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FITNESS RESPONSES OF A CARNIVOROUS PLANT IN CONTRASTING ECOLOGICAL SCENARIOS

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Abstract. This paper reports the results of a two-year field experiment on the determinants of fitness responses in *Pinguicula vallisneriifolia* (Lentibulariaceae), an endemic carnivorous plant of southeastern Spain. For the first time, in this experiment, we have considered irradiance as a factor, in combination with animal prey, for an array of natural field conditions. The goal was to determine how carnivory translates to fitness within different radiation regimes. For this, it was necessary to quantify an array of plant responses, such as survival, growth, reproduction (sexual and vegetative reproduction), as well as responses related to carnivory investment (leaf shape and mucilage secretion). Both irradiance and animal food supply proved to be important limiting factors for *P. vallisneriifolia* under field conditions. Plants clearly decreased in performance from the sunny habitat to the deep-shade one, with plants growing in the least irradiance registering the lowest values for all variables analyzed. The clearest response to prey was the production of axillary buds. Most vegetative and reproductive responses depended heavily on the initial biomass of the plant before the experiments, the largest plants bearing the most leaves, flowers, stolons, and axillary buds. A gradient from less to more mucilage secretion appeared from deep-shade to sunny habitat, and within each habitat from prey exclusion to prey addition levels. Trapped prey stimulated digestive secretions in a positive feedback (the more prey, the more mucilage secretion) under all irradiance conditions. The curled, more secretory leaves of the sunny plants, in comparison with the nearly flattened, less secretory leaves of the deep-shade plants, illustrate the constraints imposed by differing scenarios on the capture of both prey and photons.

With nonlimiting water availability, more irradiance and prey results in more survival, growth, and sexual and vegetative reproduction. Nevertheless, it is not usual to find an optimum combination of resources (i.e., irradiance, prey, and water) available in the same microhabitat during the Mediterranean summer. The spatial uncoupling of limiting resources progressively increased towards the extremes of the irradiance gradient (sunny and deep-shade habitats, respectively), and therefore the dual photosynthetic and carnivore functions of *P. vallisneriifolia* leaves were not equally efficient in all habitats. These opposing resource gradients determined all vegetative and reproductive plant responses. The perennial character of this endemic plant, together with its vegetative form of propagation, allows the possibility of resisting extinction even in the absence of seedling recruitment or when vegetative growth is strongly limited.

Key words: carnivorous plants; ecological heterogeneity; field experiment; growth and survival; irradiance; leaf shape; Lentibulariaceae; Mediterranean ecosystems; mucilage secretion; Pinguicula vallisneriifolia; *prey capture; sexual and vegetative reproduction.*

INTRODUCTION

Carnivorous plants use entrapped animal tissues for nutrition, reversing the normal animal–plant interaction and representing a great anomaly in the usual trophic order of life (Thompson 1981). These plants inhabit almost every region of the world, and ~500 species have been described to date, belonging to 19 genera and nine families (Juniper et al. 1989). Some genera, such as *Drosera, Utricularia,* and *Pinguicula,* are widespread (Juniper et al. 1989). Despite these broad geographical distributions, almost all species of carnivorous plants are restricted exclusively to nutrient-poor habitats, where carnivores are frequently among the most abundant plants of early succession (Givnish 1989). Carnivory in the plant kingdom evolved independently at least six times (Albert et al. 1992); thus, the bizarre carnivorous traits represent different evolutionary solutions to the same ecological problem: exploitation of nutrient-poor habitats. The larger question in studying this evolutionary convergence under similar ecological situations is to discover why carnivorous plants are restricted to a certain type of environment, and how this ecological scenario drives this common carnivory response in plants.

Analyses of these restricted distributions must consider the energy costs and benefits of carnivory in various habitats (Givnish et al. 1984). Traditionally, nu-

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trient availability has been considered the main limiting factor for carnivorous plants (Darwin 1875). For this reason, studies have centered predominantly on analysis of the carnivorous habit as a source of nutrients, especially nitrogen (Dixon et al. 1980, Aldenius et al. 1983, Schulze et al. 1991) and phosphorous (Chandler and Anderson 1976, Karlsson and Carlsson 1984). However, most of these studies have been conducted under laboratory conditions, with very few in the field (but see Thum 1988, 1989, Karlsson and Pate 1992*a*). As a result, scant information, even anecdotal, is available on plant responses under a range of natural environmental conditions. One exception is the study by Knight and Frost (1991) on *Utricularia* carnivory investment over a range of chemical conditions.

Until recently, there has been little recognition that most carnivorous plants species, although found in nutrient-poor habitats, also occupy sunny and moist habitats at least during the growing season (Givnish 1989). All carnivorous plants need light for photosynthesis (Luttge 1983), yet, surprisingly, no study has so far considered irradiance as a major factor in determining carnivorous plant responses. Clearly, these plants have been studied more as carnivores than as green plants.

The present paper reports the results of a two-year field experiment on the determinants of fitness responses in *Pinguicula vallisneriifolia* (Lentibulariaceae) over environmental gradients, considering the importance of light absorption by leaves in relation to prey trapping. This carnivorous Mediterranean species inhabits wet, rocky habitats with a broad range of irradiance regimes. This provides an opportunity to analyze the degree of variability in growth and reproductive responses, the variability in carnivory investment of the plant under different ecological conditions, and the factors causing this variability. This study provides the first experimental analysis of irradiance as a factor influencing carnivory over a range of natural field conditions.

The goal of the present study was to define how carnivory translates to fitness within different radiation regimes. The two-year duration of the study enabled us to analyze long-term survival, growth, and reproductive responses (both sexual and asexual), testing the following specific questions experimentally under field conditions. (1) Are prey and irradiance, under natural conditions, limiting factors for plant survival, growth, or reproduction? (2) In what time frame are the responses to these factors most clearly manifested (short term, one year, two years)? (3) Do traits associated with the carnivore habit (leaf shape and mucilage secretion) vary with environmental conditions? (4) To what extent can the experimental results be applied to wild populations?

General Methodology

Pinguicula vallisneriifolia, an endemic carnivorous plant of southeastern Spain, grows typically on wet limestone rock walls and cliffs, anchored into small rocky crevices (Zamora et al. 1996). This perennial herb overwinters as buds, which start to grow normally in April. The first 5–7 leaves sprout in spring and form a rosette, but later distal leaves differ in morphology and spatial distribution, being larger, much longer than wide, and overhanging the wall perpendicular to the basal rosette. Fruiting occurs from July to August, and leaves senesce during September with the formation of the winter buds. In July and August, plants reproduce by stolons (1–10 cm in length) and, from September to October, axillary buds develop in the outer leaf axils of the winter bud.

Fieldwork was conducted in the Sierra de Cazorla y Segura, Spain, at the headwaters of a small spring surrounded by a cliff (~50 m high and 150 m long) situated in the center of the geographical distribution area of *P. vallisneriifolia.* In this population, during January 1992, we collected winter buds of different sizes that had fallen from the rocky walls due to snow and ice. In the laboratory, the fresh winter buds were weighed to 0.01 mg and planted in individual pots (5 × 5 × 5 cm, one winter bud per pot), using a standardized rooting substrate (a mixture of cotton plus nutrient-free silica sand).

Winter buds were placed in their natural field habitat and were individually labeled in early March 1992 before plants started to grow. The potted plants were situated on a homogeneous bed of nutrient-free silica gravel within plastic trays, with the bottoms of the pots at water level so that the rooting substrate, by capillary uptake, remained constantly wet but not waterlogged. The pots were kept in trays and given a constant water flow (spring water) by means of drip irrigation (medical drip system) from 10-L plastic deposits connected to the trays. This system ensured precise water regulation, providing 1–2 L/d (depending on evapotranspiration) until the deposit needed refilling. The experimental plant/irrigation ensembles were suspended from the rocky wall, 2–4 m above the ground, among wild conspecific plants (see Fig. 1).

Experimental design

We planted 216 winter buds according to a full-crossed bifactorial design, with two main sources of variation, Prey and Irradiance, three levels per factor (nine treatments), and 24 plants per treatment.

