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Variability in floral traits and reproductive success among and within populations of *Berberis microphylla* G. Forst., an underutilized fruit species

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ABSTRACT

Berberis microphylla G. Forst. (calafate) is an evergreen and spiny shrub considered as a non-timber patagonian forest product, that is relevant for diversification of agrifood production, particularly interesting since its black–blue fruits are rich in phenolic compounds. The objective of this research is to analyze the variability in floral traits and reproductive success of *Berberis microphylla* G. Forst. among and within three populations of Tierra del Fuego along three growing seasons. The presence of variability in some floral traits as well as in the reproductive success of *B. microphylla* among and within three Tierra del Fuego populations was observed, in agreement with the environmental conditions i.e. mean daily temperatures and accumulated rainfall for the three populations and the three growing seasons. Flower dry weight and gynoecium area are good indicators of flower quality (i.e. ovule number), with positive and significant correlations between them (flower dry weight with gynoecium area, $r = 0.551$; $p = < 0.001$, and flower dry weight with ovule number, $r = 0.407$, $p < 0.001$). Pollen/ovule, seed/ovule, fruit/flower and fecundity indices are also good indicators not only of flower quality but also of the reproductive success. The multivariate analysis allowed to analyze jointly the whole measured variables, and explored the influence of annual climatic variability in the response of plants and populations. The variables with great changes among years were those representing quantities (the numbers of ovules and pollen grains) as well as some of the related with size (gynoecium elongation and pollen grain size). Likewise, the influence of each variable in the population split was highlighted at each growing season, which helps to understand the drivers of the differences among them. Plants with a highlight performance were detected and could be selected for their clonal propagation and ex-situ evaluation for the beginning of a breeding program.

1. Introduction

Presence of plant phenotypic variability leads to plant phenotypic plasticity, that is the capacity of a single genotype to change its phenotype in response to the environment, determines the range of conditions under which an individual can survive and reproduce (Atlan et al., 2015). Phenotypic plasticity can be present in different plant organs and functions and could be observed through its changes in phenology, morphology, anatomy, composition, and the three major functions that are basic for plant growth and development like photosynthesis, respiration and transpiration. Phenotypic plasticity in plant functional traits is thought to assist rapid adaptation to new living conditions and provide a buffer against rapid environmental changes (Dai et al., 2017). However, morphological traits are useful for preliminary assessment because they facilitate fast and simple evaluation and can be used as a general approach for assessing genetic diversity among morphologically distinguishable accessions. Morphological characterization combined with multivariate statistical methods, such as principal component analysis (PCA), the most commonly applied, are useful tools for screening accessions (Čolić et al., 2012).

Recently, plasticity in plant reproductive traits has received substantial attention in the context of climate change. These studies have indicated that floral traits such as flowering phenology and duration, floral size as well as mating pattern, could shift in accordance with changes in environmental conditions (Dai et al., 2017). However, relatively few studies have investigated how geography, environmental factors, and genetics affect floral trait variation (Lankinen et al., 2017), as was observed for *Polygala vayredae* (Castro et al., 2008) and *Vaccinium meridinale* (Chamorro and Nates-Parra, 2015). Exploring plasticity of reproductive traits in perennial woody species is difficult because it

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requires the growth of sexually mature plants, which can take several years, and necessitates also long-term monitoring of potentially large individuals in controlled environments (Atlan et al., 2015).

Berberis microphylla G. Forst. (calafate) often grows in differentiated environments in Tierra del Fuego such as coastal scrubs, *Nothofagus* forest margins and clearings, moister areas in grass steppes, and along streams and rivers (Moore, 1983). It is an evergreen, spiny and erect medium size shrub, with a reproductive pattern based on both seedling recruitment and clonal development by rhizomes (Arena and Radice, 2014), that belongs to the so-called group of minor or underutilized fruit tree species that are relevant for diversification of agrifood production. It is classified as a non-timber forest product (Tacon Clavaín, 2004), particularly interesting since its black–blue fruits are rich in phenolic compounds (Arena et al., 2012; Ruiz et al., 2013, 2014; Ramirez et al., 2015; Reyes-Farias et al., 2015) and can be consumed fresh and processed in marmalades and jams, in non-alcoholic beverages and in ice creams. Also, *B. microphylla* is considered an excellent ornamental shrub for their foliage, quality of its flowers and abundant flowering, ideal for protecting gardens and orchards (Bottini, 2000). A recent research indicated that it is appreciated by local rural populations also as fuelwood (Cardoso et al., 2015). At present, commercial barberry orchards are being planned due to its economic potential related to flavour, taste and nutraceutical properties of the fruits. Some aspects of the phenological phases, flower anatomy, fruit composition, postharvest and production, and the annual cycle together with the vegetative morphological variation were already studied in natural populations of this species (Arena et al., 2003; Arena and Curvetto, 2008; Arena et al., 2011, 2013a, 2013b, 2017; Arena and Radice, 2014; Radice and Arena, 2017; Rodoni et al., 2014; Giordani et al., 2016). The objective of this research is to analyze the variability in floral traits and reproductive success of *Berberis microphylla* G. Forst. among and within three populations of Tierra del Fuego along three growing seasons.

2. Materials and methods

2.1. Plant material and growing conditions

Plants growing near Ushuaia city (*US*) ($n = 12$), bordering Fagnano lake (*FL*) ($n = 12$) and central area of the Tierra del Fuego island (*CI*) ($n = 10$) were selected and the height, maximum diameter, shape (domed, rounded, broadly rounded, semi rounded and cushion-like, according to Lenard, 2008), reproductive area, proximity to another plants (0 = low proximity, > 3 m); 1 = medium proximity, between 1 and 3 m; 2 = high proximity, < 1 m), shading (0–100%) and geographical position were registered (Table 1). The mean air daily temperatures, mean environmental relative humidity and cumulative rainfall were also registered for every situation since October to March for the 2014–2015, 2015–2016 and 2016–2017 growing seasons (Table 2). The soil nitrogen (N) was determined using the Kjeldahl technique using a Böchi K350 (Böchi, Flawil, Switzerland), while carbon (C) and phosphorus (P) soil concentration were determined with a plasma emission spectrometry (ICPS 1000 III, Shimadzu, Kyoto, Japan).

