Pollination strategies of *Berberis microphylla* G. Forst, a Patagonian barberry

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Abstract

Calafate (*Berberis microphylla* G. Forst) is a spontaneous shrub of Patagonia. Fruits are black-blue berries rich in antioxidant compounds such as flavonols and anthocyanins. Blooming occurs in spring and flowers at anthesis show bright yellow sepals and petals, a receptive stigma, non-dehiscent anthers and high nectar production that results in a pleasant aroma. Like all the species of the genus *Berberis* the stamens are retractable, and dehiscence occurs after mechanical stimulation. The objective of this study was to understand the different mechanisms developed to achieve effective flower pollination. Thus, floral offering was quantified at full bloom, and anther movement, nectar production, sugar concentration and interaction with visitor insects were studied in detail.

Keywords: calafate, small fruit, aromatic nectar, *Berberis*, retractable stamen, insects

INTRODUCTION

Calafate (*Berberis microphylla* G. Forst) is a spontaneous shrub grown in the Patagonian region (Orsi, 1984), considered as a non-timber forest product (Tacón Clavaín, 2004). *B. microphylla* is an important source of alkaloids, i.e., berberines, and phenolic compounds such as anthocyanins which offer medicinal and tinctorial applications (Shaffer, 1985; Orsi, 1984; Fajardo Morales et al., 1986; Fajardo Morales, 1987; Mokhber-Dezfuli et al., 2014; Arena et al., 2012). Its black-blue fruits are of economic value (Arena and Curvetto, 2008), and are traditionally harvested for different purposes. It can be consumed fresh and processed in marmalades and jams, in non-alcoholic beverages, and in ice creams.

Flowers are solitaries and yellow with a pleasant aroma. The flowering period is about 21 days between the months of October and November (Arena et al., 2011). Flowering is very abundant and the offering of flowers during this period was calculated on 21.2 flower dm^{-2} (Suárez, 2015).

Berberis genus has a feature called seismonasty. Anthers are tactile sensitive that promote a retractable movement. That is anything that touches the filament causes it to spring toward the pistil. This particularity of the genus had already been observed by Benthley (1873). The movement of the anthers could improve the contact of pollen from the anthers with pollinators but could also be considered to facilitate self-pollination (Ren, 2010). The morphology and anatomy of the irritable filament is not uniform from species to species, as has been assumed since Linnaeus, therefore neither the self-pollination nor the cross-pollination can be discarded for this species. Pollinator-dependent crops are increasingly grown to provide food, fiber, and fuel as well as micronutrients essential to human health. The yield and quality of these crops benefit to varying degrees from flower visitation by animals. Wild and managed bees are well documented as effective pollinators of global crops of economic importance. However, the contributions by pollinators other than bees like *Syrphidae*, have been little explored despite their potential to contribute to crop production and stability in the face of environmental change (Rader et al., 2016). In *Berberis* the participation of bees in the pollination has been observed (Angulo et al., 2014). However, in *B. microphylla* growing in Tierra del Fuego, the presence of syrphids during the flowering period has been referred (Suárez, 2015).

The objective of this work was to study all the peculiarities of the *B. microphylla*

flowers and their interaction with the environment, to know the syndrome of pollination of this species.

MATERIALS AND METHODS

Geographic and climatic conditions

This experiment was made in a representative area located near Ushuaia city 54°48'S, 68°19'W, 30 m a.s.l. (Tierra del Fuego, Argentina), where *B. microphylla* grows spontaneously. Climatic data were collected by the meteorological station located at the Centro Austral de Investigaciones Científicas (CADIC) from October 15 to November 15 of 2014 and 2015 in coincidence with the flowering period (Figure 1).

