

Analysis of the Biotic Factors Affecting the Seed Set of a Rare Pacific Northwest Endemic: *Sidalcea malvaeflora* (D.C.) Gray ex Benth. *elegans* (Greene) C.L. Hitchc.

Abstract

Populations of *Sidalcea malvaeflora* ssp. *elegans*, a plant on the Oregon Natural Heritage Data Base's 1987 Review List, were investigated to determine factors that limit its seed production.

Viable, undeveloped and weevil damaged seeds from 100 racemes were monitored throughout the reproductive cycles of 1987 and 1988. Experiments were conducted to examine potential limitation to seed production: hand cross-pollinations tested pollen limitation and water/nutrient treatments tested resource limitation.

Production of viable seed was low (3.3% of total ovules in 1987 and 7.1% in 1988), although 16.9 percent and 15.1 percent of ovules (in 1987 and 1988 respectively) had dispersed before collection and could have contributed to viable seed set. The high incidence of undeveloped (aborted or unfertilized) ovules (41.1% in 1987 and 51.5% in 1988) suggested seed set was limited. Seed production was reduced by weevil predation (by 38.7% of all ovules in 1987 and 26.5% in 1988). Viable seed production in hand pollinated fruit was higher than in insect pollinated fruit. Racemes receiving extra water produced more viable seeds than controls. Resource availability and insufficient pollination appear to affect seed production in *S. malvaeflora* ssp. *elegans*.

Introduction

The intensive study of rare and endangered plant species is a relatively new scientific pursuit. In plants which have a low reproductive output, it is necessary to manipulate experimentally, not simply monitor, populations. Such experiments in the field provide the best evidence for factors that may crucially limit rare plant populations. Moreover, without such experimental evidence, legal protection by the state is impossible. Oregon Senate Bill 533, passed in 1987, states that to make a determination on a species' endangerment the reproductive potential must be in danger of failure due to (1) limited population numbers, (2) disease, (3) predation, or (4) other natural or man-made factors affecting continued existence. Moreover, such data help tailor management plans on a species by species basis.

Data collected in 1987 showed that *Sidalcea malvaeflora* (D.C.) Gray ex Benth. ssp. *elegans* (E. Greene) C. L. Hitchc. plants produced many more flowers than fruits (and thus seeds). Predation, pollination limitation, and resource limitation are some of the many hypotheses explaining why plants may produce more flowers than fruits (Stephenson 1981, Horvitz and Schemske 1988). The purpose of this study is to determine which of the above three factors play a part in limiting seed set in this rare plant.

The most common way to test for pollination limitation is through experimental cross pollinations

(e.g., Lee and Bazzaz 1982, Stephenson 1984, Garwood and Horvitz 1985). If fertilizations are limiting, the number of viable seeds produced in hand pollinated fruit should be higher than in insect pollinated fruit. A higher percentage of fruits should develop from hand pollinated flowers (less fruit abortion) if pollen is a limiting resource.

Resource limitation may be tested in a variety of ways. Adding water and/or nutrients tests whether resources limit plant size (Lee and Bazzaz 1982), plant survival (Grieg-Smith and Sagar 1981), fruit production (Lloyd 1980, Willson and Price 1980, Stephenson 1981, Stephenson 1984, Lee and Bazzaz 1982, Gorchoff 1988) or seed production (Willson and Price 1980, Stephenson 1984; Gorchoff 1988). Others have tested resource limitation of fruit and seed set through defoliation (Willson and Price 1980, Stephenson 1981, Stephenson 1984, Gorchoff 1988), shading (Willson and Price 1980, Stephenson 1981), fruit thinning (Lloyd 1980, Gorchoff 1988), girdling (Stephenson 1981, Gorchoff 1988) and removal of competitors (Grieg-Smith and Sagar 1981).

In this study I conducted one type of manipulation, resource addition, to test for resource limitation. Plants receiving extra water and/or nutrients should have more resources to mature viable seed if reproduction is limited by resources (Lee and Bazzaz 1982, Stephenson 1984).

