Effect of different pollination treatments on *Berberis microphylla* G. Forst, a Patagonian barberry

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Abstract

Calafate (*Berberis microphylla* G. Forst) grows on Tierra del Fuego in extreme climates where low temperatures and strong winds prevail throughout the year. Due to these conditions *Apidae* do not survive during the flowering season of this species. Some syrphids were observed at anthesis collecting nectar. Although the disposition of floral pieces, humid stigma and retractable stamens could suggest that the species is self-compatible, results of controlled treatments of self- and cross-pollination compared with those after open-pollination performed during three different periods (2010, 2011 and 2014), do not support this hypothesis. Thus, self-pollination resulted only in pollen germination on the stigmas but the pollen tubes were not able to reach the ovules.

Keywords: calafate, self-incompatibility, retractable stamen, pollinating insect, syrphids

INTRODUCTION

Information on the reproductive biology of sub utilized plants is crucial for breeding programs and domestication. Pollination is an important link of successful plant reproduction, and is often dependent on mutualistic interactions with animals. A reduction in pollinator activity can directly influence reproductive output, decreasing the quantity and/or quality of fruit and seed set and promoting self-pollination in self-compatible species (Rodríguez-Pérez, 2005).

B. microphylla G. Forst, commonly named "calafate", has the largest distribution, from Neuquén (37°S) to Tierra del Fuego (54°8'S). Flower structure and floral biology have been described by Arena et al. (2011) as well as the phenological stages (Arena et al., 2013) and flower bud differentiation (Arena and Radice, 2014). Nevertheless, pollination was not clear until now. Fertilization of Patagonian *Berberis* has been classified as cross-pollination by Orsi (1984). On the contrary, Hegi (1958) and Romeo et al. (2005), consider this species as autogamous justifying this assumption with the postulate that in the absence of visiting insects, flower wilting creates a possibility of self-pollination. On the other hand, floral movements have been appointed as mechanisms to avoid self-pollination (Darwin, 1862).

Stamen movement has been documented in a few plant families, among them *Berberidaceae* (Lechowski and Bialczyk, 1992). In *B. microphylla* in particular it was just discussed by Radice et al. (2019), so the objective of this work was to present the results obtained from different pollination treatments carried out during the floral period of calafate on 2010, 2011 and 2014.

MATERIALS AND METHODS

Pollination treatments

The treatments tested were natural self-pollination (NSP), cross-pollination (CP) and open-pollination (OP) i.e., spontaneous natural pollination. Calafate flowers on pre anthesis stage (phase E) (Arena et al., 2011) were employed in all the cases. Flowers employed for CP treatment were emasculated and then manually pollinated by pollen tacked from different

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Acta Hortic. 1231. ISHS 2019. DOI 10.17660/ActaHortic.2019.1231.13
Proc. II Int. Workshop on Floral Biology and *S*-Incompatibility in Fruit Species
Eds.: E. Ortega Pastor, J. Halász and P. De Franceschi

flowers. Branches with the selected flowers were identified with ribbons, while for treatments NSP and CP they were bagged with cloth bags. Each treatment was made with n=50 flowers and the experiments were conducted during flowering (October and November) of the years 2010, 2011 and 2014.

Pollination and fertilization control

During the first time (2010), five pistils from each treatment were collected at 3, 7, 10, 14 and 18 days after the start of the experiment. They were fixed in FAA solution (100 mL formaldehyde, 500 mL ethyl alcohol, 50 mL acetic acid, 350 mL distilled water). Then, to study the pollen tube growth, they were observed with the addition of aniline blue (Martin, 1959) using an IMT-2 Olympus epi-fluorescence microscope (Tokyo, Japan).

Presence of pollen grains on the stigma (%), portion of the pistil reached by pollen tubes (stigma, style, ovary) and time that the pollen tube reached the ovule were registered. In subsequent tests (2011 and 2014), the material was collected only after 10 and 14 days.

Fruits produced in each treatment were collected after 100 days. The ratios between fruits produced and flowers tested for each treatment were calculated as percentage.

Insects in captivity

Branches with flowers in anthesis stage and previously bagged to prevent pollination were suspended from a mouth of Erlenmeyer of 5-L of capacity (Figure 1A-C). Two of them were used to test the activity of two different syrphids (S_1 and S_2), while the other was used as a test treatment (T). A paper sheet was added to the bottom of the Erlenmeyer to absorb moisture for syrphids. The three containers were closed by a cloth to allow airflow and maintained under natural environmental conditions without direct sunlight. Flowers were collected after 48 h and pistil were treated.