2.2. Sampling and determinations

Yellow flower buttons on phase 59 according to Arena et al. (2013a) ($n = 50$) were collected from the North, East, South and West sectors of each plant and were kept refrigerated until their use for the following determinations:

Flower dry weight: yellow flower buttons ($n = 10$) were dried in an oven at 50 °C for 7–10 days until constant weight was reached.

Gynoecium measurements: pistils were taken from the yellow flower buttons ($n = 10$) and then they were scanned to obtained the gynoecium area, gynoecium perimeter and gynoecium elongation (ratio of the length of the major axis to the length of the minor axis) using the UTHSCSA Image Tool software (San Antonio, TX, USA) (Giordani et al., 2016).

Ovule number: number of ovules in each scanned pistil ($n = 10$) was counted.

Pollen grain size: equatorial and polar diameters of the pollen grains ($n = 20$, randomly selected), were measured for each studied genotype using a Leica DM 2500 microscope. The average of the two parameters for each pollen grain was then calculated, according to Radice and Arena (2016a).

Pollen grain germination: the pollen grain germination was registered ($n = 500$) according to Radice and Arena (2016a), during 2015 and 2016 springs. Pollen grains were put on micro drops of a saline solution composed of 2×10^{-3} M H_3BO_3 and 6×10^{-3} M Ca $(NO_3)_2$ added with sucrose 30 g L. Micro drops were placed on the inside of the lid of a petri dish in which 3 ml of water were added in the base to create a humid chamber. Incubation was at 21 ± 2 °C. The number of germinated and aborted pollen grains was recorded under optic microscope 24 h after the test started.

Pollen grain number: the Neubauer hemocytometer was used to count the pollen grains following Godini (1981) ($n = 3$). Briefly, 6 anthers per flower were macerated with 1 ml of water and centrifuged at 2000 rpm during 10 min. Then, 10 µl of the supernatant were introduced into the Neubauer camera.

Pollen grain number/ ovule number (pollen/ovule): this ratio was calculated ($n = 3$) using the pollen grain number per flower and the mean ovule number per ovary.

Seed number/ ovule number (seed/ovule): this ratio was calculated using the mean number of seeds per fruit when the fruits were harvested and the mean number of ovules per ovary.

Fruit number/ flower number (fruit/flower): one-year-old shoots ($n = 8$) were chosen taking into account the plant and shoot orientation (North, South, West or East), according to Arena et al. (2011).

Fecundity rate: this value is the product of two ratios according to Cruden (1972): seed/ ovule and fruit/ flower (Silva and Pinheiro, 2009).

2.3. Statistical analysis

The results were analyzed for each population and each year by ANOVA and Tukey Test ($p < 0.05$). Correlations between pairs of variables were also made. Principal Component Analysis (PCA) was performed to explore multivariate relations between populations and plants at the three growing seasons, evaluating the influence of seven measured variables (flower dry weight, gynoecium area, gynoecium perimeter, gynoecium elongation, ovule number, pollen grain size and pollen grain number) over the whole sample distribution in an ordination space. PCA analysis included Monte Carlo permutation test (n = 999) to assess the significance of each axes. We selected correlation coefficients among columns to obtain the cross-products matrix. PCA was conducted in PCORD version 5.01 (McCune and Mefford, 1999).

3. Results

3.1. Plant material and growing conditions

Size of *FL* plants (1.9 m height and 4.8 m maximum diameter) were highest than *CI* (1.4 m height and 2.9 m maximum diameter) and *US* (1.3 m height and 4.8 m maximum diameter) plants (Table 1). Indeed, productive area was maxima in *CI* plants (69%) than in *US* (52%) and *FL* (41%) plants. In accordance, 58% of *US* plants presented the shrub shapes typical of small shrubs like semi rounded and cushion-like shapes, while only showed the semi rounded shape the 25 and 20% of *FL* and *CI* plants, respectively.

Mean temperatures in *FL* among October to March of 2014–2015 (8.6^0C) and 2015–2016 (8.1^0C) were higher than in *CI* (8.0^0C for

Table 1
Height (H) (m), maximum diameter (MD) (m), shape (SP), productive area (PA) (%), proximity to other plants (PR) and shade (%) (SD) in the north (N), south (S), east (E) and west (W) orientations, and geographical position (GPS) at south latitude (SL) and west longitude (WL) of *B. microphylla* plants growing at Ushuaia (US), Fagnano Lake (FL) and central area of theTierra del Fuego island (CI) populations (P).

