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Responses of a carnivorous plant to prey and inorganic nutrients in a Mediterranean environment

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Abstract We have analysed the effect of prey and fertilization by inorganic nutrients on the survival, growth, reproduction (sexual and vegetative) and mucilage secretion of *Pinguicula vallisneriifolia* (Lentibulariaceae), a carnivorous plant inhabiting rocky substrates of southern Spain. We tested the hypothesis that carnivorous plants are more prey dependent when root access to nutrients is strongly limited by (1) analysing the importance of the carnivorous habit to the fitness of *P. vallisneriifolia* in its natural rocky habitat, and (2) determining whether the effect of trapped prey varies with soil nutrient levels. Our 2-year experimental results indicated prey to be limiting to *P. vallisneriifolia* growth on its natural rocky substrate. Animal food supply substantially increased the chance of survival, growth, vegetative propagation, sexual reproductive success and mucilage secretion. The differences between prey levels were more evident at the end of the experiment when all the surviving Prey-exclusion plants had lost weight, and the probability of sexual reproduction and of vegetative propagation by axillary buds had accordingly diminished. Furthermore, there were clear benefits from carnivory at the population level, since both the expected individual life span and the lifetime vegetative and sexual output correlated positively with the quantity of prey trapped. Application of insects to non-fertilized plants stimulated growth, but similar application to fertilized plants grown on a complete nutrient solution failed to enhance growth. There was no obvious benefit from the provision of a balanced mineral nutrient solution (alone or with prey). The greatest absolute growth and sexual and vegetative output resulted from providing a surplus of insects to plants on their natural rocky substrate. The strong dependence of *P. vallisneriifolia* on prey can therefore be considered a useful preadaptation enabling colonization of rocky substrates.

Key words Prey dependence · Fertilizing · Fitness responses · Mediterranean carnivorous plants · *Pinguicula vallisneriifolia*

Introduction

Carnivorous plants can trap, digest and absorb nutritionally beneficial substances from animal prey (Juniper et al. 1989). The extent to which a plant can be carnivorous depends on the importance of the nutrients derived from its prey in comparison with those taken up by the roots. Some species appear to be highly dependent on insect prey, whereas others can survive to maturity and even flower in the absence of any nutrient of animal origin (Lüttge 1983; Givnish 1989). In fact, laboratory and field experimentation has indicated that several *Pinguicula* and *Drosera* species are facultative carnivores, their growth being stimulated both by prey and mineral nutrients (Chandler and Anderson 1976; Aldenius et al. 1983; Karlsson and Carlsson 1984; Karlsson et al. 1991).

Carnivorous plants appear to have gained the ability to compete successfully in nutrient-poor environments (Givnish 1989). For this reason, it might be hypothesized that the benefits from prey are greater for plants growing in poorer substrate than for plants growing on richer soils (Givnish et al. 1984). This hypothesis has been tested by Karlsson et al. (1991) for *Drosera rotundifolia*, *Pinguicula vulgaris*, *P. alpina* and *P. villosa* growing on bog tundra soils. These authors found that although all species respond to prey, most responses did not significantly differ between varying soil nutrient levels. On the other hand, recent experiments by Karlsson and Pate (1992a) with several pygmy Australian *Drosera* spp. clearly indicated that the animal food supply is a major limiting factor under field conditions. The nutrient-deficient sandy soils where these pygmy species occur may

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have intensified specialization towards carnivory, even eventually leading to a loss of the ability to utilize mineral elements from the rooting substrate.

In this study, we analyse the responses to prey of *P. vallisneriifolia*, an endemic carnivorous plant species living on rocky substrates in the limestone mountains of Sierra de Cazorla y Segura (south-eastern Spain). Like most *Pinguicula* species, *P. vallisneriifolia* is restricted to habitats which remain wet during the growing season (spring and summer). However, unlike many other species of the same genus living on bog soils, this species is found typically on wet escarpments and cliffs, where plants anchor into small crevices by means of perennial roots. Under these conditions, nutrient uptake by roots is presumed to be severely limited because the rooting medium is rock. For these reasons, the species is a highly appropriate candidate for testing the hypothesis that carnivorous plants may be more prey dependent when the access to nutrients by their roots is strongly limited.

In relation to this hypothesis, this study addressed two main issues: (1) the importance of carnivory to the fitness of *P. vallisneriifolia* in its natural rocky habitat, and (2) whether the effect of trapped prey varies with substrate nutrient levels. The duration of the study (2 years) allowed us to analyse long-term survival, growth and reproductive responses, as well as the significance of the carnivorous habit for population performance.

Materials and methods

P. vallisneriifolia is a perennial herb that overwinters as buds. The first five–seven leaves, developing in spring, form a rosette which lies flat against the rock face; however, subsequent leaves differ in morphology and spatial distribution, being larger (up to 30 cm long), much longer than wide and overhanging the cliff face perpendicular to the basal rosette. During the June–August period, each plant develops about four–ten distal leaves. Leaves senesce during September with the formation of winter buds. Plants flower in May–June, produce stolons in July–August and, during September–October, develop axillary buds (gemmae) in the outer leaf axils of the winter bud.