1. *Irradiance.*—Using the natural range of sunlight, we defined three distinct habitats: "Sunny," where plants grew on a west-facing section of wall, receiving ~6 h of direct afternoon and evening sunlight; "Shade," situated in the central, north-facing part of the wall, where plants received no direct sunlight; and "Deep shade," where the plants grew on an overhang situated at the bottom of the north-facing wall. Sunny and Deep-shade habitats represent the opposing environmental distribution limits for *P. vallisneriifolia,* whereas the Shade habitat represents a midpoint and,

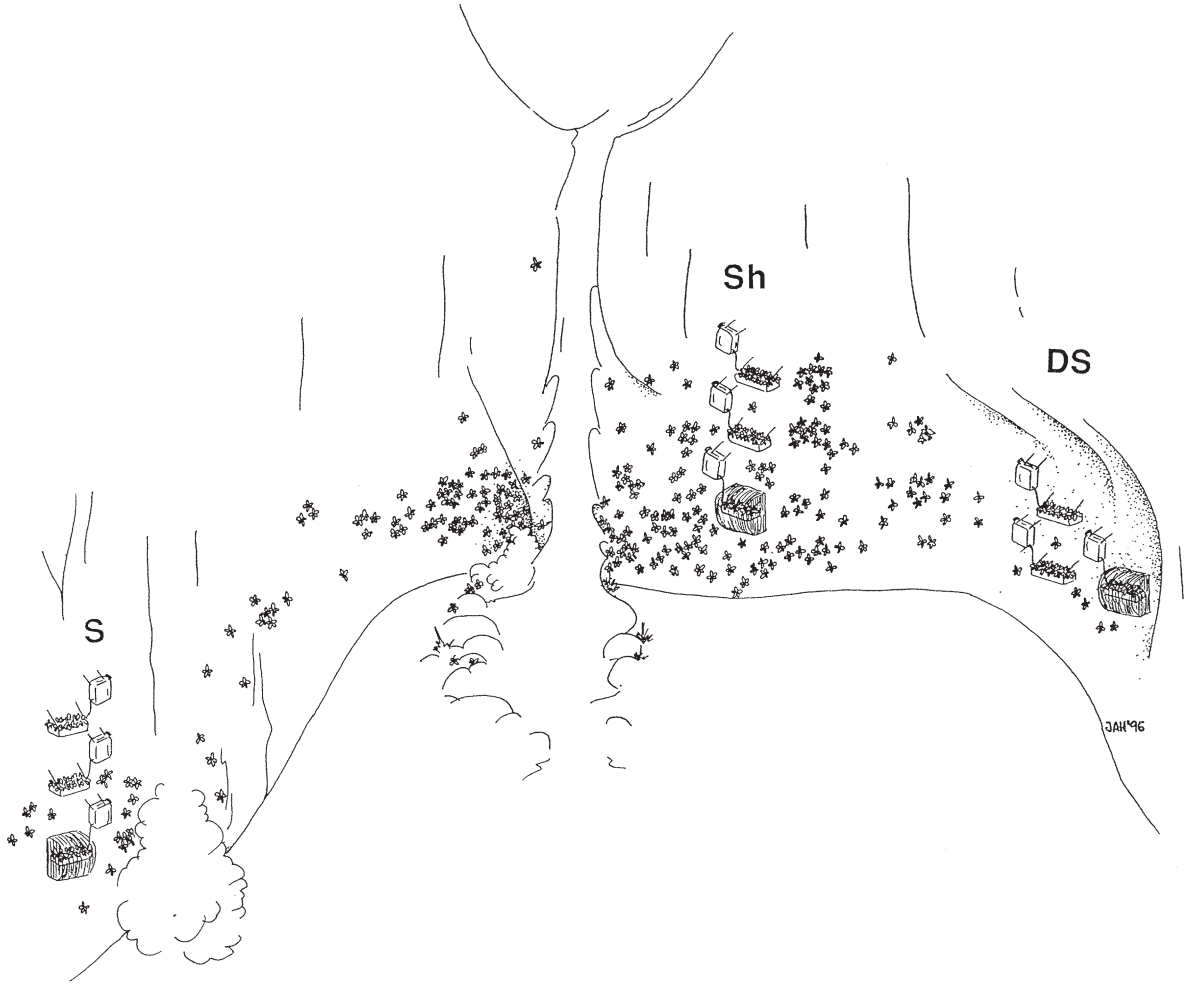

FIG. 1. Schematic diagram of the field experiment and the study site for *Pinguicula vallisneriifolia.* Sunny (S), Shade (Sh), and Deep-shade (DS) habitats are shown.

thus, the typical setting for most *P. vallisneriifolia* populations (Zamora 1995). The three habitats were located close to each other (see Fig. 1), although they differed markedly in mean photosynthetically active radiation, PAR (Sunny habitat: 337.1 ± 99.7; Shade habitat: 67.9 ± 8.1; and Deep-shade habitat: 7.8 ± 0.6 μmol photons, mean ± 1 SE, corresponding to the average values from sunrise (0800) to sunset (2100) during a typical sunny day, 30 July 1992). PAR differences were due to the differences in exposure and degree of cliff coverage ($F_{4,61} = 30.78$, $P = 0.0001$). Irradiance data were collected using a LI-COR Quantum (PAR) sensor connected to a Li-1000 data logger (LI-COR, Lincoln, Nebraska, USA).

2. *Prey.*—We considered the following levels of prey: "Prey exclusion," shielding plants from airborne insects by fine-mesh (0.25-mm mesh) screens; "Prey control," allowing the plants to capture wild insects; and "Prey addition," allowing the plants to capture insects, and also placing two flies (*Drosophila melanogaster,* wild race) per week, from the onset of mucilage secretion in the first leaves until leaf senescence. Because no plant received nutrients via the roots, animal prey constituted the only nutrient source.

The total biomass trapped by the plants belonging to the Prey control and Prey addition treatments was quantified each year at the end of the trapping season. 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 body dry mass (Hódar 1996). All prey adhering to the leaves of the labeled 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. We have restricted the consideration of "prey" to insects <5 mm long (Zamora 1995). The number of flies added in the Prey addition treatment ensured that the biomass obtained by these plants invariably exceeded that obtained in the Prey control treatment; thus, these two treatments registered highly

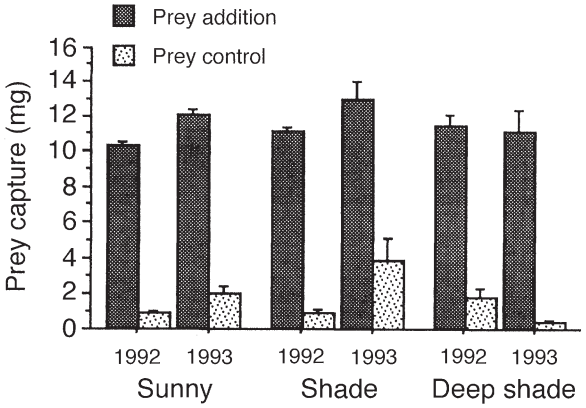

FIG. 2. Prey capture (dry mass, mean + 1 SE) per plant over the season in 1992 and 1993. Sunny, Shade, and Deep shade refer to the three habitats.

significant statistical differences in all three habitats considered (1992: $F_{1,128} = 1284.26$, $P < 0.0001$; 1993: $F_{1,60} = 111.01$, $P < 0.0001$; see Fig. 2).

Plants were randomly mixed among treatments with respect to size. Consequently, experimental treatments started with similar winter bud sizes (Irradiance: $F_{2,213} = 1.26$, $P = 0.29$; Prey: $F = 0.001$, $P = 0.99$, $n = 216$). Because of practical field limitations, all plants belonging to the same treatment were placed together within the same plastic tray (Fig. 1). However, individualized pots served to avoid root competition between plants. Moreover, size and form of the trays, rooting substrate, and interplant distances of potted plants were all standardized variables. To ensure uniform treatments, the watering system provided an ad libitum water supply for all experimental plants. For this reason, the planting medium was standardized both between and within trays, avoiding location effects (Hairston 1989). In addition, all plants belonging to the same habitat received similar irradiance (Fig. 1). In view of this, very fine Prey exclusion mesh was used to allow maximum light passage (PAR reduction was 18% in the three habitats).