Study Area and Plant Description

Sidalcea malvaeflora ssp. *elegans* occurs from southern Douglas County through Curry and Josephine Counties in southwestern Oregon to Del Norte County in northeastern California. Eighteen small, localized populations are documented from throughout this range. The study area, Oak Flat, is near the confluence of the Rogue and Illinois Rivers in Siskiyou National Forest, southwestern Oregon. The dominant overstory consists of *Quercus garryana* and *Q. kelloggii*; the understory is mostly a mixture of introduced annual grasses.

This subspecies is distinct in that it is the only clonally-growing member of the *Sidalcea malvaeflora* group. Clonal mats range in area from 1 to 8 m² and, in the study population, are spatially segregated into 7 patches of plants. Patches produced from 1 to 31 racemes in 1988. Because of the clonal nature of this plant, it is difficult to determine how many racemes each plant erects. In the following experiments, I assume that racemes act independently of one another. There is a possibility, however, that manipulation of one raceme will affect the next because interconnected shoots may compete for and/or share resources. Racemes emerge in early April and flowering occurs throughout June. These plants are obligate outcrossers; self-pollinated plants produced no seed (pers. obs.).

Methods

Non-Experimental Data

In 1987 and 1988 I made systematic, non-experimental observations, the purpose of which were to gather baseline data on reproductive biology. These data were used to monitor predation, floral abortion rates and numbers of viable seeds produced by single and multi-fruited racemes. Baseline data collected in 1988 were also used as controls for 1988 experiments.

I marked 100 racemes each year using numbered wooden stakes and recorded number of buds per plant, flowers reaching anthesis (1988 only), fruits initiated (1988 only), and fruits matured each week from April-July. In 1987 2 plant markers were lost because of anthropogenic disturbance, making the total racemes monitored 98. Racemes were collected and dried. Fates of ovules were categorized as: missing (dispersed, viability unknown), aborted and/or undeveloped, attacked (presumably by the weevil *Macrorhoptus niger*) and

viable. Viability was determined through germination tests in which seeds were placed in petri dishes to allow imbibition, scarified with a sterile knife and allowed to germinate. The resulting seedlings were grown and donated to the local botanic garden.

Predation was analyzed to determine whether single or multi-fruited racemes were preferentially attacked by weevils. A Chi-square test was used to analyze whether there was a difference in the number of ovules attacked per raceme on single and multi-fruited racemes. All racemes from the baseline population which did not have dispersed ovules were used for this analysis.

Experimental Data

Pollination Limitation

Experimental pollinations were conducted in 1988 to test whether seed production in *Sidalcea malvaeflora* ssp. *elegans* is pollinator limited. I randomly selected ten racemes with a total of 33 flowers. If stigmas were extended on my weekly visit, I cross-pollinated the flower using a small paintbrush and pollen from another clone (patch). Racemes were bagged when not being hand pollinated to discourage weevil infestation and to recover all ovules. I collected and dried racemes and categorized ovules as above.

A Chi-square test was used to analyze whether there was a difference in the proportion of hand and open pollinated fruit bearing viable seed. Controls were all racemes in the baseline population which experienced no seed predation (comparable to bagged racemes) and which had no dispersed seeds (so that the fates of all seeds were known).

Resource Limitation

Water and/or nutrients were added to 30x30 cm plots to determine if additional resources would increase viable seed production. Placement of plots was biased; they were set up in areas where plants grew vegetatively during the beginning of the field season. On average 0.5 racemes were produced per plot (range 0-2). There were seven replicates of each of the four following plot treatments: (1) control (no addition), (2) water (1 liter per week), (3) a one-time nutrient addition (19 g fertilizer, time-released 14% N/14% P/14% K per plot), and (4) water + nutrient (initial fertilizer plus 1 liter water per week). All treated racemes were collected, fruits were dried, and ovules categorized.

Results

Non-Experimental Data

Baseline information on production of reproductive parts was obtained from 98 racemes in 1987 and 100 racemes in 1988: number of buds per raceme averaged 4.62 (range 1-12), number of flowers averaged 3.70 (range 1-10), and number of fruits averaged 1.55 (range 0-7). The number of ovules per fruit was the most constant, averaging 6.0 (range 4-9).