To assess the extent of *P. vallisneriifolia* carnivory in its natural rocky habitat, we tagged winter buds of 72 plants of different size in January 1992 in a population located in the centre of the geographical distribution area of *P. vallisneriifolia* (1250 m above sea level). Plants grew 2–3 m above the ground, in a sector of the escarpment (shade habitat in Zamora 1995) with homogeneous characteristics in terms of level of irradiance, substrate wetness and plant density.

The winter buds were divided arbitrarily into three groups of 24, each group assigned to one of the following three treatments: (1) Prey-exclusion, excluding the plants from airborne insects by fine-mesh (0.25-mm mesh) screens attached to the rocky wall; (2) Prey-control, allowing the plants to capture wild insects; (3) Prey-addition, allowing the plants not only to capture insects, but additionally providing two fruit flies (*Drosophila melanogaster*, wild race) per week, commencing when the first leaves had started to secrete mucilage and terminating upon senescence of all leaves. To minimize prey loss to kleptoparasitism, we placed the flies at the apex of the leaf, the part least accessible to any kleptoparasite (Zamora and Gómez 1996).

The total biomass trapped by the plants belonging to the Prey-control and Prey-addition levels was quantified in 1992 and 1993 at the end of July. For this, the biomass of each prey taxon was estimated by means of regression equations that accounted for the allometric relationships between body length and dry body weight (Hódar 1996). All prey adhering to the leaves of the labelled plants belonging to Prey-control and Prey-addition levels were identified and measured in the field using a hand microscope (×10), equipped with a micrometer. Prey size was defined as body length, excluding the appendages (Zamora 1995).

To determine whether plants respond to prey even when supplied with soil nutrients, we performed an additional experiment by collecting 72 winter buds of different size, dislodged from the rock by ice, from the same population in January 1992. These individuals were divided into three groups of 24 each, assigned to each of the three prey levels defined above. Winter buds were thoroughly cleaned, weighed to 0.01 mg and then planted in individual pots (5 × 5 × 5 cm in size, one winter bud per pot, using cotton and sand for rooting anchorage) within trays in early March 1992 before plants started to grow. The potted plants were situated on a bed of calcareous gravel obtained from the natural rocky substrate. The bottom of the pots, situated at water level, kept the roots constantly wet. Every 3 weeks, potted plants were fertilized with a complete nutrient solution with macronutrients – ammonium phosphate 75 mg/l; ammonium nitrate, 50 mg/l; potassium nitrate, 125 mg/l – in combination with traces of the micronutrients boron, copper, iron, manganese, zinc and nickel. Nutrients were supplied with the irrigation water (5 ml/4 l spring water) to ensure that all plants within a tray had the same opportunity for uptake. The trays with potted plants were provided with a constant water flow by means of a drip system from 10-l plastic deposits connected to the trays. The experimental plant/irrigation ensembles were placed hanging from the cliff face, sharing the same sites as the wild tagged plants. Weekly, we refilled the plastic deposits with water from the nearest spring, and checked the watering system of the fertilized plants. The plastic container and the bed of calcareous gravel were cleaned monthly.

The nitrogen content of the rocky rooting substrate was determined by CNH analysis, whereas total nitrogen and total phosphorus of the spring water that soaked the wall were analysed using the ultraviolet method after persulphate digestion, and the molybdenum blue method after persulphate digestion, respectively.

We collected data from experimental plants in summer 1992 and 1993, when the plants were vegetatively and reproductively fully developed, and in winter 1992 and 1993, when the plants consisted of winter buds. The response variables analysed were the following.

(1) Probability of survival to the end of the experiment.

(2) Summer and winter biomass. The biomass of rock-dwelling plants (both full-grown summer plants and winter buds) was estimated by linear equations relating distal leaf area (independent variable) to plant weight (dependent variable) for summer biomass, and relating the maximum and minimum diameter of the winter bud (independent variable) to winter weight (dependent variable) for winter biomass:

$$\text{summer biomass} = 0.0002837(\text{leaf area}) - 0.084,\ r^2 = 0.821,\ n = 283$$

$$\text{winter biomass} = 0.010(\text{major} \times \text{minor diameters}) - 0.100,\ r^2 = 0.882,\ n = 264$$

Both equations used for estimating the fresh weight of rock-dwelling plants were formulated using weight and size data of cultivated plants. To quantify the summer and winter biomass of the fertilized plants, we extracted them carefully from the pots and weighed them on a field scale (0.01 g), afterwards returning the plants to the pots within trays. Because the fertilized plants bore very slender, short roots ($x = 14.66 \pm 1.29$ mm, $n = 29$), and handling required only a few seconds for the weighing process, root damage was deemed to be negligible as shown by no evidence of stress or loss of leaf turgor following planting.

(3) Number of distal leaves.

