WHY DOES *PINGUICULA VULGARIS* L. TRAP INSECTS?

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SUMMARY

Growth and nutrient content were studied when various nutrients were fed to the carnivorous plant *Pinguicula vulgaris* L. through its leaves. Agar blocks containing different combinations of three nutrient solutions [N, P and micronutrients (M), i.e. all other essential nutrients] simulating insects were supplied in a factorially designed experiment. Plants not exposed to any treatment, and plants supplied with insects, were used as controls. Only P had a significant effect on plant biomass. The root/leaf ratio increased as a result of N treatment and decreased as a result of the NPM interaction. The NPM interaction also markedly affected the N and P content of the plants. No treatment had any significant effect on the concentration or total content of micronutrients in tissues.

It is concluded that P is the most important nutrient gained by *P. vulgaris* through carnivory. The interactive effect of a complete nutrient solution is also important for nutrient relations.

Key words: *Pinguicula vulgaris*, carnivory, growth, nutrients, insects.

INTRODUCTION

It has commonly been supposed that the principal benefit from the capture and digestion of animal prey by a plant is a supplemental supply of N (Heslop-Harrison, 1978; Thompson, 1981). Even so, in several cases, N gained from trapped insects has been shown to improve growth significantly (Harder & Zemlin, 1966; Chandler & Anderson, 1976; Christensen, 1976), several investigations have shown that the effects of carnivory are fairly complex.

Drosera binata grown on N-free substrate and supplied with insects showed greater growth than when supplied with N in the substrate (Chandler & Andersson, 1976). *Utricularia gibba* supplied with *Paramecium* utilized Mg and K from the animals (Sorensson & Jackson, 1968). On the basis of the soil chemistry of sites of American pitcher plants, Plummer (1963) assumed that metallic ions gained from trapped insects could be more important than N. Folkerts (1982) suggested that micronutrients, such as Mo, which have a very low solubility in acid soils because they form complexes with iron (Jones, 1957), may play an important role in the carnivorous habit.

We reported previously an interaction between insect trapping and nutrient uptake by roots of *Pinguicula vulgaris* L (Aldenius, Carlsson & Karlsson, 1983). In comparison with controls, plants supplied with insects showed a larger increase in N content than could have been due to withdrawal from the insects. We hypothesized that a nutrient other than N, withdrawn from the insects, promotes N uptake by the roots.

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Table 1. *Composition and source of nutrients supplied to* Pinguicula vulgaris

Nutrient	Source	Dose	Total amount supplied
N	Soil	20–30 μg	120–180 μg
	Agar	2–3 μg	12–18 μg
P	Soil	4–6 μg	24–36 μg
	Agar	0·5–0·8 ng	3–5 ng
M	Soil	$0{\cdot}8–1{\cdot}2 \times 10^{-12}$ mol	$4{\cdot}8–7{\cdot}2 \times 10^{-12}$
Ca	Agar	$0{\cdot}01–0{\cdot}015 \times 10^{-6}$ mol	$0{\cdot}06–0{\cdot}09 \times 10^{-6}$ mol
Fe, Mn, Zn	Agar	$0{\cdot}06–0{\cdot}2 \times 10^{-9}$ mol	$0{\cdot}4–1{\cdot}2 \times 10^{-9}$ mol
Mg, K	Agar	$0{\cdot}02–0{\cdot}1 \times 10^{-6}$ mol	$0{\cdot}12–0{\cdot}15 \times 10^{-6}$ mol
S	Agar	$1{\cdot}3–1{\cdot}9 \times 10^{-9}$ mol	$7{\cdot}5–11{\cdot}4 \times 10^{-9}$ mol
Mo	Agar	$2–3 \times 10^{-9}$ mol	$12–18 \times 10^{-9}$ mol
Cu	Agar	n.d.	?

M, micronutrients (cf. Methods); other substances are called by their chemical symbols; n.d., not detectable.

The aim of this study was to investigate further the effects of various nutrients taken up through the leaves on growth and, also, the possible interaction between nutrients taken up through leaves and those taken up through roots.

