INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
18–0530/2018/20–10–2221–2229
DOI: 10.17957/IJAB/15.0768
http://www.fspublishers.org

Full Length Article

Berberis microphylla: A Species with Phenotypic Plasticity in Different Climatic Conditions

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Abstract

Berberis microphylla G. Forst., commonly called as "calafate" produces small fruits with high content of carbohydrates, phenols and antioxidants. The objective of this work was to characterize the vegetative and reproductive cycle of *Berberis microphylla* cultivated on Moreno (Buenos Aires province), Argentina in comparison with the results obtained in Ushuaia (Tierra del Fuego) which is its place of origin. Vegetative growth was very different in the two localities. Moreno plants grew with more lax branches than Ushuaia. In effect, length of the shoots was significantly higher for Moreno than Ushuaia plants. Flowering period in Ushuaia plants was concentrated in November while in Moreno it happens earlier and over a longer period. Pollen grains collected from Moreno flowers had a diameter of ~60 μm, significantly different to pollen grain from Ushuaia (57.11 μm). Nevertheless, pollen grain vitality was superior in Ushuaia flowers (75%) as compared to Moreno flowers (52%). On the other hand, fruit harvested in Moreno was at 60 days from full bloom while in Ushuaia plants at 120 days. Although the size and compounds measured in the fruits of Moreno were lower than those of Ushuaia, results obtained indicate that *B. microphylla* grown on Moreno is an interesting option to obtain another nutraceutical fruit near the centers of mass consumption. © 2018 Friends Science Publishers

Keywords: Calafate; Barberry; Underutilized species; Annual cycle; Argentina

Introduction

Berberis microphylla G. Forst., is a Patagonian native shrub commonly named "calafate", with a large distribution from Neuquén (37º SL) to Tierra del Fuego (54º 8´ SL) (Orsi, 1984). *B. microphylla* is an evergreen shrub that may be semi-evergreen where winters are particularly cold and harsh, as it occurs in Tierra del Fuego. It is a spiny and erect shrub up to 4 m high, often growing in the magellanic subpolar forest Eco region (World Wildlife Fund *et al*., 2008), in coastal scrub, *Nothofagus* forest margins and clearings, moister areas in grass steppes, and along streams and rivers (Moore, 1983).

In recent years, there has been an increased demand for the fruits of *Berberis sp.*, both for fresh consumption and for the elaboration of products such as candies and jellies, pulp for making ice creams, beverages without alcohol and cosmetic products. Its fruits have a high content of carbohydrates, phenols and antioxidants (Arena and Curvetto, 2008; Arena *et al*., 2011; 2013b). In fact, fruits native to the Andean region as *Berberis*, have been included in the Argentine Food Code, authorizing its use in food products like sweets, jams, liqueurs, ice cream and candies (www.infoleg.mecon.gov.ar). But this species has other properties besides fruit production. In fact, in most of the species of the genus are assigned medicinal properties due to the presence of the alkaloids called berberine and berbamine (Shaffer, 1985; Fajardo Morales *et al*., 1986, 1987; Mokhber-Dezfuli *et al*., 2014). Berberine has been cited as a toxic agent for insects and vertebrates, inhibits the growth of bacteria, fungi and viruses because it acts on many receptors (Jatimliansky and Sivori, 1974; Schemeller *et al.*, 1997). Also, berberine has significant antitumor activities against many types of cancer cells (Kuo *et al*., 2012). Berberine is used clinically at present in patients with severe heart disorders (Bagade *et al*., 2017), in patients with HIV as gastrointestinal infections appear to control due to the wide antifungal, antimicrobial and antispasmodic spectrum (Schneider, 1996). Traditionally it has been used in the treatment of jaundice, diarrhea and other ailments. In addition to all properties mentioned, this shrub has an important ornamental value. Its yellow and perfumed flowers are very attractive as a whole and its prickly branches make this species very useful for performing live fences.

Studies were conducted on the genetic and morphological analysis of spontaneous accessions in natural populations of *B. microphylla* grown on Tierra del Fuego

To cite this paper: Radice, S., M. Alonso and M.E. Arena, 2018. *Berberis microphylla*: A species with phenotypic plasticity in different climatic conditions. *Int. J. Agric. Biol*., 20: 2221–2229

(Giordani *et al*., 2016), as well as the changes in form and leaf anatomy due to weather conditions (Radice and Arena, 2015). On the other hand, flower bud differentiation (Arena and Radice, 2014), pollen structure and physiology (Radice and Arena, 2016), like the phenological stages (Arena *et al*., 2013a), flower structure and floral histology have already been reported (Arena *et al*., 2011). More recently, a comprehensive study of flower anatomy related to blooming development was published (Radice and Arena, 2017).