(4) Mucilage volume per unit of leaf surface. This variable was estimated in 1992 by multiplying the volume of the spherical droplets by the number of stalked glands per unit of leaf

surface. For this, we counted the glands in a portion of a functional distal leaf from each experimental plant (a total of 144 leaves, each from a different plant) immediately after leaf collection, using a binocular microscope equipped with a graticule in one eyepiece, and a micrometer in the other (for counting the glands and measuring the diameter of the spherical secretion droplets, respectively).

(5) Percentage of plants producing stolons, and number of stolons per plant.
(6) Percentage of plants producing axillary buds and number of axillary buds per plant.
(7) Percentage of flowering plants and number of flowers per plant. In 1993, we hand-pollinated the flowers of every experimental plant, using a fine brush to transfer pollen from several donors. Full ripe capsules were harvested from experimental plants and the number of contained seeds and aborted embryos or ovules was counted in the laboratory with a binocular microscope. By so doing, we quantified (for 1993) the following reproductive variables:
(8) fruit set (proportion of flowers setting fruits);
(9) seed:ovule (S:O) ratio (proportion of ovules setting seeds in each mature fruit);
(10) percentage of plants setting seeds;
(11) plant female fecundity, estimated as the total number of developed seeds of a given plant.

Statistical analyses

To analyse the response of non-fertilized plants to Prey treatments over the 2 years, we applied repeated-measure ANOVAs (rmANOVAs) to compare quantitative plant traits, and logit models to compare categorical variables. We introduced the Initial biomass of the plants as a covariant, since this variable can affect plant responses to treatments. In the first and especially the second experimental year, there were zero values in both the sexual and vegetative reproductive characteristics, since a number of plants failed to flower or to reproduce vegetatively. Such data produced non-normal distributions and as such were not suitable for a parametric rmANCOVA (Mead 1988). For this reason, in the rmANCOVA test of sexual and vegetative reproductive characteristics, we included only the plants that had flowers or stolons. These rmANCOVAs indicated whether treatments affected the number of flowers and stolons of the plants that actually reproduced.

To test specifically whether the response to prey depended on fertilization at the end of the experiment, we used two-way ANCOVAs for 1993 data. Both factors, Fertilization and Prey, were considered fixed (Bennington and Thayne 1994). Analyses were initially performed with full models. However, when interactions were not significant ($P > 0.05$), we used a pooling procedure (Zar 1996) to improve estimates of error variance and increase error degrees of freedom. We used type III sum squares, due to the unbalanced nature of the data (Shaw and Mitchell-Olds 1993).

When more than one comparison was made using the same analytical model, the sequential Bonferroni technique was used to select the critical probability level in order to prevent type I errors (Rice 1989). We show both the original significance after running the statistical procedures and the significance after the application of sequential Bonferroni correction at a table-wide level of $\alpha = 0.05$. Statistical analyses were performed using the computer software JMP 3.1.5 (SAS 1995).

Results

One- and 2-year responses to prey of plants growing in the natural rocky habitat

The number of flies added to the plants of the Prey-addition level assured that the biomass available to these plants was greater than that of prey captured naturally by the plants ($F_{1,66} = 72.54$, $P < 0.0001$, rmANOVA). The average values for annual prey biomass (expressed as the mean ± SE mg dry weight) obtained per experimental group were: Prey-addition 18.72 ± 1.29 in 1992 and 36.91 ± 3.13 in 1993; Prey-control 12.75 ± 4.17 in 1992 and 12.12 ± 2.81 in 1993.

Survival was clearly affected by prey ($G = 8.16$, $df = 69$, $P < 0.17$), since half of the Prey-excluded plants had died by the end of the experiment (54% surviving plants in Prey-exclusion versus 83% Prey-addition and 87% Prey-control). All variables related to plant growth (Summer and Winter biomass as well as Leaf number) were affected by the Prey factor and by the Initial biomass, Year affecting only Winter biomass (Table 1). For plants surviving through the second year,

Table 1 Summary of the rmANCOVAs on plant growth, *N* stolons and *N* flowers for non-fertilized plants during 1992 and 1993 (*SS* type III sum of squares, *P** *P* value corrected with sequential Bonferroni procedure, R^2 variance explained by the model)

	Source	*df*	SS	*F*	*P*	*P**
Winter biomass	Year	1	0.54	7.52	0.007	s
R^2 0.67	Prey	2	5.06	35.02	0.000	s
	Initial biomass	1	8.91	123.42	0.000	s
	Error	116	8.38			
Summer biomass	Year	1	0.36	3.95	0.049	ns
R^2 0.55	Prey	2	2.82	15.38	0.000	s
	Initial biomass	1	9.01	98.43	0.000	s
	Error	120	10.98			
Leaf number	Year	1	0.07	6.06	0.015	ns
R^2 0.49	Prey	2	0.78	34.11	0.000	s
	Initial biomass	1	0.47	41.02	0.000	s
	Error	126	1.44			
N flowers	Year	1	0.09	7.03	0.009	ns
R^2 0.46	Prey	2	0.08	3.10	0.050	ns
	Initial biomass	1	1.07	80.14	0.000	s
	Error	96	1.28			
N stolons	Year	1	0.03	1.11	0.295	ns
R^2 0.44	Prey	2	0.71	14.39	0.000	s
	Year × Prey	2	0.70	14.15	0.000	s
	Initial biomass	1	0.14	5.81	0.018	ns
	Error	87	2.14			

Prey-control plants and, above all, Prey-exclusion plants diminished in size over time (Fig. 1). In contrast, the Winter biomass of Prey-addition plants increased from the beginning to the end of the experiment (Fig. 1). Furthermore, Prey-addition plants produced more leaves than did Prey-control or, especially, Prey-exclusion plants (Fig. 1).

