

REPRODUCTIVE EFFORT AND HERBIVORY TIMING IN A PERENNIAL HERB: FITNESS COMPONENTS AT THE INDIVIDUAL AND POPULATION LEVELS¹

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We experimentally investigated how pollinator- and herbivore-induced changes influence the performance of the long-lived herb *Primula veris*. Eight treatments that corresponded to natural factors normally affecting this species were designed to enhance or reduce reproductive success and resource availability (flower removal, supplementary pollination, defoliation). During the experimental season and in the following year we quantified responses in terms of survival, growth, and seed production of reproductive plants. Matrix population models were used to calculate population growth rate using the demographic parameters recorded in permanent plots and respective treatment groups. Seed production was not limited by pollen availability, and we found no evidence of a cost of reproduction. Leaf removal had either no effect or a negative effect on future performance, depending on the timing of removal. Defoliation early in the season reduced current seed production and future growth, whereas removal during fruit development affected performance in the following year. Demographic models suggest that leaf damage has a smaller negative impact than flower removal on overall performance in this population. Our results suggest that the source-sink paths vary over the season and that the timing of herbivory may influence the extent to which effects are carried over to subsequent reproductive seasons.

Key words: fitness components; flower and leaf removal; hand pollination; life table response experiment (LTRE) analysis; matrix population models; *Primula veris*; short- and long-term effects; source-sink path.

A central assumption in life-history theory is that organisms have a limited amount of resources that can be partitioned into different functions. As a consequence, trade-offs are predicted. One important trade-off is the cost of reproduction, where increased current offspring production is assumed to result in reduced future survival, growth, or fecundity (Stearns, 1989). Contrary to animals, reproductive individuals of most plant species show a clear division between energy sources (photosynthetic parts) and sinks (for example, reproductive organs), although photosynthesis by flowers and fruits can contribute to the carbon costs of reproduction (e.g., Bazzaz, Carlson, and Harper, 1979). Therefore, experimental analysis of the cost of reproduction using plants has the advantage of allowing manipulation of sources and sinks relatively independently.

Phenotypic costs of reproduction can be examined experimentally by manipulating reproductive effort through pollen addition or flower removal and recording the effects on vegetative growth and reproduction within the same season, or survival, growth, and reproduction in the following years. So far, experimental studies in this field have produced very mixed results. Whereas some have found evidence of a cost (Snow and Whigham, 1989; Primack and Hall, 1990), others

have failed to do so (Horvitz and Schemske, 1988; Calvo, 1990; Jennersten, 1991; Ramsey, 1997). Similarly, only some studies manipulating leaf sources have demonstrated significant effects on future performance (Obeso, 1993a, b; Escarré, Lepart, and Sentuc, 1996). Further, photosynthetic tissue losses might reduce different fitness components depending on the species (Ågren, 1989; Lehtilä and Syrjänen, 1995b; Niesenbaum, 1996; Mothershead and Marquis, 2000).

According to the theory of natural selection, organisms maximize their lifetime fitness. Fitness has traditionally been assessed from reproductive parameters such as fruit or seed production, but this is clearly an incomplete method for iteroparous species, as current reproduction is only one component of the overall fitness of an individual. When the objective is to compare how a factor enhances or reduces individual fitness, the effect throughout the whole life cycle must be evaluated. Hence, in order to examine how effects on different components of fitness are translated into effects on overall fitness, it is necessary to experimentally investigate the effects not only on current performance, but also on lifetime seed production. Nonetheless, even when flower and leaf removal have been demonstrated to influence future performance of plants, effects on lifetime reproduction have generally remained unexamined because of a lack of the information necessary to compare changes in current seed production with changes in future performance.

In long-lived organisms with overlapping generations, average overall fitness of a group of individuals with similar life histories can be estimated by the population growth rate of this group (Brandon, 1978; McGraw and Caswell, 1996). The population growth rate, in turn, can be calculated for the whole population from the fecundity, growth, and survival of all individuals belonging to it (e.g., Caswell, 1989a). The pioneering proposal of Calvo and Horvitz (1990) to use transition matrix models to calculate population growth rate and to as-

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sess the net effect of a change in any of the traits involved in a trade-off on lifetime seed production has been followed by only a few studies. Using this approach, the reproductive cost was argued to be irrelevant to the population dynamics of an orchid (Calvo, 1993), whereas long-term seed production in the legume *Lathyrus vernus* was shown to be resource limited in spite of pollen-limited seed production within a season (Ehrlén and Eriksson, 1995). Additionally, a few studies have used population simulation models to assess long-term effects of herbivory (Doak, 1992; Ehrlén, 1995).