The field installation was checked every 3–5 d from the beginning to the end of plant growth (March–October) in 1992 and 1993. During each field visit, we routinely: (1) refilled the plastic deposits with water from the nearest spring and checked the watering system, adjusting the drip volume to the differential evapotranspiration of each habitat; (2) inspected the plants belonging to the Prey exclusion treatment for the efficiency of the screen; (3) placed two flies per plant in the Prey addition treatment. In addition, the plastic trays and the gravel beds where potted plants grew were cleaned monthly.

Target variables

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 were in the form of winter buds. The response variables measured were the following:

1) Plant survival, measured as the probability of survival at the end of the experiment.

2) Plant biomass and growth, determined as: (i) summer biomass, quantified by extracting each experimental plant from the rooting substrate, weighing it on a field scale (0.01 g), and immediately returning it to the substrate (note: the slender roots showed no damage and no plant lost leaf turgor after this manipulation); (ii) winter biomass, quantified following the same procedure; (iii) number of distal leaves per plant; and (iv) leaf length of the longest extended distal leaf.

3) Sexual reproduction, determined as (i) percentage of flowering plants; (ii) number of flowers per plant; and (iii) plant fecundity (only in 1993), the total number of developed seeds per plant. For this, we hand-pollinated the unwithered flowers of each experimental plant by transferring pollen from several pollen donors. Ripe capsules were harvested and seeds were counted in the laboratory.

4) Vegetative reproduction, estimated as: (i) percentage of plants producing stolons; (ii) number of stolons developed per plant; (iii) percentage of plants producing axillary buds; and (iv) number of winter axillary buds per plant.

5) Leaf shape, estimated as (i) leaf curling, quantified as the ratio between the maximum and natural width (at the middle of the leaf) of the same distal leaf, the former quantified with the leaf flattened and the latter with the leaf in its natural curved shape; and (ii) leaf roundness, quantified as the ratio between leaf length and maximum width.

6) Mucilage secretion, determined in 1992 as: (i) droplet size; and (ii) stalked-gland density. Both variables were counted in a portion of a functional distal leaf belonging to each experimental plant (a total of 216 leaves, each from a different plant) immediately after leaf collection. We measured the mucilage droplet diameter of 40 stalked glands and counted the number of stalked glands in 10 1-mm^2 quadrats per leaf, using a binocular microscope equipped with a graticule in one eyepiece and a micrometer in the other. In addition, we estimated (iii) mucilage volume secreted per unit of leaf surface by multiplying the volume of the spherical droplets by the number of stalked glands per unit of leaf surface.

7) Retention capacity of leaves, determined by placing living flies on functional leaves and recording the escape rate. Fly size was divided into three categories: small (*Drosophila melanogaster,* wild race, 2.2 mm), medium (*D. melanogaster, virilis* race, 3.3 mm), and large (native flies of 5 mm collected from the surrounding vegetation). The experiment was carried out using 10 plants per experimental treatment (one functional leaf per plant). Seven flies were placed on each leaf:

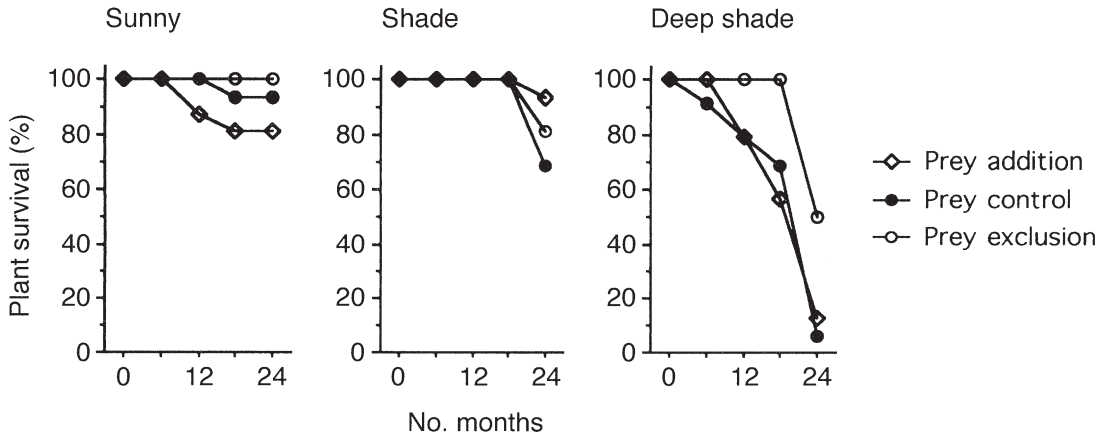

FIG. 3. Survival curve for experimental plants, by habitat, over the two years of experiments.

three small, three medium, and one large fly. Flies were placed in a natural landing position on the leaf. If the insect remained fixed for >1 h, it was considered "trapped."

Statistical analyses

We used two-way ANCOVAs to analyze the effect of the factors on plant response variables in both years. Both factors, Irradiance and Prey, were considered fixed variables because their levels in the experiment were specifically set by the researchers, and the same levels of each effect were used during both experimental years (Bennington and Thayne 1994). We introduced the Initial biomass of the plants as a covariant, because this variable can affect plant responses to treatments. Variables were log-transformed to improve normality and homoscedasticity. We used Type III sum of squares, due to the unbalanced nature of the data (Shaw and Mitchell-Olds 1993). Analyses were initially performed with full models. However, when interactions were not significant ($P > 0.05$), we used a pooling procedure (Zar 1996). In the first and especially the second experimental year, there were zero values in both the sexual and vegetative reproductive characteristics, because a significant number of plants neither flowered nor reproduced vegetatively. Such data provide both quantitative and qualitative information, and thus are not suitable for a parametric ANCOVA (Mead 1988). For this reason, in the ANCOVA test of sexual and vegetative reproductive characteristics, we included only the plants that had flowers or stolons. We performed logit analyses to test whether treatments af-

TABLE 1. Summary of the two-way ANCOVAs on summer and winter biomass for *Pinguicula vallisneriifolia*. R^2 refers to the variability of the response variable explained by the whole model. Nonsignificant interaction terms have been pooled in Error SS.

Dependent variable	Sources	df	SS†	*F*	*P*	*P**‡
Summer biomass						
1992 (R^2 = 0.57)	Irradiance	2	5.76	26.70	0.0000	S
	Prey	2	0.28	1.29	0.278	NS
	Initial biomass	1	25.17	233.33	0.0000	S
	Error	208	22.44			
1993 (R^2 = 0.49)	Irradiance	2	7.38	33.40	0.0000	S
	Prey	2	0.46	2.09	0.129	NS
	Initial biomass	1	5.88	53.24	0.0000	S
	Error	114	12.60			
Winter biomass						
1992 (R^2 = 0.58)	Irradiance	2	10.21	40.25	0.0000	S
	Prey	2	0.23	1.10	0.335	NS
	Initial biomass	1	21.43	202.54	0.0000	S
	Error	197	20.84			
1993 (R^2 = 0.35)	Irradiance	1	0.61	5.30	0.024	NS
	Prey	2	1.66	7.23	0.001	S
	Initial biomass	1	3.07	26.77	0.0000	S
	Error	78	8.96			

† Type III sum of squares.
‡ The probability of *F* after Bonferroni correction: S, significant; NS, nonsignificant.