The percentage of plants setting flowers did not differ between Prey levels in 1992 ($G = 4.81$, $df = 71$, $P = 0.09$), but did differ in 1993 ($G = 14.36$, $df = 61$, $P = 0.0008$, log-likelihood ratio), with more than 90% of the Prey-addition plants flowering in both years (Fig. 2). Larger plants produced more flowers (Table 1), and Prey-control and Prey-exclusion plants bore less flowers in 1993 than 1992 (Fig. 2).

The percentage of plants producing stolons differed between Prey levels both in 1992 ($G = 10.64$, $df = 68$, $P = 0.005$) and in 1993 ($G = 5.89$, $df = 58$, $P = 0.05$), nearly all the plants of the Prey-addition treatment producing stolons both years (Fig. 2). Number of stolons depended on Prey level and the Prey × Year Interaction (Table 1). Prey-addition plants bore more stolons per plant than did Prey-control or Prey-exclusion plants, particularly in 1993, when the number of stolons per plant doubled the 1992 rate (Fig. 2). Similarly, the percentage of plants producing axillary buds differed between Prey levels (Fig. 2), both in 1992 ($G = 11.95$, $df = 67$, $P = 0.003$) and 1993 ($G = 37.50$, $df = 53$, $P < 0.0001$). Nearly all Prey-addition plants produced axillary buds in both years, whereas no Prey-exclusion plants produced axillary buds in 1993 (Fig. 2). With respect to the number of axillary buds produced per plant, Prey-exclusion plants produced fewer axillary buds than did the other plants in 1992 ($F_{2,64} = 22.04$, $P < 0.0001$, one-way ANCOVA; Fig. 2).

Effect of prey and fertilization on plant growth and vegetative reproduction at the end of the experiment

All plants, whether fertilized or not, started with a similar sample of winter bud sizes (Fertilization: $F = 0.08$, $P = 0.78$; Prey: $F = 0.27$, $P = 0.76$; $n =$

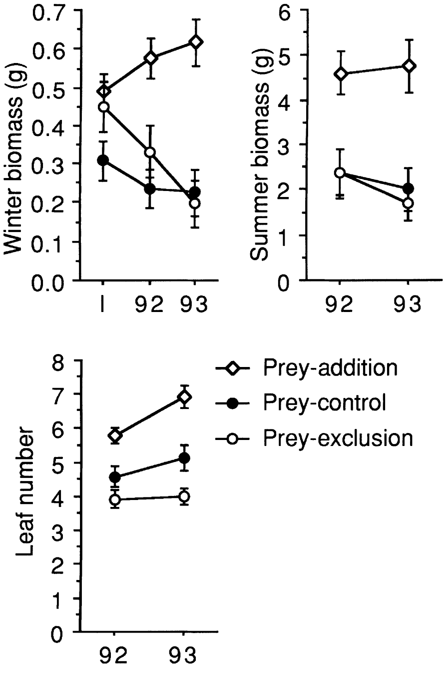

Fig. 1 Between-treatment growth differences for non-fertilized plants in the natural rocky habitat during the 2 years of the study. Posthoc tests (Bonferroni-Dunn) with 1993 values always showed significant differences between Prey levels ($P < 0.001$), except Prey-control vs. Prey-exclusion for Summer and Winter biomass. Values are the mean ± 1 SE