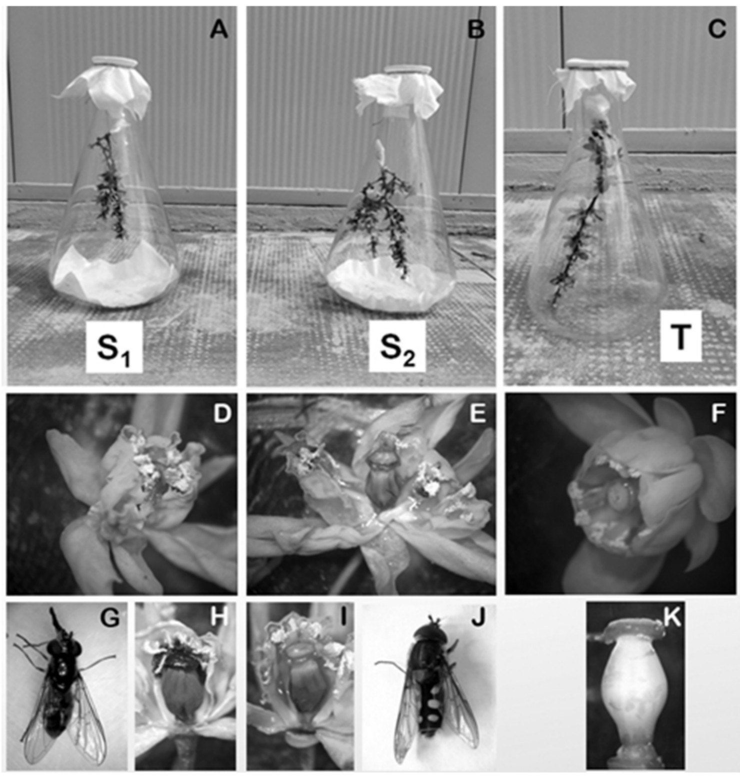

Figure 1. Insect in captivity with *Berberis microphylla* (calafate) flowers in anthesis stage. A-C) Erlenmeyers at time 0; D-F) calafate flower after 48 h; G-K) *Syrphidae* S_1; detail of pistil collected from S_1 treatment; detail of pistil collected from S_2 treatment; *Syrphidae* S_2; detail of pistil collected from T treatment.

RESULTS AND DISCUSSION

Pollen tube growth was well revealed by the treated pistils collected from different days after manual pollination (cross-pollination). In fact, seven days after pollination of the flowers the pollen tubes reached the ovary (Table 1). Some pistils of OP treatment showed the same growth while on the NSP flowers pollen tube growth was not observed (Table 1). Ten days later, pistils of CP showed pollen tube growth surrounding the ovules (Table 1; Figure 2) and probably some of them were already fertilized. Pistils of the other two treatments suffered a normal delay because they are dependent on different factors that allow arrival of pollen on the stigma. In effect, the wind and cloudy days influence the activity of insects (Suárez, 2015). Similar results were obtained in pistils treated in 2011 and 2014 (data not shown).

Table 1. Results obtained from different pollination treatments realized in 2010. Pollen grains present on the stigma (PPS) and site reached by the pollen tubes (PT). Pistils were treated according to Martin (1959) and observed with a fluorescence microscope.

Time after treatment (days)		Pollination treatments NSP	CP	OP
3	PPS (%)	0	100	0
	PT		Stigma	
7	PPS (%)	20	100	100
	PT	Stigma	Ovary	Style/ovary
10	PPS (%)	60	100	72
	PT	Style	Ovules	Style/ovary
14	PPS (%)	50	100	83
	PT	Style	Ovules	Style/ovary

NSP: natural self-pollination; CP: cross pollination; OP: open pollination.