As has already been mentioned, majority of the works above cited were done with plants grown spontaneously in Tierra del Fuego, Patagonia. Therefore, for all these reasons it was thought to introduce *B. microphylla* cloned plants to the Buenos Aires province in order to carry out experimental studies, *i.e*. evaluate its phenotypic plasticity and the possibility of fruit production. The objective of this work was to characterize the vegetative and reproductive cycle of *B. microphylla* cultivated on Moreno (Buenos Aires province), Argentinain comparison with the results obtained in Ushuaia (Tierra del Fuego) which is its place of origin.

Materials and Methods

Plant Material and Measurements

The plants of *B. microphylla* were obtained through clonal propagation from a natural populationgrown in Ushuaia (Tierra del Fuego, 54°48' SL, 68°19' WL, 30 m a.s.l.). At the beginning of 2013, 60 plants were taken to Moreno (Buenos Aires) and cultivated in the experimental field of the Faculty of Agriculture and Agrifood Sciences, University of Morón (34° 39' SL, 58° 47' WL, 14 m.a.s.l.). Same methodology for all variable studied were applied on a spontaneous homogenous lot of shrubs of *B. microphylla* grown in its origin place (See: P1 Campo PR CADIC, Ushuaia in Giordani *et al*., 2016) (Fig. 1).

Climatic Classification and Weather Conditions

Moreno's climate is classified as warm and temperate. According to Köppen (1936), this climate is classified as Cfa i.e., temperate rainy climate. Moreno is a city with significant rainfall without driest month. Average annual temperature was 16.2°C and rainfall was 1033 mm approximately. On the other hand, Ushuaia has a tundra climate although it is close to the limit with the sub-polar oceanic climate since the average temperature of the warmest month is slightly below 10ºC. It is also characterized by not too cold winters (with an average temperature in the coldest month slightly above 1°C) and by quite cool summers. Also, is called cold oceanic climate, or oceanic sub-polar. Average temperatures and rainfall per month for the two sites studied were taken from the accuweather. On the other hand, cumulative chilling hours were calculated according to Mota (1957).

Fig. 1: Plants of *B. microphylla* tested on Moreno and Ushuaia sites

Flower Measurements and Insect Visitation

Data on number of floral organs, size and morphology were collected (n= 6) at random. In addition equatorial and polar diameters of the pollen grains (*n= 300*, randomly selected) and their viability was calculated (*n=500*) according to Radice and Arena (2016). Total flower produced were registered on Moreno plants but for Ushuaia plants, eight branches oriented toward the four cardinal points were selected as random. In addition, different insects that visited the flowers were collected to their identification.

Vegetative Growth, Floral Development and Phenology

Floral development for every plant (Moreno *n= 45*; Ushuaia *n= 12*) were registered weekly during 2015 to 2016 but the vegetative growth was measured only in Moreno in the form of the differentiated sprouts from branches or rhizomes. Results were expressed as percentage respect to the total plant tested on monthly basis. Following phenological phases were registered according to Arena *et al.* (2011): button flower, anthesis and fruit.

Fruit Production

Total fruit produced for every plant were counted from Moreno plants and total fruit produced on every branch marked at ripening time on Ushuaia plants. Fruit set was calculated as the ratio of total fruits/total flowers produced.

Physical Properties of Fruits

Fruit physical attributes: Total fruits produced on 2016 year were collected. Fresh and dried weight *(n= 10)* were registered from fruits and seeds as well as the number of seeds per fruit. Fruit size was estimated through the equatorial and polar diameters taken with a digital caliper Mitutoyo Model 500-196. Color of fruit skin was evaluated according to Arena (2016).

Soluble solids: Soluble solids were determined in fruit juice using an ATAGO N1--α refractometer with 0–32°Brix measurement range with 0.2 Brix increments, and with temperature compensation at 20°C (Arena, 2016).