In this study we investigated both short- and long-term impacts of changes in reproductive success and resource availability resulting from animal interactions in the long-lived iteroparous plant *Primula veris*. We manipulated photosynthetic sources and reproductive sinks and evaluated current and future performance of individuals. We also assessed the impact on finite population growth rate, an integrative parameter based on the entire life cycle of an average genotype under specific environmental conditions. The following specific questions were addressed: (1) To what extent does pollen availability limit current female fecundity and determine reproductive costs in terms of future performance? (2) Is current or future performance affected by a reduction in leaf sources? If so, is the timing of source reduction important? (3) How do differences in reproductive effort and leaf damage during one season translate into effects on lifetime seed production?

MATERIALS AND METHODS

The species and plant-animal interactions—*Primula veris* L. (Primulaceae) is a long-lived herb (Inghe and Tamm, 1988) that is common in meadows and forest openings of the Northern Hemisphere. Early in spring, plants produce a rosette of several preformed leaves and one (rarely two or more) flowering stalks that bear about 5–15 yellow distylic flowers disposed in an umbel. Blooming normally takes place in May, when new leaves are still growing. The fruit is a capsule and ripens in about 2 mo. The fruits are often damaged before dispersal by the larva of the plume moth, *Amblyptilia punctidactyla*, but postdispersal losses appear to be smaller. Seeds lack obvious mechanisms for dispersal but may be dispersed by wind over short distances. By July, no more leaves are produced or enlarged, and by August, leaves turn yellow and senesce. Only the rhizome stays alive to the next year. Clonal propagation through side rosettes sometimes occurs in *P. veris*, but is rare at the study site.

Plant-animal interactions of several types may influence the relationship between leaf sources and fruit sinks in *P. veris*. Insects or mollusks eat individual flowers, and cattle sometimes damage entire inflorescences. Fruit set has been demonstrated to be pollen limited in some populations (Syrjänen and Lehtilä, 1993). Leaves are eaten by insect or mollusk herbivores during a large part of the growing season (estimated loss of photosynthetic area ranged between 0 and 80% in 1995 and 1996 at the time of recording reproductive plants in this study).

Experimental design—Rather than designing the experimental treatments to achieve controlled levels of source and sink perturbations, we carried out manipulations that corresponded to natural variation in the four biotic interactions that we considered most relevant to sink and source strength in this species: flower damage, inflorescence removal, pollen availability, and leaf herbivory. Flower removal treatments were used to decrease sink strength and reproductive effort. Supplemental hand pollination was used to investigate pollen limitation, and if pollen limitation occurred, to increase reproductive effort. Defoliation treatments were conducted on three different occasions to decrease source strength and investigate the importance of timing of damage. The experimental design thus resulted in eight treatments: (1) control (no treatment), (2) removal of all flowers (No fl.), (3) removal of 50% of the flowers (Half fl.), (4) supplemental hand pollination of all flowers (Pollen),

(5) removal of 50% of the flowers and hand pollination of the remaining flowers (Half fl.+pol), (6) removal of 50% of the leaves during flowering (Early D), (7) removal of 50% of the leaves during early fruit development (Middle D), and (8) removal of 50% of the leaves during fruit maturation (Late D).

The study was performed in a managed meadow in South Sweden containing several hundred reproductive plants. A long-term demographic monitoring of the species was taking place simultaneously at the same place.

Experimental manipulations—In May 1995, a total of 397 reproductive plants were randomly assigned to treatments (46–51 plants per treatment), mapped, and marked with a numbered tag. Flowers and buds were recorded every 3 d. *Primula veris* is distylic and strictly self-incompatible, so flower morph was recorded for each plant. Removal of flower buds was performed by scissors before flower opening. Pollination avoided within-morph crosses and was conducted using a brush as soon as flowers opened. This was repeated every 3 d until flowers withered. At least two pollen donors located >2 m away were used for every receptive flower. To simulate leaf herbivory, a variable number of leaves was removed to achieve an approximately 50% reduction of photosynthetic area. Manipulation of sources took place at one of three different times: during blooming (late May), fruit initiation (beginning of June), or fruit maturation (end of June).