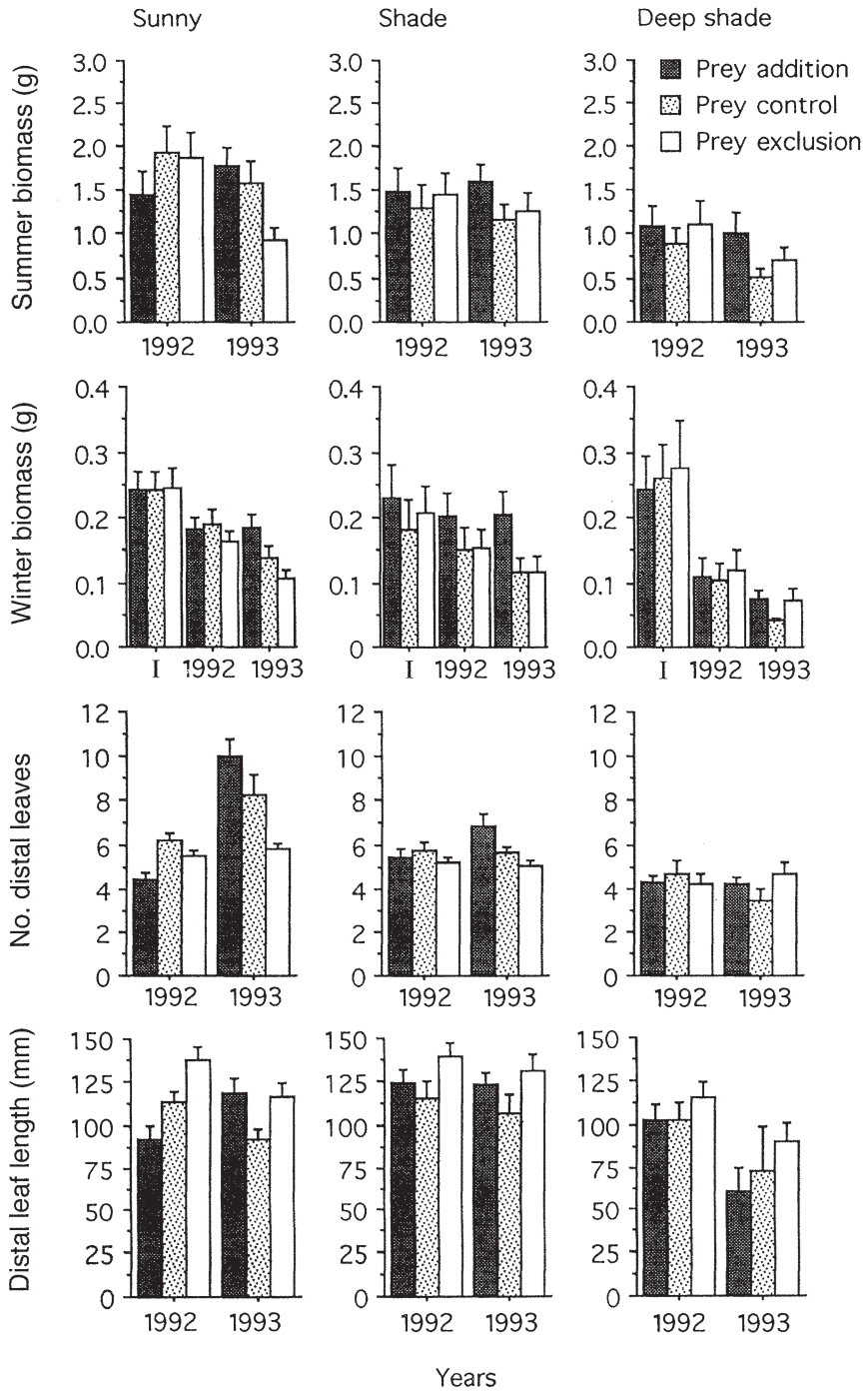

FIG. 4. Differences among treatments for winter and summer biomass, number of distal leaves, and leaf length during 1992 and 1993. The "I" in the *x*-axis refers to the Initial biomass of the experimental plants. Data are expressed as mean + 1 SE.

fected the proportion of plants reproducing or surviving, using the same bifactorial statistical model with covariate as in parametric analyses.

We used a multivariate type of repeated-measure ANOVA (rmANOVA; Potvin et al. 1990) for analyzing the year effect of the experimental factors on the quantitative plant responses (growth and biomass variables). We performed this test only with the sample of plants surviving until the second year (Gurevitch and Chester 1986). Only within-subjects effects are shown, because we were interested only in the effect of year (Von Ende 1993).

TABLE 2. Summary of the two-way ANCOVAs on number and length of distal leaves for *P. vallisneriifolia.*

Dependent variable	Sources	df	SS	*F*	*P*	*P**
No. distal leaves						
1992 (R^2 = 0.35)	Irradiance	2	0.67	19.22	0.0000	s
	Prey	2	0.13	3.68	0.027	s
	Initial biomass	1	1.05	60.57	0.0000	s
	Error	207	3.59			
1993 (R^2 = 0.61)	Irradiance	2	1.22	64.95	0.0000	s
	Prey	2	0.17	8.99	0.0002	s
	I × P	4	0.29	7.70	0.0000	s
	Initial biomass	1	0.47	49.49	0.0000	s
	Error	110	1.03			
Leaf length						
1992 (R^2 = 0.37)	Irradiance	2	0.51	11.47	0.0001	s
	Prey	2	0.44	9.89	0.0001	s
	Initial biomass	1	2.14	96.06	0.0000	s
	Error	208	4.63			
1993 (R^2 = 0.43)	Irradiance	2	1.40	31.37	0.0000	s
	Prey	2	0.26	5.94	0.004	s
	I × P	4	0.26	2.96	0.023	s
	Initial biomass	1	0.76	34.08	0.0000	s
	Error	110	2.45			

Notes: Nonsignificant interaction terms have been pooled in Error SS. See Table 1 for an explanation of statistics.

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 obtained after running the statistical procedures and the significance after applying Bonferroni correction at a table-wide level of $\alpha = 0.05$. Statistical analyses were performed using the computer software JMP 3.1.5 (SAS Institute 1995).

At the end of the first winter, we arbitrarily collected eight winter buds per experimental treatment for a parallel study on nutrient economy. For this reason, the sample size of the experimental plants was reduced to 16 per experimental cell at the beginning of the second experimental year.

RESULTS

The phenology of the experimental plants was virtually indistinguishable from that of wild ones. Also, cultivated plants showed the same normal growth and reproductive pattern as did wild plants, with all plants starting to grow simultaneously within treatments and all plants senescing in September–October. In addition, growth and reproduction of experimental plants were independent of the size of neighboring plants within the same tray. This was estimated by an a posteriori overgrowth index of competition, calculated as the sum of the summer biomass of the three nearest neighboring plants. There was no significant covariance between plant vegetative or reproductive responses and the neighboring plant size; thus each plant could be considered as an independent estimate of the treatment effect (Hairston 1989).

Survival and growth

Survival probability differed across treatments at the end of the second experimental year, with Irradiance being the only factor to explain statistical differences (Wald $\chi^2 = 20.31$, df = 2, $P < 0.0001$), whereas Prey and Initial biomass proved nonsignificant ($P > 0.1$ in both cases, using multivariate analysis of contingency). Much higher plant mortality occurred in the Deep-shade treatment (Fig. 3), whereas most plants survived in the Sunny and Shade treatments. However, 12 plants from the Prey addition treatment in the Sunny habitat suffered dehydration (April 1992) due to a failure in the drip system. Because damage occurred at the very beginning of the experiment, we replaced these plants. Furthermore, three Prey addition and five Prey control plants from the Deep-shade habitat died accidentally in 1993.

Irradiance was the main factor in explaining summer biomass differences during both 1992 and 1993 (Table 1), with Deep-shade plants growing the least (Fig. 4). With respect to winter biomass, Irradiance was significant only during the first year, whereas Prey was significant only during the second year (Table 1). However, Initial biomass strongly affected both the summer and winter biomass of the experimental plants, especially during the first year (Table 1).

The number of leaves borne by plants and the leaf length depended on both Irradiance and Prey factors, as well as on Initial biomass (Table 2); that is, large plants produced higher numbers and larger leaves than did small ones (Fig. 4). Deep-shade plants had fewer and shorter leaves than did the other experimental plants, whereas plants excluded from prey consistently produced the longest leaves in all habitats (Fig. 4).