Total monomeric anthocyanins: Total monomeric anthocyanins were quantified by the pH differential method as described by Arena *et al*. (2008, 2012; 2017). Samples (5 g) of initially frozen fruits were extracted for 24 h in 50 mL 0.1% HCl–MeOH solution at 4°C. Then, aliquots were diluted from 1:5 to 1:80 with either a 0.025 *M* KCl (pH 1) or 0.4 M sodium acetate (pH 4.5) buffer. Absorbance measurements were made at 510 and 700 nm with a Shimadzu 1203 UV–Vis. spectrophotometer. Anthocyanin fruit tissue content was determined on the basis of a molar extinction coefficient of 26,900 and a molecular weight of 449.2 for cyanidin 3-glucoside. Values were expressed in terms of mg of anthocyanin/100 g of fresh-frozen fruit.

Total polyphenols: Total polyphenols were quantified as reported by Makkar *et al*. (1993). Samples (n = 3.0 g each one) were extracted for 24 h in 30 mL 80% MeOH-H2O at 4°C. Aliquots (15 µL) were adjusted to 500 µL with deionized water, and then 250 µL of 50% of the Folin-Ciocalteu reagent (Sigma-Aldrich) and 1.25 mL of 20% (w/v) aqueous sodium carbonate solution were added. After 40 min standing at 24°C, the absorbance at 725 nm was measured. A calibration curve was prepared using tannic acid (Sigma) and the results were expressed as mg tannic ac acid equivalents/100 g of fresh-frozen fruit.

Statistical Analysis

Values obtained were analyzed by χ^2test for discontinuous variables and ANOVA with Tukey Test for continuous values with SPSS 23.0 software.

Results

Weather Conditions

In Moreno, both years of study showed similar average temperatures with a slightly warmer winter for the year 2015 (Table 1). Particularly, during the winter period of 2015, the number of days with minimum temperatures below 7°C was 17 days in June and 17 days in July (data not shown). Cumulated chilling hours were 420 and 657 for 2015 and 2016 year respectively (Table 1). Rainfall was intense in August of 2015 and in April and July of 2016 before bloom, although moderately at blooming time that is, it was not an impediment to the activity of pollinating insects.

On the other hand, average temperatures recorded in Ushuaia during the same period were lower and rains more abundant than in Moreno region, and particularly more volumes were recorded in the year 2015. Cumulated chilling hours were 2060 and 1800 for 2015 and 2016 year respectively (Table 1). These references highlight that the studies of the species were done in very diverse climatic situations.

Table 1: Climatic data for mean air daily temperatures and cumulative rainfall for 2015 and 2016 growing seasons for Moreno and Ushuaia, and January to March 2017 for Ushuaia

Growing season	MORENO		USHUAIA	
	Temperature (°C)	Rainfall (mm)	Temperature (°C)	Rainfall (mm)
Jan 2015	nd	nd	9.52	69.39
Feb 2015	nd	nd	8.92	73.02
Mar 2015	nd	nd	9.19	70.61
Apr 2015	nd	nd	5.94	42.00
May 2015	16	28.00	3.25	130.10
June 2015	12	49.00	1.26	198.80
July 2015	11	41.00	0.39	77.00
Aug 2015	14	146.00	1.96	119.90
Sep 2015	14	51.00	2.54	145.90
Oct 2015	16	57.00	6.41	65.67
Nov 2015	19	112.0	7.96	67.54
Dec 2015	24	42.00	8.15	70.62
Cumulative chilling hours	420		2060	
Jan 2016	25	24.00	9.63	69.82
Feb 2016	25	89.00	9.23	72.31
Mar 2016	21	79.00	5.87	74.77
Apr 2016	17	178.0	5.10	71.50
May 2016	12	33.00	4.22	14.60
June 2016	10	46.00	5.37	22.10
July 2016	10	138.0	2.70	78.90
Aug 2016	13	58.00	2.79	54.90
Sep 2016	14	44.00	8.00	17.90
Oct 2016	17	84.00	8.01	69.36
Nov 2016	20	34.00	8.23	73.25
Dec 2016	nd	nd	8.41	75.45
Cumulative chilling hours	657		1800	
Jan 2017	--	--	9.58	72.04
Feb 2017	--	--	10.75	71.75
Mar 2017	--	--	8.78	76.29

Floral Morphology

Flowers sprouted on spring on Moreno place showed six yellow sepals distributed in two cycles, and six yellow petals with two nectarines on the basal portion near the insertion with the receptacle. Six stamens and gynoecium by a pistil with a noticeable stigma and ovary without style was also observed. Ovary had numerous ovules inserted on its basal sector. Some plants of Moreno area formed flowers in the autumn period but their morphology was particular. It was observed flowers with fewer flower pieces i.e. 4 sepals (Fig. 2A), 5 petals. In addition, stamens with filaments linked together in number of 3 instead of 6 with anomalous anthers (Fig. 2C) and pistil with necrotic stigma were found (Fig. 2B).

