New Phytol. (1983) 93, 53–59

EFFECTS OF INSECT TRAPPING ON GROWTH AND NUTRIENT CONTENT OF *PINGUICULA VULGARIS* L. IN RELATION TO THE NUTRIENT CONTENT OF THE SUBSTRATE

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(*Accepted 30 August 1982*)

SUMMARY

The significance of the carnivorous habit of *Pinguicula vulgaris* L. was studied when plants were fed with insects and/or supplied with complete nutrient solution in the substrate. In most cases, plants supplied with insects alone or in combination with fertilizer showed higher values for dry wt, number and length of leaves, and concentrations of nitrogen and phosphorus. The amount of nitrogen absorbed when insects were added to fertilized plants was larger than the insects contained. It is inferred that *P. vulgaris* uses both nitrogen and phosphorus from the insect. It is hypothetized that some other substance is also obtained from the insects and used for the uptake by roots or utilization of nitrogen. No major differences in response were found between plants from the two sites studied, i.e. one relatively rich and one relatively poor mire in the Torneträsk area, North Sweden.

INTRODUCTION

There are about 450 species of carnivorous plants distributed among six families (Lloyd, 1942). To be considered carnivorous, a plant should fulfil the following three criteria: it should be able to (1) catch or trap the prey, (2) absorb metabolites from it and (3) utilize these metabolites in its growth and development (Chandler and Anderson, 1976).

Individual features of a carnivorous species, such as secreting and absorbing glands, pitcher-shaped leaves and attractive odours occur throughout the plant kingdom but a carnivorous plant must have an assemblage of several of these individual adaptations (Lloyd, 1942).

Since all carnivorous species of plants are photosynthetic, the carnivorous habit is more likely to be a way of providing mineral nutrients than carbohydrates (Denffer *et al.*, 1976). Another common characteristic of carnivorous plants is the weakly developed root system, the absorptive function of which may be compensated by the nutrients supplied through the digestion of insects (Schmucker and Linnemann, 1959). The habitats of carnivorous plants are often moist, poor in nutrients and/or with a low degree of competition. One reason for the connection with moist habitats may be that the glands which are the sites of enzyme secretion and absorption have evolved from hydathodes responsible for guttation by many species living under moist conditions (Schmucker and Linnemann, 1959).

That catching insects promotes growth of carnivorous plants has been shown by,

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0028-646X/83/010053+07 $03.00/0

among others, Darwin (1878) for *Drosera rotundifolia*, Harder and Zemlin (1967) for *Pinguicula lusitanica*, and Chandler and Anderson (1976) for *Drosera binnata* and *D. whittakeri*. Harder and Zemlin (1967) also showed that normal development and flowering in *P. lusitanica* could take place without any addition of nutrients to the leaves, provided the plants were raised on a substrate with a complete nutrient medium.

Pinguicula vulgaris L., a perennial herb with a basal rosette of leaves and weakly developed roots, occurs in moist grasslands and fens. In southern Sweden, it prefers calcareous soils but, in the north, it also occurs commonly in nutrient-poor soils (Hultén, 1960). On the upper surface of the leaves, there are stalked and sessile glands (Heslop-Harrison and Knox, 1971). Both types contain the following digestive enzymes: phosphatase, esterase, protease and ribonuclease. The stalked glands also contain amylase but their major function is to secrete a sticky droplet of mucopolysaccharides to which small insects adhere. The sessile glands are more active in the digestion and resorption of the released metabolites.

Darwin (1875) noticed that, when an insect was applied to the margin of a *P. vulgaris* leaf, a temporary rolling-up of the leaf occurred. This presumably enables a larger number of glands to come in contact with the prey, thereby promoting digestion.

In boreal areas, where plants have a short growing season, nutrient deficiency in poor habitats may be reinforced by a low rate of decomposition and mineralization (Haag, 1974; Chapin, Barsdate and Barel, 1978). In addition, the absorption of phosphorus is adversely affected by low soil temperatures (Chapin and Bloom, 1976).

The hypotheses tested by the present study were that the growth and development of *P. vulgaris* are enhanced by trapping insects and that the species benefits most from its carnivorous habit when growing on a relatively nutrient-poor substrate.

MATERIALS AND METHODS

The experiment was carried out at Abisko Scientific Research Station in northern Sweden (68° 21′ N, 18° 49′ E). The plant material originated from two subalpine mires, at Abisko and at Katterjåkk, 24 km west of Abisko. On the basis of the vegetation, the former was assumed to be richer in nutrients than the latter. The mires differ also in that a more oceanic climate prevails at Katterjåkk than at Abisko (Sonesson, 1967). Each plant was dug up together with substrate of suitable size to fit into a plastic pot measuring 8 × 8 × 6 cm. Soil samples were collected at the same time and stored at −15 °C until analysed. Other plants in the pots were cut down continuously during the experiment. Any insects on the leaves were removed and the pots were placed in an insect-proof greenhouse.