MATERIALS AND METHODS

The experiment was carried out in a greenhouse at Abisko Scientific Research Station in northern Sweden (68° 21′ N). The plant material originated from a subalpine mire at Abisko, the same site as that of the Abisko plants in Aldenius *et al.* (1983). On 17 June 1982 *P. vulgaris* plants were dug up together with a piece of the substrate to fit in a plastic pot measuring 8 × 8 × 6 cm. Ten groups with 20 plants in each group were randomly selected from the collected material. One untreated group was left as a reference (O), one was supplied with insects (I), and the other were supplied with 'artificial insects' consisting of agar blocks (5 % agar) with different combinations of nutrients added. Three different nutrient solutions were used at two levels, present or absent, in a factorially designed experiment: (1) ammonium nitrate, with an N concentration of 0·5 %, (2) sodium hydrogen phosphate, containing 0·1 % P and (3) 'micronutrients' (M), i.e. all other essential nutrients: 6 μM K and 0·2 μM each of Ca, Mg, Zn, Cu, Mn, Fe, Mo and B. EDTA was also added to the M solution. The exact ion composition was as described by Moore (1981), and amounts supplied to the plants through the agar blocks are shown in Table 1. Thus, the procedure gave eight different groups – AO (agar blocks without any additional nutrients), N, P, M, NP, NM, PM, and NPM.

The plants were supplied with agar blocks or insects once a week. Each agar block weighed approximately 4 to 6 mg. A nutrient solution of the same composition as used by Aldenius *et al.* (1983), but diluted to a concentration of 5 mg N l^{-1}, 1·3 mg P l^{-1} and 4·6 mg K l^{-1} was supplied to the soil three times per week.

On 3 and 4 August the plant material was harvested, since some of the plants showed an incipient fungal infection. The plants were carefully extracted from the substrate and all remains of insects or agar blocks were removed. The plants were separated into roots, leaves, buds and reproductive organs, i.e. flower stalk and seed capsule. All tissues were dried at 70 °C for 24 h and then weighed. Ten of the plants were used for N analysis by Kjeldahl digestion, the ammonium concentration being

Table 2. *Biomass, proportions and chemical composition of* Pinguicula vulgaris *after different nutrient treatments*

Parameter	Treatment									
	O	AO	N	P	M	NP	NM	PM	NPM	I
Biomass (mg)*	36·6	37·3	41·1	53·9	42·1	47·9	36·6	39·4	50·1	49·7
	(4·6)	(5·0)	(4·5)	(3·7)	(3·9)	(4·9)	(4·0)	(3·4)	(7·3)	(5·3)
Root wt (mg)*	2·22	2·21	2·82	3·47	2·53	3·27	2·68	2·46	2·95	3·11
	(0·24)	(0·27)	(0·29)	(0·26)	(0·21)	(0·29)	(0·26)	(0·20)	(0·44)	(0·56)
Leaf wt (mg)**	21·7	18·0	24·2	38·0	28·6	32·4	20·9	23·8	32·4	32·8
	(3·0)	(2·7)	(2·9)	(3·2)	(3·2)	(3·7)	(2·5)	(2·8)	(5·6)	(4·9)
Reprod. wt (mg)*	13·7	12·3	13·3	14·0	10·3	17·3	9·7	13·4	13·6	13·8
	(3·5)	(1·8)	(1·4)	(1·8)	(1·2)	(2·8)	(1·3)	(1·6)	(1·3)	(1·4)
Root/leaf*	0·15	0·12	0·13	0·10	0·10	0·12	0·14	0·12	0·10	0·10
	(0·01)	(0·02)	(0·01)	(0·01)	(0·01)	(0·01)	(0·01)	(0·01)	(0·01)	(0·01)
N conc. (mg g^{-1})***	5·12	4·82	6·00	4·16	5·14	4·01	6·02	3·69	6·45	3·63
	(0·35)	(0·27)	(0·54)	(0·33)	(0·26)	(0·24)	(0·52)	(0·26)	(1·13)	(0·26)
P conc. (mg g^{-1})**	0·94	1·29	1·37	1·36	0·99	1·13	0·86	1·00	1·97	1·34
	(0·08)	(0·18)	(0·29)	(0·13)	(0·08)	(0·14)	(0·11)	(0·08)	(0·26)	(0·20)
Total N (μg)**	116	103	174	181	156	146	146	120	182	150
	(15)	(14)	(12)	(12)	(15)	(17)	(11)	(18)	(19)	(17)
Total P (μg)**	48·4	58·9	64·2	84·0	53·1	67·5	41·9	45·2	113	78
	(8·4)	(9·7)	(9·0)	(9·9)	(7·7)	(13·7)	(4·7)	(4·7)	(19)	(14)

Each value is a mean followed by a standard error in brackets.

