6 × 6 × 6 cm), placed in a tray filled with distilled water to a depth of 1–2 cm was used. Daily temperatures fluctuated between 15 and 35 °C, relative air humidity was 50–100 % and maximum daily irradiance was 1500 μmol m^{-2} s^{-1} PAR (photosynthetically active radiation). Young, medium-sized non-flowering plants (5–6 months old) of similar size were used in our experiments.

As a model prey, fruit flies (*Drosophila melanogaster*) were used. They were cultured from eggs in a carbohydrate-rich medium and were provided by the Department of Genetics, Faculty of Natural Sciences in Bratislava. Adult flies were narcotized with ether for better manipulation and were alive when placed on the leaf surface. A parallel set of 30 fruit flies were placed on the leaves to estimate the percentage dry weight decrease of the flies due to digestion and nutrient absorption.

Prey attraction experiments

The typical form of *D. capensis* has red tentacles but the Alba form has white tentacles. This makes *D. capensis* an ideal plant for studying the hypothesis that the red colour of the tentacles is attractive to prey. One plant of each form was enclosed in a 20 L aquarium and 50 fruit flies were introduced. The plants were of similar size and had the same number of leaves. After 4 days, the number of prey captured was counted for each form and the experiment was repeated five times.

Enzyme assays

Enzymatic activity was used as measure of the plant's digestive capability and 30 plants were used for the experiment. Depending on leaf size, one young leaf of each plant was fed with three or four narcotized fruit flies for enzyme induction and three or four polystyrene balls (diameter 3–4 mm) were placed on the tentacles in the middle part of another leaf to provide mechanical irritation with no prey. After 24 h, 30 unfed (control), fed and mechanically irritated leaves were cut off using a scalpel and all leaf blades of a given variant (with tentacles) were submerged in sequence in 4 mL of 50 mM sodium acetate buffer solution (pH 5·0) for 3 min to collect the exudates. Then, the pH was checked again. We used the four chromogenic substrates *bis*(4-nitrophenyl) phosphate, 4-nitrophenyl phosphate, 4-nitrophenyl β-D-glucopyranoside and 4-nitrophenyl N-acetyl-β-D-glucosaminide (all from Sigma-Aldrich) to estimate the activity of phosphodiesterases, acid phosphatases, β-D-glucosidases and N-acetyl-β-D-glucosaminidases (NAGs), respectively. These substrates were prepared in 50 mM acetate buffer (pH 5·0) at concentrations of 5, 5, 5 and 2 mM, respectively. Fifty microlitres (for acid phosphatases), 150 μL (for phosphodiesterases) and 400 μL (for β-D-glucosidases and NAGs) of the collected fluids were added to 500 μL (for acid phosphatases), 400 μL (for phosphodiesterases) and 150 μL (for β-D-glucosidases and NAGs) of the acetate buffer and mixed with 400 μL of the particular substrate. As a control, 400 μL of each substrate solution was added to 550 μL of buffer. Mixed samples were incubated at 25 °C for 30 min (for acid phosphatases), 2 h (for phosphodiesterases) and 4 h (for β-D-glucosidases and NAGs). Thereafter, 160 μL of 1·0 M NaOH was added to terminate the reaction. Absorbance was measured at 410 nm with a Jenway 6705 UV/Vis spectrophotometer (Bibby Scientific, Essex, UK). The calibration curve was determined using 4-nitrophenol and the activities were expressed in μmol mg^{-1} protein h^{-1}. The total protein content was determined using a Bicinchoninic Acid Kit for Protein Determination (Sigma-Aldrich, St Louis, MI, USA) and the absorbance was measured at 562 nm.

The proteolytic activity in leaf exudates was determined by incubating 150 μL of the sample with 150 μL of 2 % (w/v) bovine serum albumin (BSA) in 200 mM glycine–HCl (pH 3·0) at 37 °C for 1 h. The reaction was stopped by the addition of 450 μL of 5 % (w/v) trichloroacetic acid. Samples were incubated on ice for a further 10 min and centrifuged at 20 000 *g* at 4 °C for 10 min. Absorbance of the supernatant at 280 nm was measured spectrophotometrically (Jenway 6705 UV/Vis). One unit of proteolytic activity was defined as an increase of 0·001 in the absorbance at 280 nm per min (Matušíková *et al.*, 2005). All enzyme activities

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were measured on the basis of five independent collections of digestive fluid.

As phosphatase had the highest activity of all the studied enzymes, we used an enzyme-labelled fluorescence technique for its visualization. ELF®97 phosphate (Endogenous Phosphatase Detection Kit, Molecular Probes, Eugene, OR, USA) forms insoluble fluorescent precipitates of ELF–alcohol at sites of phosphatase activity. ELF®97 phosphate was diluted 20× in a detection buffer or in distilled water for the visualization of endogenous and exogenous phosphatase activity, respectively. Fifty microlitres of the ELF®97 phosphate solution was added to the leaf surface of control, 24 h-fed and mechanically irritated plants and evaluated under a fluorescence magnifying microscope (Leica M165FC, Heerbrugg, Switzerland) equipped with green fluorescent protein (GFP1; $\lambda_{exc} = 425$ nm, $\lambda_{emis} = 505$ nm) and cyan and red fluorescent protein (CFP/DsRED; $\lambda_{exc} = 420$ and 550 nm, $\lambda_{suppression} = 490$ and 645 nm) filters.