Pollen Analysis

Pollen grains collected from Moreno flowers had a diameter variable between 57.94 and 64.03 µm with an average value of 60 µm, while Ushuaia flowers produced pollen with an average diameter of 57.11 µm (Table 2). Registered measures differ statistically according to the selected germplasm but also according to the growth site.

Table 2: Measure of pollen grain of *B. microphylla* grown on Moreno and Ushuaia places, collected on the flowering period of 2015

MORENO		USHUAIA	
No. of plants	Mean diameter (μm)	No. of plants	Mean diameter (μm)
		109	58.50bc
12	57.94bc	123	57.80bc
37	64.03a	124	57.05bc
44	56.38c	176	54.30c
56	61.76ab	202	57.90bc
Average	60.03		57.11
F (place)	*12.051*	*F (plant)*	*6.173*
p (place)	*0.01*	*p (plant)*	*<0.001*

Different letters show significant differences Tukey ($p<0.05$)

Table 3: Viability of pollen grain of *B. microphylla* grown on Moreno and Ushuaia places, collected on the flowering period of 2015

Moreno		Pollen viability (%)	
Plant	Viable	Sub-viable	Dead
2	48.97c	16.61	34.,42a
12	61.92ab	25.66	12.42ab
37	49.73b	29.93	20.34ab
44	48.50c	26.90	24.60ab
56	52.45b	14.35	33.20^{a}
Average	52.06	23.91	24.03
Ushuaia	Pollen Viability		
Plant	Viables	Sub viable	Dead
109	81.84ab	12.35	5.81c
123	71.93ab	20.18	7.8c
124	85.24a	8.33	6.43c
176	73.13ab	14.34	12.53ab
202	72.64ab	22.64	4.72c
Average	74.89	15.57	7.46
F (place)	*30.908*	*3.758*	*27.357*
p (place)	*<0.001*	*0.56*	*<0.001*
F (plant)	*4.706*	*2.033*	*4.795*
p (plant)	*<0.001*	*0.47*	*<0.001*

Different letters in the same column show significant differences Tukey ($p<0.05$)

Pollen viability was 52.06% on average (Table 3). There were significant differences between values obtained from flowers or different plants (Table 3). In fact, poorer results were observed on plant number 2 and 44 respect of the other three tested. Pollen grains vitality of Ushuaia flowers was 74.89% on an average (Radice and Arena, 2016). Statistical analysis showed significant differences in the site and the selected plant but values obtained in Ushuaia were superior to Moreno.

Insect Visitation

Abundant flowers, yellow color, nectar production and scent are important rewards to attract insect's pollinator. Presence of bees and syrphids in active nectar search was observed on Moreno flowers at blooming time. In effect, *Apis melifera* and *Allograpta* spp., *Platycheirus sp.* and *Toxomerus sp.* as representative *Shyrfidae* family were observed in this

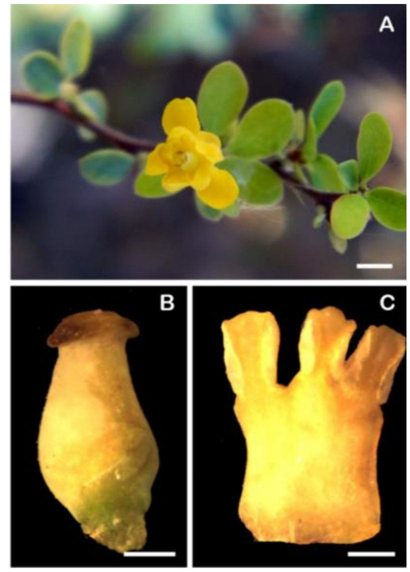


Fig. 2: *B. microphylla* abnormal flower collected on March 2015 from plants cultivated on Moreno (Buenos Aires, Argentina). **A**, View of flower on anthesis phase grown on the brunch; **B**, Pistil with necrotic stigma; **C**, Anther with filament linked together. Bars= **A**, 1 cm; **B**-**C**, 1 mm

Fig. 3: Plant of *B. microphylla* grown on Moreno (Buenos Aires, Argentina) **A**, Leaves grown up on new sprouts on March of 2014 (arrow); **B**, Plant with new sprouts grown from rhizomes (arrow) on September of 2014

place. On the other hand, there are no bee populations in Tierra del Fuego given the climatic conditions. Flowers of Ushuaia were very visited by the syrphids *Allograpta* spp. and *Platycheirus* spp.