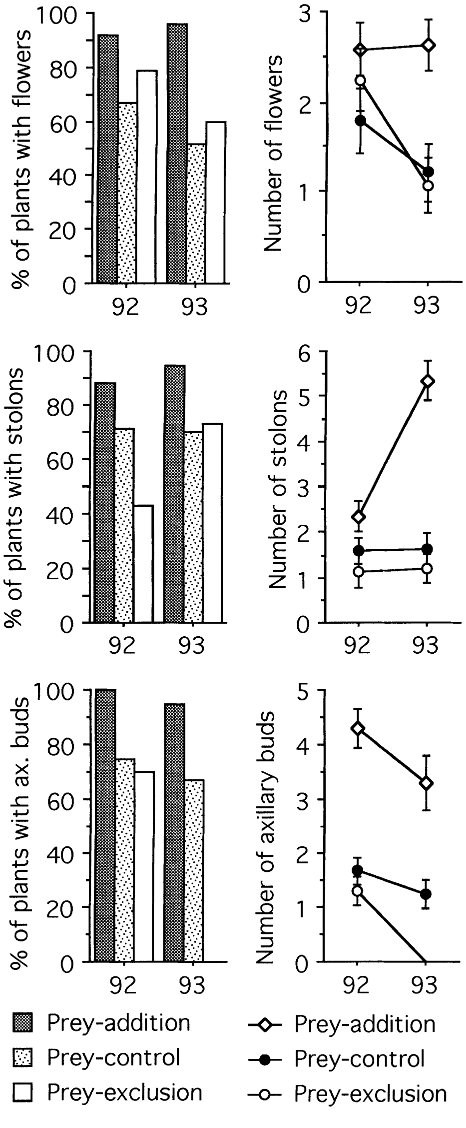

Fig. 2 Between-treatment differences in sexual and vegetative reproduction for non-fertilized plants during the 2 years of study. Posthoc tests (Bonferroni-Dunn) with 1993 values always showed significant differences between Prey levels ($P < 0.001$) except Prey-control vs. Prey-exclusion for Number of flowers and Number of stolons. Values are means (±1 SE error bars)

144). Furthermore, the phenology, growth and reproductive behaviour of the fertilized plants were very similar to that of non-fertilized ones: they started to grow at the end of March and all plants senesced in September. As for non-fertilized plants, there were differences between treatments in prey capture for fertilized, potted plants ($F_{1,89} = 48.53$, $P < 0.0001$, rmANOVA), with Prey-addition plants capturing 10.53 ± 0.20 (mean ± SE in mg) in 1992 and 11.04 ± 0.34 in 1993 and Prey-control capturing 2.08 ± 0.37 in 1992 and 4.92 ± 1.25 in 1993. The percentage of surviving plants at the end of the experiment in fertilized plants was similar at the three Prey levels ($G = 0.03$, $df = 28$, $P = 0.99$), and lower than for non-fertilized plants; 37% surviving plants in Prey-addition, 56% in Prey-control and 44% in Prey-exclusion. Furthermore, Fertilization affected Winter and Summer biomass (Table 2), since non-fertilized plants (especially Prey-addition ones) grew more than fertilized ones (Fig. 3). This difference was due mainly to the strong growth of Prey-addition non-fertilized plants (Fig. 3). Winter biomass of fertilized plants decreased from the beginning of the experiment to the second year (from 0.430 ± 0.088 to 0.179 ± 0.067 g in Prey-addition, from 0.483 ± 0.147 to 0.113 ± 0.024 g in Prey control and from 0.396 ± 0.096 to 0.142 ± 0.036 g in Prey-exclusion; see Fig. 3 for final biomass data). Together with Fertilization, Initial biomass proved significant for Summer biomass, whereas Initial biomass and Prey significantly affected Winter biomass in 1993 (Table 2). However, Fertilization was the only factor non-significant for Leaf number (Table 2).

A lower proportion of fertilized plants bore stolons (Prey-addition 37.5%, Prey-control 25.0%, Prey exclusion 44.4%) with respect to non-fertilized plants (see Fig. 2). The percentage of plants producing stolons depended on Fertilization (Wald $\chi^2 = 15.89$, $df = 1$, $P = 0.0001$) but not Prey (Wald $\chi^2 = 3.61$, $df = 2$, $P = 0.16$) or Initial biomass (Wald $\chi^2 = 1.84$, $df = 1$, $P = 0.17$). With respect to axillary buds, there was no effect of Fertilization (Wald $\chi^2 = 1.62$, $df = 1$, $P = 0.20$) or Initial biomass (Wald $\chi^2 = 1.67$, $df = 1$, $P = 0.20$), only Prey having an effect (Wald $\chi^2 = 14.33$, $df = 2$, $P < 0.001$). The percentage of fertilized plants producing axillary buds was 16.7% at the Prey-addition, 44.4% at Prey-control and 42.9% at Prey-exclusion levels (see Fig. 2 for non-fertilized plants).

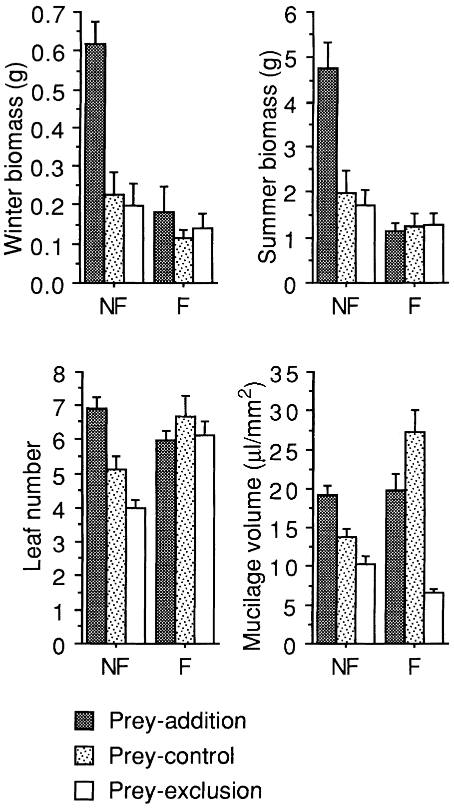

Fig. 3 Comparison between non-fertilized (*NF*) and fertilized (*F*) plants for the final values on Summer and Winter biomass, Leaf number and Mucilage volume. Values are the mean ± 1 SE

