

M. E. Torres, C. Ruiz, J. M. Iriondo, & C. Pérez

Pollination ecology of *Antirrhinum microphyllum* Rothm. (Scrophulariaceae)

Abstract

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In this work, the pollination effectiveness in two populations of *Antirrhinum microphyllum*, endemic to Central Spain, were investigated. The mean number of flowers per plant (54) and the percentage of flowers that produced seeds was high at both locations (84% and 92%). The floral advertisements (ultraviolet pattern) and rewards (volume and sugar concentration of nectar), and pollinators were also studied. The flowers produced an average of 3 µl nectar with 40-45% sugar. The most frequent flower visitor was a solitary bee, *Rhodanthidium sticticum*. It is active only on sunny days between 9 a. m. and 4 p. m., solar time, with a maximum between 11 a. m. and 1 p. m. The percentage of flowers visited per day in a plant ranged from 6-24%. The behaviour of nectar-collecting is described. The results indicate that the narrow distribution of this species cannot be explained by factors linked to the pollination process.

Introduction

Antirrhinum microphyllum Rothm. (Scrophulariaceae) is an endemic species located in the provinces of Guadalajara and Cuenca (Central Spain) that grows in the cracks of vertical calcareous cliffs. It is classified vulnerable under IUCN criteria due to its narrow area of distribution and its restricted rupicolous habitat. Reproductive success is a primary factor of study in the assessment of the viability of threatened populations. *A. microphyllum* is self-incompatible (unpublished data), and depends on insect pollination for fruit formation, like many other *Antirrhinum* species. The factors that influence the foraging behaviour of these animals include inflorescence size, floral colour, nectar volume and concentration, and amount of pollen (Kevan & Baker 1983). In this work, several aspects of pollination ecology of *A. microphyllum* were investigated including 1) pollination effectiveness, 2) floral advertisement and rewards, and 3) the pollinators and their nectar-pollen-collecting behaviour.

Materials and Methods

- Floral biology.-

Field work was carried out during the flowering period in 1997. 150 plants of two popu-

lations (Bolarque & Entrepeñas) were marked and visited at approximately weekly intervals. In these plants, the number of opened flowers and fruits were counted. Pollination effectiveness was evaluated as the percentage fruit set (number of fruits/number of flowers) in each population.

- *UV patterns in flowers.*

Sampled flowers were illuminated at a 365 nm wavelength with a mercury vapour lamp, rich in UV and the resulting images were captured using a CCD camera with a 8-bit resolution.

- *Nectar*

Volume of nectar accumulated during one day in the cavity around the ovary was measured from 44 bagged flowers from 10 different plants using a 5 µl micropipette. Sugar concentration was estimated as sucrose equivalent in % w/w with a temperature-compensated hand refractometer in 89 flowers from Buendía population and 69 flowers from Entrepeñas population.

- *Flower visitors*

The activity of *Rhodanthidium sticticum* (Fab.) (the most important pollinator) was recorded for a total of 90 h., during April when most plants were in bloom. The observations were made for periods of 15 min. each hour between 7 a. m. and 6 p. m. (solar time). Other different visitors that were observed probing the corolla, the stigmas or the anthers were captured and their family or subfamily level was determined.

Results and Discussion

The total number of flowers produced per plant varied between 1 and 316 with a mean value of 54. Fruit set, as a result of successful natural pollination, was high at both loca-

Table 1. Production of flowers and fruits in wild plants from two *Antirrhinum microphyllum* populations. n: number of plants, n° fl: number of flowers, n° fr: number of fruits produced, %fl/fr: percentage of flowers which develop fruits. Mean value per plant and standard deviation are given in parenthesis.