Predation by the weevil, *Macrorhoptus niger*, and nondevelopment were the most common fates of ovules at Oak Flat in 1987 and 1988 (Table 1). It was clear that most of the seed destruction was due to the weevil because adult beetle pairs were found in *Sidalcea* flowers and larval and pupated forms were found in dried fruits during ovule analysis. Numbers of dispersed seeds were similar in both years. When rainfall was greater during fruit and seed maturation (1988), viable seed set doubled.

The pattern of weevil predation was analyzed to determine whether weevils preferentially attacked racemes with more fruits. The number of attacked ovules per raceme from 21 single-fruited racemes (62 flowers, 35 attacked/120 total ovules) was compared to the number of attacked ovules per 27 multi-fruited racemes (113 flowers, 65 fruits and 144 attacked/383 total ovules). Multi-fruited racemes experienced a much higher attack rate ($\chi^2_{1df} = 42.5$, $p < .005$). However, when predation rate was analyzed per fruit (number of attacked ovules per fruit), there was no difference in ovular attack rate in single and multi-fruited racemes ($\chi^2_{1df} = 2.33$ n.s.).

The pattern of abortion on floral parts from bud to mature fruit was examined on the baseline populations in both 1987 and 1988. Only about 10 percent of racemes matured all fruits they initiated (12.2% in 1987 and 9.0% in 1988), while nearly

30 percent aborted all fruits (34.7% in 1987 and 30.0% in 1988). In 1988 abortion rates between stages were more thoroughly monitored to determine at which stage(s) abortion took place; between bud and anthesis 19.9 percent of buds were aborted, between flowering and fruit initiation 50.6 percent were aborted, and between fruit initiation and maturation abortion was 15.4 percent.

Pollination

The ten racemes that were hand pollinated produced thirty-three flowers. Only eighteen flowers were receptive to pollination during my visits; seven fruits and 12 viable seeds out of 39 total ovules matured. Twenty-three unmanipulated open pollinated racemes, serving as the control group, produced 36 fruits, 73 flowers and 32 viable seeds out of 207 total ovules. There was a difference in the number of viable seeds produced per fruit in hand and open pollinated fruit ($\chi^2_{1df} = 3.91$, $p < 0.050$).

Resource Limitation

In Oak Flat *Sidalcea* in 1987 I compared single and multi-fruited racemes of 100 baseline plants and found that 70% of the viable seeds produced were from single-fruited racemes (of the 62 racemes which produced fruit, 32 were single-fruited). In 1988, the 100 baseline single-fruited and multi-fruited racemes produced roughly proportional numbers of viable seed; 24 single fruited racemes (34% of total racemes) produced 36.0 percent of total viable seeds while 46 multi-fruited racemes (66% of total racemes) produced 64.0 percent of total viable seeds.

The purpose of resource additions was to partition effects of water and/or nutrient limitation on viable seed set. The results may be compared but the sample sizes are small, and thus too variable, to detect any statistical differences. Water appears

TABLE 1. Percentages of ovules collected from control racemes in 1987 (98 racemes) and 1988 (100 racemes).

	Viable* (%)	Undeveloped** (%)	Missing (%)	Attacked (%)	Total Ovules	Total Flowers	Matured Fruit
1987	3.3	41.1	16.9	38.7	302	—	105
1988	7.1	51.5	15.1	26.3	930	370	155

*Ovules were deemed viable if they germinated after laboratory treatment.

**Includes aborted and unfertilized seeds.

TABLE 2. Fate of ovules in fruit receiving additional water and/or nutrients as compared to fruit from the natural environment.

Treatment	Total Racemes	Total Fruit	Total Seeds	Viable Seed/ Fruit	Ovules Eaten/ Fruit	Ovules Undeveloped/ Fruit
Water	2	5	29	2.00	1.40	2.40
Nutrient	3	4	21	0.00	2.75	2.50
Water/Nutrient	2	5	30	0.60	1.40	4.00
Control	100	155	931	0.43	1.58	4.00

to be a limiting factor; fruits receiving extra water produced over four times the number of viable seeds per fruit than controls (Table 2). The effect of additional nutrients may decrease viable seed set because no viable seeds were produced.