Vegetative Growth, Floral Development and Phenology

Plants on Moreno place stopped growing for a variable period of time, some of them lost part of their leaves and when growth restarted, the leaves showed different morphology (Fig. 3A). Then, the normal growth resumed through an active growth in shoots and new sprouts from rhizomes (Fig. 3B) among January to May in 2015 and among September to April (2015–2016) and August to December of 2016. That is, plants ceased growth in the winter period i.e. June to August for 2015 and May to July for 2016 (Fig. 4). More than 60% of the plants presented shoots from branches among January to May and September to December of 2015, while nearly 60% of the plants presented shoots from rhizomes during January to May and lower values for shoots from rhizomes from September to December for the same year. Plants observed in 2016 stopped its growth earlier than in 2015, from April to August, although there were no significant differences in the percentage of plants with shoot between both years.

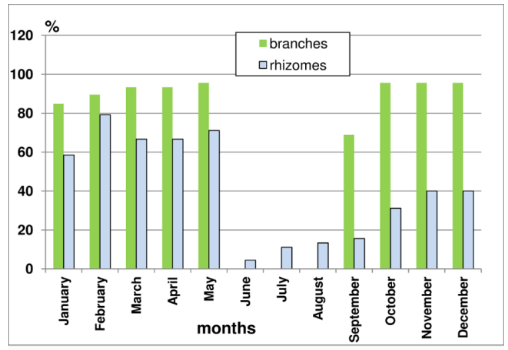
Length of the shoots was significantly higher for Moreno plants than Ushuaia plants (Table 4). Nevertheless, number of nodes and vegetative buds formed did not differ between both places. Although number of total and mixed buds formed per branch, the relationship between nodes and length of branch, total buds and nodes and mixed buds with total buds were higher in Ushuaia than in Moreno plants (Table 4).

Flowering Phenology

It was observed that 25 of 42 tested plants flowered in Moreno, i.e. 59.52%, during the year 2015. Blooming started on 22 September while full of bloom was observed between the days 9 and 14 October (Fig. 5A). Flowering was not continuous, in fact there were three peaks in the emergence of buds and more than three in the flowers on anthesis phase (Fig. 5A). End of flowering was noted on October 27 (Fig. 5A) i.e. the flowering period was 35 days approximately. Results during 2016 were somewhat different. In this year, 29 plants flowered i.e. 69.04% of total tested plant. Start of bloom was noted on September 10–12 while full bloom occurred on September 20 (Fig. 5B). In this year, the emergence of button flowers occurred discontinuously but with only two picks while anthesis was continuous until the 20th of September and then gradually fell. End of bloom was estimated between days 5–8 of October so the flowering period was 25 days approximately. Notably, eight plants did not flower in any of the two years of studies. All the studied plants in Ushuaia site formed flowers. First button flower was observed on October 21 and then they grew quickly until they started the anthesis on November 5. In this situation, blooming period followed a normal curve model especially during the month of November in Ushuaia site (Fig. 5C).

Number of flowers per plant in Moreno site was variable between 10.40 and 10.76 in the two years studied, although significant differences were not observed (*p=0.835*) among years and plants (Table 5). On the other hand, flowers produced per branch on Ushuaia site were similar on the two years of study and the value obtained from Moreno plants (Table 5).

Fruits were harvested on Moreno site on December 18 in 2016. It was observed that some plants increased the fruit



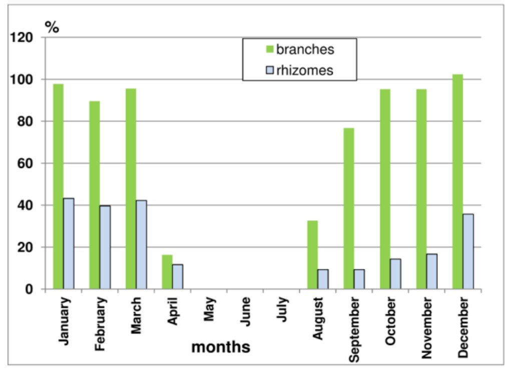


Fig. 4: Vegetative growth of *B. microphylla* plants grown on Moreno (Buenos Aires, Argentina). Records were tacked from branch buds or rhizomes registered on years 2015 (**A**) and 2016 (**B**). Values are expressed in percentage respect to the total plants tested

production respect to the previous year with a contribution of more than 12% of the total harvested fruit. Previous year, unfortunately fruits were not harvested because we did not understand that the cycle was too short in this place and consequently the fruits fell by themselves. Fruit set in Moreno plants was variable between 22.87 to 17.26% in 2015 and 2016 respectively and 3.67 to 2.82 for Ushuaia plants in the same years. Significant differences (*p<0.001*) were observed only between sites (Table 5).