Significance levels after each parameter show the probability of significant differences between the groups (except O and I) as obtained by a one-way ANOVA. Treatments: O, control; AO, agar-control; N, agar + nitrogen; P, agar + phosphorus; M, agar + micronutrients; NP, agar + nitrogen and phosphorus; NM, agar + nitrogen and micronutrients; PM, agar + phosphorus and micronutrients; NPM, agar + nitrogen, phosphorus and micronutrients, and I, plants supplied with insects.

* $P < 0{\cdot}05$, ** $= P < 0{\cdot}01$, *** $= P < 0{\cdot}001$.

determined by a Bifok FIA analyser (Bifok AB, Upplands-Väsby, Sweden). The other 10 plants in each treatment group were digested in nitric acid and hydrochloric acid (1 : 4) for analysis of P, K, Ca, Mg, Fe and Mo. Phosphorus was determined by the lactate method (Egner *et al.*, 1960), and the metallic ions were determined by atomic absorption spectrophotometry. The agar was also analysed for its nutrient content.

A one-way analysis of variance (Sokal & Rohlf, 1969) was used to test whether there was any significant variation between the eight groups included in the factorial experiment (Table 2). The variation was then analysed by a 'factorial' analysis of variance according to Snedecor & Cochran (1973). This analysis assumes an equal number of replicates in each treatment group. To attain this, the missing values, resulting from deaths in the plant material, were replaced by the mean for the respective group. In the entire material a total of 10 plants was lost during the experiment and consequently replaced by mean values in the final statistical analysis. The degree of freedom for the residual (error) was decreased by the number of missing values to reduce the overestimation of the probability for separation of groups (Montgomery, 1976). By this analysis, tests of the mean effect of the individual factors, i.e. N, P and M, and of the interactions between them, were obtained. Table 3 shows the result of the second analysis of factor and interaction effects.

The chemical analysis showed that the agar contained considerable amounts of

Table 3. *Mean factor and interaction effects obtained from a factorial analysis of variance on biomass, proportions and chemical composition of* Pinguicula vulgaris

Parameter	Factor effects			Interaction effects			
	N	P	M	NP	NM	PM	NPM
Leaf d. wt (mg)	−0·55	7·77***	−2·65	2·05	0·97	−4·44*	6·07**
Root d. wt (mg)	0·26	0·47**	0·29	−0·11	0·06	−0·38*	0·29
Reprod. d. wt (mg)	0·60	2·85**	−2·8*	1·14	−0·83	0·66	−0·73
Root/leaf	0·015*	−0·012	0·00	0·012	−0·001	0·004	−0·015*
Biomass (mg)	−0·25	9·60**	−1·99	2·62	2·85	−4·17	5·46
N conc. (mg g^{-1})	1·17***	−0·92**	0·58	0·14	0·65*	0·41	0·80**
Total N (μg)	21·7*	12·4	0·2	−8·4	4·4	−12·0	44·2***
P conc. (mg g^{-1})	0·12	0·19	−0·13	−0·15	0·20	0·27*	0·30*
Total P (μg)	11·3	22·8**	−5·4	14·2	16·9*	8·6	25·1***

Significance levels as in Table 2. Missing values exchanged by means as described in Materials and Methods.

several micronutrients (Table 1). Accordingly, an alternative 'factorial' analysis of variance was performed. In this analysis, the AO group was used as 'M', and the reference group (O) as 'AO'.