TABLE 3. Multivariate repeated-measures ANOVAs (Wilks' λ statistic) on biomass and vegetative growth of *P. vallisneriifolia*. Significant effects after Bonferroni correction are shown in boldface. Nonsignificant interaction terms have been pooled in Error SS.

	Year		Year × Irradiance		Year × Prey		Year × Prey × Irradiance	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Winter biomass	**177.6**	0.000	2.28	0.135	**7.48**	0.001	0.57	0.57
Summer biomass	**130.2**	0.000	**14.52**	0.000	0.98	0.380	**9.40**	0.000
No. distal leaves	3.22	0.075	**27.43**	0.000	**11.53**	0.000	**14.17**	0.000
Leaf length	**75.44**	0.000	**23.92**	0.000	2.00	0.141	**13.72**	0.000

The rmANOVAs clearly indicated that growth and biomass varied over time (Table 3). Winter biomass diminished over time in all habitats, the greatest decrease being in Deep-shade plants (Fig. 4). Plant biomass diminished from the first to the second summer, with the exception of plants belonging to the Prey addition treatment in the Sunny and Shade habitats (Fig. 4). Plants produced more leaves in the second than the first year in the Sunny habitat, especially in the Prey addition treatment (Fig. 4).

Sexual reproduction

The percentage of plants bearing flowers depended only on Initial biomass in 1992 (Table 4), with ~35% of the plants flowering in every habitat (Fig. 5). However, the percentage of plants flowering in 1993 depended on both Irradiance and the Initial biomass (Table 4). In this year, only 3.6% (n = 28 plants) of the plants flowered in Deep-shade habitat, whereas 72.7% (n = 44 plants) flowered in the Sunny and 39.6% (n = 48 plants) in the Shade habitats. Thus, flowering increased over time in the Sunny habitat, but decreased in the Deep-shade habitat (Fig. 5). With respect to the number of flowers per plant, both Initial biomass and Irradiance affected both years (Table 4, Fig. 5).

As a whole, 59.1% of the plants growing in the Sunny habitat, 25% of the plants in the Shade habitat, and only 3.6% (one plant) in the Deep-shade habitat produced seeds in 1993. Plant fecundity (mean ± 1 SE) was 193 ± 60 seeds/plant in Prey exclusion (n = 10), 193 ± 54 in Prey control (n = 11), and 147 ± 38 in Prey addition (n = 7) treatments for plants growing in the Sunny habitat (Table 4). In the Shade habitat, there were 227 ± 122 seeds/plant in Prey exclusion (n = 2),

TABLE 4. Summary of the multivariate contingency analyses (Wald χ^2 values) of percentage of plants flowering and the two-way ANCOVAs (F values) on number of flowers per plant and fecundity.

	Sources	df	SS	χ^2 or F	P	P*
Percentage of plants flowering						
1992 (R^2 = 0.48)	Irradiance	2		1.73	0.42	NS
	Prey	2		7.22	0.027	NS
	I × P	4		18.08	0.001	S
	Initial biomass	1		41.99	0.0001	S
	Error	200		73.35		
1993 (R^2 = 0.41)	Irradiance	2		18.95	0.0001	S
	Prey	2		3.57	0.170	NS
	Initial biomass	1		15.50	0.0001	S
	Error	113		48.22		
No. flowers/plant						
1992 (R^2 = 0.41)	Irradiance	2	0.182	6.35	0.003	S
	Prey	2	0.024	0.83	0.438	NS
	Initial biomass	1	0.712	49.72	0.0001	S
	Error	73	1.045			
1993 (R^2 = 0.45)	Irradiance	2	0.144	10.84	0.002	S
	Prey	2	0.059	2.23	0.118	NS
	Initial biomass	1	0.427	32.26	0.0001	S
	Error	46	0.609			
Fecundity						
1993 (R^2 = 0.27)	Irradiance	1	3.75	6.38	0.015	NS
	Prey	2	0.41	0.35	0.709	NS
	I × P	2	7.78	6.62	0.003	S
	Initial biomass	1	2.78	4.73	0.035	NS
	Error	41	24.10			

Notes: Deep shade habitat was excluded in the analysis of 1993 data (except for percentage of plants flowering), because of sample size limitation. Nonsignificant interaction terms have been pooled in Error SS. See Table 1 for an explanation of statistics.

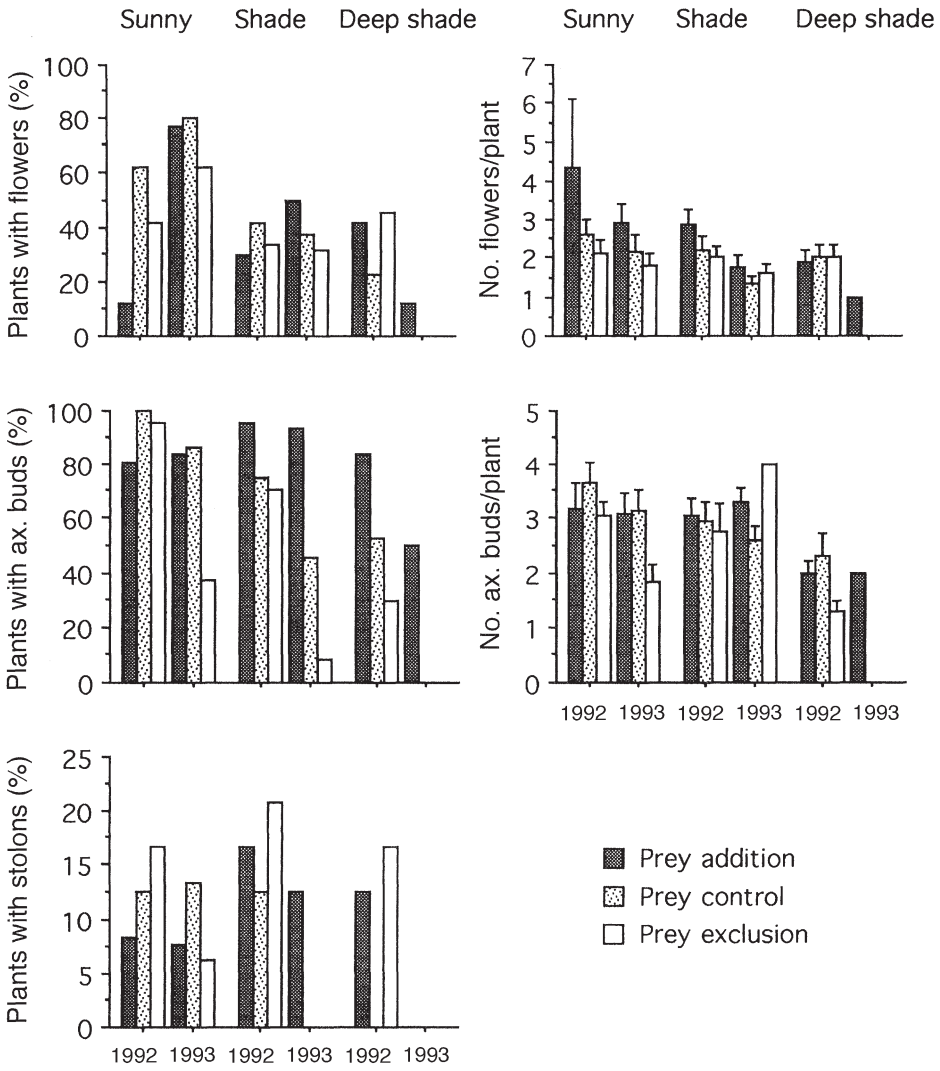

FIG. 5. Sexual and asexual reproduction in *P. vallisneriifolia*: differences among treatments in the percentage of plants bearing flowers, stolons, and axillary buds, and the number of flowers and axillary ("ax.") buds per reproductive individual (mean + 1 SE) during 1992 and 1993.

141 ± 71 in Prey control (n = 3), and 169 ± 44 in Prey addition (n = 7). Individual seed mass was similar between treatments (mean mass 0.01 mg; $P > 0.3$ in all cases).