Fruit Analyses

Fresh and dry weight of Ushuaia fruits (0.3 and 0.1 g, respectively) were significantly higher than Moreno fruits (0.2 and 0.05g, respectively), as well as the dry fruit weight as percentage of fresh weight (28.47 and 24.64% for Ushuaia and Moreno, respectively) (Table 6). However, seed number did not differ significantly between both places (4.33 and 3.5 for Ushuaia and Moreno, respectively), as occurred with dry seed weight (0.032 and 0.022 g for Ushuaia and Moreno, respectively). As it was expected, equatorial and polar diameters were the highest in Ushuaia fruits (8.37 and 8.30 mm, respectively), as well as the fruit surface with purple color (100%).

Soluble solids were significantly higher in fruits from Ushuaia than Moreno (19.7 and 11.5 °Brix, respectively)

Table 4: Values of branch measurements. Length, node numbers, total buds (TB), vegetative buds (VB), mixed buds (MB) and the relationship between node number and branch length, total buds and node number and mixed buds and total buds. ANOVA analysis was applied

Site	Length cm	Nodes No	TB No	VB No	MB No	Nodes/Leng. No/cm	TB/Nodes	MB/TB%
Moreno	29.86a	21.4	18.6b	15.0	9.0b	0.85b	0.98b	40.5b
Ushuaia	17.05b	18.4	27.3a	13.3	17.3a	1.24a	1.61a	64.6a
F	*23.245*	*1.223*	*13.836*	*0*	*9.809*	*41.993*	*29.014*	*9.164*
p	*<0.001*	*0.274*	*<0.001*	*0.99*	*0.003*	*<0.001*	*<0.001*	*0.004*

Different letters in the same column show significant differences Tukey (p<0.05)

Table 5: Average number of flowers and fruits produced by *B. microphylla* grown on Moreno and Ushuaia sites in two years consecutives (2015 – 2016) and Fruit set calculated

Year	Site	No. of flowers	No. of fruits	Fruit set (%)
2015	Moreno	10.40	2.80a	22.87a
2016	Moreno	10.76	3.02a	17.26a
2015	Ushuaia	10.84	0.45b	3.67b
2016	Ushuaia	14.05	0.63b	2.82b
	F (site)	*0.949*	*21.403*	*45.387*
	p (site)	*0.332*	*<0.001*	*<0.001*
	F (year)	*0.782*	*0.716*	*0.151*
	p (year)	*0.378*	*0.399*	*0.699*

Different letters in the same column show significant differences Tukey (p<0.05)