Simultaneous measurement of gas exchange and chlorophyll a fluorescence

To determine the benefit of prey digestion and nutrient absorption, we measured photosynthetic activity in fed and unfed plants. Ten medium-sized plants were chosen, with five being fed on fruit flies and five as controls (unfed, but with the possibility of occasionally catching some small prey in the greenhouse). The fed plants were given 5–10 fruit flies per week during a 10-week feeding period, giving 90–100 fruit flies per plant. After 10 weeks, five young fully developed leaves were used for simultaneous measurements of gas exchange (Ciras-2, PP Systems, Hitchin, UK) and chlorophyll *a* fluorescence (Fluorcam FC1000LC, Photon Systems Instruments, Brno, Czech Republic). The apical part of the leaf blade (covered with tentacles) was enclosed in a PLC6 (PP Systems) leaf cuvette connected to a Ciras-2 infrared gas analyser. The leaf was dark-adapted for 30 min and then minimal fluorescence (F_0) was measured (0·1 µmol m^{-2} s^{-1} PAR, $\lambda = 455$ nm) with a fluorocamera attached to the PLC6 leaf cuvette. Maximum fluorescence (F_m) was then measured using a saturation pulse (3000 µmol m^{-2} s^{-1} PAR, 800 ms duration, $\lambda = 620$ nm) and the maximum quantum yield (variable/maximum fluorescence, F_v/F_m) was calculated as $(F_m - F_0)/F_m$. An induction curve of 15 min duration was then obtained by switching on an actinic light of 100 µmol m^{-2} s^{-1} PAR. For analysis of the quenching mechanism, ten saturation pulses were triggered to estimate maximum chlorophyll fluorescence in the light-adapted state (F_m', 3000 µmol m^{-2} s^{-1} PAR, 800 ms duration, $\lambda = 620$ nm). The actinic light was then turned off and F_0' was measured. The effective photochemical quantum yield of photosystem II (Φ_{PSII}), photochemical quenching (qP) and non-photochemical quenching (NPQ) were calculated according to Maxwell and Johnson (2000). Irradiance of 70 µmol m^{-2} s^{-1} PAR was then applied and photosynthetic light response curves were determined. The light intensity was increased stepwise in seven steps of 4-min irradiation periods until 1800 µmol m^{-2} s^{-1} PAR was reached. Saturation pulses were applied at the end of each light intensity for calculation of Φ_{PSII}. Thus, light response curves of A_N and Φ_{PSII} were recorded simultaneously at a CO_2 concentration of 380 µmol mol^{-1}, a temperature of 25 ± 1 °C and a relative air humidity of 65–70 %. After these measurements, the actinic light was switched off and the dark respiration rate (R_D) was measured for 5 min after 10 min in the dark. As the measured leaf did not cover the entire cuvette area, the photosynthetic and respiration rates were recalculated to account for the leaf area enclosed in the leaf cuvette as measured by calibrated Fluorcam software. Results are the means of five independent measurements.

Chlorophyll and carotenoid extraction and quantification

One young fully developed leaf was collected from each of five fed and five unfed plants and half of the leaf was dried at 70 °C to determine the dry weight percentage. Remaining portions of the leaves were ground in a mortar and pestle with a small amount of sand and extracted with 80 % (v/v) chilled acetone with $MgCO_3$ to avoid acidification and phaeophytinization of the pigments. The samples were centrifuged at 8000 *g* at 4 °C for 5 min. Assimilation pigment concentration in the supernatant was determined spectrophotometrically (Jenway 6705 UV/Vis) at 663·2 nm for chl *a*, 646·8 nm for chl *b* and 470 nm for carotenoids. Chlorophyll and total carotenoid concentrations (mg L^{-1}) were calculated according to Lichtenthaler (1987) and expressed in mg chl $a + b$ (or carotenoids) g^{-1} dry weight.

Elemental analysis

After the feeding experiments, the plants, fresh fruit flies used for feeding and their spent carcasses (collected from the leaves after digestion) were dried at 70 °C and the aboveground plant biomass was weighed and ground to a fine powder. Samples (1 mg) were packed into tin capsules and the nitrogen content was determined in a Vario MICRO Cube (Elementar, Hanau, Germany) Elemental Analyzer. A connected continuous-flow IRMS Delta plus XL (Thermo Finnigan, Bremen, Germany) was also used to analyse the stable isotope ratios of nitrogen to assess the plant's nitrogen origin. The $\delta^{15}N$ values (‰) were measured against a laboratory reference gas (pure N_2, which had $\delta^{15}N$ −2·4 ‰) calibrated by the N1 standard [$(NH_4)_2SO_4$, IAEA, Vienna, Austria]. The $^{15}N/^{14}N$ ratios of plant samples, *R*p, were referenced to the $^{15}N/^{14}N$ ratio of atmospheric N_2 (*R*s) and expressed as $\delta^{15}N = [(Rp/Rs) - 1] \times 1000$ in ‰. The precision of the IRMS, based on multiple measurements of the N1 standard, was 0·08 ‰.

The samples were then mineralized using concentrated acids, diluted and analysed for P, K, Ca and Mg content (for all analytical details see Adamec, 2002). For P analyses, 1·8–2·4 mg dry weight was mineralized with $HClO_4$ and 6–7 mg with HNO_3 for metallic cation analyses. Analyses of P were performed colorimetrically with an automatic FIAstar 5010 Analyzer (Tecator, Sweden), while metallic cation concentrations were estimated by atomic absorption flame spectrometry using a Varian AA240FS (Agilent, Santa Clara, CA, USA) atomic absorption flame spectrometer. Five samples were used for each variant type of foliar material. Due to the low biomass of spent or unspent fruit flies, they were pooled into one mixed sample for each treatment. The results of tissue nutrient content experiments are expressed in dry weight percentages. Due to the absorption of their macroelements by the plants, spent flies are much lighter than live ones and this difference (estimated from parallel plants) can influence the calculation of the percentage of absorbed nutrients from prey. The results are the means of five measurements (except fruit flies analysis).