Discussion

In both 1987 and 1988 viable seed set in *Sidalcea malvaeflora* ssp. *elegans* was very low (3.3% and 7.1% of the total number of ovules respectively). Some of the missing seeds may have been viable; the actual seed set may have been higher. Arnold (1982) cites low seed sets for other self-incompatible perennials: *Dicentra canadensis* (2-7%), *Erythronium albidum* (6-11%) and *Linaria vulgaris* (1.4%).

There are two levels on which this population of plants seems to be producing, and subsequently aborting, floral parts. The first is a common overproduction of floral structures (buds, flowers, fruits) that may be caused by genetic or developmental abnormalities or by a response to limited resources (Stephenson 1981). Some abortion occurred between bud and anthesis (19.9%) and between fruit initiation and maturation (15.4%), but the bulk of floral structure loss took place between flowering and fruit initiation (50.6%). The high incidence of floral abortion between anthesis and fruit initiation may be seen as an adaptation either to respond to environmental stress or to eliminate fruits containing many aborted or unfertilized ovules (Udovic and Aker 1981).

The second level on which *Sidalcea* is producing and aborting floral parts is ovular. Harper (1977), Lloyd (1980) and Stephenson (1981) suggest some type of internal regulation is responsible for this abortion and Salisbury (1942) suggests "internal competition," both of which imply resource limitation. Gorchov (1988) points out that unfertilized ovules and fertilized ovules that subsequently abort must be distinguished because the

first type of abortion is primarily determined by lack of fertilization and the latter may be determined by lack of resources. The large proportion of undeveloped (unfertilized and aborted seeds lumped together) seeds in this *Sidalcea*, 41.1 percent in 1987 and 51.5 percent in 1988, suggests that seed initiation may be limited by the pollination system. In 1988 mean viable seed production per fruit in *Sidalcea* was higher in hand extraclonally pollinated fruit than insect pollinated fruit (1.71 vs. 0.89). Two explanations for the lack of development might be that the system is limited by the number of pollinators or, more plausibly, much of the pollen received by flowers from insects is intraclonal and thus incompatible. Although seed set was higher in hand pollinated racemes, fruit set was not. Thus, pollination limitation may affect the distribution of seeds among fruits but not total fruit set.

Seed predation caused the second greatest limitation of seed set. Seeds of this plant experienced high predation from the weevil, *Macrorhoptus niger*, as evidenced by its presence in all its developmental forms within *Sidalcea* flowers and fruits. Predation rates such as those seen in *Sidalcea*, 38.7 percent and 26.5 percent, are not uncommon. Lee and Bazzaz (1982) cite a range of 0-49 percent of seeds attacked by weevil larvae in *Cassia fasciculata*. Multi-fruited racemes were preferentially attacked by weevils (5.33 seeds/raceme vs. 1.67 seeds/raceme on single-fruited racemes), but predation was not significantly different per fruit (2.21 seeds/fruit vs. 1.67 seeds/fruit on single-fruited racemes). Even though a clone which spreads out its fruit in several single-fruited racemes might escape predation (per raceme) better than a clone which produces a single multi-fruited raceme, the production of more fruits for weevils to choose from on multi-fruited racemes seems to negate any advantage in escaping predation single-fruited racemes might have.

Preliminary evidence suggests that resources also limit viable seed set. Single-fruited racemes from the baseline population produced more viable seed than multi-fruited racemes during 1987, but when more water was available, viable seed set was roughly equal. Gorchov (1988) found that treatments that reduced the number of plant parts competing for resources produced higher fruit sets than controls.

Although I attempted to partition the suggested resource limitation into water and/or mineral nutrients, both predation and small sample sizes complicate interpretation of results. However, the fact that racemes receiving extra water produced more viable seeds than controls suggests that water may be a limiting factor.

In Oak Flat *Sidalcea*, three factors seem to be acting to limit seed set: lack of pollination, limited water and seed predation. Because this species is rhizomatous, plants may spread throughout current habitats through asexual reproduction. However, seed production is necessary for establishment of

new populations. Because established populations may persist indefinitely through clonal growth, I suggest simply monitoring the density of emergent leaf clumps per meter squared in clonal patches of selected populations to determine whether population size is increasing or decreasing. If population sizes decline, I suggest a combination of three management strategies to increase seed set: cross pollinations, water additions and/or predator exclusion.

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