Length and width of the largest leaf and number of flowers were recorded for all plants twice in 1995 (May and July) and once in 1996 (July). In July each year, the number of fruits was recorded and mature intact fruits were collected and brought to the laboratory where ovules (1995) and seeds (1995 and 1996) were counted. The ratios of mature fruits to flowers (fruit set) and seeds to ovules (seed set), as well as the total seed production, were calculated for every individual (for the two latter parameters, only plants with <20% of fruits preyed were used). For treatments involving 50% flower removal, both ratios were calculated from the flowers and ovules present after treatment. We recorded the number of leaves at each visit, but we did not include this information in plant size estimation, as leaves often turned yellow and withered during the season. Hence, we used the area of the largest leaf, calculated from the length and width by applying the phenologically appropriate formulas in Syrjänen and Lehtilä (1993), as an estimate of plant size. Ten plants initially included in 1995 could not be identified with certainty in 1996 and were excluded from analyses.

Six demographic plots of 0.6 m² were randomly distributed within the experimental population, but outside the area where manipulations took place. All individuals in the demographic plots were mapped in May 1995, and new seedlings (or previously dormant individuals) were added after 1 yr. At each census, the number of developed leaves, length and width of the largest leaf, number of flowers, and number of intact and preyed fruits were recorded. A total of 490 individuals were followed within these plots from 1995 to 1996.

Statistical analysis—The following fitness components were analyzed in experimental plants in the year of treatment and 1 yr later by one-way ANCOVA: growth of the largest leaf (absolute change in area; not compared within the year of manipulation for the three leaf-removal treatments), number of flowers (only for reproductive plants in 1996), fruit set, seed set (only in 1995), and total seed production per plant. Initial leaf area (May 1995) was used as the covariate. Appropriate transformations were performed after check with normal probability plots. Homogeneity of slopes was tested for all models. Dunnett's tests served to assess the effect of experimental manipulation compared to the control group. Survival between years was examined by logistic regression. The effect of treatment on flowering probability 1 yr later was examined by a *G* test of independence and seven unplanned pairwise comparisons (each treatment against control), using an adjusted experiment-wise error rate of $\alpha' = 0.0073$.

Lifetime seed production—To calculate total effects on lifetime seed production we used matrix models (Caswell, 1989a). This model is based on the decomposition of the entire life cycle into different steps or "life-history transitions" that an individual goes through to complete its life cycle. Except for seeds, which formed a clearly delimited class, we used three criteria to group

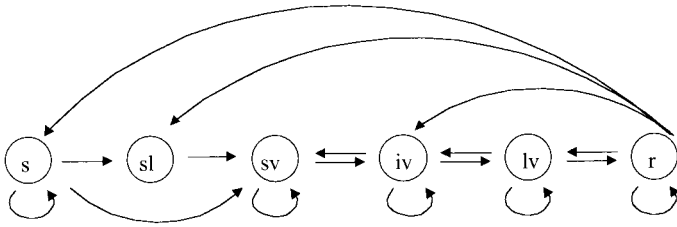


Fig. 1. Life-cycle diagram showing the classes used for the matrix model and the transitions observed during the study. Abbreviations: s = seeds in the seed bank; sl = seedlings 1-yr-old; sv, iv, and lv = small, intermediate, and large vegetative plants, respectively; r = reproductive plants.

plants: age (younger or older than 1 yr), reproductive status of individuals (flowering or vegetative), and size. Vegetative rosettes older than 1 yr were divided into three classes according to the length of the largest leaf. We used the maximum length of leaves attained by 1-yr-old plants (17.5 mm) as the lower limit, and the threshold size for reproduction (43.5 mm) as the upper limit, to get three classes with a similar number of individuals.

This classification resulted in six distinct classes: seeds in the seed bank, seedlings (younger than 1 yr), small vegetative (≤ 17 mm length), intermediate vegetative (18–43 mm length), large vegetative (≥ 44 mm), and flowering individuals (Fig. 1). The probabilities of nonreproductive individuals remaining in the same class or changing to a different class were calculated from censuses of marked plants in the demographic plots. We used these transition probabilities of nonreproductive individuals in matrices for all treatments. Transition probabilities and fecundities for the reproductive class were, however, calculated separately for each treatment. Reproduction involved transitions to both seedlings (if seeds germinate within 1 yr from release) and to seeds in the seed bank. Fecundity was calculated as the product of the mean seed production of all individuals within a particular treatment category and the seed fate probabilities derived from seed sowing experiments. In these experiments we tried to mimic the natural seed dispersal process as closely as possible. Seeds were collected at maturation just outside the study area and immediately sown into six 10×10 cm plots. Fifty seeds were dispersed over each plot, and the vegetation was gently shaken to allow seeds to settle, but no other treatment was applied. The sowing density was higher than the natural seed rain (13.5 ± 13.2 seeds/dm², $N = 6$ permanent plots). This was to make a careful examination of germination possible and to increase sample sizes. Comparison of recruitment in similar sowing experiments in several populations during several years with natural recruitment rates suggests that density-dependent interactions are not important at these densities (K. Lehtilä, M. B. García, and J. Ehrlén, unpublished data). Germination probability was calculated as the number of seedlings emerging in sowing plots minus the number of seedlings emerging in unsown control plots, divided by the number of seeds sown. Survival in the soil seed bank was calculated by carrying out identical sowing experiments at the same site in consecutive years and assuming that germination probabilities were similar for different cohorts of sowed seeds (K. Lehtilä, M. B. García, and J. Ehrlén, unpublished data).