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Statistical analysis

Throughout this paper, the mean and standard error are provided wherever possible. A paired *t*-test was used for the evaluation of significant differences in the prey attraction experiments. To evaluate the significance of differences between fed and unfed plants, Student's *t*-test (Microsoft Excel) was used. For enzymatic activities, one-way ANOVA followed by an LSD test was used (Statgraphics Centurion XV, Warrenton, VA, USA). The statistical significance of the relationship between nutrient content and A_N was evaluated using Statgraphics Centurion XV. Prior to the statistical tests, the data were analysed for normality and homogeneity of variance. When a non-homogeneity was present, a *t*-test was employed with the appropriate corrected degrees of freedom.

RESULTS

Prey attraction experiments

The hypothesis that red-coloured tentacles are more attractive to fruit flies was not confirmed as, during the course of 4 days, the white plants captured an average of 9·6 ± 2·3 and the red plants 8·6 ± 2·2 flies. These means are not significantly different ($P = 0·662$, $n = 5$).

Induction of enzyme activities

The activities of acid phosphatases and phosphodiesterases and total proteolytic activity increased significantly in response to feeding. Activities of β-D-glucosidases and N-acetyl-β-D-glucosaminidases were not detected in fed or unfed plants. Twenty-four hours after the polystyrene balls were placed on the leaves to induce mechanical irritation, the activity of acid phosphatases and phosphodiesterases had increased significantly, but no proteolytic activity was induced (Table 1).

Endogenous phosphatase activity in cell walls of the tentacle head was observed but there were no differences between control and fed (or mechanically irritated) plants (Fig. 1). The highest exogenous phosphatase activity was detected in leaves fed on fruit flies, followed by mechanically irritated plants. Exogenous phosphatase activity was barely detectable in control leaves (Fig. 2).

Nutrient uptake from prey and leaf nutrient content

The dry weight of aboveground biomass increased significantly in fed plants (186 ± 18·8 vs. 90·6 ± 19·1 mg, $P = 0·007$), as did the number of leaves (19·6 ± 1·6 vs. 12·6 ± 1·3, $P = 0·012$). Digestion reduced the dry weight of unspent flies by 41·9 %; this reduction could result in underestimation of the efficiency of absorbed nutrients from the prey. The most efficiently absorbed nutrient from fruit flies was P, followed by K and N. It seems that Mg was not absorbed from prey but, owing to the biomass decrease in spent flies, low Mg uptake efficiency can be suggested. Calcium was transferred into the prey (Table 2). This is consistent with the high and inducible activities of acid phosphatases, phosphodiesterases and total proteolytic enzymes (Table 1). The foliar P and N contents increased significantly in response to the uptake of nutrients from prey (Table 3). The foliar content of P doubled and N slightly increased, while the contents of other nutrients slightly decreased (significantly only for K). Fruit flies are thus a rich source of N and P, the content of which exceeds that in leaf tissue by several times, and these elements are markedly absorbed. The $\delta^{15}N$ values were not significantly different between fed and unfed plants (4·51 ± 0·38 vs. 3·78 ± 1·94, $P = 0·322$) and overlapped with the $\delta^{15}N$ of fruit flies (3·15): this did not allow us to calculate the percentage of leaf nitrogen coming from the insect prey. Prey addition significantly altered both the leaf nutrient content and the stoichiometry of the critical elements N, P and K. In fed plants, the N:P ratio significantly decreased, N:K increased and K:P decreased in comparison with unfed plants (Table 4).

Benefit from prey capture in term of increased photosynthesis

Feeding the plants with fruit flies for 10 weeks increased both the dark (enzymatic) and light reactions of photosynthesis (Fig. 3). A_N increased almost linearly with increasing irradiance at intensities less than about 200 μmol photon $m^{-2} s^{-1}$ PAR and then reached saturation, while it did so in unfed plants at lower light intensities. A_{Nmax} of leaves from unfed plants was only about 50 % of that of fed leaves at saturating irradiance (Fig. 3A). Φ_{PSII}, which measures the proportion of light absorbed by chlorophylls associated with photosystem II and is used in photochemistry, typically decreased with increasing irradiance and was also significantly higher in fed plants (Fig. 3B). Chlorophyll fluorescence parameters from photosynthetic

TABLE 1. *Activity of acid phosphatases (APs), phosphodiesterases (PDs), N-acetyl-β-D-glucosaminidases (NAGs) and β-D-glucosidases (BGs) and total proteolytic activity (PA) in mucilage of* Drosera capensis *24 h after feeding with* Drosophila melanogaster *or mechanical irritation*

Enzyme activity	Control plants	Mechanical irritation	Fed plants
APs (μmol mg protein^{-1} h^{-1})	24·7 ± 0·53[a]	65·4 ± 6·0[b]	297 ± 10·1[c]
PDs (μmol mg protein^{-1} h^{-1})	2·04 ± 0·10[a]	4·07 ± 0·36[b]	11·6 ± 0·54[c]
NAGs (μmol mg protein^{-1} h^{-1})	ND	ND	ND
BGs (μmol mg protein^{-1} h^{-1})	ND	ND	ND
PA (U mg protein^{-1})	363 ± 25·4[a]	297 ± 16·7[a]	2000 ± 48·8[b]

Data are means ± s.e. ($n = 5$).
Different letters in rows indicate significant ($P = 0·05$) differences (one-way ANOVA, LSD test).
ND, activity not detected.

