Pollen morphology and reproductive biology of *Omphalodes brassicifolia* (Lag.) Sweet: an endangered iberian endemism

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Illustration 1: *Andrena sp.* pollinating *O. brassicifolia* flower.

Background

Omphalodes brassicifolia (Lag.) Sweet. (Boraginaceae), is an annual herbaceous plant whose distribution is restricted to the lower parts of the western Central System, namely the north of the province of Cáceres and the south of the province of Salamanca, where it is rare and scarce. It is, therefore, an Iberian endemism with a notable degree of threat, included in the Red List of Spanish Vascular Flora (Bañares et al., 2010), within the category "Endangered", and in the regulations of The Junta de Castilla y León (Decree 63/2007, of 14 of June), with the category of "Endangered". This herbaceous plant can reach 1 meter height, has amplexicaudes leaves and has a glaucous green color. Its flowers (see Illustrations 1 to 3) have a white actinomorphic collora with dimensions of 5-5,5 x 7-8,5 mm. Its fruits are dark brown nodes with a bent toothed wing. This species displays entomophilous pollination, being its main pollinators of the genus *Andrena*, *Apis* and *Bombus*, among others, as discovered in a recent study performed by the Germoplasm Bank of the University of Salamanca (BG-USAL) in 2016 (see Illustration 1)

The following work aims to provide information on the pollen grain morphology between two different populations and the strategies of reproductive biology presented by the species. In addition, these results could be useful to arrange conservation strategies.

Methods

Samples of dry pollen coming from 4 populations (2 from Castilla y León, 2 from Extremadura) were acetolysed according to Erdtman's method (1960) and observed by light microscope (see Illustrations 5 and 6). Pollen grains were prepared in glycerine jelly with basic fuchsin and measured (polar axis length -P-, equatorial diameter -E-, P/E ratio) using a Leica software LAS V 3.8. These results were analysed making a one-way analysis of variance (ANOVA) among the populations.

Reproductive biology was studied by means of hand pollination techniques and four mating systems: agamospermy, spontaneous autogamy, obligated autogamy and hand cross-pollination.

Table 1: Results of the reproductive biology experiment.

Treatment	NF	NFF	NS	%V
Agamospermy	140	1	2	0
Spontaneus autogamy	152	1	1	100
Obligated autogamy	334	76	174	80
Hand cross-pollination	1112	929	1832	93

NF: number of flowers treated; NFF: number of flowers fecundated; NS: number of seeds produced; %V: viability percetage..

Illustration 4: Reproductive biology assay flowers.

Illustration 2: *O. brassicifolia* flower.

Illustration 3: Details of an *O. brassicifolia* flower.

All flowers were hand treated, marking them with a number (see Illustration 4) and daily monitoring the fecundation process.

Differences between treatments were analysed by Generalized Linear Models (GLM) using a Logit function as link function. All data analyses were performed using PASW Statistics version 22. The seeds harvested from this reproductive biology experiment were pretreated for 24 hours with 500 ppm gibberellic acid and germinated in 1% agar petri plates in chambers programmed at 15 ° C with a photoperiod of 16 hours of light and 8 hours of darkness. The final germination obtained was used in order to verify the viability of the seeds obtained with the autogamy and agamospermy treatments.

Results

The size and shape of the 6-zono-heterocolpate pollen grains in the studied populations (Extremadura: P= 6.87 – 10.16; E= 3.59 – 5.02; P: E= 1.55 – 2.64; Castilla y León: P= 7.53 – 9.75; E= 3.32 – 4.90; P: E= 1.75 – 2.71) revealed no significant differences using one-way ANOVA. These results coincide with those obtained by Coutinho et al. (2010).

The reproductive biology assay displayed lower fecundation percentages with agamospermy and autogamy treatments than the results appeared in hand pollination flowers as it can be seen in Figure 1. The GLM analysis showed significant differences between all the treatments (*p*-values<.05) with hand cross-pollination and no differences (*p*-value=1) between agamospermy and autogamy used systems. Final results on the germinative assay performed on the seeds collected can also be seen in Table 1. In spite of the percentage of final germination obtained in those seeds from the obligate autogamy treatment, the seedlings obtained had little vigor and more than 50% died within a few days of germination. This also happened in the case of spontaneous autogamy whose single seedling obtained stopped its growth within a few days of germination.