Vegetative reproduction

Few plants produced stolons in any of the treatments (Fig. 5). For example, 10% of plants in Deep-shade (n = 70 plants), 16.7% in Shade (n = 72 plants), and 12.5% in Sunny habitat (n = 72 plants) produced stolons in 1992. Moreover, only 5% of all experimental plants produced stolons in 1993 (Fig. 5), with a very low number of stolons per plant.

Contrary to the scarce vegetative reproduction via stolons, most plants produced axillary buds (Fig. 5). The percentage of plants with axillary buds in 1992 depended mainly on Irradiance, Initial biomass, and the interaction term between Irradiance and Prey (Table 5). By contrast, only Prey was a significant factor in 1993. Similarly, all factors significantly affected the number of axillary buds produced per plant in 1992, whereas only Prey did in 1993 (Table 5). Nearly all of the plants in the Prey addition treatments of the Sunny and Shade habitats produced axillary buds in both years (Fig. 5). In contrast, Deep-shade plants consistently bore fewer axillary buds than did the Sunny and Shade plants, especially in the second winter. No plant belonging to the Prey control and Prey exclusion Deep-shade treatments bore axillary buds (Fig. 5). Prey exclusion plants bore fewer axillary buds than did Prey control and Prey addition plants, irrespective of irradiance level (Fig. 5).

TABLE 5. Summary of the multivariate contingency analyses (Wald χ^2 values) of percentage of plants bearing axillary (ax.) buds and the two-way ANCOVAs (F values) on number of axillary buds per plant.

	Sources	df	SS	χ^2 or F	P	P*
Percentage of plants with ax. buds						
1992 (R^2 = 0.53)	Irradiance	2		19.13	0.0001	S
	Prey	2		6.52	0.038	NS
	I × P	4		15.33	0.0041	S
	Initial biomass	1		21.73	0.0001	S
	Error	190		49.74		
1993 (R^2 = 0.33)	Irradiance	1		3.76	0.052	NS
	Prey	2		20.33	0.0001	S
	Initial biomass	1		3.13	0.077	NS
	Error	76		37.00		
No. axillary buds						
1992 (R^2 = 0.43)	Irradiance	2	3.53	39.76	0.0000	S
	Prey	2	0.50	5.61	0.004	S
	I × P	4	0.90	5.06	0.001	S
	Initial biomass	1	2.08	46.96	0.0000	S
	Error	193	8.57			
1993 (R^2 = 0.43)	Irradiance	1	0.14	2.78	0.100	NS
	Prey	2	2.50	24.21	0.0000	S
	I × P	2	0.38	3.64	0.031	NS
	Initial biomass	1	0.31	5.99	0.017	S
	Error	75	3.87			

Notes: Deep-shade habitat was excluded for 1993 because of sample size limitation. Nonsignificant interaction terms have been pooled in Error SS. See Table 1 for an explanation of statistics.

Leaf shape and mucilage secretion

Leaf curling depended mainly on Irradiance (Table 6); that is, Deep-shade plants bore nearly flattened leaves, whereas Sunny plants were characterized by a high degree of curling (Fig. 6). On the other hand, leaf roundness depended on both Prey and Irradiance factors (Table 6). Prey addition plants bore proportionally short leaves (~11–17 times longer than wide), whereas Prey exclusion plants bore elongated leaves (~17–22 times longer than wide; Fig. 6). No estimate of leaf shape depended on plant size (Table 6).

The density of stalked glands was only affected by the Prey factor (Table 7), whereas droplet size strongly depended on both Irradiance and Prey, the Initial biomass being nonsignificant (Table 7). As a consequence,

TABLE 6. Summary of the two-way ANCOVAs on *P. vallisneriifolia* leaf curling and roundness.

Sources	df	SS	F	P	P*
Leaf curling (R^2 = 0.46)					
Irradiance	2	1.08	87.63	0.0000	S
Prey	2	0.06	5.11	0.007	S
Initial biomass	1	0.00	0.19	0.667	NS
Error	207	1.27			
Leaf roundness (R^2 = 0.34)					
Irradiance	2	0.80	25.30	0.0000	S
Prey	2	0.91	28.74	0.0000	S
I × P	4	0.17	2.69	0.032	NS
Initial biomass	1	0.01	0.62	0.431	NS
Error	204	3.22			

Notes: Only 1992 data are analyzed. Nonsignificant interaction terms have been pooled in Error SS. See Table 1 for an explanation of statistics.

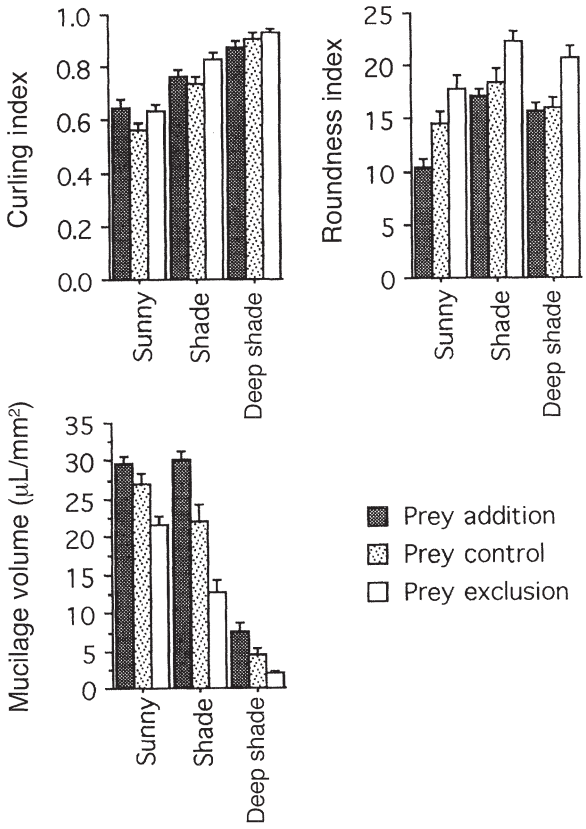

FIG. 6. Differences among treatments in leaf curling, roundness, and mucilage volume during 1992. Data are expressed as mean + 1 SE.

TABLE 7. Summary of the two-way ANCOVAs on *P. vallisneriifolia* mucilage variables.

Sources	df	SS	*F*	*P*	*P**
Gland density (R^2 = 0.07)					
Irradiance	2	0.01	1.31	0.273	NS
Prey	2	0.05	5.33	0.006	S
I × P	4	0.05	2.85	0.025	NS
Initial biomass	1	0.00	0.59	0.442	NS
Error	205	0.94			
Droplet size (R^2 = 0.73)					
Irradiance	2	4.24	242.44	0.0000	S
Prey	2	0.75	42.94	0.0000	S
I × P	4	0.13	3.58	0.008	NS
Initial biomass	1	0.04	5.03	0.026	NS
Error	205	1.79			
Mucilage volume (R^2 = 0.73)					
Irradiance	2	37.89	243.89	0.0000	S
Prey	2	5.64	36.33	0.0000	S
I × P	4	1.45	4.67	0.002	S
Initial biomass	1	0.32	4.18	0.042	NS
Error	205	15.92			

Notes: Only 1992 data are analyzed. Nonsignificant interaction terms have been pooled in Error SS. See Table 1 for an explanation of statistics.

mucilage volume depended primarily on Irradiance and Prey; Deep-shade plants secreted very little, and Sunny plants produced the largest volume of mucilage (Fig. 6). In each habitat, Prey exclusion plants consistently secreted less mucilage than did Prey control plants, and far less than did Prey addition plants (Table 7, Fig. 6).

The experimental placement of flies on leaves indicated that Deep-shade plants had less retention capacity than did Shade and, above all, Sunny plants (Fig. 7). Retention capacity strongly depended on Irradiance (Wald χ^2 = 107.97, P = 0.0000), although Fly size and Prey also had a significant effect (Wald χ^2 = 16.96, P = 0.0002, and Wald χ^2 = 48.64, P = 0.0000, respectively; multivariate analysis of contingency). Prey addition plants had the most retention capacity at all Irradiance levels. As a whole, all small and medium flies, as well as most of the large flies, remained trapped in Sunny habitat, whereas only some of the small and medium flies were retained by Deep-shade plants (Fig. 7).