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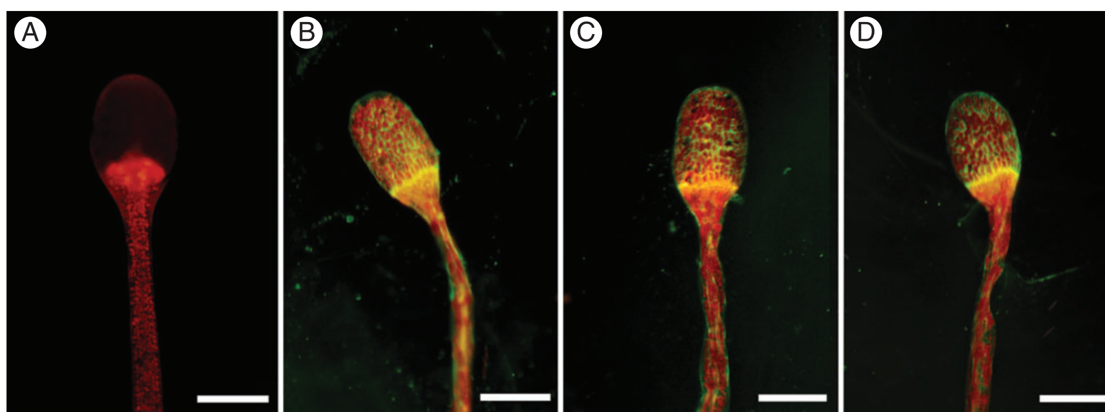

FIG. 1. Visualization of endogenous phophatase activity in the tentacles of *Drosera capensis*. Control tentacle in water (A) and endogenous phosphatase activity labelled by ELF97 phosphate diluted in detection buffer in control (B), mechanically irritated (C) and fed (D) tentacles. Yellow–green colour under the cyan and red fluorescent protein filter set in cell walls of the tentacle head indicates phosphatase activity. Scale bars = 200 µm.

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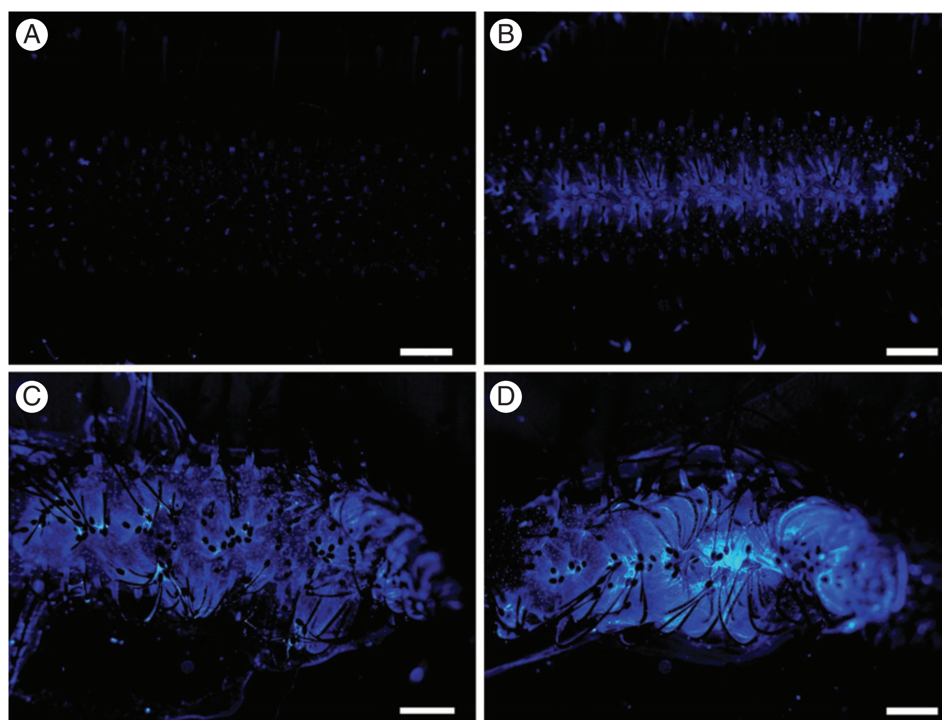

FIG. 2. Visualization of exogenous phophatase activity in leaves of *Drosera capensis* 24 h after mechanical stimulation or feeding. (A) Control, unfed plants with water (negative control). (B) Control, unfed plants labelled with ELF97 phosphate. (C) Mechanically stimulated leaf labelled with ELF97 phosphate. (D) Fed plants labelled with ELF97 phosphate. Exogenous phosphatase activity is shown in blue under the green fluorescent protein filter set. Scale bars = 1 mm.

induction measurements revealed that nutrient stress in unfed plants was overcome by feeding, as indicated by the maximum photochemical quantum yield (F_v/F_m). Non-photochemical quenching was higher in unfed plants as a consequence of less light energy being used in photochemistry and through greater heat dissipation by the xanthophyll cycle pigments (Table 5).

The content of chl $a + b$ also increased significantly in response to feeding (Fig. 4A). Although the content of xanthophylls and carotenoids ($x + c$) was not changed in response to feeding, the unfed plants had a significantly lower (chl $a + b$)/($x + c$) ratio. The chl *a*/*b* ratio did not change significantly (Fig. 4B).