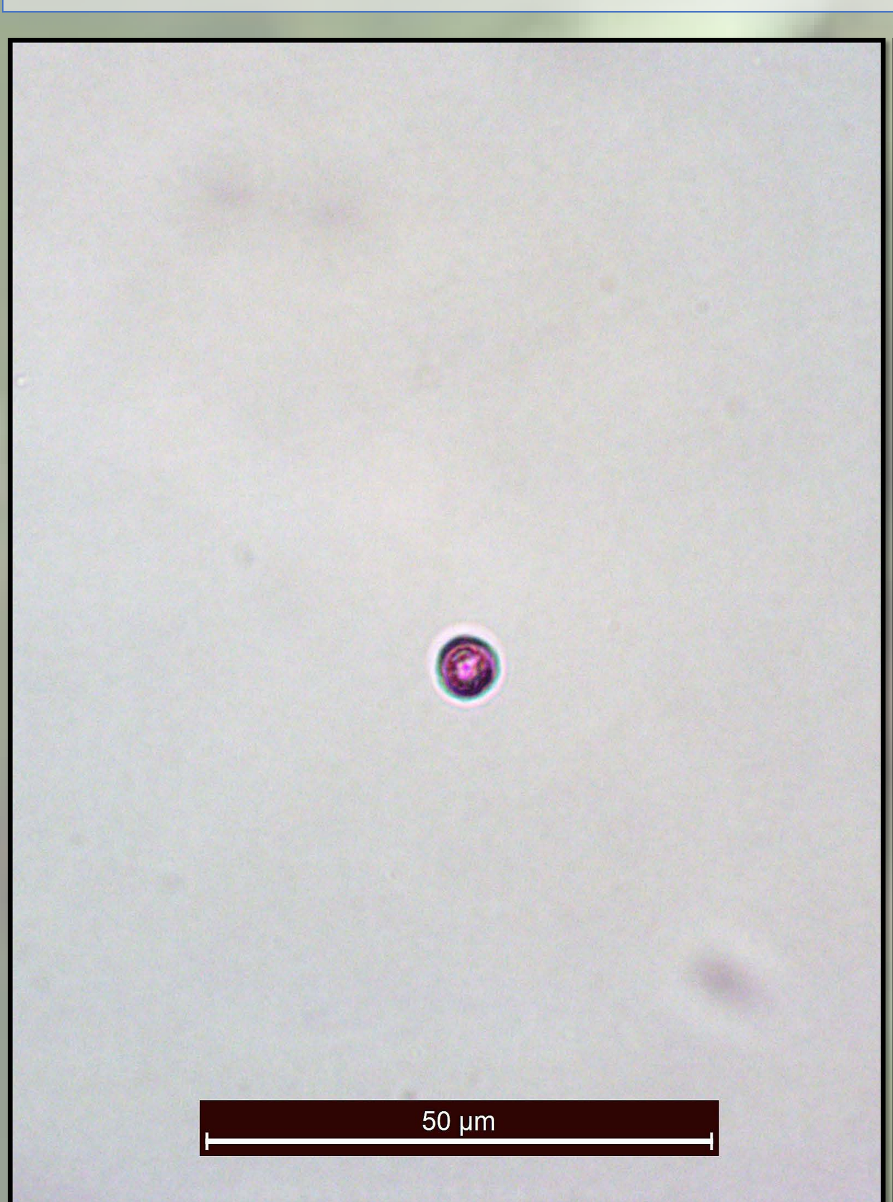

Illustration 5: Polar view of *O.brassicifolia* pollen grain on the light microscope (1000X).

Illustration 6: Equatorial view of *O.brassicifolia* pollen grain on the light microscope (1000X).

Conclusions

There were no significant differences in the pollen grains measurements among population samples. In order to improve the information on the species pollen grains, it would be advisable to carry out a study on the viability of the grains to test differences among populations.

Hand pollination was the most effective mating treatment, what could indicate that cross-pollination should be the preferred mating system of this species. This fact may be reinforced with the limited viable seeds produced by agamospermy and unforced autogamy indicating a lack of spontaneous self-pollination as reported in other endangered Iberian species (Amat et al., 2011). Due to the weakness observed on the plants obtained from autogamy treated flowers, only cross-pollination is recommended as *ex situ* seed production method.

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