DISCUSSION

Experimental plants differed morphologically, physiologically, and ecologically according to environmental gradients. To interpret these results while taking into account natural variation in the field, we must first consider the nature and extent of variability of the factors (Irradiance and Prey). The three irradiance levels constituted a broad gradient, with Sunny and Deep-shade treatments representing the natural limits of the distribution of *Pinguicula vallisneriifolia*. With respect to Prey, however, potted plants captured less prey than did plants growing in the natural rocky substrate. Leaves of the wild plants projected horizontally from the vertical wall, thereby enhancing prey capture (Zamora 1995), whereas leaves of the potted plants grew upwards, hardly expanding laterally. In fact, captures by wild plants (12.75 ± 4.18 mg, mean ± 1 SE; Zamora et al. 1997) were closer to captures by Prey addition than to Prey control plants (Fig. 2). Thus, the Prey addition level corresponded to an average number of natural captures, whereas Prey control registered a low Prey level. For this reason, the Prey gradient is smaller than the Irradiance one. With more prey, plants growing in the cliff responded much more (Zamora et al. 1997).

Growth responses

Carnivorous plants might move from autotrophic toward heterotrophic ways of obtaining carbon (Givnish et al. 1984). Were this hypothesis true, *P. vallisneriifolia* plants in Prey addition treatments should have grown vegetatively at similar rates in the Sunny, Shade, and Deep-shade habitats, because these plants received similar quantities of flies (and, potentially, of heterotrophic carbon). In contrast, plants clearly showed a

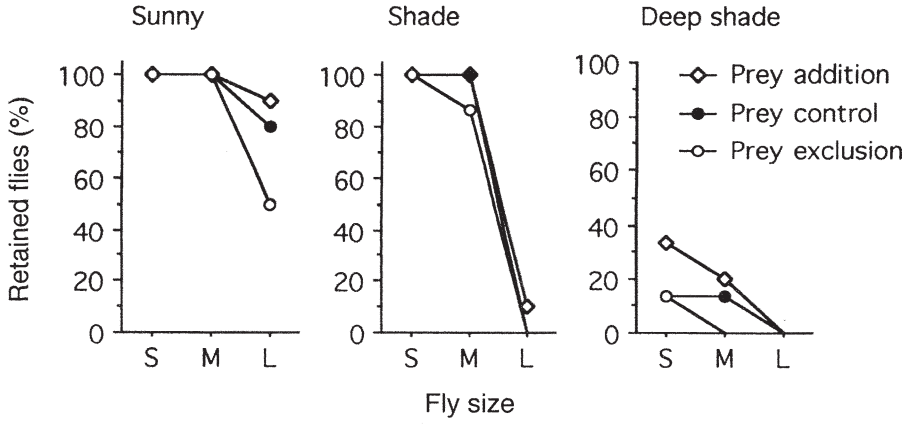

FIG. 7. Differences among treatments in the percentage of flies remaining after 1 h on the leaves of experimental plants, as a function of insect body size. S, M, and L represent the different fly sizes (small, medium, and large, respectively).

decreasing gradient of performance from the Sunny to the Deep-shade habitat; plants growing in the lowest irradiance registered the lowest values for all variables analyzed. Furthermore, all plants in Deep-shade habitat grew similarly, irrespective of the Prey level. Thus, successful carnivory cannot compensate for diminished photosynthesis. These experimental results agree with the physiological evidence provided by Luttge (1983), characterizing *P. vallisneriifolia,* like all carnivorous plant species analyzed to date, as a green, C_3-photosynthesizing, carbon autotrophic plant. According to Chandler and Anderson (1976), *Drosera* that were fed prey in a darkened room showed little growth, implying a negligible role for carbon heterotrophy.

Another possibility is that carnivory may enhance photosynthesis as a result of nutrient absorption from prey. This is the most commonly cited benefit from prey capture (Aldenius et al. 1983, Luttge 1983, Givnish 1989), and our results showed that prey availability determined vegetative growth. The field studies of Thum (1988, 1989), Schulze and Schulze (1990), and Karlsson and Pate (1992*a*) demonstrated marked growth responses to artificial feeding of insects in several European and Australian species of *Drosera* (see also Watson et al. 1982, Karlsson et al. 1991). Prey digestion can provide essential nutrients (mainly nitrogen, phosphorous, sulphur, and oligoelements; Chandler and Andersson 1976, Karlsson and Carlsson 1984) for both photosynthetic and digestive enzymatic activity. This benefit is more evident when irradiance is nonlimiting (Sunny habitat) than when irradiance is clearly limiting (Deep-shade habitat). Pooling all treatments, we find that the lowest plant performance appeared, irrespective of the Prey level, in the Deep-shade habitat. Because of the nutrient-free rooting medium and the oligotrophic nature of the irrigation water (Zamora et al. 1997), the only source of nutrients for our experimental plants was prey. This fact also precluded a potential prey stimulation of mineral absorption from the soil via roots (Aldenius et al. 1983, Hanslin and Karlsson 1996).

One-year and two-year responses

Most vegetative and reproductive plant responses depended heavily on the Initial biomass of the plant before the experiments, the largest plants bearing the most leaves, flowers, stolons, and axillary buds (see also De Ridder and Dhont 1992, Worley and Harder 1996). This size effect was far clearer in the plant responses during the first year, when the Initial biomass even exceeded Irradiance and Prey as determining factors of plant responses. The time-delayed response after experimental manipulations, in the *Pinguicula* genus, is related to the fact that the winter bud serves as a nutrient reservoir, containing the incipient leaves and flowers for the next vegetative cycle (Sorensen 1941). This also explains why the decrease in plant performance after prey exclusion is a gradual process that is accentuated with time, rather than being an immediate response. In this respect, the differences between Prey levels are more evident in the second year, when all plants in the Prey exclusion treatment lost mass in comparison with the first year, and both sexual and vegetative reproductive investment diminished dramatically, above all in the Deep-shade habitat (Figs. 4 and 5). Despite the negative tendency, no plant in the Sunny Prey exclusion treatment, and only 8% of plants in the Shade Prey exclusion treatment died at the end of the second year. An important question, implicit in these facts, is how plants that are excluded from prey and, consequently, are without external nutrients (because of the nutrient-free rooting substrate) can survive over time. A parsimonious explanation appears to be that *P. vallisneriifolia,* an herbaceous perennial species, has a highly efficient system of nutrient reallocation, being able to survive for a time (even two years) on stored nutrients. This represents an advantage, considering that *P. vallisneriifolia,* a prey-limited carnivore under natural conditions (Zamora et al. 1997), has no attraction mechanisms; thus, it has microsite-dependent capture probabilities (Zamora 1995). Under these nutrient-limiting conditions, it may be more important to recycle internal resources efficiently than to maximize nutrient gain (Chapin et al. 1993). In fact, reallocation of resources from old leaves appears to be as essential as insect prey capture for *Drosera rotundifolia* (Schulze and Schulze 1990). Some noncarnivorous perennial species without access to nutrients can also maintain vegetative growth by using stored reserves (Jonasson and Chapin 1985, Jonasson and Widerberg 1988).

Leaf shape and mucilage secretion

In our experiments, leaf shape changed at different irradiance levels; that is, plants growing in the Deep-shade habitat bore nearly flattened leaves, whereas plants growing in the Sunny habitat bore strongly curled leaves, apparently to diminish evapotranspiration. In most species of *Pinguicula,* leaf margins curve inward, often indicating a response to prey capture (Darwin 1875, Zamora 1990). However, the margin of the distal leaves of *P. vallisneriifolia* curve in the opposite way, with the glandular surface outwards, thus improving the capture probability (Zamora 1995). The flatter leaves borne by the plants growing in the Deep-shade habitat would maximize photon intake under irradiance limitation.