Figure 5 summarizes the significant linear relationship between foliar nutrient content and A_N. There was a significant correlation between N and A_N ($P = 0{\cdot}042$, $r^2 = 0{\cdot}422$, Fig. 5A) and between P and A_N ($P = 0{\cdot}016$, $r^2 = 0{\cdot}540$, Fig. 5B), but not between K and A_N ($P = 0{\cdot}207$, $r^2 = 0{\cdot}191$, not shown), Ca and A_N ($P = 0{\cdot}363$, $r^2 = 0{\cdot}104$, not shown) and Mg and A_N ($P = 0{\cdot}504$, $r^2 = 0{\cdot}058$, not shown).

TABLE 2. *Mean tissue nutrient content in spent and unspent* Drosophila melanogaster *flies and percentage of nutrients absorbed by* Drosera capensis

Element (% dry weight)	Unspent flies	Spent flies	Absorbed nutrients (%)
N	9·86	5·88	40·4
P	0·987	0·094	90·5
K	0·991	0·271	72·7
Ca	0·071	0·293	−313[a]
Mg	0·113	0·113	0·0

Results of one analysis from one pooled sample are shown.
Note that the nutrient contents in spent flies were not corrected for the decrease in the organic biomass in spent flies, so that these values are therefore overestimated, while those of absorbed nutrients are somewhat underestimated.
[a]The negative sign indicates accumulation of nutrients in the prey.

TABLE 3. *Tissue nutrient content in leaves of* Drosera capensis *in response to feeding with* Drosophila melanogaster

Element (% dry weight)	Unfed plants	Fed plants	*P*
N	2·13 ± 0·12	2·81 ± 0·10	**0·0022**
P	0·047 ± 0·006	0·094 ± 0·005	**0·0004**
K	1·81 ± 0·26	1·01 ± 0·06	**0·0431**
Ca	0·802 ± 0·120	0·572 ± 0·024	0·1332
Mg	0·752 ± 0·071	0·586 ± 0·032	0·0754

Values are means ± s.e. ($n = 5$).
Significantly different ($P < 0{\cdot}05$) values are in bold (Student's *t*-test).

TABLE 4. *Ratios of element content (N, P, K) in foliar tissues of* Drosera capensis

Element ratio (%/%)	Unfed plants	Fed plants	*P*
N:P	47·6 ± 3·9	30·1 ± 1·9	**0·0070**
N:K	1·2 ± 0·1	2·8 ± 0·2	**0·0008**
K:P	38·8 ± 3·0	10·7 ± 0·5	**0·0007**

Values are means ± s.e. ($n = 5$).
Significantly different ($P < 0{\cdot}05$) values at are in bold (Student's *t*-test).
Nitrogen limitation is usually implied by N <2·0 % dry weight (N:P <14·5, N:K <2·1), P limitation by P < 0·1 % dry weight (N:P > 14·5, K:P > 3·4) and K limitation by K <0·8 % dry weight (N:K >3·1, K:P <3·4).

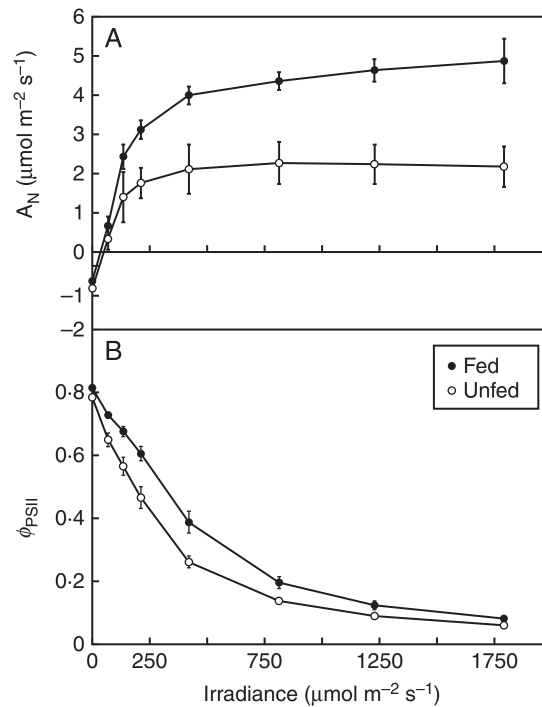

FIG. 3. Net photosynthetic rate (A_N, *A*) and effective photochemical quantum yield of photosystem II (Φ_{PSII}, B) in response to irradiance in fed and unfed *Drosera capensis* plants (as indicated in the key). Values are means ± s.e. ($n = 5$).

TABLE 5. *Chlorophyll* a *fluorescence parameters from photosynthetic induction measurements at actinic light intensity of 100* µ*mol m*$^{-2}$ *s*$^{-1}$ *PAR* in Drosera capensis

Parameters	Unfed plants	Fed plants	*P*
F_v/F_m	0·785 ± 0·007	0·815 ± 0·006	**0·0110**
Φ_{PSII}	0·608 ± 0·026	0·706 ± 0·010	**0·0171**
*q*P	0·872 ± 0·006	0·896 ± 0·010	0·0889
NPQ	0·472 ± 0·082	0·192 ± 0·024	**0·0218**

Values are means ± s.e. ($n = 5$).
Significantly different ($P < 0{\cdot}05$) values are in bold (Student's *t*-test).

DISCUSSION

In this study we investigated traits of the complex carnivorous syndrome in *D. capensis*, i.e. prey attraction and digestion as well as the benefit from prey in terms of increased photosynthesis, according to the Givnish *et al.* (1984) hypothesis *sensu stricto*. Our greenhouse study represents a considerable simplification of many ecological factors and highlights the potential ability of carnivorous plants to take up nutrients from prey and soil and utilize them in photosynthetic performance rather than just showing the ecological importance of carnivory.