A 6×6 projection matrix was constructed for each treatment group. Each matrix element, a_{ij} , represents the probability of an average individual in class j at time t to be in class i at time $t + 1$. Iterations of these matrices yielded values of the finite population growth rate (λ ; Caswell, 1989a). To assess the extent to which differences in λ among the experimental groups represent treatment effects, we calculated the sampling variance and standard error of λ for the control group using an analytical method based on a Taylor series expansion (Alvarez-Buylla and Slatkin, 1994, formula 3). We used the observed variances for fecundities and sampling variances according to the binomial distribution for transition probabilities. Sampling covariances between pairs of transitions were estimated according to the multinomial distribution. All transitions from nonreproductive classes were identical among treatment groups and therefore their sampling variance was set to zero. Correlations, including fecundities, were assumed to be zero, because identification of non-zero correlations (e.g., costs of reproduction) was one of the objectives of the present study. Population growth rate from treatments was considered to be

significantly different from the control if its value was outside the 95% confidence interval for control.

The elasticity matrix (de Kroon et al., 1986) was calculated to examine the relative importance of each matrix element to population growth rate. We used life table response experiment (LTRE) analysis (Caswell, 1989a, b; Horvitz, Schemske, and Caswell, 1997) to decompose the total effect of a treatment on λ into contributions from the different life-cycle transitions. These contributions were calculated by multiplying the differences in each matrix element (a_{ij}), between the control matrix and each respective experimental matrix, by the sensitivity of λ to that element. Sensitivities were evaluated at matrices midway between the mean matrix and the two original matrices (Caswell, 1989a).

RESULTS

Performance of individuals in the treatment year—No plants died during the first study season. Leaf area in July was not significantly affected by flower removal and/or hand pollination, but was strongly correlated to pretreatment size in May (Fig. 2, Table 1).

Neither fruit set nor seed set was significantly increased by pollen addition or flower removal, although the average values were higher than in control plants (Fig. 2). Early defoliation had a significant negative effect on fruit set (Fig. 2). Total seed production was correlated with plant size and differed among treatments (Table 1). Early defoliation significantly reduced seed output ($\bar{X} \pm 1$ SD = 101.4 ± 110.4 vs. 182.1 ± 142 for control plants; Fig. 2).

Performance of individuals in the year following treatment—Mortality during the year following experimental manipulation was very low and was not affected by treatment or plant size (logistic regression: $\chi^2 = 7.03$, $df = 7$, $P = 0.53$; Fig. 3). Leaf size was strongly correlated to the initial size in the previous year (Table 1). Treatment significantly affected leaf growth, but only plants that were exposed to early and middle leaf removal were significantly smaller than controls (Fig. 2).

A large proportion of individuals that were reproductive in 1995 became vegetative in 1996 (Fig. 3). Only defoliation during fruit development had a significant negative effect on flowering probability ($G = 41.3$, $df = 7$, $P < 0.0001$ from a two-way contingency table; all the comparisons of treatment vs. control provided $P > 0.1$ except “middle defoliation” with $P < 0.0001$; Fig. 3). The number of flowers produced by reproductive individuals during the second year was only marginally affected by treatment (Table 1). Neither fruit set nor total seed production differed significantly among treatments.

Total effects—The largest elasticity values in the control matrix corresponded to transitions from intermediate vegetative plants to remain in the same class and for large vegetative plants to become reproductive (Table 2). In contrast, values for reproductive plants to seeds and seedlings were very low.

Treatments resulted in decreases in λ (1.21) of between 1 and 20% (Table 3). The values for the three treatments involving removal of flowers as well as early and intermediate leaf removal were below the calculated 95% confidence interval for the controls (1.18–1.25), suggesting that these treatments resulted in significant decreases in overall fitness. Pollen addition to all flowers, and late defoliation, did not significantly affect population growth rate compared to the control.