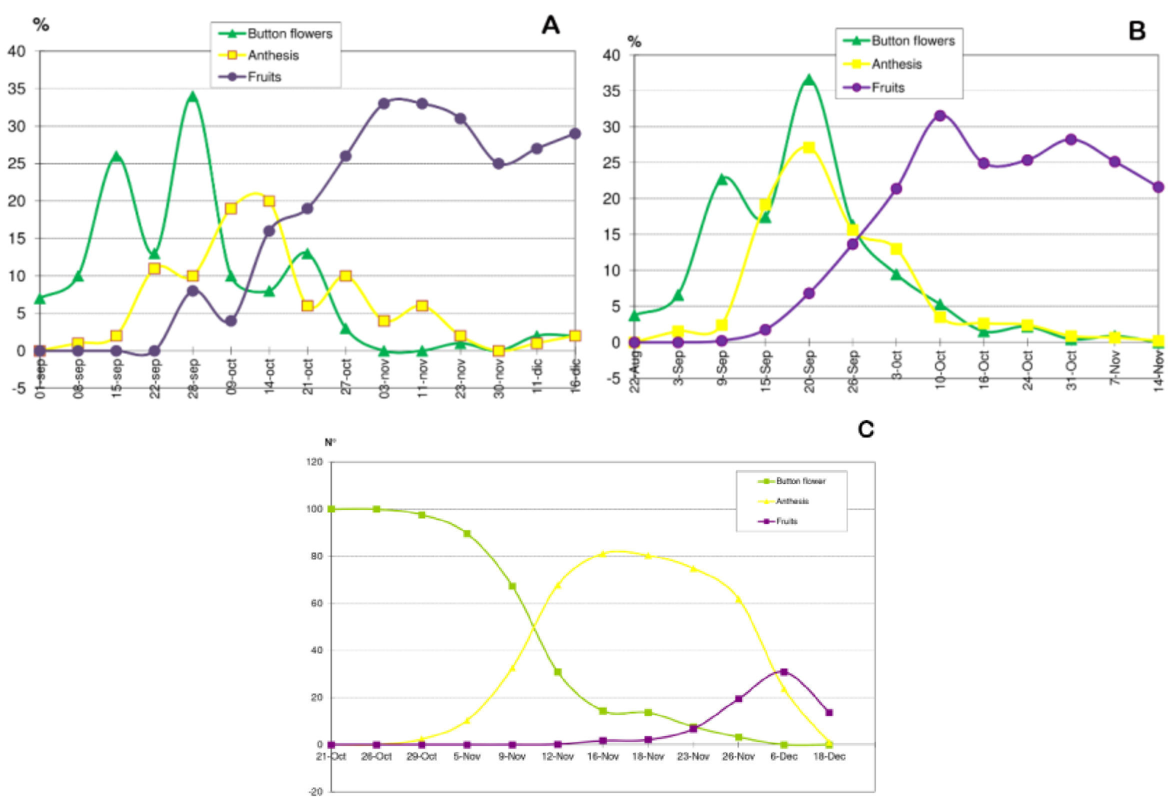


Fig. 5: Flowering phenology of *B. microphylla* grown on Moreno (Buenos Aires, Argentina) during spring of years 2015 (**A**) and 2016 (**B**), and Ushuaia (**C**)

(Table 7). A similar behavior was observed in anthocyanin concentration (313.4 and 118.4 mg cyanidin-3-glucoside/100 g fresh fruit weight) and total phenol concentration (713.0 and 560.5 mg tannic acid/100 g fresh fruit weight).

Discussion

Climatic conditions were very different in the two sites of study. In effect, tundra climate or sub-polar oceanic climate is a natural site of *Berberis* growth while Moreno area is a

Table 6: Fresh fruit weight (FFW), dry fruit weight (DFW), dry fruit weight as percentage of fresh weight (DFWP), seed number (SN), dry seed weight (DSW), dry seed weight/dry fruit weight (DSW/DFW), polar fruit diameter (PFD), equatorial fruit diameter (EFD) and fruit surface with purple (C) of *B. microphylla* grown in Moreno and Ushuaia sites

Site	FFW g	DFW g	DFWP %	SN No	DSW g	DSW/DFW	EFD mm	PFD %	C %
Moreno	0.198b	0.049b	24.637b	3.50	0.022	41.807	7.421b	7.205b	95b
Ushuaia	0.309a	0.088a	28.469a	4.33	0.032	34.309	8.373a	8.303a	100a
F	*11.840*	*16.820*	*10.096*	*1.384*	*4.392*	*1.842*	*6.549*	*21.878*	*2.250*
p	*0.003*	*0.001*	*0.005*	*0.255*	*0.051*	*0.191*	*0.020*	*<0.001*	*<0.001*

Different letters in the same column show significant differences Tukey ($p<0.05$)

Table 7: Soluble solids (% Brix) (SS), anthocyanin concentration (mg cyanidin-3-glucoside/100 g fresh fruit weight) (A) and total phenol concentration (mg tannic acid/100 g fresh fruit weight) (P) of *B. microphylla* fruits grown in Moreno and Ushuaia sites

Site	SS	A mg	P mg
Moreno	11.50b	118.37b	560.55b
Ushuaia	19.66a	316.36a	712.97a
F	*343.00*	*53.726*	*35.813*
p	*<0.001*	*0.002*	*0.004*

Different letters in the same column show significant differences Tukey (p<0.05)

temperate rainy climate (Köppen, 1936). Despite these important climatic differences and the few accumulated cold hours, *B. microphylla* in Moreno place could differentiate in spring, normal flower buds and flowers showed the same morphology as described by Arena *et al*. (2011). Nevertheless, some abnormal flowering was observed in autumn period. Abnormal structures could be due to different climatic condition in which these plants were cultivated. In fact, temperatures of Moreno are warmer and the relation of temperature and photoperiod is very different respect from those observed in Ushuaia. Uncharacteristic environmental conditions and particularly a sudden change to an unfavorable environment affect the transition from vegetative to reproductive activity and could induce various degrees of phyllody in flowers (Roberts and Struckmeyer, 1938). In fact, pollen grains of Moreno flowers were bigger than Ushuaia. In this regard, Ejsmond *et al*. (2011) reported that number and size of pollen grains produced was closely connected to environmental temperature.