The plants were divided at random into six groups, with approximately 25 in each group, according to the following scheme: AO Abisko control; AI Abisko insects only; AN Abisko extra nutrients only; AIN Abisko insects and nutrients; KO Katterjåkk control; KI Katterjåkk insects only.

The insects, applied every 4 to 6 days, consisted of small Diptera, collected in the surrounding mountain-birch wood immediately before each application. One insect was applied per plant to the healthiest leaf in the rosette of each feeding time.

To keep the water content of the substrate uniform, the pots were placed in trays in which the water level was held constant. The water in the trays was changed at regular intervals. The plants were watered with tap-water, with added nutrient solution for groups AN and AIN. The nutrient solution contained the following concentrations of nutrients when diluted: 420 mg l^{-1} nitrogen, 110 mg l^{-1} phosphorus, 390 mg l^{-1} potassium, 42 mg l^{-1} sulphur, 40 mg l^{-1} magnesium and 20 mg l^{-1} calcium. In addition, lesser amounts of the following microelements were included: boron, copper, zinc, iron, molybdenum and manganese.

As the plants started to flower, they were pollinated with the aid of a brush. This was done separately for the Abisko and the Katterjåkk plants. The plants were harvested after 45 days since they had been attacked by mould. Anthesis was then complete but the seed capsules were not yet fully developed. On harvesting, the number of leaves in the rosette of each plant was counted and the length of the longest leaf as well as that of the peduncle were measured. The number of plants that had flowered in each group was recorded. The plants, including the roots, were separated from the substrate and the undigested insect remains were removed. The plants were dried at 85 °C for 48 h and then weighed. Soil samples were taken from each group.

Total nitrogen in the plants was estimated by Kjeldahl digestion (Balsberg, 1975) and total phosphorus according to the vanadate method (Rauterberg, 1951; Balsberg, 1975). Because of the low weights of the individual plants, between two and six plants had to be taken together for analysis of phosphorus. The soil samples were analysed for pH (KCl), phosphate (lactate method, Egner, Riem and Domingo, 1960; Balsberg 1975), nitrate and ammonium with a BIFOK FIA-analyser.

The significance of the differences between the groups was assessed using Student's *t*-test.

RESULTS

The nutrient content and pH of the soil at the Abisko site were, as assumed, higher than in the Katterjåkk site, and the nitrogen and phosphorus contents were three to five times higher at the former (Table 1). During the course of the experiment, the nitrate and phosphate contents of the soil decreased in the groups supplied with tap-water only; while the total nitrogen content of the soil of the fertilized groups was 25 times higher than initially. There was a corresponding approximately sixfold increase in phosphorus content.

The Abisko plants responded positively when insects were added to the leaves. The differences between AO and AI were significant in both nitrogen and phosphorus content, in dry wt (Figs 1 and 2) and in the morphological parameters measured (Table 2). Also, the peduncle was significantly longer in AI than in AO. The frequency of flowering was not affected by supply of insects. The same pattern obtained between the control group, AO, and the fertilized group, AN, but the differences between AO and AN were two to five times greater than the corresponding differences between AO and AI. The group that was supplied with both fertilizer and insects, AIN, did not show any significant change in concentration of nitrogen when compared with AN, while the concentration of phosphorus decreased by 25 %. The AIN group had a significantly higher plant dry wt and longer leaves than the AN group. The plant dry wt of the AIN plants was three times higher than that of AO, and 50 % higher than AN.

The Katterjåkk plants responded to a somewhat lesser degree to the insect supply than the Abisko plants. Only the number and length of leaves (Table 2) differed significantly between KO and KI. However, the means of all parameters were

Table 1. *Nutrient status of the soil before and after the experiment*

Sample	*n*	pH(KCl)	NO_3^- (μg g^{-1} dry wt)	NH_4^+ (μg g^{-1} dry wt)	PO_4^{3-} (μg g^{-1} dry wt)
Abisko*	8	5·6 ± 0·2	12·0 ± 1·2	310 ± 23	360 ± 60
Katterjåkk*	4	4·1 ± 0·1	4·8 ± 0·8	54 ± 4·8	180 ± 45
AO and AI†	4	6·0 ± 0·1	7·9 ± 1·8	165 ± 53	285 ± 83
AN and AIN†	4	6·7 ± 0·1	5600 ± 325	2570 ± 335	2020 ± 300

* Before experiment. † After experiment.
Mean ± s.e. n is the number of replicates. See 'Materials and Methods' for notation of soils.