	H	MD	SP	PA	PR				SD				GPS	
P					N	S	E	W	N	S	E	W	SL	WL
US														
109	1.65	8.10	D	50	1	1	1	1	0	25	0	25	54 49 43 0	68 19 02 2
110	1.30	8.50	D	60	2	1	1	1	50	0	25	25	54 49 42 3	68 19 02 1
111	1.15	3.20	BR	50	1	1	1	1	50	25	0	25	54 49 43 5	68 19 00 1
121	1.32	4.20	SR	60	1	1	1	1	25	25	25	25	54 49 41 5	68 19 02 3
122	1.25	4.30	SR	40	2	1	2	1	25	25	50	25	54 49 40 9	68 19 04 1
123	1.60	4.70	SR	40	2	2	1	1	25	25	0	25	54 49 42 4	68 19 07 1
124	1.55	6.60	SR	30	1	2	2	1	0	25	25	25	54 49 42 8	68 19 04 2
125	1.10	4.30	SR	40	1	1	0	2	50	25	0	50	54 49 46 1	68 19 00 6
126	0.95	3.40	CL	50	1	1	2	1	50	50	50	50	54 49 45 4	68 18 58 7
149	1.00	2.40	BR	80	1	2	1	1	0	50	50	0	54 49 50 5	68 19 17 8
200	1.40	3.10	BR	70	0	2	0	1	0	50	0	50	54 49 50 4	68 19 21 5
202	1.30	4.40	SR	50	2	2	1	0	0	50	50	0	54 49 51 2	68 19 20 1
FL														
81	1.70	3.00	BR	30	1	0	1	0	100	50	100	50	54 36 00 7	67 38 05 1
82	1.70	4.40	BR	40	1	0	1	1	50	50	100	0	54 36 01 8	67 38 03 8
83	1.65	2.40	BR	60	1	1	1	0	0	50	0	0	54 36 00 4	67 38 07 9
84	2.35	4.10	D	20	1	1	0	1	50	0	50	0	54 36 00 3	67 38 08 7
85	1.40	2.40	R	10	1	1	1	1	50	0	100	0	54 36 00 4	67 38 09 1
86	2.20	5.90	SR	30	2	1	2	1	100	50	100	50	54 36 00 7	67 38 09 7
87	1.85	6.00	SR	70	2	0	1	1	50	0	50	0	54 36 00 5	67 38 10 8
146	1.55	5.70	SR	40	1	0	0	0	50	50	50	50	54 36 02 5	67 37 59 8
148	2.20	2.70	SR	70	0	1	0	1	50	50	0	50	54 36 02 7	67 38 08 7
172	1.95	3.50	D	60	1	0	0	0	0	0	0	0	54 35 74 8	67 38 41 1
183	2.10	3.60	D	35	2	1	1	1	100	50	100	50	54 35 74 3	67 38 41 5
184	2.10	4.60	BR	30	0	0	1	0	0	0	0	0	54 35 67 9	67 38 33 9
CI														
72	1.60	4.00	BR	80	1	1	1	1	0	25	25	0	54 28 04 1	67 33 52 9
73	1.70	5.00	BR	60	1	1	1	0	0	25	25	50	54 28 03 5	67 33 52 4
74	1.40	1.60	R	50	1	1	1	1	25	235	25	0	54 28 03 1	67 33 51 9
77	1.30	1.70	R	90	0	1	0	1	25	0	25	0	54 28 01 0	67 33 52 7
78	1.40	2.00	R	90	0	1	0	1	0	25	0	25	54 28 00 9	67 33 52 8
150	1.80	2.20	R	80	0	1	0	0	0	25	25	25	54 27 76 1	67 34 03 3
171	1.10	2.00	BR	40	1	0	1	0	100	75	75	100	54 27 81 2	67 34 03 3
180	1.70	3.50	BR	70	0	1	1	1	0	25	25	25	54 27 78 1	67 34 08 0
181	1.50	3.80	SR	60	1	1	1	2	100	50	75	100	54 28 02 9	67 33 51 9
182	1.00	3.50	SR	50	1	1	1	1	0	25	25	0	54 27 82 5	67 34 11 6

Shrurb shapes: D = domed; R: rounded; BR = broadly rounded; SR = semi rounded and CL = cushion-like.
Proximity to another plants (0 = low proximity, > 3 m); 1 = medium proximity, between 1 and 3 m; 2 = high proximity, < 1 m).

2014–2015 and 2015–2016 growing seasons) and *US* (8.1 and 7.9^{0}C for 2014–2015 and 2015–2016 growing seasons, respectively) (Table 2). The warmest months in the three sites were January and February, with mean air daily temperatures of 10.0, 10.4 and 11.2^{0}C in *FL* site for 2015, 2016 and 2017, respectively. At the same time, accumulated rainfalls in *US* among October to March were higher (280.7, 240.9 and 235.4 mm for the 2014–2015, 2015–2016 and 2016–2017 growing seasons, respectively) than *FL* (172.7, 104.4 and 130.4 mm for the mentioned growing seasons, respectively) and *CI* (75.4, 127.6 and 122.2 mm for the mentioned growing seasons, respectively).

Soil nitrogen content in *US* population (0.56%) was close to the triple compared to *FL* (0.18%) and *CI* (0.19%), as well as the carbon content (33.79, 9.66 and 13.50%, respectively), which conduce to C/N relations of 60.15, 55.1 and 71.15 for *US*, *FL* and *CI* populations, respectively. Phosphorous contents were of 12.5, 5.4 and 3.0 ppm for *US*, *FL* and *CI* populations, respectively. The soil pH of the sites varied between 5 and 5.6, classified as medium acidic.

3.2. Flower morphology and pollen grain germination

Population significantly affected the flower dry weight, gynoecium area, gynoecium elongation, ovule number, pollen grain size, pollen grain number and pollen grain germination (Table 3). Flower dry weight of *FL* plants was significantly higher (11.1 mg) compared with *US* and *CI* plants (10.7 and 9.9 mg, respectively). However, gynoecium area was maxima for *US* plants (9.2 mm^2) compared with *FL* and *CI* plants (8.8 and 8.5 mm^2, respectively). Gynoecium elongation followed the same behavior as the flower dry weight, being maxima for *FL* plants (0.5). Ovule number was significantly higher for *FL* plants (10.0) than for *US* and *CI* plants (8.9 and 8.5, respectively). Pollen grain size in *CI* site (51.1 µm) was higher than in *US* and *FL* sites (47.6 and 47.4 µm, respectively). Pollen grain number was maximum in *FL* site (10,030.2), being significantly higher than in *CI* and *US* sites (8830.7 and 8512.1, respectively). Pollen grain germination in *FL* (79.8%) and *CI* (82.5%) plants were significantly higher than in *US* plants (71.2%).

Growing season significantly affected the flower dry weight, gynoecium area, gynoecium perimeter, gynoecium elongation, ovule number and pollen grain size (Table 3). Flower dry weight in *2014–2015* growing season was significantly higher (11.6 mg) compared with *2015–2016* and *2016–2017* growing seasons (10.5 and 9.8 mg, respectively). Gynoecium areaand perimeter were also maxima for *2014–2015* growing season (9.2 mm^2 and 14.1 mm, respectively), while gynoeciumelongation in 2015–2016 growing season (0.5)). Ovule number was significantly higher in *2014-2015* growing season (9.6) respect to *2015–2016* and *2016–2017*growing seasons (9.1 and 8.7, respectively). Pollen grain size in the *2015–2016* growing season

Table 2
Climatic data for mean air daily temperatures (T) (°C), mean environmental relative humidity (H) (%) and cumulative rainfall (R) (mm) from October to March for the 2014–2015, 2015–2016 and 2016–2017 growing seasons for Ushuaia, Fagnano Lake and central area of the Tierra del Fuego island sites.

