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Effect of pollen limitation, additional nutrients, flower position and flowering phenology on fruit and seed production in Salvia verbenaca (Lamiaceae)

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Variation in fruit set, seed/ovule ratio and mean single-seed weight among plants, and within plants among inflorescences, was investigated in a population of *Salvia verbenaca* in northwest Spain. Mean single-seed weight varied significantly among plants which flowered at about the same time and mean single-seed weight was significantly greater for plants that flowered late in the season than for plants flowering early in the season. Within individual plants, mean single-seed weight increased steadily between the earlier opening basal inflorescences and the later opening upper inflorescences. However, neither fruit set nor seed/ovule ratio varied significantly among inflorescences. Previous work on this population indicates that outcrossing improves mean single-seed weight, so I suggest that the observed pattern of among-inflorescence variation in mean single-seed weight may be attributable to a gradual increase in nonself pollen receipt as the flowering season progresses.

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Introduction

A general problem encountered by plants during a reproductive episode concerns the coordination of fruit and seed number with the available resources (Stephenson 1981). When resources are limiting, plants typically favor some flowers or ovules over others, probably with consequent effects on progeny quantity and quality. Numerous studies have detected within-plant variation in fruit set, seed set and/or seed weight and such variation may be related to the position of the flower or ovule on the plant (Maun & Cavers 1971, Schaal 1980, Waller 1982, Stanton 1984, McGinley et al. 1987, Nakamura 1988, McGinley 1989, Rocha & Stephenson 1990, Gonzalez Rabanal et al. 1994) or to a "trade-off" between seed size and seed number (Janzen 1982, Wilken 1982, Stanton 1984, Broyles & Wyatt 1991, Cipollini & Stiles 1991, Obeso 1993).

However, resource limitation is not the only cause of variation in fruit and seed production; for example, such variations may reflect pollen limitation (Galen et al. 1985, see Burd 1994, for review) or variability in pollen source (Schemske & Pautler 1984, Galen et al. 1985, Marshall & Ellstrand 1986, Redmond et al. 1989, Thompson & Pellmyr 1989, Manasse & Stanton 1991).

The aim of the present work was to further understand the causes of variation in fruit and seed production. Specifically, I studied a population of the herb *Salvia verbenaca* L. (Lamiaceae), with the aim of identifying the causes of within- and between-plant varia-

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tion in fruit set, seed/ovule ratio and mean single-seed weight. Potential causes considered to explain variation were resource limitation, flowering phenology, flower and fruit position on the plant, number of seeds per inflorescence and plant and pollen source.

Materials and methods

The plant and the study area

Salvia verbenaca is a 30 - 60 cm high biennial herb with bluish purple flowers. Flowering commences in mid-April and finishes towards the end of May. The corolla is 6 - 10 mm long, enclosed within a green calyx 4 - 8 mm in length. The flowers are arranged in verticillaster-type inflorescences, each of which generally contains six flowers. Each plant typically bears 8 - 15 inflorescences and these are close together on the stem at flowering, but move further apart at fruit set. The flowers are protandrous and flowering order is acropetal. Nutlet fruits, each containing 1 - 4 seeds, are produced. The fruits mature (allowing seed release) about two weeks after senescence of the flower. Insect visits are not necessary for fruit production, but supplementary pollination with nonself pollen improves seed weight, but not seed number (Navarro 1997). Pollination is largely by the honey bee *Apis mellifera* and Bombyliidae flies such as *Bombylius major* (unpublished data). Each flower produces 0.6 - 1.5 µl of nectar per day (Petanidou & Vokou 1993).

Salvia verbenaca is typically found on stony slopes, pasture and verges. The population studied in the present work is located in an area of semi natural pasture on limestone, near the village of Chana, in the far western part of the El Bierzo region, close to the Galicia-León border in northwest Spain (42° 29'N, 6° 44'W). The population was monitored in spring 1993 and spring 1994.

Experimental design

Among- and within-plant variation in fruit/ seed production

In order to investigate whether reproductive success, defined as fruit set, seed/ovule ratio and mean single-seed weight, vary among the inflorescences of a plant, and/or among the plants of a population, I selected ten plants along a 1 × 100 m transect in April 1993 (all clearly defined individuals of height 30 - 40 cm) and monitored them over the flowering and fruiting period. During the fruiting period I visited the plants daily, recorded fruit set for each inflorescence, and collected all mature fruits. I subsequently determined (for all inflorescences and all plants) final fruit set, seed/ovule ratio and the weight of all individual seeds.