Prey attraction

While many carnivorous plants use olfactory signals (Jürgens *et al.*, 2009; Di Giusto *et al.*, 2010), it has been suggested that they also use visual signals to attract prey (Moran *et al.*, 1999, 2012). Many traps of carnivorous plants have red pigmentation, but red is considered cryptic to most insects (Briscoe and Chittka, 2001). Instead, UV as a primary colour is most attractive to almost all arthropods and certain traps show conspicuous UV patterns (Juniper *et al.*, 1989; Moran *et al.*, 1999). On the contrary, red trap pigmentation might be non-adaptive, since the production of anthocyanins is often related to N deficiencies (Moran and Moran, 1998; Ichiishi *et al.*, 1999). Recently, Schaefer and Ruxton (2008) reported that prey captured by *Nepenthes ventricosa* increased with increasing red coloration on pitchers. However, their study has been questioned by Bennet and Ellison (2009), who found that the pitcher plant *Sarracenia purpurea* does not attract insects by pitcher colour, but by nectar. We did not find any adaptive value of red tentacle coloration either, as the red and anthocyanin-free forms of *D. capensis* did not differ in prey capture rates. Juniper *et al.* (1989) suggested that adhesive traps of sundews may attract insects by the glistening droplets

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secreted by stalked glands. They are easily detected from a distance as they reflect both direct and indirect light across the colour spectrum, including UV. Olfactory signals in adhesive traps are poor, as revealed by Jürgens *et al.* (2009) in *Drosera binata*. The authors suggested a camouflage idea, i.e. that positioning of the sticky trap in the flight path of potential prey might be efficient enough to catch prey at random. The link between trap coloration and prey capture needs further study.

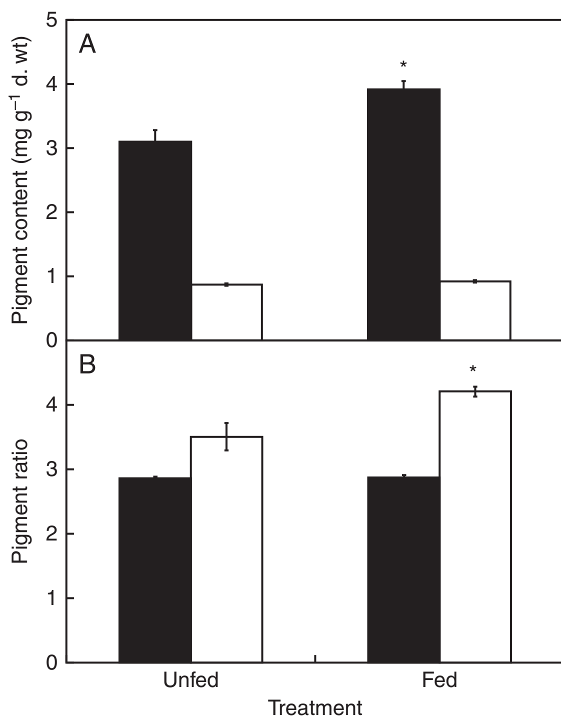

FIG. 4. (A) Assimilation pigment content (chl $a+b$ – black bars; carotenoids – white bars) and (B) pigment ratio (chl a/b – black bars; (chl $a+$ b)/(xanthophylls + carotenoids) – white bars) in response to feeding in *Drosera capensis*. Data are means ± s.e. ($n = 5$). Asterisks denote significant differences between fed and unfed plants ($P < 0{\cdot}05$, Student's *t*-test).

Prey digestion

After the prey is captured by the sticky mucilage produced by the tentacle head, the process of digestion begins (Heslop-Harrison, 1975). We found low basal enzyme activity in the control, non-stimulated mucilage. Rost and Schauer (1977) found ions and polysaccharides, but not structural proteins, in the mucilage of *D. capensis*. Therefore, we cannot exclude the possibility that the enzymes from tentacle heads (Fig. 1) were partially flushed away into the buffer from cell walls during sampling. However, this sampling procedure was very short and was necessary to obtain a sufficient amount of digestive fluid. McNally *et al.* (1988) also found an accumulation of phosphatases in cell walls in the secretory cells of stalked glands in *D. rotundifolia*. Mechanical irritation resulted in increased phosphatase and phosphodiesterase activities; no other enzymatic activity tested was upregulated (Fig. 2, Table 1). In addition to these two enzymes, live prey also induced proteolytic activity (Table 1). It can be concluded that gentle mechanical irritation is sufficient for upregulation of the enzymatic activity responsible for phosphate digestion and uptake. However, intensive mechanical stimulation, together with chemical stimulation by insect bodies, is probably important for leaf movement (Nakamura *et al.*, 2013) and the induction of proteolytic activity. Induction of proteolytic activity in response to proteins applied to *Drosera* leaves was also documented by Chandler and Anderson (1976*a*), Clancy and Coffey (1977) and Matušíková *et al.* (2005). Takahashi *et al.* (2011, 2012) found that the cysteine endopeptidase droserain is involved in protein digestion. Several other papers document proteolytic activity in the Australian sundews *D. peltata*, *D. whittakeri*, *D. binata* and *D. auriculata*; unfortunately leaf extracts rather than tentacle secretions were used, raising the question of possible contamination by intracellular enzymes from whole leaves similar to those found in non-carnivorous plants (Amagase *et al.*, 1972; Chandler and Anderson, 1976*a*; Juniper *et al.*, 1989). The inducible enzyme activities in *Drosera* are in contrast to the constitutive enzyme production in the aquatic, carnivorous, *Utricularia* (Sirová *et al.*, 2003; Adamec *et al.*, 2010).