Growing season	USHUAIA			FAGNANO LAKE			CENTRAL AREA OF THE ISLAND		
	T	H	R	T	H	R	T	H	R
Oct 2014	6.08	69.19	58.10	5.88	68.40	9.91	5.54	69.17	13.89
Nov 2014	6.82	69.20	38.80	7.64	63.94	3.30	7.19	65.66	14.18
Dec 2014	8.03	73.58	61.80	9.20	65.69	82.29	9.06	65.53	19.33
Jan 2015	9.52	69.39	29.80	10.02	63.18	20.32	9.75	65.14	11.40
Feb 2015	8.92	73.02	44.20	9.61	66.40	26.92	9.36	68.00	13.80
Mar 2015	9.19	70.61	48.00	9.11	70.11	29.97	7.09	73.27	2.80
	8.09	70.83	280.7	8.58	66.29	172.7	8.00	67.79	75.40
Oct 2015	6.41	65.67	37.00	5.55	89.25	0.00	4.98	69.87	4.80
Nov 2015	7.96	67.54	63.10	7.71	88.59	14.48	7.75	66.19	18.80
Dec 2015	8.15	70.62	48.70	7.81	77.81	44.71	7.82	69.64	40.40
Jan 2016	9.63	69.82	30.20	10.42	75.13	14.40	9.46	64.64	8.40
Feb 2016	9.23	72.31	60.40	9.28	75.79	20.40	9.21	67.50	45.00
Mar 2016	5.87	74.77	1.50	7.93	79.23	10.40	8.61	72.09	10.20
	7.88	70.12	240.9	8.12	80.97	104.4	7.97	68.49	127.60
Oct 2016	8.01	69.36	11.20	5.97	79.64	15.20	6.61	73.39	10.80
Nov 2016	8.23	73.25	52.40	7.60	76.48	20.00	8.29	69.47	14.40
Dec 2016	8.41	75.45	65.20	8.05	76.34	31.00	8.71	69.21	23.80
Jan 2017	9.58	72.04	36.40	9.65	73.75	27.60	9.36	70.70	19.80
Feb 2017	10.75	71.75	42.20	11.20	72.66	11.60	9.83	74.70	26.40
Mar 2017	8.78	76.29	28.00	9.25	75.4	25.00	8.94	76.10	27.00
	8.96	73.08	235.4	8.62	75.7	130.4	8.62	72.29	122.20

(59.9 µm) was higher than in *2016–2017* and *2014–2015* (43.3 and 42.8 µm, respectively).

Significant interactions were found between factors for most of the studied variables. Indeed, differential increments were verified in the values between the main factors and combinations (Table 3). *FL* flowers presented the highest flower dry weight in relation with the highest gynoecium elongation and ovule number, although in *2014–2015* the flower dry weight was similar to *US* flowers. Gynoecium area of *FL* flowers was higher than *CI* and *US* in *2016–2017* growing season, being also maxima with respect *2015–2016* and *2014–2015* growing seasons. Ovule number in *CI* flowers during *2016–2017* was higher than *US* flowers, while ovule number of *CI* flowers did not present differences among the growing seasons. Pollen grain size of *CI* flowers was higher than *FL* and *US* flowers in *2015–2016* growing season, while pollen grain number of *FL* flowers was higher than *CI* and *US* flowers in *2015–2016* and *2016–2017* growing seasons.

Table 4
ANOVA for the pollen grain number/ovule number (P/O), fruit seed number/flower ovule number (S/O), fruit number/flower number (FR/FL) and fecundity rate (F) of the yellow button flowers considering the plants growing at Ushuaia (US), Fagnano Lake (FL) and central area of the Tierra del Fuego island (CI) populations/sites and the 2014–2015, 2015–2016 and 2016–2017 growing seasons as main factors.

Factor	P/O	S/O	FR/FL	F
Population (P)				
US	966.77b	56.419b	4.781c	0.030c
FL	1003.70ab	65.235a	15.306a	0.104a
CI	1089.90a	66.570a	11.025b	0.072b
F	*2.94*	*7.947*	*18.925*	*17.461*
p	*0.050*	*< 0.001*	*< 0.001*	*< 0.001*
Growing season (GS)				
2014-2015	1006.29	58.292b	13.158a	0.085
2015-2016	985.32	63.661ab	9.918ab	0.065
2016-2017	1068.76	66.271a	8.036b	0.055
F	*1.41*	*3.968*	*3.961*	*2.777*
p	*0.24*	*0.019*	*0.005*	*0.063*
Interaction P x GS (F)	*2.30*	*3.522*	*5.638*	*4.385*
Interaction P x GS (p)	*0.06*	*0.007*	*< 0.001*	*0.002*

F = Fisher test; p = significance. Different letters at each column indicate significant differences at $p < 0.05$ using Tukey test.

3.3. Reproductive indices

Population significantly affected the pollen/ovule, seed/ovule, fruit/flower and fecundity indices (Table 4). Pollen/ovule in *CI* (1089.9) was higher than *US* population (966.8). Seed/ovule in *CI* and *FL* populations (66.6 and 65.2, respectively) were higher than *US* population (56.4). Fruit/flower in *FL* population (15.3) was higher than in *CI* (11.0) and *US* (4.8) populations. Fecundity rate was higher in *FL* flowers (0.10) compared with *CI* (0.07) and *US* (0.03) flowers.

Growing season significantly affected the seed/ovule and fruit/flower indices (Table 4). In the *2016–2017* growing season the seed/ovule (66.3) was higher than in *2014–2015* growing season (58.3). Fruit/flower was maxima in *2014–2015* (13.2) respect to *2016–2017* (8.0).

Table 3
ANOVA for the flower dry weight (FDW) (mg), gynoecium area (GA) (mm^2), gynoecium perimeter (GP) (mm), gynoecium elongation (GE), ovule number (ON), pollen grain size (PGS) (µm), pollen grain number (PGN) and pollen grain germination (PGG) (%) of the yellow button flowers considering the plants growing at Ushuaia (US), Fagnano Lake (FL) and central area of the Tierra del Fuego island (CI) populations and the 2014–2015, 2015–2016 and 2016–2017 growing seasons as main factors.