Seasonal variation in fruit/seed production

To investigate the possible effect of flowering period on fruit set, seed/ovule ratio and mean seed weight, comparisons were made between plants which flowered early and plants which flowered late in the season. During the second half of April 1993 I marked five randomly selected plants (hereinafter referred to as "early-flowering plants") which were about to commence anthesis. During the second half of May, I marked another five randomly selected plants ("late-flowering plants") which were likewise about to commence anthesis. Both groups were monitored until fruiting.

Effects of resource supplementation on fruit/ seed production

To investigate the possible effects of resource limitation on reproductive success, I selected twenty plants along a 1 × 150 m transect in spring 1994. All selected individuals were of height 30-35 cm. Ten of these plants received abundant water (50 l m-2) and NPK fertilizer (Nitrophoska Azul Especial; 250 g per plant); the remaining ten plants were not treated. Again, both groups were monitored until fruiting.

Data analysis

To investigate whether fruit set, seed/ovule ratio and/or mean seed weight varied significantly among plants or among inflorescences, I used nested analysis of variance. The percentage fruit set data and seed/ovule ratio data were first subjected to an arcsine transformation. In addition, I used Student's t test to investigate whether mean seed weight differed significantly between the upper two inflorescences and the basal two inflorescences of each plant. Spearman's rank correlation analysis was used to investigate whether the number of seeds produced by a plant was correlated with mean seed weight. Finally, I used analysis of covariance to investigate the effects of fruit position on each of the different verticillaster-type infrutescences (position) (meaning position 1 to the lowermost infrutescence, position 2 to the second, etc.) and of flowering subperiod (early-flowering and late-flowering plants) on the studied variables with flowering subperiod as covariate. The effects of position on the plant (position) and resource-supplemented treatment (treatment) on fruit set, seed/ovule ra-

Table 1. Seed and flowering characteristics (total or mean and standard deviation) of ten individuals of *Salvia verbenaca* (MSSW = Mean single-seed weight).

Parent	Total no. inflorescences	Total no. flowers	Total no. seeds	Fruit set (%)	Seed/ovule ratio	MSSW (mg)
1	13	77	243	97.4 ± 6.0	0.792 ± 0.069	1429 ± 777
2	10	53	181	86.7 ± 19.4	0.860 ± 0.087	1708 ± 688
3	12	63	207	82.6 ± 10.5	0.830 ± 0.105	2046 ± 680
4	10	60	190	100 ± 0	0.792 ± 0.065	1615 ± 524
5	12	70	231	97.2 ± 6.2	0.824 ± 0.060	1639 ± 553
6	14	83	276	98.8 ± 4.3	0.830 ± 0.075	1961 ± 765
7	12	71	228	98.6 ± 4.6	0.792 ± 0.068	2136 ± 622
8	10	60	209	100 ± 0	0.871 ± 0.076	1810 ± 526
9	11	65	211	97.0 ± 6.4	0.813 ± 0.074	1535 ± 589
10	8	48	164	100 ± 0	0.854 ± 0.063	2004 ± 685

tio and seed weight were investigated by two-way analysis of variance.

Results

Among- and within-plant variation

Mean seed weight ($F_{9, 2028} = 29.9$, $p < 0.0001$) varied significantly among plants which flowered at about the same time in April 1993. Fruit set ($F_{9, 89} = 1.82$, n.s.) and seed/ovule ratio, on the other hand, did not vary significantly among these plants ($F_{9, 537} = 0.71$, n.s.) (Table 1).

Neither fruit set ($F_{13, 89} = 0.99$, n.s.) nor seed/ovule ratio ($F_{13, 537} = 0.94$, n.s.) varied significantly with vertical position of the inflorescence on the plant; however, there was a strong variation in mean seed weight ($F_{13, 2028} = 49.2$, $p < 0.0001$), which increased steadily up along the shoot (Fig. 1). Thus, mean seed weight for the uppermost two (i.e., last-opening) inflorescences was significantly greater than for the lowermost two (i.e., earliest-opening) inflorescences ($t = -7.011$, d.f. = 38, $p < 0.001$).

At the plant level, mean seed weight was not significantly correlated with either number of flowers (rs = -0.11, p = 0.7427) or total number of seeds (rs = -0.20, p = 0.5485).