Table 2 Summary of the two-way ANCOVAs, for non-fertilized and fertilized plants, on Winter and Summer biomass and Leaf number in 1993 (see Table 1 for legend)

	Source	*df*	SS	*F*	*P*	*P**
Winter biomass						
R^2 0.44	Fertilization	1	1.76	12.86	0.001	s
	Prey	2	1.78	6.48	0.003	s
	F × P	2	0.98	3.56	0.034	ns
	Initial biomass	1	1.86	13.58	0.001	s
	Error	69	9.45			
Summer biomass						
R^2 0.28	Fertilization	1	2.22	13.54	0.001	s
	Prey	2	1.81	5.52	0.006	ns
	Initial biomass	1	2.17	13.26	0.001	s
	Error	83	13.60			
Leaf number						
R^2 0.40	Fertilization	1	0.07	5.90	0.017	ns
	Prey	2	0.15	6.30	0.003	s
	F × P	2	0.17	7.13	0.002	s
	Initial biomass	1	0.13	11.42	0.001	s
	Error	81	0.94			

Effect of prey and fertilization on mucilage secretion

Mucilage volume depended on Prey ($F_{2,132} = 47.76$, $P = 0.0000$) and the Fertilization × Prey Interaction ($F_{2,132} = 14.54$, $P = 0.0000$, two-way ANCOVA), with both non-fertilized and fertilized Prey-exclusion plants producing less mucilage (Fig. 3).

Effect of prey and fertilization on sexual reproduction

The percentage of plants flowering in 1993 was similar in fertilized and non-fertilized plants (Wald $\chi^2 = 1.36$, $df = 1$, $P = 0.24$). Fruit set depended only on Initial biomass, non-fertilized plants setting the same average quantity of fruit as fertilized plants (Fig. 4). However, the two treatments with either prey or inorganic nutrients produced the most fruit per flower. The SO ratio marginally depended on the interaction between Fertilization and Prey (Table 3). Almost all non-fertilized plants belonging to the Prey-addition treatment set seeds, whereas less than 40% of the surviving plants in any other treatment produced seeds (Fig. 4). Furthermore, the total number of seeds produced per plant (Female fecundity) depended both on Initial biomass and the interaction term (Table 3). The average number of seeds per plant was much higher in non-fertilized Prey-addition plants than in other treatments (Fig. 4). Individual seed weight was similar between treatments, regardless of Fertilization level ($P > 0.1$ in all cases).

Discussion

Importance of carnivory for *P. vallisneriifolia* in its natural habitat

Our experimental results indicated that prey capture affected plant survival, as well as Summer biomass, Winter biomass and Leaf number, of plants growing on the cliff. Flowering depended mainly on plant size (Initial biomass), as did the number of flowers per plant. Moreover, prey captured during the summer positively influenced the size of buds the next winter, these serving as a nutrient reservoir. Because Prey-addition plants increased progressively in size, and the larger plants bore more flowers, prey exerted a positive, time-delayed effect on flowering (Fig. 3) and, consequently, on seed production (Fig. 4). Furthermore, prey enhanced vegetative reproduction by stolons and, even more so, by axillary buds (common vegetative reproduction in the *Pinguicula* and *Drosera* genus; see Karlsson and Pate 1992b; Wor-

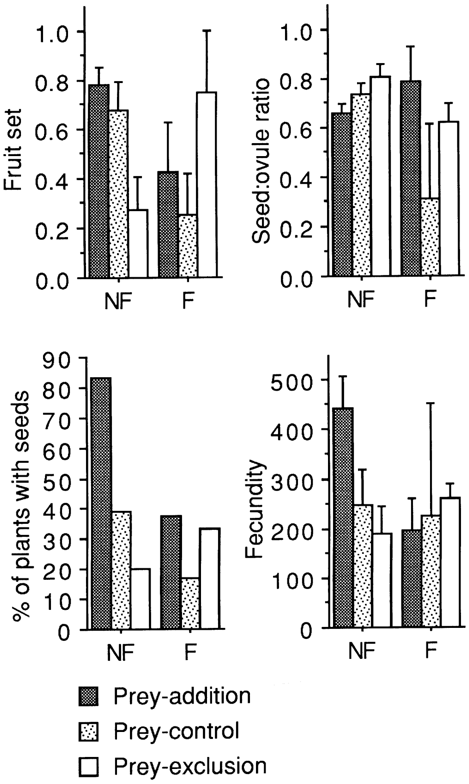

Fig. 4 Between-treatment differences in Fruit set and Seed:ovule ratio, Percentage of plants setting seeds and Fecundity, in 1993, for non-fertilized (*NF*) and fertilized (*F*) plants. Values are means (±1 SE error bars)

Table 3 Summary of the two-way ANCOVAs, for non-fertilized and fertilized plants, on sexual reproduction in 1993 (See Table 1 for legend)