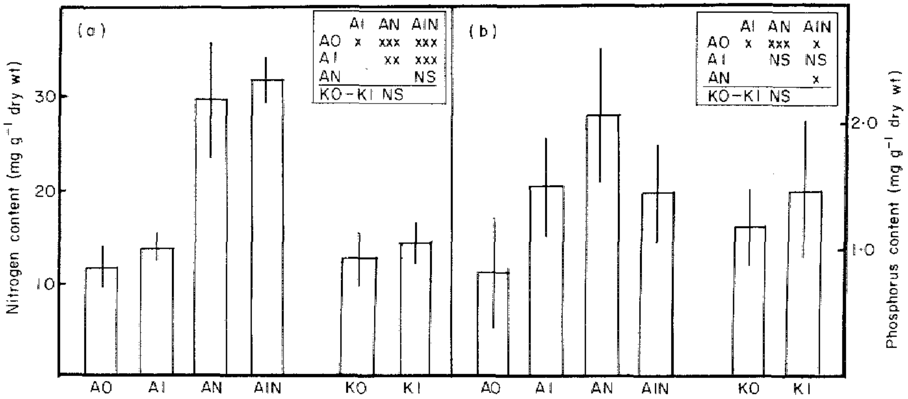

Fig. 1. The nitrogen (a) and phosphorus (b) content in *P. vulgaris* L. supplied with fertilizer and insects in different combinations; the significance levels of differences between means are given. The bars indicate 95 % confidence limits of the mean. Symbols: A, Abisko plants; K, Katterjåkk plants; O, control; I, supplied with insects; N, supplied with fertilizer; *** significant at 0·1 % level; ** significant at 1 % level; * significant at 5 % level; NS not significant at 5 % level.

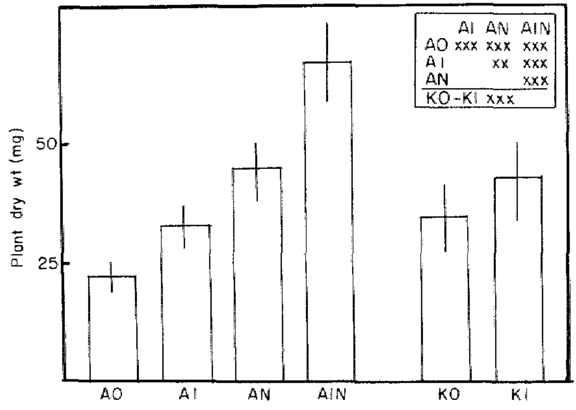

Fig. 2. The dry wt of *P. vulgaris* L. supplied with fertilizer and insects in different combinations; the significance levels of differences between means are given. The bars indicate 95 % confidence limits of the mean. Symbols as in Figure 1.

Table 2. *The number of leaves and length of the longest leaf in* P. vulgaris *L. supplied with fertilizer and insects in different combinations*

		Number of leaves					Leaf length (mm)				
				Significance of difference between groups					Significance of difference between groups		
Plant origin	Treatment	Mean	C.L.	I	N	IN	Mean	C.L.	I	N	IN
Abisko	O	4·9	0·8	*	***	***	20·4	2·2	***	***	***
	I	5·6	0·7	—	***	***	24·3	3·7	—	N.S.	***
	N	7·5	1·3	—	—	N.S.	28·3	3·9	—	—	*
	IN	7·9	1·3	—	—	—	32·8	5·2	—	—	—
Katterjåkk	O	5·9	0·9	*	—	—	22·1	3·7	***	—	—
	I	6·4	1·1	—	—	—	27·0	3·4	—	—	—

O, Control. I, Supplied with insects. N, Supplied with fertilizer. C.L., 95 % confidence limits:
* Significant at 5 % level. *** Significant at 0·1 % level. N.S. not significant at 5 % level.

higher for KI than for KO. In all parameters measured, the Katterjåkk plants had higher values than the corresponding Abisko group.

DISCUSSION

The results obtained support the hypothesis that growth and development of *P. vulgaris* are enhanced by trapping insects. The groups supplied with insects showed, in all but two of the measured parameters, an increase when compared with the corresponding groups without insects. The deviating results were: (1) A lower concentration of phosphorus in AIN than in AN. This probably reflects a luxury consumption (Smith, 1962) of phosphorus in AN combined with an increased growth in AIN, rather than a lower uptake of phosphorus. The amount of phosphorus per plant was 99 μg for the AN and 107 μg for the AIN group. (2) The frequency of flowering showed no significant difference between the control group and the others. This is probably because establishment of the flowering buds occurred before the start of the experiment. In some arctic plants, flowering buds are initiated several years before they flower (Sörensen, 1941). A similar result was obtained by Harder and Zemlin (1967) in an experiment with *P. lusitanica*, where an increase in dry wt, number of leaves and length of peduncle was observed when the plants were supplied with egg-white.