RESULTS

Biomass was largest for plants (P group) supplied with agar blocks containing only P (Table 2). The NPM, NP and I groups were slightly smaller, but the differences were not significant. The pair O and AO had smaller, almost identical biomasses. The groups supplied with only N or M in the agar blocks showed a small, non-significant increase in biomass in comparison with the agar control (AO).

The factorial analysis of biomass showed a significant effect only for P; plant biomass was increased by more than 25% by addition of P to the agar blocks (Table 3). Both leaf and root weights were negatively affected by the PM interaction. The ratio between root weight and leaf weight was positively affected by N and negatively affected by the complete-nutrient (NPM) interaction effect.

The concentration of N in the plant increased most in plants supplied with agar blocks containing N (Table 3). The factorial analysis of plant N concentration showed, in addition to the positive effect of N, a negative effect of P and a positive interaction effect between NM and NPM (Table 3). The total N content of the plants was twice as strongly affected by the NPM interaction as by N alone.

The concentration of P in the plant showed an increase as a result of the PM and NPM interaction effects only. Phosphorus content of the total plant increased as a result of the P treatment and of the interaction effects of NM and NPM.

The other nutrients analysed – K, Fe, Mg and Ca – caused no significant effect. The amount of Mo was too low for accurate analysis. Only traces were found in the M and NPM groups.

Insect trapping elicited a response pattern similar to that caused by the complete agar blocks (Table 2). Biomass increased and the root/leaf ratio decreased when insects were added to the plants. Concentrations of N, Mg and Fe were lower in plants supplied with insects in comparison with those supplied with complete-nutrient agar.

A comparison between the control (O) and the agar control (AO) groups showed no significant difference (Table 3). Because of the small differences between the O and AO groups, the alternative statistical analysis (described in Materials and Methods, but not shown here), using the AO group as 'M' and the O group as 'AO' also gave similar results, although there were slightly stronger effects and more distinct statistical differences between groups in the second analysis.

Discussion

The NMP agar blocks seemed to have had an effect on the plants similar to that of insects. It is notable that the O and AO groups also showed similar results, and there was no indication of any substantial effect of agar itself, although the agar contained a considerable amount of micronutrients. An important difference between the AO and the M treatments is, however, the presence of EDTA in the M agar blocks.

From the biomass results (Tables 2 and 3) it appears that the most important nutrient withdrawn from the insects is P. Neither N, nor the micronutrients, had any significant effect on biomass. The increased weight ratio between roots and leaves after the N-treatment may indicate that the plant was attempting to compensate for a deficiency of nutrients by allocating a larger part of its carbon resources to the root system (cf. Thornley, 1976). An opposite response was detected as a result of the NPM-interaction effect, and possibly for the effect of P, but the latter was not significant.

It may be difficult to distinguish the effects of N or P from those resulting from the interaction effects NM and PM because of the micronutrient content of the agar. The large difference between the P and PM effects indicates that there are some important micronutrients not available in the agar. It could be the presence of EDTA in the micronutrients making the difference in response. The presence of EDTA makes Fe, at least, much more easily accessible for the plants. The amount of Fe available in the soil could be low due to the high pH of 5·6 (Aldenius *et al.*, 1983).

Most of the increases of plant N and P content were smaller than the amounts of nutrients supplied through the agar block (Tables 1 and 3). However, the NM-interaction effect produced an increase in P content although no P was supplied.

Thus, P seemed to be the most important single nutrient gained through the leaves. No effect of micronutrients alone was detected in either the factorial experiment or the comparison between the reference plants (O) and the agar control (AO). Regarding interactions between nutrient gain through leaves and root uptake, the interaction between N and micronutrients found in this study seems to promote P uptake. In addition, there is a marked interaction effect of the complete nutrient supply (NPM) through the leaves on plant N and P content.

The interactive effects between insect trapping and root uptake of N, noted in our previous study (Aldenius *et al.*, 1983), were observed only at very high soil nutrient concentrations. It is possible that this interaction is not detectable, or does not exist, at lower, more ecologically relevant, soil nutrient concentrations. It seems clear, however, that the role of carnivory in *P. vulgaris* is not simply to provide N or P. Although micronutrients alone did not affect the plants, they seemed to play an important role in plant nutrient relations when combined with N and P.

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