Factor	FDW	GA	GP	GE	ON	PGS	PGN	PGG
Population (P)								
US	10.71b	9.18a	12.86	0.51ab	8.90b	47.58b	8512.13b	71.182b
FL	11.07a	8.80b	12.78	0.52a	10.05a	47.38b	10,030.18a	79.806a
CI	9.89c	8.53b	12.69	0.50b	8.47c	51.08a	8830.66b	82.524a
F	*31.84*	10.85	*0.79*	*3.78*	*73.87*	136.42	*7.69*	*9.644*
p	*< 0.001*	*< 0.001*	*0.453*	*0.023*	*< 0.001*	*< 0.001*	*0.001*	*< 0.001*
Growing season (GS)								
2014-2015	11.58a	9.24a	14.08a	0.50b	9.59a	42.84b	9266.97	——
2015-2016	10.53b	8.64b	11.20b	0.52a	9.14b	59.90a	8838.89	79.166
2016-2017	9.79c	8.69b	12.25b	0.51ab	8.75c	43.29b	9294.61	76.508
F	*80.05*	10.99	*145.89*	*6.16*	*18.06*	3157.96	*0.772*	*1.445*
p	*< 0.001*	*< 0.001*	*< 0.001*	*0.002*	*< 0.001*	*< 0.001*	*0.463*	*0.234*
Interaction P x GS (F)	*6.04*	17.12	*9.24*	*4.95*	*7.87*	60.60	*5.63*	*7.970*
Interaction P x GS (p)	*< 0.001*	*< 0.001*	*< 0.001*	*0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*0.001*

F = Fisher test; p = significance. Different letters at each column indicate significant differences at $p < 0.05$ using Tukey test.

Some significant interactions were found between factors. Indeed, differential increments were verified in the values of seed/ovule, fruit/flower and fecundity indices between the main factors and combinations (Table 4). Seed/ovule was maxima in *2016–2017* growing season for *FL* and *CI* flowers, while for *US* flowers it was maxima in *2015–2016* growing season. Fruit/flower was also maxima in *2014–2015* in *FL* plants. Fecundity index of *FL* plants was higher than *CI* and *US* plants in the *2014–2015* and *2015–2016* growing seasons. Also, correlations were detected between some pairs of variables, thus flower dry weight was strong to moderate, positive and significantly correlated with gynoecium area ($r = 0.551$; $p = < 0.001$), gynoecium perimeter ($r = 0.605$, $p < 0.001$), ovule number ($r = 0.407$, $p < 0.001$), and moderate, negative and significantly correlated with seed/ovule ($r = -0.300$, $p < 0.002$). Ovule number was weakly, positive and significantly correlated with pollen grain number ($r = 0.221$; $p = 0.026$), and weakly to moderately, negative and significantly correlated with pollen/ovule ($r = -0.429$, $p = < 0.001$) and seed/ovule ($r = -0.223$; $p = 0.024$).

3.4. Flower morphology, pollen grain germination and reproductive indices in relation to the plant

Plant significantly affected the flower dry weight, gynoecium area, gynoecium perimeter, gynoecium elongation and ovule number for the three studied sites, while the pollen grain size in *FL* flowers and pollen grain number and pollen grain germination in *US* and *FL* flowers (Table 5). Also, plant significantly affected the pollen/ovule in the three populations, while the seed/ovule in *US* and *FL* flowers and the fruit/flower and fecundity indices in *FL* and *CI* flowers (Table 6).

In *US* population, the maximum flower dry weight was found in plant 124 (12.8 mg), while the minimum in plants 149, 109 and 122 (9.7, 9.7 and 9.6 mg, respectively). Plant 111 attained the maxima gynoecium area (10.7 mm^2), while the minimum value was obtained in plant 122 (8.0 mm^2). However, the highest gynoecium perimeter was observed in plants 109 and 123 (14.0 and 13.9 mm, respectively), and the minimum in plants 149 and 122 (12.0 and 11.9 mm, respectively). Maximum gynoecium elongation was found in plant 202 (0.6) and the minimum in plants 109, 126 and 200 (0.5). Highest ovule number was found in plant 202 (9.8) and the lowest value in plant 126 (7.5). Maxima value for the pollen grain number was found in plant 202 (11,600.0), while the minimum in plant 111 (6029.2). Maxima pollen/ovule value was found in plant 126 (1464.3) while the minimum in plant 149 (721.5) and plant 111 (696.2). Also, the highest seed/ovule was observed in plants 126 and 149 (70.5 and 70.8, respectively), although without significant differences respect to the lowest value of plants 122 and 202 (44.4 and 44.2, respectively). Highest fecundity index was found in plant 126 (0.10), while the lowest in plant 111 (0.006).

In *FL* site, the maximum flower dry weight was found in plant 183 (14.0 mg), while the minimum in plant 84 (8.6 mg). Plant 183 also attained the maxima gynoecium area and perimeter (10.9 mm^2 and 14.7 mm, respectively), while the minimum values were obtained in plant 85 (7.6 mm^2 and 11.5 mm, respectively). Maximum gynoecium elongation was found in plant 146 (0.6) and the minimum in plant 148 and 83 (0.5). Highest ovule number was found in plant 183 (12.5) and the lowest in plants 86 and 82 (9.1 and 8.8, respectively). Highest pollen grain size was observed in plant 81 (50.4 μm), while the lowest in plant 183 (44.6 μm). Maxima pollen grain number was obtained in plant 83 (14,096.3), while the minimum values in plants 84, 86, 146, 183 and 148 (9229.2, 9000.0, 8629.6, 8274.1 and 8096.3, respectively). Maxima pollen/ovule was found in plant 83 (1340.7) while the minimum in plant 183 (685.2). Also, the highest seed/ovule were observed in plants 84, 86, 172 and 184 (79. to 73.8), with the lowest value for plant 183 (45.0). Highest fruit/flower was found in plant 83 (32.2), while the lowest in plant 183 (3.9). Maxima fecundity index was observed in plant 172 (0.2) while the minimum value in plant 81 (0.007).

In *CI* site, the maximum flower dry weight was found in plant 182 (12.1 mg), while the minimum in plant 74 (7.5 mg). Plant 182 also attained the maxima gynoecium area together with plant 171 (9.5 mm^2), while the minimum value was obtained in plant 74 (7.7 mm^2). Maximum gynoecium perimeter was observed in plant 171 (14.1 mm) and the minimum values in plants 78, 180 and 74 (12.0, 11.9 and 11.6 mm, respectively). Highest gynoecium elongation was found in plant 150 (0.6) and the minimum in plant 180 (0.5). Highest ovule number was found in plant 171 (11.5) and the lowest in plant 180 (5.9, respectively). Maxima pollen/ovule was found in plant 180 (1564.6), while the highest fruit/flower and fecundity values were found in plant 171 (27.1 and 0.2, respectively).