	Source	*df*	SS	*F*	*P*	*P**
Fruit set	Fertilization	1	1.02	2.93	0.093	ns
R^2 0.28	Prey	2	0.71	1.02	0.368	ns
	F × P	2	2.22	3.20	0.049	ns
	Initial biomass	1	3.28	9.44	0.004	s
	Error	54	18.80			
Seed:ovule ratio	Fertilization	1	0.17	3.34	0.077	ns
R^2 0.19	Prey	2	0.37	3.65	0.037	ns
	F × P	2	0.61	6.03	0.006	ns
	Initial biomass	1	0.01	0.16	0.690	ns
	Error	33	1.67			
Fecundity	Fertilization	1	4.39	7.89	0.007	ns
R^2 0.54	Prey	2	4.63	4.17	0.022	ns
	F × P	2	8.93	8.03	0.001	s
	Initial biomass	1	9.69	17.43	0.0001	s
	Error	45	25.02			

ley and Harder 1996), and the mean number of axillary buds per plant.

The different Prey levels generated a performance gradient in plants growing on the rocks from Prey-exclusion (the minimum) to Prey-addition (the maximum; Figs. 1, 2). Consequently, Prey-addition plants showed a steadily increasing fitness, while Prey-exclusion plants either died or drastically diminished over time in terms of size and reproductive investment. Despite this negative trend, half of the Prey-exclusion plants survived until the end of the experiment. This may have been because *P. vallisneriifolia,* a herbaceous perennial, can survive for a time by exclusively reallocating nutrients from senescing leaves (see also Schulze and Schulze 1990), and/or because some nutrients could be taken up by the roots. Fitness differences between Prey levels were more evident at the end of the experiment, when all the surviving Prey-exclusion plants had lost weight, and reproduction by seeds and axillary buds diminished sharply (in fact, no surviving Prey-exclusion plant bore axillary buds entering the second winter). Thus, prey are an important limiting factor for *P. vallisneriifolia* growing on its natural rocky substrate, and an animal food supply may indeed improve the chance of survival, and increase growth and vegetative as well as sexual reproductive success. The field studies of Thum (1988, 1989), Schulze and Schulze (1990) and Karlsson and Pate (1992a) also demonstrated significant growth and reproductive responses to artificial feeding of insects in several *Drosera* species. In this respect, the prey value for *P. vallisneriifolia* appears to be more critical than for several subarctic *Pinguicula* species (Karlsson et al. 1996).

From a demographic perspective, a hypothetical population of plants in a suitable environment but unable to capture prey (e.g. Prey-exclusion plants) should become extinct in a few years through a steady decline in performance. In contrast, a population of successful prey-capturing individuals (e.g. Prey-addition plants) in the same environment should survive over a longer period due to both the perennial habit and vegetative propagation. Furthermore, these successful plants should also be better colonizers because of the high percentage of reproductive individuals and great numbers of seeds produced per plant and year. Analysing the results of plants obtaining natural captures (Prey-control), we found that individual plants differ markedly in their success as carnivores, ranging from 0.40 mg to 82.93 mg of prey captured per plant and year (see Karlsson et al. 1994 for similar results with *P. vulgaris*). That is, our population contained a few individuals that trapped numerous prey and others that trapped few prey. These differences reflect trap size, a positive relationship being found between plant size and prey biomass captured (Fig. 5). Because *P. vallisneriifolia* plants act as passive traps (Zamora 1995), the larger the leaves, the greater the insect mass trapped. Furthermore, trapping success was temporally constant, the most and the least successful trappers being the same individuals in this study in both years (r^2 = 0.841, n = 23, P < 0.0001). Only the largest plants were able to obtain major amounts of prey in both years and, consequently, contributed disproportionately to the next generation by means of a substantial output of seeds, stolons and axillary buds. In contrast, over half of the individuals (the smallest) trapped only minor quantities of prey (Fig. 5), this translating into negative growth in Prey-control plants (Fig. 1). For this reason, there are clear benefits gained from carnivory at the population level, because both the expected individual life span and the lifetime vegetative and sexual output are positively correlated with the quantity of prey trapped.

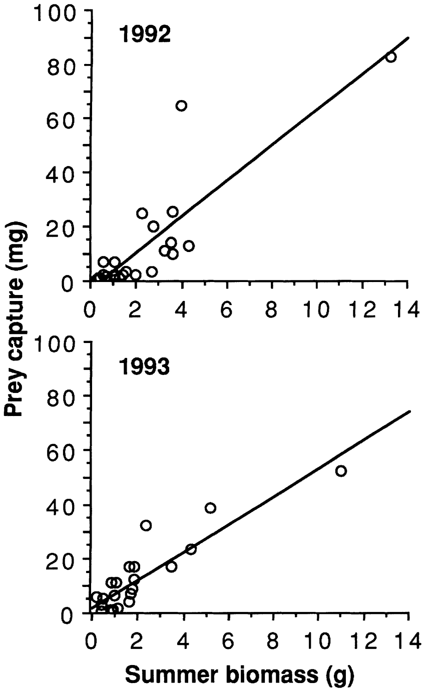

Fig. 5 Relationships between Summer biomass and Prey capture of non-fertilized Prey-control plants. 1992: R^2 = 0.716, P < 0.0001, n = 24; 1993: R^2 = 0.781, P < 0.0001, n = 23