The origin and activity of chitinases in carnivorous plants have long remained uncertain. Although Amagase *et al.* (1972), Chandler and Anderson (1976*a*) and Libantová *et al.* (2009) found chitinolytic activity in the *Drosera* genus, they again

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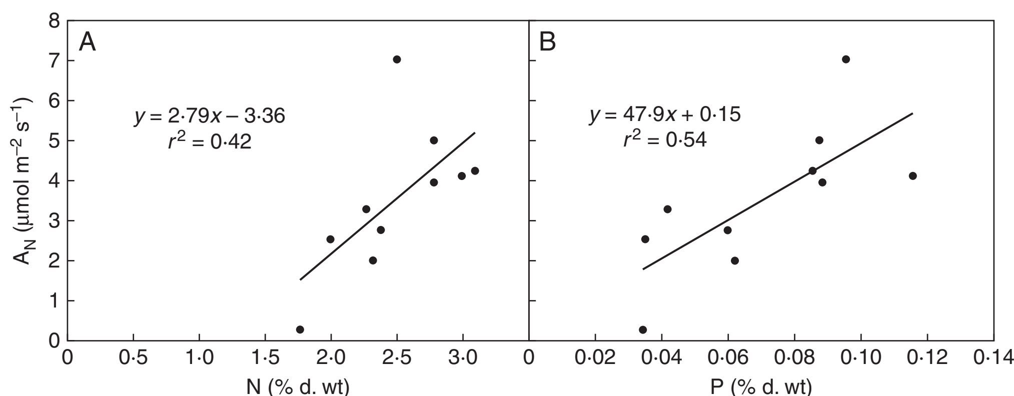

FIG. 5. Net photosynthetic rate (A_N) in relation to (A) leaf nitrogen and (B) phosphorus content in *Drosera capensis*. The linear relationship was significant (significance test for linear regression) for both nitrogen ($P = 0{\cdot}042$) and phosphorus ($P = 0{\cdot}016$). d. wt, dry weight.

used leaf extracts rather than tentacle secretions. As the activity was not detected in plants grown *in vitro*, the authors concluded that the activity may be of a microbial origin. This uncertainty was removed by the study by Matušíková *et al.* (2005), who found chitinase mRNA after chitin induction in the tentacle heads in *D. rotundifolia* grown *in vitro* by *in situ* hybridization. Furthermore, chitinase expression was also confirmed in exudates of pitcher plants of the genus *Nepenthes* (Eilenberg *et al.*, 2006; Rottloff *et al.*, 2011; Ishisaki *et al.*, 2012; Hatano and Hamada, 2012). The uncertainty about the presence or absence of chitinolytic activity may lie in the substrate specificity of chitinases. Plants contain seven classes of chitinases, which differ strongly in their cleavage patterns (Brunner *et al.*, 1998). In our study, we did not find any exochitinase activity in the tentacle secretions in stimulated or non-stimulated glands. This is not in contrast with the study by Matušíková *et al.* (2005), because their putative class 1 chitinase from *D. rotundifolia* belongs to the endochitinases. Low exochitinase activity in *D. rotundifolia* leaves was also found by Libantová *et al.* (2009); however, this was again from a plant tissue extract rather than a gland secretion.

Nutrient uptake from prey

The mineral nutrient content of prey and leaf tissue confirmed that the prey is a good potential source of N and P, but not of K, Ca and Mg (Tables 2, 3; cf. Adamec, 1997, 2002). This is in accordance with high and inducible proteolytic, phosphatase and phosphodiesterase activities (see above and Table 1), which led to significant increases in leaf tissue N and P content, but not K, Ca and Mg (Table 2). The natural abundance of stable isotopes showed that prey-derived nitrogen accounted for around 50 % of the total nitrogen in various *Drosera* species (Schulze *et al.*, 1991; Millett *et al.*, 2003). The nutrients are probably taken up by specific carriers or by endocytosis (Schulze *et al.*, 1999; Adlassnig *et al.*, 2012). Stoichiometric relationships among nutrients can be used to determine if their relative contents limit plant growth. Nitrogen limitation is usually implied by N $<$2·0 % dry weight (N:P $<$14·5, N:K $<$2·1), P limitation by P $<$0·1 % dry weight (N:P $>$14·5, K:P $>$ 3·4) and K limitation by K $<$0·8 % dry weight (N:K $>$3·1, K:P $<$3·4; Olde Venterink *et al.*, 2002, 2003; Ellison, 2006). Our unfed *D. capensis* plants were apparently P-, but not N- and K-limited, because the P content was much lower than 0·1 %, N:P was much higher than 14·5 and K:P was higher than 3·4 (Table 4). Although *D. capensis* took up both N and P from added prey (Table 3), P uptake from prey appeared to be more effective (Table 2). Thus, the N:P ratio decreased in the fed plants relative to the unfed controls. The effective P uptake from prey improved the P status almost to a non-limiting content. Adamec (2002) suggested that less effective N uptake is due to the possibly large proportion of N in insect chitin exoskeletons (as poly-N-acetylglucosamine), which is then not available for absorption. This is in accordance with the absence of N-acetyl-β-D-glucosaminidase activity in this study. As much lower portions of K, Ca and Mg are present in prey when compared with N and P (Table 2), the growth enhancement due to carnivory could theoretically be limited by the shortage and dilution of leaf K, Ca and Mg by organic substances derived from increased photosynthesis. This may explain why the content of these elements decreased in fed plants. The decrease in foliar K content and the increase in N:K ratio in fed plants indeed tends towards K limitation (Table 4). A similar response to feeding was found in *Sarracenia purpurea* by Wakefield *et al.* (2005). Adamec (1997, 2002) suggested that the uptake of P and N from seasonally caught prey is of principal importance for the plant's nutrient economy and that catching prey can stimulate K, Ca and Mg uptake by the roots. Whereas the natural growth of the majority of carnivorous genera is P-limited or P- and N-co-limited (Ellison, 2006), carnivorous pitcher plants of the genus *Nepenthes* are only N-limited (Osunkoya *et al.*, 2007; Pavlovič *et al.*, 2011*b*; Brearley and Mansur, 2012; He and Zain, 2012).