3.5. Multivariate relations between populations and plants at the three growing seasons

When PCA was performed for the whole data, the *2015–2016* growing season constituted a clear different group from the other two growing seasons, but patterns were not found among populations. Eigenvalues for the first three axes were 2.608 ($p = 0.001$), 1.284 ($p = 0.147$) and 1.051 ($p = 0.830$), explaining 37.3%, 55.6% and 70.6% of the accumulative variation of total dataset, respectively. The split among growing seasons was highlighted in the graphic of Axis 2 vs. Axis 3 (Fig. 1). The variables highly correlated with Axis 2 were gynoecium elongation and ovule number; and those for Axis 3 were pollen grain number and pollen grain size.

When separated PCAs were performed for each growing season data, population groups better split, being *CI* a more conspicuous and differentiated group at *2014–2015* and *2015–2016* growing seasons than *US* and *FL* (both intermingled between themselves). Meanwhile, *FL* was the most differentiated from *CI* and *US* at *2016–2017* growing season. Likewise, variables slightly changed their influence over the ordination space (Fig. 1). Axes 1 were mainly directed by gynoecium perimeter, gynoecium area and flower dry weight at the three growing seasons. On the other hand, Axes 2 were mainly influenced by ovule number, pollen grain size and gynoecium elongation in *2014–2015*, by gynoecium perimeter, gynoecium elongation, ovule number and pollen grain size in *2015–2016*, and by pollen grain number and ovule number in *2016–2017*. The eigenvalues and cumulative variance for Axes 1 were: 2.610 and 37.3% at *2014–2015*, 2.780 and 39.7% at *2015–2016* and 2.859 and 40.8% at *2016–2017*. The eigenvalues and cumulative variance for Axes 2 were: 1.586 and 59.9% at *2014–2015*, 1.586 and 59.9% at 2015–2016, and 1.474 and 61.9% at *2016–2017*.

It is important to note that some plants with similar response in the studied variables remain closer one to each other in different growing seasons in the ordination space (remarked with dotted line in Fig. 1 lower graphics) independently of the population they belong, while other plants showed extremely different responses with time.

4. Discussion

Bloom of several fruit species occurs at different times because it is a species characteristic. However, the beginning, length and intensity of the bloom period are strongly influenced by ecological factors. Respect to the weather conditions, chilling requirements during dormancy period and heat necessities determine the start of blooming while temperature, solar radiation, humidity and frost condition influence full bloom and duration of blooming. On the other hand shrub vigor, flower bud formation and different applied managements to the orchard are decisive in the flower and fruit production (Nyéki and Soltész, 1996). Flower and/or ovary size have been shown to impact the final fruit size in a number of species like as peach (*Prunus persica*), rabbiteye blueberry (*Vaccinium ashei*), olive (*Oleo europea*) and pomegranate (*Punica granatum*), as well as the fruit set (Wetzstein et al., 2013). Flower size can vary widely within species with immediate consequences also on reproductive success. An important future goal will be to elucidate the

Table 5
ANOVA the flower dry weight (FDW) (mg), gynoecium area (GA) (mm), gynoecium perimeter (GP) (mm), gynoecium elongation (GE) (mm), ovule number (ON), pollen grain size (PGS), pollen grain number (PGN) and pollen grain germination (PGG) of the yellow button flowers for the plants in each population of Ushuaia (US), Fagnano Lake (FL) and central area of the Tierra del Fuego island (CI) and in the 2014–2015, 2015–2016 and 2016–2017 growing seasons.

Factor	FDW	GA	GP	GE	ON	PGS	PGN	PGG
US Plants								
109	9.68d	9.90abc	13.99a	0.48c	7.93de	47.90	7662.96abcd	66.08
110	11.12 bc	9.42abcd	13.34abc	0.49bc	8.90abcd	45.29	10122.22abc	80.08
111	10.85bcd	10.75a	13.73ab	0.55ab	8.50bcde	47.83	6029.17d	66.00
121	11.23bc	8.96bcd	12.59abc	0.53abc	9.59ab	46.07	7577.78abcd	76.72
122	9.62d	7.96d	11.88c	0.50bc	8.73abcde	49.10	7407.41bcd	61.59
123	11.50ab	10.39ab	13.93a	0.53abc	9.70ab	48.63	8851.85abcd	58.55
124	12.78a	9.50abcd	13.08abc	0.51abc	9.50ab	46.78	8308.33abcd	81.04
125	10.64bcd	8.95 bcd	12.56abc	0.51abc	9.41ab	48.00	9142.86abcd	78.40
126	10.17cd	8.71bcd	12.56abc	0.48c	7.53e	46.50	10985.19ab	71.72
149	9.72d	8.48cd	12.02c	0.54abc	9.20abc	47.97	6711.11cd	66.21
200	10.64bcd	8.22cd	12.21bc	0.48c	8.03cde	48.02	7985.19abcd	76.74
202	10.63bcd	8.91bcd	12.39abc	0.57a	9.82a	48.81	11,600.00a	71.04
F	*10.65*	*5.23*	*4.16*	*5.68*	*8.40*	*1.47*	*3.83*	*0.526*
p	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*0.14*	*< 0.001*	*0.852*
FL Plants								
81	11.31bc	7.81de	12.64bcd	0.52cde	9.70bcde	50.40a	9608.33ab	81.10
82	12.20b	9.57bc	13.15bc	0.50de	8.80e	48.14ab	10141.67ab	81.54
83	9.02ef	8.12de	11.87cd	0.47e	10.52bc	46.00ab	14,096.30a	75.99
84	8.61f	8.13de	12.06cd	0.58ab	9.20de	49.43ab	9229.17b	82.67
85	9.61de	7.56e	11.52d	0.56abc	10.45bcd	46.77ab	10308.33ab	76.46
86	9.52ef	7.99de	12.11cd	0.49de	9.10e	48.87ab	9000.00b	84.99
87	11.860bc	8.43cde	12.77bcd	0.50de	9.90bcde	48.63ab	10481.48ab	76.16
146	10.78cd	8.90bcd	12.36cd	0.62a	10.93b	45.50ab	8629.63b	73.42
148	9.98de	8.64bcde	12.53bcd	0.47e	9.77bcde	45.67ab	8096.30b	87.52
172	12.12b	9.75ab	13.75ab	0.52cde	9.52cde	46.81ab	10066.67ab	76.94
183	14.01a	10.86a	14.70a	0.52cde	12.48a	44.60b	8274.07b	72.21
184	12.46b	9.51bc	13.21bc	0.53bcd	10.50bc	48.17ab	12325.00ab	88.67
F	*40.96*	*12.61*	*8.84*	*13.49*	*13.31*	*2.42*	*3.05*	*0.549*
p	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*0.006*	*0.002*	*0.835*
CI Plants								
72	8.84d	8.42ab	12.55abc	0.47cd	7.53e	50.27	8407.41	88.00
73	9.154d	8.56ab	12.39bc	0.49bcd	7.53e	53.90	8916.67	79.89
74	7.54e	7.74b	11.59c	0.53ab	9.00cd	52.17	7155.56	85.42
77	9.77cd	8.62ab	12.87abc	0.51abcd	10.23b	48.27	10022.22	81.21
78	11.03abc	8.23ab	12.05c	0.52abc	7.90de	49.93	9866.67	82.65
150	9.82cd	8.300ab	12.43bc	0.55a	8.77cd	48.97	7948.15	74.80
171	11.15ab	9.47a	14.08a	0.52abc	11.53a	53.13	8800.00	83.80
180	9.93bcd	7.64b	11.93c	0.46e	5.93f	51.93	8783.33	79.64
181	9.46d	8.75ab	13.20abc	0.51abcd	6.93ef	50.20	9104.58	83.17
182	12.14a	9.54a	13.79ab	0.49bcd	9.33bc	52.00	9333.33	86.66
F	*20.94*	*4.33*	*5.04*	*5.12*	*42.86*	*1.66*	*0.86*	*0.488*
p	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*0.095*	*0.562*	*0.852*