Measure and vitality of pollen grains of *B. microphylla* grown in Ushuaia site, are according to those observed by Radice and Arena (2016) in previous studies. Lower pollen vitality of Moreno's flowers could be due again to the high temperatures combined high humidity since it is known this condition affects both pollen viability and vigor (Xue *et al*., 2000; Shivanna, 2003).

Yellow color, nectar production and odor are sources of attraction of insect (Yang *et al*., 2016). So, *Apidae* and *Shyrfidae* species were observed in Moreno site but only *Shyrfidae* insect are efficient in the pollination of Patagonian *Berberis* (Suárez, 2015; Radice *et al*., 2016). Activity of insects is closely related to climatic conditions (Torres Días *et al.*, 2007). Although the syrphids are insects of strong and agile flight, they are not favored by cold, humid, or windy weather (Lewis, 1965) but *Platycheirus* genus are very resistant to cold climates and start their activity with temperatures at ~ 10ºC (Lewis, 1965).

Monitoring of vegetative growth weekly was only done in Moreno site but dimorphic leaves were similar to the new leaves observed on sprouts grown from rhizomes of Ushuaia plants (Arena, oral communication). Period of the vegetative growth was longer than that observed for the same plants in Ushuaia (Arena and Radice, 2014). The changes in the phenological phases of Moreno plants i.e. duration and peaks in relation to Ushuaia plants could be attributed to the higher temperatures and different photoperiod regime (Cleland *et al*., 2007) in Moreno.

Smaller sizes of fruits harvested in Moreno than in Ushuaia could be due to the shorter growth period that these fruits have in this locality until maturity. In fact, mature fruits were obtained 60 days from full bloom in Moreno, while 98 days from full bloom were necessary as a minimum time to harvest ripe fruits in Ushuaia (Arena and Curvetto, 2008). Soluble solids reached in Ushuaia were comparable to previous results (18.5°Brix at 98 days from full bloom: Arena and Curvetto, 2008), as well as anthocyanin concentration in fruits harvested in Ushuaia, (ranging from 200 mg cyanidin-3-glucoside/100 g fresh fruit weight at day 98 from full bloom to 800 mg cyanidin-3-glucoside/100 g fresh fruit weight at day 126 from full bloom) (Arena and Curvetto, 2008; Arena *et al*., 2012). Concentration of total phenols was similar to the results of Arena *et al*. (2012) which ranged from 700 mg tannic acid/100 g fresh fruit weight at day 98 from full bloom to 1550 mg tannic acid/100 g fresh fruit weight at day 126 from full bloom. It is important to mention that genetic and epigenetic factors, i.e. fruit ripening stages, environmental conditions during fruit growth and cultural practices all influence the synthesis of fruit phenolic compounds (Kähkönen *et al*., 1999; Ferreyra *et al*., 2007; Zoratti *et al*., 2014). Particularly, biosynthesis of anthocyanins is dependent upon exposure to sunlight, UV radiation, temperature and water availability, which produce qualitative and quantitative changes in this group of phenolic compounds. Temperature is a key factor affecting the synthesis of anthocyanins. Temperatures near to 25◦C promote the synthesis of anthocyanins in *Vitis*, whereas temperatures around 35◦C affect their accumulation and favor their degradation. However, high night temperatures inhibits the accumulation of anthocyanins (He *et al*., 2010), which highlights the role of the daily temperature range in the accumulation of these compounds, and explains the lowest anthocyanin and soluble solids concentration in fruits from Moreno.

The changes in the vegetative and reproductive traits observed in Moreno leaves could indicate that the plants are trying to adjust its morphophysiology to the new culture conditions i.e. higher temperatures and lower irradiance, as was seen previously in leaf morphology and structure (Radice and Arena, 2015).

Conclusion

B. microphylla showed a marked phenotypic plasticity in most of the variables studied, which allow its cultivation in Moreno. Climatic differences condition the architecture of the plant, number of buds per branch, floral phenology evolution, ripening time like content of anthocyanins, total phenols and antioxidant activity of fruits but the culture of *B. microphylla* in Moreno is an interesting option to obtain another nutraceutical fruit near the centers of mass consumption.

Acknowledgements

The authors acknowledges the finacial grant from CONICET (PIP 0314)

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(Received 07 April 2018; Accepted 20 April 2018)