For the treatment with the largest decrease in λ (complete flower removal), as well as for the other two treatments in-

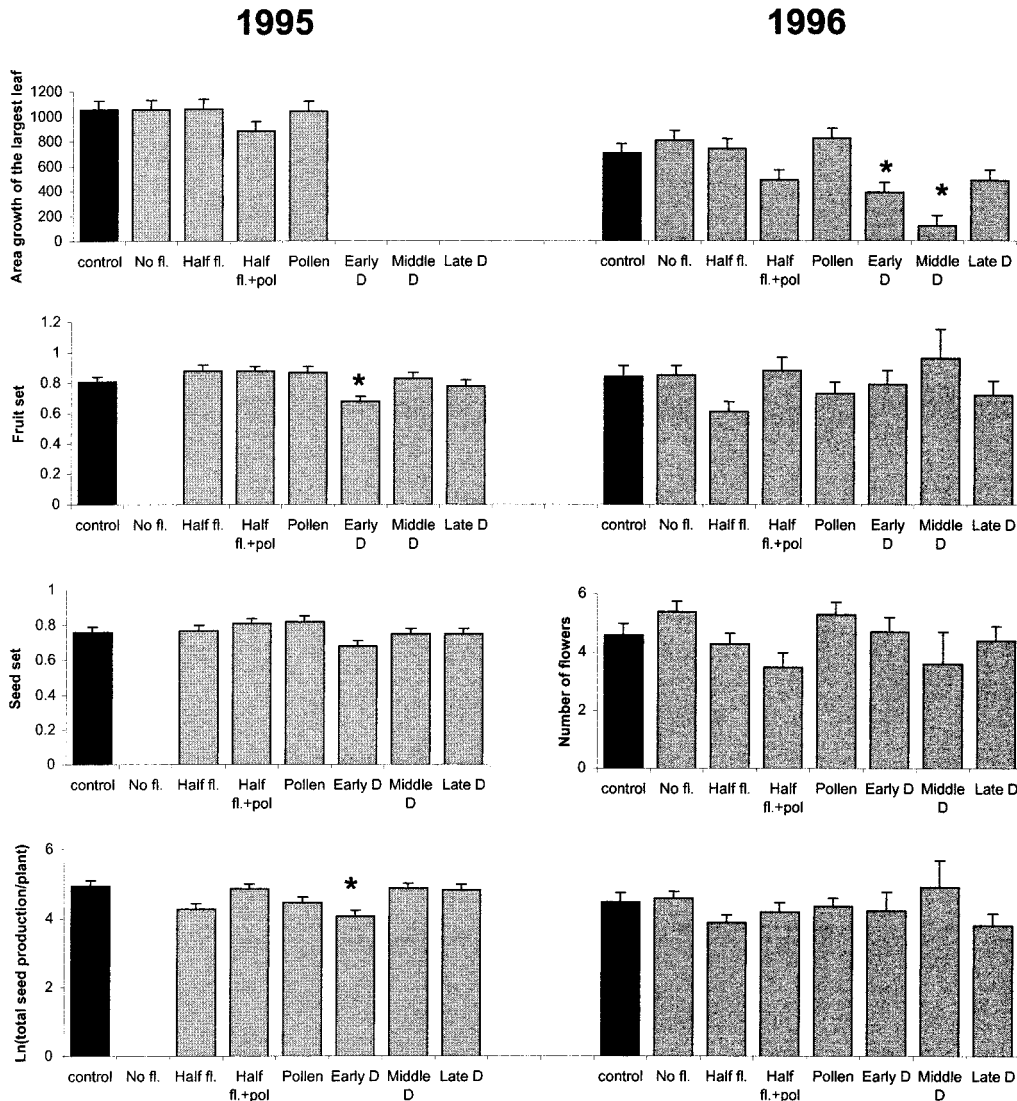


Fig. 2. Least squares means (+SE, using the area of the largest leaf in 1995 as covariate) of different fitness components during and 1 yr after treatment application. Asterisk indicates significant differences between each treatment and the control ($\alpha = 0.05$) by Dunnett's test. The growth of the largest leaf is estimated as the difference of leaf area (in square millimeters) of the largest leaf, measured at two different times (May–July of 1995 and July of 1995–1996). For an explanation of treatment abbreviations, see MATERIALS AND METHODS.

volving partial flower removal, seedling production was the life-cycle transition that contributed the most to that reduction (Table 3). The negative effects of leaf removal were largely due to a change in the probability of staying reproductive in the next year, and, for early removal, also due to reduced seedling production (Table 3).