While N, P and K were effectively absorbed from prey, in accordance with previous studies on *Drosera* (Chandler and Anderson, 1976*b*; Schulze and Schulze, 1990; Karlsson and Pate, 1992; Kováčik *et al.*, 2012; for review, see Adamec, 1997), we also found that Mg was not effectively absorbed from prey. In contrast, efficient Mg absorption in the genus *Drosera* was found by Adamec (2002) and Kováčik *et al.* (2012). The reason for the low efficiency of Mg absorption from prey is unclear and we can only speculate that it was caused by the relatively high foliar Mg content. Surprisingly, the Ca content in spent flies similarly increased, as previously reported for two *Drosera* species (Adamec, 2002). Rost and Schauer (1977) found a high concentration of Ca (22 mM) in the mucilage secretion and it can be assumed that the high Ca concentration is also maintained during prey digestion and absorption.

Benefit from prey capture

Undoubtedly, the growth of carnivorous plants is enhanced by carnivory, as has been confirmed by many studies. However, an increased growth rate alone is an inadequate criterion for photosynthetic benefits, because of possible carbon uptake from prey (Ellison, 2006). In this study, we measured both the aboveground biomass as well as the dark and light reactions of photosynthesis in response to prey addition. The results support the classical intepretation of the Givnish *et al.* (1984) cost/benefit model: feeding enhanced A_N as well as Φ_{PSII} (Fig. 3, Table 5) and increased the aboveground biomass. The F_v/F_m ratio, which is used as a sensitive indicator of plant photosynthetic performance (Maxwell and Johnson, 2000), increased above 0·800 in response to feeding, indicating that nutrient stress was almost completely alleviated. Moreover, chl $a + b$ also increased. The unfed plants had a significantly lower (chl $a + b$)/($x + c$) ratio (Fig. 4B), indicating a protective role of xanthophylls in the thermal dissipation of excessive energy absorbed by the leaf measured as NPQ. This suggests that increased thermal dissipation by the xanthophyll cycle protects photosystem II against photoinhibition, at least at lower light intensities (measured at 100 μmol m^{-2} s^{-1} PAR), because the reduction state of the primary electron acceptor plastoquione A (Q_A), measured as qP, was not significantly different between fed and unfed plants (Table 5). Recently, the increased A_N in response to prey addition was documented in ten *Sarracenia* species (Farnsworth and Ellison, 2008), three *Nepenthes* species (Pavlovič *et al.*, 2009, 2011*b*; He and Zain, 2012) and in aquatic *Aldrovanda vesiculosa* (Adamec, 2008). Moran and Moran (1998) found a decrease in chlorophyll content in *Nepenthes rafflesiana* deprived of prey in comparison with control plants which could catch insect prey. On the other

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hand, Méndez and Karlsson (1999) did not find any increase in A_N in response to feeding *Drosera rotundifolia* and three *Pinguicula* species. We suggest that in their study the feeding period was very short (only 1 week) and the dose of fruit flies was low (five fruit flies) and was therefore not sufficient to have any significant effect on A_N. Wakefield *et al.* (2005) showed no increase in A_N following prey addition in *Sarracenia purpurea*, but A_N was measured directly on fed leaves. The photosynthetic rate may increase only in new leaves that are produced after feeding, due to effective nutrient translocation into the new growth (Butler and Ellison, 2007; Farnsworth and Ellison, 2008), but *Nepenthes talangensis* also showed increased A_N in fed leaves (Pavlovič *et al.*, 2009). In this study, young leaves of *D. capensis* produced after feeding were used in the measurements. In all studies where the positive effect of feeding on A_N in terrestrial carnivorous plants was documented, a positive correlation between foliar N content and A_N was found, confirming the original hypothesis of Givnish *et al.* (1984). As around 50 % of leaf N is incorporated into photosynthetic proteins, it is tempting to assume that N absorption from prey and increased leaf N content can stimulate A_N. The importance of P was neglected by Givnish *et al.* (1984) and its role in the cost/benefit model was recently highlighted by Ellison (2006) and Farnsworth and Ellison (2008). In this study, we found an even stronger relationship between P and A_N ($P = 0{\cdot}016$, $r^2 = 0{\cdot}540$, Fig. 5B) than between N and A_N ($P = 0{\cdot}042$, $r^2 = 0{\cdot}422$, Fig. 5A), which confirms the important role of P in the cost/benefit model.

Conclusions

In this study we unequivocally showed that in *Drosera capensis*: (1) red tentacle coloration is not attractive for fruit flies; (2) enzyme activities are induced in response to mechanical irritation (phosphatases, phosphodiesterases) or prey capture (phosphatases, phosphodiesterases, proteases); (3) there is efficient uptake of N, P and K, but not Ca or Mg, from prey; (4) leaf N and P contents increase in response to feeding; (5) photosynthetic efficiency is increased in fed plants. Our study confirmed the original cost/benefit hypothesis proposed by Givnish *et al.* (1984) that there is a benefit in terms of increased photosynthetic efficiency in response to nutrient uptake from captured prey.

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