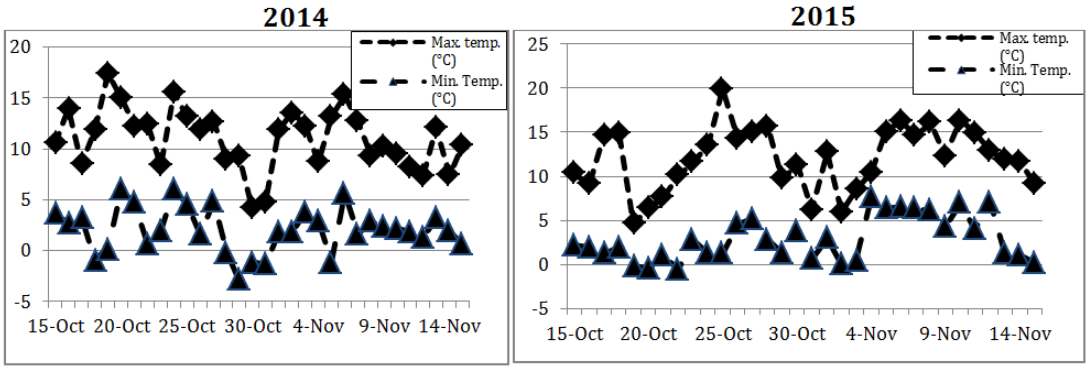

Figure 1. Minimum and maximum daily temperatures near Ushuaia city, Argentina.

Plant material

Flowers of *B. microphylla* on anthesis phase (F1, F2 and F3 according to Arena et al., 2011) were selected on different shrubs to study anther movement, nectar production and the insect visit (Figure 2).

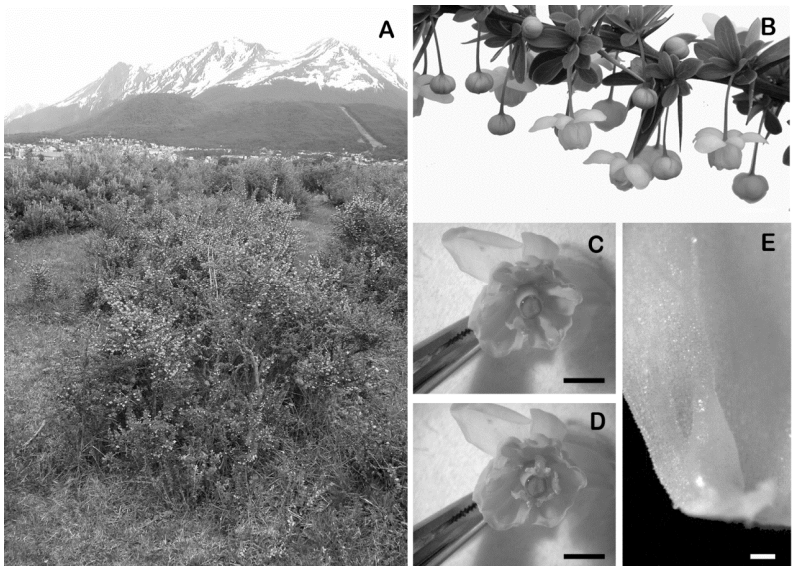

Figure 2. *Berberis microphylla* (calafate) grown spontaneously in Tierra del Fuego and in flower phase. A) view of the environment where it grows, B) branch with calafate flowers in different flower stages, C-D) detail of a flower in anthesis phase, C) anthers attached to the petals, D) anthers attached to the pistil, E) detail of the petal nectary. Bars B-D = 1 cm, E = 1 mm.

Flower measurements

Flowers (*n*=15) of different branches were selected to promote the clash of the anthers against the pistil. This effect was induced by puncturing the base of the petals with a calafate thorn. This action was repeated many times until the fall or flower senescence. Number of times flower^{-1} that anthers struck the pistil, and the time taken by the anthers to resume its initial position was recorded. Values obtained were related to phenological phase and different climate components. The extraction of the nectar was tacked from collected flowers on anthesis phase by a modified capillary (1 mm diameter) coupled with a Pasteur pipette. Extracting nectar was done in the laboratory (25°C) and field (5°C). Sugar concentration was measured by a hand-held refractometer Atago N-2E Brix 28~62%. Volume was measured by an electronic caliper and calculated using the formula: volume of nectar = measure of nectar (mm) × total volume of the capillary (µL)/ total length of the capillary (mm).

Insect activity

The type of insect visitors, time spent on the visit and its activity (search or collection) were recorded. Insect presence and activity were correlated with climatic conditions. Careful quantification was performed in 2014 while in 2015 insect visitors were estimates.