F = Fisher test; p = significance. Different letters at each column indicate significant differences at $p < 0.05$ using Tukey test.

genetic basis of flower size variation in natural plant populations (Krizek and Anderson, 2013). *B. microphylla* flowers collected in the *FL* population had the highest flower dry weight in relation to the highest gynoecium elongation and number of ovules, and a significant and positive correlation was found between the dry weight of the flower and the number of ovules. These results coincide with those observed in pomegranate (*Punica granatum*), between the size of the flower and the ovule in relation to the flower position (Wetzstein et al., 2013). Ovule number also varied in flowers according to the vertical stratum in the shrub of *Adesmia tristis* (Ferreira et al., 2014), demonstrating that other factors affect plant physiology, such as light reception and nutrient translocation in the drain and source operations, and hormonal actions act synergistically to enhance branch vigor, increasing the number of seeds in the upper stratum. Flower size is often correlated with other floral traits that increase pollinator visitation rates, i.e. large flowers generally contain more nectar rewards and are more conspicuous than smaller flowers. Thus, pollinators tend to be more attracted to larger than smaller flowers both within and between plant species (Krizek and Anderson, 2013).

Pollen availability and quality are two principal determinants of female reproductive success and pollen limitation has been shown to be widespread, especially in animal-pollinated species. Furthermore, the available resources and resource allocation also play a major role in the final female reproductive success (Castro et al., 2008). *CI* flowers presented the highest pollen size, while the *FL* flowers presented the highest pollen grain number particularly in *2015–2016* and *2016–2017* growing seasons. The highest pollen grain germination values were found in *CI* and *FL* flowers. Perhaps the interannual differences in the size of the pollen grain could be attributed to hormonal and nutritional causes that occur in the phase of the formation of floral buds and those deficit conditions partially inhibit their growth (Nyéki and Soltész, 1996). This could explain the larger size of pollen grains during the *2015–2016* growing season. Pollen grain number of *B. microphylla* was not correlated with pollen size, in contrast with the results observed by Willmer (2011). Environment has an important role in the pollen production since insolation affect pollen production within and between plants. Plants grown under high light produce more pollen grains per flower than those grown under low light. However, the response is usually genotype-specific; some individuals respond little to changes in light availability while others substantially reduce pollen production.

Pollen/ovule adjustments have been probed at various levels, i.e., between populations, races and within genera, tribes and families.

Table 6
ANOVA for the pollen grain number/ovule number (P/O), fruit seed number/flower ovule number (S/O), fruit number/flower number (FR/FL) and fecundity rate (F) of the yellow button flowers for the plants in each population of Ushuaia (US), Fagnano Lake (FL) and central area of the Tierra del Fuego island (CI) and in the 2014–2015, 2015–2016 and 2016–2017 growing seasons.

Factor	P/O	S/O	FR/FL	F
US Plants				
109	953.99bc	68.087a	4.759	0.0315ab
110	1141.83abc	63.444a	5.327	0.0390ab
111	696.17c	54.261a	0.947	0.0057b
121	796.35bc	50.857a	4.722	0.0099ab
122	855.03bc	44390a	1.797	0.0106ab
123	906.45bc	56.890a	3.802	0.0151ab
124	866.44bc	49.002a	5.320	0.0331ab
125	985.97bc	48.691a	2.139	0.0113ab
126	1464.30a	70.517a	12.472	0.1014a
149	721.47c	70.853a	5.238	0.0351ab
200	991.29bc	53.248a	2.981	0.0212ab
202	1183.85ab	44.253a	8.861	0.0274ab
F	*5.40*	*2.377*	*1.209*	*1.651*
p	*< 0.001*	0.008	0.231	*0.086*
FL Plants				
81	1000.46abc	56.167abc	1.681b	0.0077b
82	1146.39abc	69.362abc	6.095b	0.0619ab
83	1340.74a	72.953ab	32.197a	0.1971ab
84	1008.51abc	79.711a	14.338ab	0.1460ab
85	972.32abc	69.217abc	16.217ab	0.1579ab
86	975.67abc	78.864a	12.972ab	0.0594ab
87	1060.94abc	65.400abc	22.008ab	0.1521ab
146	798.07bc	46.014bc	11.691ab	0.0407ab
148	832.55bc	57.832abc	22.407ab	0.1192ab
172	1051.80abc	74.556a	22.187ab	0.2036a
183	685.22c	44.965c	3.858b	0.0249ab
184	1193.92ab	73.837a	13.118ab	0.0896ab
F	*3.30*	*4.404*	*2.734*	*2.628*
p	*0.001*	*< 0.001*	0.001	*0.004*
CI Plants				
72	1145.84ab	64.275	8.127b	0.0443b
73	1180.34ab	78.051	7.034b	0.0787ab
74	796.44b	68.292	17.320ab	0.1418ab
77	992.38b	77.873	10.769b	0.0602ab
78	1247.58ab	49.312	9.951b	0.0483b
150	908.48b	60.251	10.218b	0.0472b
171	763.83b	66.172	27.153a	0.1801a
180	1564.64a	76.271	11.636b	0.0491b
181	1318.51ab	65.617	4.244b	0.0458b
182	1003.13ab	67.957	8.372b	0.0426b
F	*3.99*	*1.120*	*3.345*	*2.749*
p	*< 0.001*	*0.348*	*0.001*	*0.005*