Seasonal variation

Seed weight increased as the flowering period progressed. Mean seed weight was significantly greater for late-flowering plants than for early-flowering plants, but seed/ovule ratio and fruit set did not differ (Fig. 2). Analysis of covariance with the factor fruit position and

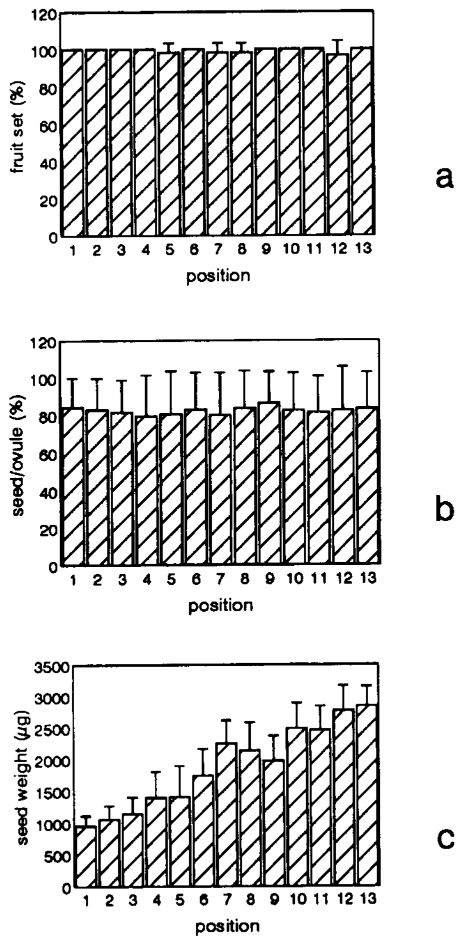

Fig. 1. Fruit set (a), seed/ovule ratio (b) and mean single-seed weight (c) for *Salvia verbenaca* inflorescences at different vertical positions on the plant (1 = lowermost position; 13 = uppermost position).

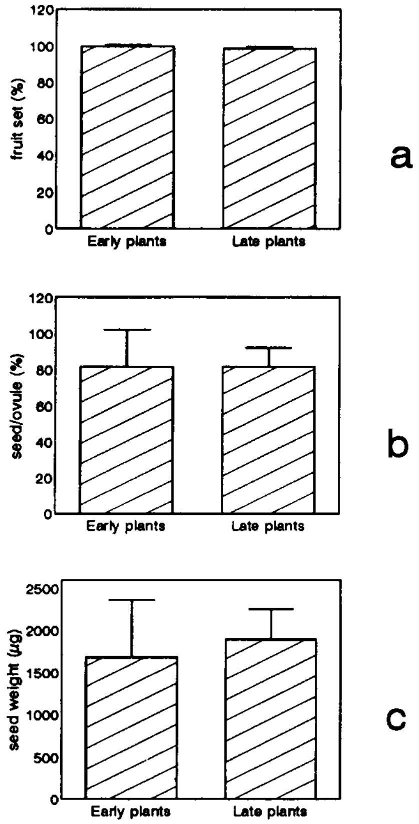

Fig. 2. Fruit set (a), seed/ovule ratio (b) and mean single-seed weight (c) for early-flowering and late-flowering *Salvia verbenaca* plants (see text).

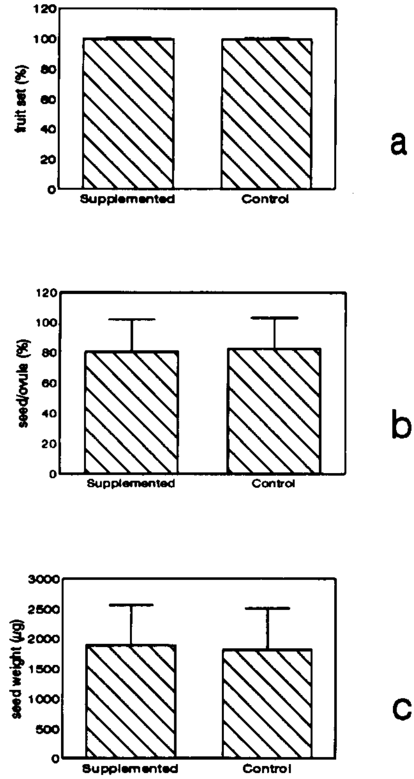

Fig. 3. Fruit set (a), seed/ovule ratio (b) and mean single-seed weight (c) for resource-supplemented and non-supplemented (control) *Salvia verbenaca* plants.

the covariate flowering subperiod indicated that flowering subperiod was a significant source of variance in seed weight ($F_{1, 2127} = 56.0$, $p < 0.0001$). Fruit position within the plant was, also, a significant source of variance in seed weight ($F_{11, 2127} = 6.6$, $p < 0.001$), even when the effect of flowering subperiod was accounted for. On the other way, no such significant differences were detected in fruit set or seed/ovule ratio.