DISCUSSION

This study has examined how alterations in reproductive effort and herbivory affect a long-lived plant over two seasons. We found no evidence of pollen limitation and were not able to detect any reproductive costs. The leaf-removal experiments suggest that herbivory have negative effects, but that source-sink paths vary over the season. As a consequence, herbivory affected different components of current and future performance depending on the timing. Only modeling the total effects, in terms of population growth rate changes, enabled us

to assess the relative importance of interactions with pollinators, flower herbivores, and leaf herbivores for plant fitness.

Reproductive effort and cost of reproduction—Supplemental hand pollination failed to significantly increase fruit set, seed set, or total seed output in our population during the year of study. Lehtilä and Syrjänen (1995a), with the same species, found that pollen limitation varied between years and populations. In our study, fruit set in control plants was 81.2% ($N = 46$), which is only slightly higher than the average recorded in 25 nearby populations during 3 yr ($75.0 \pm 27.7\%$; 50 plants per population; K. Lehtilä, M. B. García, and J. Ehrlén, unpublished data). This suggests that severe pollen limitation is not common in the area. On the other hand, total seed output was not significantly lower than in control plants after 50% flower removal with or without supplementary pollination. It appears that total seed production is mainly determined by plant size.

TABLE 1. Effect of different treatments on various fitness components during the experimental season (1995) and the year following experimental manipulation (1996), examined by ANCOVA models. Effects with a probability <0.05 are in boldface type.

Fitness component	Source of variation	df	SS	F	P
Growth 1995	Among treatments	4	920 174.1	1.01	0.4
	Size	1	28 261 328.1	12.4	0.0005
	Error	195	44 455 240		
Fruit set 1995	Among treatments	6	1.43	4.35	0.0003
	Size	1	0.02	0.46	0.5
	Error	287	15.69		
Seed set 1995	Among treatments	6	0.46	1.93	0.076
	Size	1	0.01	0.3	0.59
	Error	265	10.46		
Total seed production 1995	Among treatments	6	19.77	4.31	0.0004
	Size	1	29.52	38.64	<0.0001
	Error	199	152.04		
Number of flowers 1996	Among treatments	7	49.63	1.99	0.06
	Size	1	222.26	62.59	<0.0001
	Error	133	472.3		
Growth 1996	Among treatments	7	19 245 803	9.37	<0.0001
	Size	1	190 926	0.65	0.42
	Error	371	108 831 604		
Fruit set 1996	Among treatments	7	1.34	1.71	0.11
	Size	1	0.11	0.99	0.32
	Error	133	14.87		
Total seed production 1996	Among treatments	7	5.03	1.21	0.31
	Size	1	2.69	4.52	0.038
	Error	54	38.67		

In this study we performed pollen addition and flower removal treatments to alter current reproductive effort and investigate potential compensating responses (e.g., a cost of reproduction) in the following year. Supplementary hand pollination did not have any significant negative effects, but this lack of a clear reproductive cost is to be expected when hand pollination does not increase seed production (see also, Horvitz and Schemske, 1988; Jennersten, 1991, for other species). We also failed to find any positive effect of pollen addition on other fitness components, as has been demonstrated previously for the same species (Lehtilä and Syrjänen, 1995a). On the other hand, reduction of reproductive effort by removal of half of the flowers did not significantly increase fruit set in remaining flowers. Altogether, our results suggest that reproductive costs are small in the study population of *P. veris* despite the fact that reproductive effort appeared to be naturally near the maximum level possible. The cost of reproduction has previously been demonstrated to vary spatially in *P. veris* (Syrjänen and Lehtilä, 1993), suggesting that this trade-off depends

on environmental conditions. Several other studies have also shown that the expression of reproductive costs may differ within species (Obeso, 1993a) or between closely related species (Karlsson et al., 1990; Ågren and Willson, 1994). Hence, if reproductive effort varies in the study population, then costs may be possible to detect only in some years. The proportion of large individuals that flowered and flower number in the experimental population (59.8% [$N = 102$], 5.6 [$N = 64$], respectively) was very close to the average for 15 nearby populations during 3 yr ($50.3 \pm 9.0\%$ and 5.7 ± 0.9 , respectively; K. Lehtilä, M. B. García, and J. Ehrlén unpublished data). This, along with data on fruit set levels (see above), suggest that reproductive effort in our experiment was representative for the study area. In this study we carried out manipulations of reproductive effort during a single year. It is thus still possible that repeated manipulations could result in detectable costs. For several other perennial herbs, it has been shown that costs are expressed only after several years of manipulations (Ackerman and Montalvo, 1990; Primack and Hall, 1990; Ehrlén and van Groenendael, 2001).