Plant responses to prey and inorganic nutrients

Application of insects to plants grown on a complete nutrient solution failed to enhance growth in comparison with fertilized plants denied prey (Fig. 3). Furthermore, Prey-control and Prey-addition plants growing on the limestone cliff survived, grew and reproduced sexually and vegetatively more than Prey-control and Prey-addition plants growing on calcareous sand and raised on a completely inorganic medium (Figs. 3, 4). Thus, nitrogen-containing insect metabolites appeared to be more effective in promoting the fitness of plants growing on the rocks than on an enriched substrate. Chandler and Anderson (1976) found that the addition of inorganic nitrate to *Drosera whittakeri* and *Drosera binata* plants supplied with insects even inhibited absolute growth, and the greatest absolute growth was attained in the presence of insects at low nitrate concentrations.

A gradient of responses was also found for mucilage secretion in plants growing on the rocky wall with respect to Prey factor: from Prey-exclusion (the minimum) to Prey-addition (the maximum; Fig. 4). Thus, mucilage secretion is stimulated by prey capture in a positive feedback, i.e. the more prey, the more mucilage secretion. For this reason, Prey-addition plants, secreting more mucilage, captured more wild insects than Prey-control plants in the same rocky place. On the other hand, fertilized plants denied prey secreted, like all the other Prey-exclusion plants, the smallest mucilage quantity, whereas fertilized plants in the Prey-addition treatment secreted even less mucilage than did fertilized plants belonging to the Prey-control treatment (Fig. 4). In contrast to prey, fertilization via roots did not significantly increase mucilage secretion. The spatial distribution of the distal leaves, overhanging from the wall, increased the probability of prey capture (Zamora 1995). For this reason, non-fertilized plants growing on the rock captured more prey than did fertilized, potted ones.

Despite the similar percentage of flowering plants and number of flowers per plant in fertilized and non-fertilized treatments, most non-fertilized but few fertilized flowering plants set seed (Fig. 5). On the other hand, the quantity of trapped prey strongly determined the number of seeds produced per non-fertilized plant, with a downward gradient of response from Prey-addition, non-fertilized plants (maximum, with hundreds of seeds per reproductive plant and year) to Prey-exclusion plants (minimum seed production). Furthermore, prey-capturing plants without fertilizer produced more axillary buds and stolons than did prey-capturing plants which were fertilized. These results indicate that the ad libitum availability of mineral nutrients via roots did not translate to higher sexual or vegetative reproduction in potted plants of *P. vallisneriifolia*. Thus, reproductive success, limited by inorganic nutrients in many other plant species (Willson and Burley 1983; Lee 1988), appears to be strongly limited in *P. vallisneriifolia* by organic nutrients derived from prey.

In conclusion, the greatest absolute growth as well as sexual and vegetative output resulted from providing surplus insects to plants growing on the natural rocky substrate rather than on nutrient-enriched substrates. Karlsson et al. (1991) found that the level of prey supply explained more of the variance in winterbud weight than did the nutrient supply for *P. alpina*, *P. villosa* and *D. rotundifolia*, whereas *P. vulgaris* was more responsive to the nutrient supply. In fact, prey capture appears to enhance nutrient uptake from the soil via roots in *P. vulgaris* (Aldenius et al. 1983; Hanslin and Karlsson 1996), but not in *P. vallisneriifolia*, because of the kind of rooting substrate (rock instead of soil), and the very low nutrient availability both in the calcareous rock (Hanslin and Karlsson 1996 found 0.298–0.802% total nitrogen in the soil where *P. vulgaris* grew vs. 0.0013% of nitrogen in the rock of the Covacho population) and in the spring water that soaked the wall (total nitrogen = 719.22 ± 21.8 μg/l, total phosphorus = 3.16 ± 0.15 μg/l). In this sense, the situation of *P. vallisneriifolia* may resemble that of pygmy *Drosera* growing on oligotrophic sandy soil (Karlsson and Pate 1992a). Both the strong response to prey of plants growing on the rocky substrate together with the lack of positive response when fertilizer was provided ad libitum might reflect an increased specialization towards nitrogen-containing insect metabolites in *P. vallisneriifolia* (see Karlsson and Pate 1992a for a similar conclusion with pygmy species of *Drosera*).

Consideration of the characteristics of the Mediterranean ecological scenario where *P. vallisneriifolia* grows may provide an understanding of the ecophysiological responses of this plant. Because of the dry Mediterranean summer, the few wet ground sites are habitable for only a short duration because of successional plant displacement. The only wet gaps that remain free of plant competition for a long period are the wet rocky substrates lacking soil, unsuitable for most plant species. The carnivory of *P. vallisneriifolia* can be considered a useful preadaptation enabling colonization and even maintenance of viable populations over time on this rocky substrate. As a consequence of living under these extreme conditions, *P. vallisneriifolia* appears to have evolved a much higher level of dependence on prey than have other *Pinguicula* species that grow in the soil.

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