	n	N° fl	N° fr	% fl/fr
Bolarque	43	2189 (50.91±53.14)	2007 (41.67±48.87)	91.68 (90.6±13.84)
Entrepeñas	84	4725 (56.25±59.06)	3964 (47.19±49.46)	83.89 (81.6±23.83)
Total	127	6914	5971	86.36

tions, 84% (Entrepeñas) and 92% (Bolarque) (Table 1). Flowering plants of *A. microphyllum* are attractive to pollinators due to the large number of showy flowers opened at a time. Another visual stimulus that attracts the insect from a distance is the ultraviolet (UV) reflection and absorption pattern. UV photographs of *A. microphyllum* flowers (Fig. 1) show a λ shaped pattern on the upper lip of the corolla and a round solid patch on the internal surface of the lower lip reflecting UV radiation, while the remainder is UV-absorbing. The combination of a yellow palate and a yellow-ultraviolet tube found in *A. microphyllum* flowers is probably very attractive, as the insect eyes are highly sensitive to both wavelengths (Kevan & Baker 1983). Similar yellow-ultraviolet reflection patterns have been reported in other *Scrophulariaceae* (Lynch & Milligan 1994, Milligan & Kevan 1973). A last component of visual advertisement is the presence of parallel red stripes on the upper lip and on the inner side of the corolla tube. These can be interpreted as nectar guides, present in many other *Scrophulariaceae*, that function as orientation cues to which bees and wasps respond (Kampny 1995).

The floral nectar is an important reward offered to potential pollinators (Kevan & Baker 1983). In *A. microphyllum*, a mean volume of 3 μ l nectar with 40–45% sugar was produced which is in agreement with values for flowers visited by short-tongued bees (Kevan & Baker 1983). The differences in sugar concentration were not statistically significant from one population to another. Similar results have been reported in other *Antirrhineae* (Elisens & Freeman 1988).

The flowers of *A. microphyllum* allow the access to insects of a wide range of sizes. Ten different species were recorded: 4 species of *Megachilidae* (Hymenoptera), 4 *Apidae* (Hymenoptera), 1 *Cetoniinae* (Coleoptera) and 1 *Cleridae* (Coleoptera). However, not every visiting insect is an effective pollinator. Some of them are not large enough to come into contact with the receptive stigma and others, such as Coleoptera behave as thieves of pollen. The most frequent visitor was *Rhodanthidium sticticum* (Fab.) (Hymenoptera - *Megachilidae*). It was only active on sunny days. Its activity began at 9 a. m. and ended at 4 p. m. with a maximum between 11 a. m. and 1 p. m. The behaviour observed to collect nectar and pollen was similar to that reported for other bees in nototribic flowers (Westerkamp 1996). Usually, it lands on the lower lip of *A. microphyllum* flowers and opens the corolla with the legs. As the nectar is at the bottom, it is forced to crawl towards the flower base in order to suck nectar rubbing along stigma and stamens. This way, the pollen grains are attached onto the vertex of the bee's head and the dorsal side of the thorax. As reported in *Megachiloides fortis* (Neff and Simpson 1990), pollen collection is largely a passive process with pollen accumulating on the hairs of the scopa and on the vertex of the head. When it visits another flower it touches the stigma and the pollen is deposited. They are, therefore, potential pollinators during nectar sucking. The percentage of flowers in a plant visited per day ranged from 6–24%. At sunset and on cloudy days, *Bombus hortorum* L. were observed visiting *A. microphyllum* flowers. Sometimes they acted as legitimate pollinators entering the flower, but other times they collected the nectar directly from the pouch by making a perforation. The percentage of visits of *R. sticticum* and *B. hortorum* and the longevity of *A. microphyllum* flowers (up to 14 days) ensures the reproductive success of the species.

In conclusion, gathered data shows that *A. microphyllum* flowers have a complete set of attraction, reward and longevity features which enhances and assures a high pollination



Fig. 1. Left: Flower of *Antirrhinum microphyllum* Rotm. under human visible radiation; Right: the same flower under ultraviolet radiation (365 nm).

efficiency. In this threatened species, the viability of the populations does not seem to be constrained, at this moment, by factors linked to the pollination process.

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Address of the authors:

Torres M.E., Ruiz, C., Iriondo, J.M. & Pérez, C: Dpto. Biología Vegetal, E.T.S.I. Agrónomos, Universidad Politécnica. E-28040 Madrid, Spain.