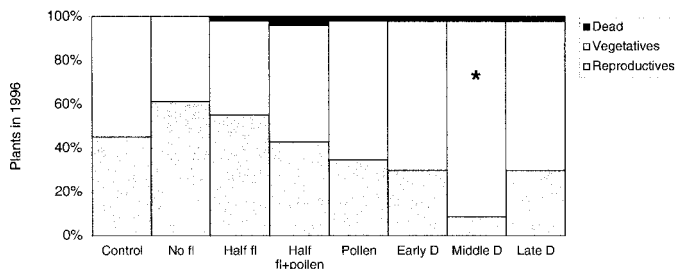


Fig. 3. State (reproductive, vegetative, or dead) in 1996 for individuals exposed to different treatments in 1995. The asterisk indicates that only defoliation during fruit development had a significant negative effect on future flowering probability ($P < 0.0001$; adjusted experimentwise error rate of $\alpha' = 0.0073$). For an explanation of treatment abbreviations, see MATERIALS AND METHODS.

Leaf damage—In *P. veris*, defoliations performed at an early phenological stage of plant development negatively affected current reproductive performance (fruit set and total seed production) and future growth, whereas removal of leaves later in the season only reduced future flowering probability and growth (middle defoliation), or did not affect any fitness component (late defoliation). These results suggest that the source-sink paths vary over the season and that the influence of herbivory might vary depending on the stage of development of the plant. The timing of herbivore damage has been shown to be important for future plant performance in several other plant species as well (Obeso and Grubb, 1994; Ehrlén, 1995; Escarré, Lepart, and Sentuc, 1996). Such phenological differences may occur because of differences in the constraints acting on the plant at the time of leaf damage (Marquis, 1992;

TABLE 2. Transition probabilities of *Primula veris* individuals between 1995 and 1996 and associated elasticity values. (A) "Control matrix," containing data from demographic plots of the population, plus transitions corresponding to reproductive plants of the "control" group. (B) Elasticity values corresponding to this "control matrix" (the two highest values are in boldface type). (C) Transition probabilities for reproductive plants exposed to seven treatments. Abbreviations: s = seeds in the seed bank; sl = seedlings 1-yr-old; sv, iv, and lv = small, intermediate, and large vegetative plants, respectively; r = reproductive plants. For an explanation of treatments abbreviations, see MATERIALS AND METHODS.

A) Transition probabilities							
	s	sl	sv	iv	lv	r.control	
s	0.11					16.90	
sl	0.14					22.53	
sv		0.57	0.53	0.10			
iv			0.36	0.82	0.12	0.02	
lv				0.04	0.41	0.53	
r					0.46	0.45	
B) Elasticities							
	s	sl	sv	iv	lv	r.control	
s	0.001					0.007	
sl	0.007					0.075	
sv		0.082	0.073	0.013			
iv			0.095	0.204	0.003	0.000	
lv				0.085	0.074	0.058	
r					0.140	0.083	
C) Transition probabilities							
	r.No fl	r.Half fl	r.Half fl + pol	r.Pollen	r.Early D	r.Middle D	r.Late D
s		10.17	16.64	10.25	10.83	15.51	18.94
sl		13.56	22.18	13.67	14.44	20.68	25.25
sv							
iv	0.02	0.02	0.06	0.04	0.15	0.22	0.09
lv	0.37	0.41	0.57	0.49	0.53	0.67	0.60
r	0.61	0.55	0.35	0.43	0.30	0.09	0.30

Lowenberg, 1994; Obeso and Grubb, 1994; Escarré, Lepart, and Sentac, 1996).

If current reproductive success relies primarily on resource availability and resource acquisition during early phases of ovary fertilization, as seems to be the case in *P. veris*, then leaf removal during late phases of fruit development may not influence seed production. Growth and flowering in one year, in contrast, also depend on the photosynthetic activity during

fruit and seed maturation during the previous year. Hence, there appears to be phenological variation in how resources are distributed between competing sinks: fruit-filling vs. storage for growth and reproduction in the following season. In a study of two species of *Geranium*, Ågren and Willson (1994) found differences between species in the cost of reproduction, despite the fact that the time required for flowering and fruit maturation was similar. They interpreted the results in terms of a "temporal overlap." A higher cost of reproduction was associated with a higher interference between allocation to reproduction and allocation to vegetative growth and storage.

Total effects—In this study we went beyond single demographic parameters and assessed the overall effects of treatments on maternal fitness through a demographic modeling approach. This method has rarely been used to evaluate the effect of isolated factors such as pollen limitation (Calvo, 1993; Ehrlén and Eriksson, 1995) and herbivory (Doak, 1992; Ehrlén, 1995) on population growth rate. Our experimental design allowed us to compare simultaneously the sensitivity of λ to different intensities of flower predation, supplemental pollination, and the timing of leaf herbivory. The estimated effect of treatments on lifetime fitness (λ) suggested reduced population growth rate for five of the seven treatments when compared to control plants.