Resource limitations

Comparison of inflorescences subjected to resource-supplemented treatment (treatment) and the corresponding control inflorescences indicated that in the year of study provision of abundant water and nutrients (treatment) did not have any signifficant effect on fruit set ($F_{1, 252} = 1.8$, $p = 0.186$) or seed/ovule ratio ($F_{1, 1274} = 0.3$, $p = 0.605$). Similarly, neither fruit position nor interaction fruit position * treatment had any significant effects on fruit set ($F_{13, 252} = 1.0$, $p = 0.430$ and $F_{13, 252} = 1.8$, $p = 0.052$ respectively) or seed/ovule ($F_{12, 1274} = 1.0$, $p = 0.464$ and $F_{12, 127}4 = 0.2$, $p = 0.999$ respectively). The resource supplemented treatment did not have any significant effect on seed weight ($F_{1, 2703} = 2.6$, $p = 0.108$). However, the factor fruit position ($F_{12, 2703} = 172.8$, $p < 0.0001$) and the interaction fruit position * treatment ($F_{12, 2703} = 9.8$, $p < 0.001$), had a significant effect on seed weight (Fig. 3).

Discussion

In *Salvia verbenaca* only mean seed weight varied significantly among plants which flowered at about the same time. In addition, mean seed weight was significantly larger for plants which flowered late in the 1993 season than for plants which flowered earlier. Within individual plants, mean seed weight increased steadily between the lowermost (i.e., earliest-opening) inflorescences and the uppermost (i.e., latest-opening) inflorescences; indeed, mean seed weight varied among inflorescences of the same plant by a factor of up to three (Fig. 1c).

Numerous studies have addressed between- and within-plant variation in female reproductive success. Such variation has generally been attributed to resource limitations, pollen limitations and/or variability in pollen source (see Navarro 1996 for review).

When resources for reproduction are limited, two factors that may influence fruit production are the location of the flower within the inflorescence or on the plant, and the time of fruit initiation. Solomon (1988) and Lee (1988) have suggested that intraplant competition between flowers for resources may explain the higher seed set (i.e., lower abortion rate) often observed in early-formed fruits or in fruits located closest to the source of nutrients and photosynthate. Thus, flowers in proximal positions in the inflorescence or on the plant often have higher fruit set or produce heavier seeds than flowers in distal positions, presumably because they are closer to the main stem (Stephenson 1981, Lee 1988, Solomon 1988). An alternative hypothesis is that phytohormones produced by a seed or fruit can inhibit the growth and development of neighbouring buds and fruits (Lee 1988).

In the present study of *Salvia verbenaca*, however, mean seed weight was lower for early-opening basal than for late-opening distal inflorescences, despite the fact that basal inflorescences can be expected to have an easier access to resources. Furthermore, provision of abundant water and fertilizer had no effect on seed production in the year of study.

It has been also suggested that many plants, under resource-limited conditions, tend to produce either a small number of large seeds or a large number of small seeds (Cipollini & Stiles 1991, Westoby et al. 1992, Obeso 1993, see however Manasse 1990). No such trade-off was detected in the present study, since there was no significant correlation between seed number and mean seed weight at the plant level. In view of these results, the observed pattern of variation in mean seed weight cannot be attributed to resource limitations.

There have been some reports of species whose seed production and/or seed weight are influenced by the source of pollen (Schemske & Pautler 1984, Galen et al. 1985, Marshall & Ellstrand 1986, Stephenson & Windsor 1986, Redmond et al. 1989, Thompson & Pellmyr 1989, Johnston 1992, Montalvo 1992, Navarro 1997). If the pollen grains deposited on the stigma show high genetic variability, gametophytic competition and/or selective abortion of seeds can be expected to occur, leading to an improvement in mean seed quality (see Hormaza & Herrero 1994).

The number of seeds per fruit in *Salvia verbenaca* did not appear to be affected by pollen limitation, since supplementary pollination with nonself pollen did not lead to any increase in seed number. However, mean seed weight was significantly enhanced by supplementary pollination with nonself pollen (Navarro 1997). Several studies have revealed a positive correlation between pollen competitive ability and offspring fitness (evaluated as seed dry weight, seed germination rate, mean seedling weight, number of leaves or root tip growth) (see Hormaza & Herrero 1994).

As noted above, mean seed weight for plants flowering late in 1993 (May) was higher than for plants flowering early in the same season (April). *Salvia verbenaca* flowers early in the year, when pollinator activity in the study area is low. However, flowers of this plant species have capacity to produce fruits and seeds without pollinators, although produced seeds are lighter than outcrossed produced seeds (Navarro 1997). In view of this result and the results of my outcrossing experiments, one possible explanation for the observed pattern of among-inflorescence variation in mean seed weight is that nonself pollen receipt was inadequate at the start of the flowering season (leading to extensive selfing), but became more common as the season progressed, coinciding with the observed increase on pollinator activity in the study area. This lead to a gradual increase in the incidence of outcrossing, and thus in mean seed weight. Such a trend in pollen receipt might reflect either changes in the abundance of pollen in the population or changes in pollinator activity.

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