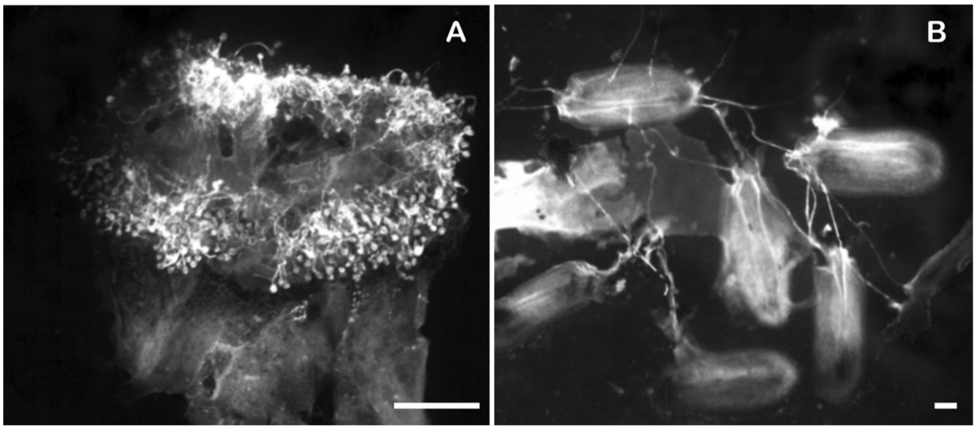

Figure 2. Pistil treated of *Berberis microphylla* shown on microscope with UV filter. A) Pollen germinated on the stigma; B) pollen tube growth surrounding ovules. Bars = 1 mm.

On the other hand, fruit harvest is described on Table 2. The CP treatment was the best treatment in the three years tested, with significant differences from the other two pollination treatments. Fruit harvest was variable among 39.73% in 2010 and 22.22% in 2014. This decrease in yield obtained in 2014 is consistent with the results of the OP treatment, which had the same tendency. It is very likely that weather conditions have affected the pollination of the species in this year.

The NSP treatment produced no fruits except in 2011, in which fruits were obtained but values were insignificant (2.07%) (Table 2). Bagging flowers for NSP treatment limits the possibility of deposition of pollen on the stigma. Lack of stimulation of the filament activity decreases the probability of contact between anther and the self-stigma; so, even being self-compatible, it would be very difficult to obtain pollination and fertilization.

Table 2. Fruit collected (%) from different pollination treatments on the tree years tested.

	Fruits collected (%)		
	Pollination treatments		
	NSP	CP	OP
2010	0 c	39.73 a	24.61 b
2011	2.07 b	30.14 a	11.05 b
2014	0 c	22.22 a	6.47 b

NSP: natural self-pollination, CP: cross-pollination; OP: open pollination.
Values followed by different letters show significant differences between treatments for each year.
Results were analyzed by X^2 test ($p \leq 0.05$).

Flowers collected from the Erlenmeyers used to test insect activity were very different according to the treatment (Figure 1 D-K). In effect, flowers collected from T treatment were turgid and they had green pistil without or with rare pollen grains on the stigma (Figure 1F, K). While pistils of *Syrphidae* treatment (S_1 and S_2) were dehydrated, they look dark and with germinated pollen grains on the stigma (Figure 1D, E, G, H, I, J; Table 3).

Table 3. Results obtained from flowers of *Berberis microphylla* collected after 48 h from S_1 and S_2 treatments and shown by UV microscope. Germinated pollen grain (GPG) and abundance of pollen grain (APG) on the stigma were evaluated as number and percentage.

Syrphidae	Pistils (no.)	GPG (no.)	APG (no.)	GPG (%)	APG (%)
S_1	16	9	4	56.25 b	25.00 b
S_2	16	16	10	100.00 a	62.50 a

Value with different letters between treatments were significant different by X^2 test ($p \leq 0.05$).

Although other *Berberis* species have a cross-pollination syndrome (Lebuhn and Anderson, 1994; Angulo et al., 2014), based on the floral morphology of *B. microphylla* and the ease with which the anthers deposit pollen on their own stigma, it can be assumed that self-pollination is possible. It is not surprising that a species may behave differently from their peers as has happened in 'Forastero' peach (Newcomer) that unlike most peach cultivars, that are self-fertile, was self-incompatible (Radice, 2005). Nevertheless, all those questions were resolved after analyzing the flowers and insects into captivity. As already mentioned, pistils were pollinated by insect and on their bodies several attached pollen grains were found (data not shown).

CONCLUSIONS

While several authors defined *B. microphylla* as self-fertile, this species is more likely cross-pollinated and *Syrphidae* insects are essential in pollen transport. Experiments developed so far support this hypothesis.

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