The highest reduction in population growth rate (almost 20%) occurred after complete flower removal. A lower but still significant reduction of λ was also seen after removal of half of the inflorescence, with or without hand pollination of the remaining flowers. Decomposition of these differences into contributions from different life cycle transitions by LTRE analysis showed that changes in λ were largely the result of a reduced seedling production. Differences in growth and survival of reproductive individuals contributed much less to differences in λ . In our model we kept transitions from vegetative plants constant. Hence, our experimental design says nothing about the relative importance of transitions from vegetative vs. reproductive individuals. However, the elasticity analysis of individuals in the control group shows that the survival and growth of larger vegetative individuals contributed most to the growth of this population.

Supplementary hand pollination had no effect on population growth rate, suggesting that fitness was not limited by pollen availability in this population. This was expected given that we did not increase seed output with hand pollinations and did

TABLE 3. Population growth rate (λ) calculated for each treatment and percentage of difference in comparison with the control matrix ($\lambda = 1.21$, 95% confidence interval [CI] = 1.18–1.25). The total effect of treatments is decomposed into contributions (proportions) by transitions from reproductives to all classes by LTRE analysis. Abbreviations: s = seeds in the seed bank; sl = seedlings 1-yr-old; sv, iv, and lv = small, intermediate, and large vegetatives, respectively; r = reproductive plants. Population growth rates from experimental treatments outside the CI of λ for the control matrix and transitions with the highest impact for each treatment (>40% of the total effect) are in boldface. For an explanation of treatment abbreviations, see MATERIALS AND METHODS.

	No fl.	Half fl.	Pollen	Fl. + pollen	Early D	Middle D	Late D
Lambda	0.97	1.17	1.19	1.16	1.14	1.16	1.20
% change from control λ	-19.79	-3.42	-1.44	-4.64	-5.74	-4.61	-0.89
Contributions from reproductives to							
s	0.07	0.05	0.00	0.07	0.05	0.01	0.02
sl	0.68	0.49	0.05	0.74	0.50	0.08	0.20
sv	0.00	0.00	0.00	0.00	0.00	0.00	0.00
iv	0.00	0.00	0.02	0.01	0.03	0.03	0.02
lv	0.10	0.20	0.18	0.09	0.00	0.17	0.16
r	0.15	0.26	0.74	0.09	0.41	0.71	0.60

not detect subsequent improved performance as found by Lehtilä and Syrjänen (1995a). In other species where supplemental pollination considerably enhanced reproductive success, matrix models were used to examine whether pollen (Calvo, 1993) or resources (Ehrlén and Eriksson, 1995) limited lifetime seed production.

The effect of herbivory differed considerably depending on timing, as the same intensity and pattern of defoliation were detrimental during fruit development and especially flowering, but had no significant effect when applied late in the growing season. The total negative effects were smaller than that of complete flower removal and similar to that of half-flower removal, and they were expressed through different paths. Early leaf removal mainly resulted in reduced seed production (as occurred with flower removal), whereas removal later during the season reduced the probability that reproductive individuals remained fertile. These results confirm the conclusions from analyses of fitness components and, again, suggest that resources necessary for seed production and storage for future performance rely on different phenological states of the plant. As demonstrated in other species, the timing of the damage is important for detecting negative or compensatory responses (Marquis, 1992; Obeso and Grubb, 1994; Lehtilä and Syrjänen, 1995a), and therefore must be taken into account when investigating the effect of herbivory. In addition to timing, the intensity and frequency of herbivory should also be included for a complete view of its real effects under natural conditions (Doak, 1992; Ehrlén, 1995).

General conclusion—Our results suggest that the broad spectra of animal interactions in which plants are involved cause a variety of effects that differ in their magnitude and time of appearance, as well as in terms of which components of fitness are affected. With respect to herbivory, no future effects after complete flower removal or current effects after half flower removal were detected when examining isolated fitness components. Furthermore, flower removal and leaf damage both reduced population growth rate, but they did this by affecting different life history stages. Long-lived iteroparous plants, however, spend a variable period as reproductive individuals, and the full consequences can be correctly assessed only when placing that period in the broader context of the entire life cycle, as demographic models do. In order to compare factors that influence different fitness components and proceed towards a more general knowledge of plant–animal interactions, it is therefore important to assess the effects in terms of universal fitness estimates.

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