The question of what it is that the plants use from the insects arises. When grown at a low soil phosphorus content, the concentration of phosphorus in the Abisko plants almost doubled when insects were added (AI compared with AO) while the Katterjåkk groups did not show any significant change. The phosphorus gained by the AI plants can be accounted for by the phosphorus content of the insects (Table 3). At high soil phosphorus content, the phosphorus concentration of the plants decreased when insects were added but the overall plant phosphorus content was unaffected. Hence, the *Pinguicula* plants did not gain extra phosphorus from insects when grown at high soil phosphorus content. A similar response regarding concentration of phosphorus in *Sarracenia flava* has been found by Christensen (1976).

Table 3. *Estimation of the total amount of nitrogen and phosphorus in the insects supplied to each plant during the experiment, compared with the total plant content of these nutrients*

		Nitrogen			Phosphorus		
Plant origin	Treatment	Plant content (μg)	Difference (μg)	Estimated insect content (μg)	Plant content (μg)	Difference (μg)	Estimated insect content (μg)
Abisko	O	320 ± 30	150	500	18 ± 5	33	60
Abisko	I	470 ± 50			50 ± 8		
Abisko	N	1300 ± 170	800		99 ± 16	8	
Abisko	IN	2100 ± 170			107 ± 15		

Each plant was supplied with nine insects during the experiment. The content of nitrogen and phosphorus in the insects was calculated assuming a mean insect weight of 1 mg, a nitrogen content of 5 % and a phosphorus content of 0·6 %. This is considered to be an overestimate rather than an underestimate since a large fraction of the insect, the exoskeleton, is not digestible and the insect nutrient content is on a dry wt basis.

Mean ± standard error is given for the plant content. For notation of treatments, see legend to Table 2.

The increase in concentration of nitrogen in the plants is much larger when nutrients are added to the soil (AN compared with AO) than when insects are added to the leaves (AI compared with AO, and AIN compared with AI). However, the nitrogen content of the insects is much lower than the soil nitrogen content. When insects were added to the fertilized plants, the total plant nitrogen content increased by a larger amount of nitrogen than the insects could have contained (Table 3). The AIN plants must have used another substance from the insects to increase the uptake of nitrogen from the soil.

Thus, *P. vulgaris* probably uses both nitrogen and phosphorus from the insects but some other substance or substances seem also to be obtained from them and used in the processes of uptake of nitrogen from the soil and its utilization. An increased uptake of one nutrient with an increase in supply of another has been shown in several cases (Chapin, 1980). Such ions could possibly be iron or molybdate ions which are needed for the metabolic reduction of nitrate (Noggle and Fritz, 1976). The availability of these ions depends on the soil pH. At low soil pH, molybdate ions are less available than at high, while iron ions are much less soluble in neutral and alkaline soils than in acid soils (Devlin, 1969).

A different response was found for *D. whittakeri* when grown in sand cultures with nutrient solutions lacking nitrogen, sulphur, phosphorus or microelements (Chandler and Anderson, 1976). With no nitrogen or sulphur in the substrate, growth was enhanced by insects while no such effect was observed in plants growing in solutions lacking phosphorus or microelements.

The hypothesis that the species benefits most from insects when grown on nutrient-poor substrates is not supported by the results. Approximately the same or a smaller response was observed by adding insects to the Katterjåkk plants (KO and KI) as compared with the Abisko plants (AO and AI), which originated from a more fertile habitat. Differences in the phenological status between the Abisko and Katterjåkk plants at the time of collection could have affected the response, making the comparison somewhat distorted. Also, when the differences in response between AO–AI and AN-AIN are compared, the response is the same or lower in the groups with low soil nutrient content. This pattern was also found for *P. lusitanica* where the effects of insect trapping increased with increasing nutrient status of the soil (Harder and Zemlin, 1967). This supports the hypothesis that some substance or substances from the insects are used to increase the efficiency of nutrient uptake from the soil.

ACKNOWLEDGEMENTS

The field work was financially supported by the Abisko Scientific Research Station and the University of Lund. The experiment was suggested by and designed jointly with Dr B. Sveinbjörnsson. Constructive criticism of the manuscript was made by Dr T. V. Callaghan, Dr P. Jensén, Professor M. Sonesson and Professor G. Tyler. Ms I. Kerstensson typed the manuscript and Mr R. Kumar revised the language. All are gratefully acknowledged.

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