Plants produced more mucilage with more light, secreting very small quantities in the Deep-shade habitat, where added flies were hardly covered by mucilage and, consequently, prey digestion should be less efficient. The contrasts between the curled, more secretory leaves of the Sunny plants and the nearly flattened, less secretory leaves of the Deep-shade plants, illustrate the constraints imposed by differing scenarios on the capture of both prey and photons. Givnish (1989) indicated that *Drosera* living in the understory of an Australian

rain forest show signs of losing the carnivorous habit, having few tentacles per leaf.

Plants excluded from prey bore longer leaves, whereas plants supplied with flies (Prey addition treatment) bore shorter leaves in all habitats (Fig. 4). A possible explanation of this fact is that the production of sticky mucopolysaccharides and enzymes necessary to digest prey might retard leaf growth, as a consequence of a trade-off resulting from the dual photosyntetic and digestive physiological function of *P. vallisneriifolia* leaves. In fact, the shorter leaves of plants in the Prey addition treatment secreted more mucilage than did the longer leaves of Prey exclusion plants (Figs. 4 and 6). Because differences in mucilage secretion between treatments depended on the volume of the secretory droplet (and not on the density of stalked glands), the plant response was more a physiological than a morphological response. In this respect, trapped prey represent a stimulus for digestive secretions, acting as a positive feedback (the more prey, the more mucilage secretion) under all irradiance conditions. Part of the nutrients derived from prey are diverted to digestive secretion (mucopolysaccharides and enzymes such as proteases, ribonucleases, and hydrolases; see Heslop-Harrison and Knox 1971). This secretory stimulation increases capture probabilities and, consequently, nutrients derived from prey. Glandular secretion in *Drosera* and *Dionaea* also occurs only in response to nitrogenous material (Darwin 1875, Luttge 1983, Juniper et al. 1989).

In short, the trapping success of *P. vallisneriifolia* leaves depended both on insect body size (the larger the size, the greater the escape possibilities) and on mucilage production and viscosity (Figs. 6 and 7; Zamora 1995). As a result, plants in the Deep-shade habitat were able to retain only the smallest insects.

Reproductive responses

Our experimental results indicate that sexual reproduction depends more on Irradiance than on the Prey factor. Other *Pinguicula* species have no clear responses in seed production to prey addition and/or fertilization treatments (Karlsson et al. 1991). In contrast, vegetative reproduction via axillary buds is strongly determined by both Prey and Irradiance factors. Despite its widespread occurrence, reproduction by axillary buds has rarely been analyzed in studies on carnivorous plant responses (but see Karlsson and Pate 1992*b*, Worley and Harder 1996). Vegetative reproduction represents the steady work of clonal spread on the already occupied wet microsite, whereas seeds represent the lottery in colonizing open, even distant, wet areas.

Moreover, vegetative propagation by budding appears to have some advantages over sexual reproduction under the current ecological conditions. Firstly, there was a threshold size (0.14 g of initial winter bud biomass) for sexual reproduction, but the size threshold for vegetative reproduction was so low (0.02 g of Initial winter bud biomass) that almost any plant, regardless of size (except seedlings), could propagate vegetatively. The absence of a clear size threshold for vegetative reproduction emphasizes the similarity between this type of reproduction and growth of other vegetative parts, as opposed to sexual reproduction (Schmid et al. 1995). Secondly, seeds are wind dispersed and mostly fall in unfavorable places on the ground, where *Pinguicula* plants cannot escape overgrowth competition with other plant species, or in dry sites of the rocky wall where germination and seedling establishment are unlikely. Axillary buds, however, result when a successful individual is able to spread vegetatively in a suitable microsite, or even leave the mother plant, dispersed by the film of water that usually flows down the entire rock walls in winter, until being caught in a crack. Thirdly, the miniscule seeds of *P. vallisneriifolia* produce tiny seedlings (cotyledons <1 mm) that frequently fail to establish themselves even in wet microsites, as in other *Pinguicula* species (Svensson et al. 1993, Karlsson et al. 1996). Axillary buds are much larger than a typical seed, and the resulting quantitative difference in stored energy and nutrients gives them an initial growth advantage over seedlings. In reproducing by axillary budding, the mother plant avoids the risks of pollination failures, seeds not germinating, or seedlings dying, and gains a reliable and predictable reproductive output. This reproductive strategy ensures permanence of the mother plant in an already colonized patch on vertical rocks, where vegetative propagation by the primary ramet allows plants to expand horizontally.

The Mediterranean ecological theater and the P. vallisneriifolia *plant population performance*

How can experimental results be translated to population performance, in view of natural resource variation? It is evident from our field experiments that both irradiance and animal food supply are important limiting factors under field conditions for *Pinguicula vallisneriifolia.* With nonlimiting water availability, increased irradiance and prey result in increased survival, growth, sexual and vegetative reproduction, and mucilage secretion. However, it is not usual to find an optimum combination of resources (i.e., irradiance, prey, and water) available in the same microhabitat under current ecological conditions, because of the hot and dry Mediterranean summer. Firstly, sunny places are also normally dry, whereas wet places are also normally shaded. Secondly, flying insects are concentrated in the shaded and wet places, being very scarce in the sunny ones (Zamora 1995). In our experiments, this spatial uncoupling of limiting resources progressively increased toward the extremes of the irradiance gradient (Sunny and Deep-shade habitats, respectively); therefore, the dual photosynthetic and carnivorous functions of *P. vallisneriifolia* leaves did not have the same efficiency in all habitats. Toward the Sunny ex-

TABLE 8. Demographic structure of wild plant populations located on cliffs in each of the three habitats sampled in July 1993 in a typical sector of 2-m^2 rocky surface per habitat near potted experimental plants. Sample sizes were 130 plants in Sunny, 191 in Shade, and 195 in Deep-shade habitats. Resource availability is indicated as: ++, high; +, low; −, limiting.

	Habitats		
	Sunny	Shade	Deep shade
Resource availability			
Irradiance	++	+	−
Prey	−	+	++
Water	−	++	++
Demographic stage			
Seedling	0	28.3	23.1
Prereproductive	23.1	7.8	25.6
Nonreproductive†	53.8	6.3	25.6
Reproductive‡	23.1	47.1	25.6
Large reproductive§	0	10.5	0

† Plants as large as reproductive ones, but nonflowering.
‡ Plants with 1–3 flowers.
§ Large plants with >3 flowers.

treme, there was a surplus of light, but very few insects and little water. Toward the Deep-shade extreme, irradiance was clearly limiting, but a wet environment and a surplus of flying insects were available; however, low mucilage secretion meant a low retention of insect prey. These opposing resource gradients determined all vegetative and reproductive responses of the experimental plants. Our next step was to translate these trends into habitat-specific responses of wild plants at the population level. For this, we used a simple stage-structured classification of wild populations in the vertical cliff as an indicator of their demographic viability (see Table 8).

Even though the three habitats were only a few meters apart, they had markedly different demographic structures, illustrating their responses to contrasting gradients. In this respect, the dryness of the rocky substrate strongly limited seedling establishment in the Sunny habitat, where there were no large plants and vegetative individuals prevailed ("remnant population" sensu Eriksson 1996). Reproductive individuals also represented a small fraction (25%) of the total plants in the Deep-shade habitat. This shady, moist habitat favored seedling establishment. However, there was a ceiling on vegetative growth because of limited light and, thus, there were no large plants ("sink population"). Large reproductive plants appeared only in the Shade habitat, where the soaked substrate favored effective population recruitment, as in Deep-shade habitat; moreover, most plants actually reproduced, only a few plants remaining in a vegetative stage ("viable source population;" see Table 8). Plants growing in Shade habitat might represent a source of colonizers because of the high percentage of reproductive plants exporting seeds to the nearest lower quality sites, such as the Deep-shade habitat.

The perennial character of this endemic plant and its vegetative form of propagation allow the possibility that *P. vallisneriifolia* may resist extinction even in the absence of seedling recruitment (e.g., Sunny habitat) or when vegetative growth is strongly limited (i.e., Deep-shade habitat). Because of this plant depends on water, the overall suitable scenario shifts toward shaded places under dry, Mediterranean conditions. These locations may not be optimal for overall growth and reproduction, but are the most feasible, as a compromise among ideal vs. essential light, prey, and water.

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