RESULTS AND DISCUSSION

Flowers of calafate are solitary, axillary, bisexual, actinomorphic and hypogynous (Figure 2B) (Arena et al., 2011). The abundance of yellow flowers in anthesis phase is a very important attraction for the visit of pollinators (Figure 2A). Furthermore the movement of the anthers in this species always promotes adhesion of pollen on the stigma (Figure 2C-D). The time spent between the firing of the anthers to the pistil and return to its normal position varies according to the phenological phase, the number of occurrence and some climatic factors. Actually, the time recorded (29 min) was higher in the intermediate phase of anthesis (F2) compared with the beginning and the end of anthesis phase, when the anthers took between 15 and 18 min (Table 1). It is also noted that the time was greater between the first and successive puncture made in the same flower, with a correlation value of 0.594 (Sig<0.001). Furthermore it is noted that the movement of the anthers was directly proportional to the heat index (heat index) Pearson 0.29 (Sig<0.001) and wind speed (wind speed) Pearson 0.297 (Sig<0.001).

Table 1. Time employed by the anthers between strikes the pistil and the return to normal position next to petal, according to phonological phase.

Phenological phase	Average time
F1	00:18:33 b
F2	00:29:48 a
F3	00:15:47 b

Different letters show significant differences by Tukey test (p<0.05). Values are expressed as hh:mm:ss.

Nectar is another important factor attracting insect visitors. The six petals of the calafate flowers have two nectarines on the base position (Figure 2E). Calafate in its natural environment produces small volumes of nectar (1.57 µL) (Table 2) with a high concentration of sugars (36.28 °Brix) (Table 2). Contrary to what happens with many other species, the nectar volume of *Berberis* decreases with increasing temperature. It was observed that at 25°C, nectar undergoes evaporation of the liquid content giving a gummy structure which is impossible to extract with the capillary. This nectar crystallization is probably due to the higher glucose concentration.

Table 2. Volume and concentration of sugar measured on the nectar extracted from *B. microphylla* flower on anthesis phase.

Sample	Place	Temperature (°C)	Volume flower^{-1} (µL)	°Brix
n=50	Inside	25	0.64 b	36.28±4.37
n=50	Outside	5	1.57 a	

Significant differences are expressed by different letters by Tukey test ($p<0.05$).

Pollination active by *Himenopthera* and *Coleoptera* in other *Berberis* species was observed (Urquieta, 2010) but bees are not found during the flowering period of calafate in Tierra del Fuego due to the local climate conditions. Between 10 and 17 h, depending on wind speed and cloud cover, the presence of three syrphids and a butterfly was recorded. Three syrphids were classified as *Allograpta, Carposcalis* (ex *Platycheirus*) and *Syrphus* genus (Fluke et al., 1945; Vockeroth and Wood, 1987) while the butterfly was *Tatochila* sp. (Pérez D'Angelo, 1996). In 2014, presence was quantified as *Carposcalis* sp. 84.86%, *Syrphus* sp. 11.35%, *Tatochila* sp. 3.78% and *Allograpta* sp. very little. On the contrary, the presence of *Syrphus* sp. was higher than *Carposcalis* sp. during flowering in 2015. Syrphids reach calafate flowers from different directions and when approaching the bushes, they switch to a hover in search of flowers for foraging. Syrphids take nectar introducing the proboscis to the bottom of the corolla. In this way; pollen is adhered to the different body parts (Suárez, 2015). Then, pollen sticks to stigmas of other flowers visited, and partly is consumed when the insect cleans their body. Nectar is the source of energy and protein requirements for insects is provided by pollen. *Tatochila* sp. flies from shrub to shrub introducing their proboscis into one or two flowers shrub^{-1}, just seconds only, but this insect is only seen on very sunny, warm and windless days in special climatic conditions, that are very sporadically.

CONCLUSIONS

Calafate has different strategies that induce both natural self-pollination as well as cross-pollination. Irritability of stamen filaments to any stimulus makes anthers deposit pollen on the stigma of the own flower. It is usually triggered by insects that visit and touch stamens. Calafate bush have abundant yellow flowers that produce nectar in anthesis stage and are very attractive to pollinators. Nectar has a high sugar content and exudes a pleasant aroma therefore this combination of effects is very attractive to insects present in the area. Several insects were recognized but the climatic conditions make the syrphids the most abundant, especially *Carposcalis* sp. and *Syrphus* sp.

According to these observations, pollination syndrome of calafate may be cross-pollination like to other species of *Berberis*.

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