F = Fisher test; p = significance. Different letters at each column indicate significant differences at $p < 0.05$ using Tukey test.

Rather than breeding system, in several taxa pollen/ovule ratio reflects better pollination mechanism or pollination efficiency (Amela García et al., 2014). Pollen/ovule values obtained in the three populations (966.77–1089.90) confirmed the previous results of the reproductive system of *B. microphylla* (Radice and Arena, 2016b), which indicate that this species is xenogamy (cross pollination), although according to Cruden (1977) this species could be considered as facultative xenogamy. Pollen/ovule values of *B. microphylla* were higher than the obtained on *Vaccinium meridionale* grown in Colombia (Chamorro and Nates-Parra, 2015), and pollen/ovule ratio was not correlated with pollen size as occurred in *Passiflora* species (Amela García et al., 2014). Pollen/ovule ratios can vary between populations within a species, but generally in a direction consistent with ecological constraints, as occurred in *B. microphylla* where pollen/ovule ratio in *CI* population (1089.90) was higher than *US* population (966.77). Pollen and ovule can vary between individuals and between flowers in an individual, but usually there is either no relationship or a positive relationship between them at this level. Despite this, a weak, positive and significant correlation between ovule number with pollen grain number was observed, and a weak to moderate, negative and significant correlation between ovule number with pollen/ovule index was found in *B. microphylla*. Both pollen and ovule number can change through a flowering season, although pollen/ovule often remains constant. Hence, the use of the pollen/ovule ratio removes some of the variation and allows more useful and meaningful comparisons.

During the reproductive process, not all flowers produce fruit nor do all ovules become seeds. Limiting factors occur at each stage of the reproductive process, reducing its efficiency. These factors include the natural condition in the area, the pollination efficiency, energy resource allocation for fruit and seed production, natural abortion rates, flower, fruit and seed predation, as well as pollen germination capacity. However, the genetic factor can be a characteristic tendency as occur in *Eugenia* spp (Silva and Pinheiro, 2009). Annual seed production depends not only on biological factors, such as pollination and maternal resource allocation, but also on environmental factors, such as mean annual precipitation and habitat fragmentation (Silva and Pinheiro, 2009). Frequent winds, rainfalls and fog in the studied area reduced pollinators activity, which, together with intrinsic plant characteristics affect allogamy rate, also known as natural crossing rates with consequent low seed production (Ferreira et al., 2014). There are several factors responsible for the selective abortion of ovules and seeds at different stages of development. Flower, fruit and seed predation is also a highly significant limiting factor for reproductive success and has a direct influence on population recruitment (Silva and Pinheiro, 2009). Seed/ovule and the fruit/flower ratios are the main parameters for evaluating species fecundity and can be used to measure the degree of reproductive efficiency of a population (Cruden, 1972). Studies focusing on reproductive success and consequent seed production are very common in commercial plants. However, studies in natural areas are scarce (Silva and Pinheiro, 2009). Seed/ovule ratios found in *B. microphylla* could be considered as high values (close to 0.63), given that woody species usually have lower seed/ovule ratios (near 0.30), as was cited for *Eugenia* spp. (Silva and Pinheiro, 2009). Seed/ovule and fruit/flower ratios varied among populations and within them too, with the highest values in *FL* population. Energetic factor could be one of the main reasons for the limited fruit-set in several species, as was found in *Eugenia* spp., since in most of the analyzed pollen grains of the stigma, a sufficient amount to fertilize most of the ovules were found. Then, a low fruit-set in these species (3.6 to 17.2%) can be considered a result of a selective pressure in favor of the most vigorous fruits, and an adjustment in the nutrient supply to sustain fruit and seed development, as was seen in *Vaccinium corymbosum* too (Silva and Pinheiro, 2009). Fruit/flower ratio in *B. microphylla* varied from 4.8 to 15.3% among sites. Previous results of the hand cross pollination and the natural fruit/flower ratio in *US* site, indicated that this ratio was double when the hand cross pollination was made, indicating the pollinator efficiency (Radice and Arena, 2016b). Also, if enough pollen grains on stigma were observed before, their source could not be determined (same flower, different flowers of the same species and/or different species). The fecundity rates obtained in *B. microphylla* (0.030 to 0.104) were higher than the obtained in *Eugenia* spp. (Silva and Pinheiro, 2009), mainly due to the higher seed/ovule ratio.

Regardless of the genetic causes and the nutritional and hormonal status that could be responsible for the differences in the studied variables among sites and plants, the obtained results could be in part related with the site conditions, as was observed in a previous study in *B. microphylla*, mostly on vegetative characteristics (Giordani et al., 2016). The highest mean temperature in *FL* along the studied growing seasons, particularly among October to March of *2014–2015* and *2015–2016*, together with the lower accumulated rainfall in *FL* and *CI* sites compared with *US* site, could determine the differences found in the floral traits and reproductive success. Indeed, rainfall could be associated with the collected solar irradiation (the annual solar irradiation in Tierra del Fuego is low, being of 1.25 MW h/m^2 in Ushuaia and

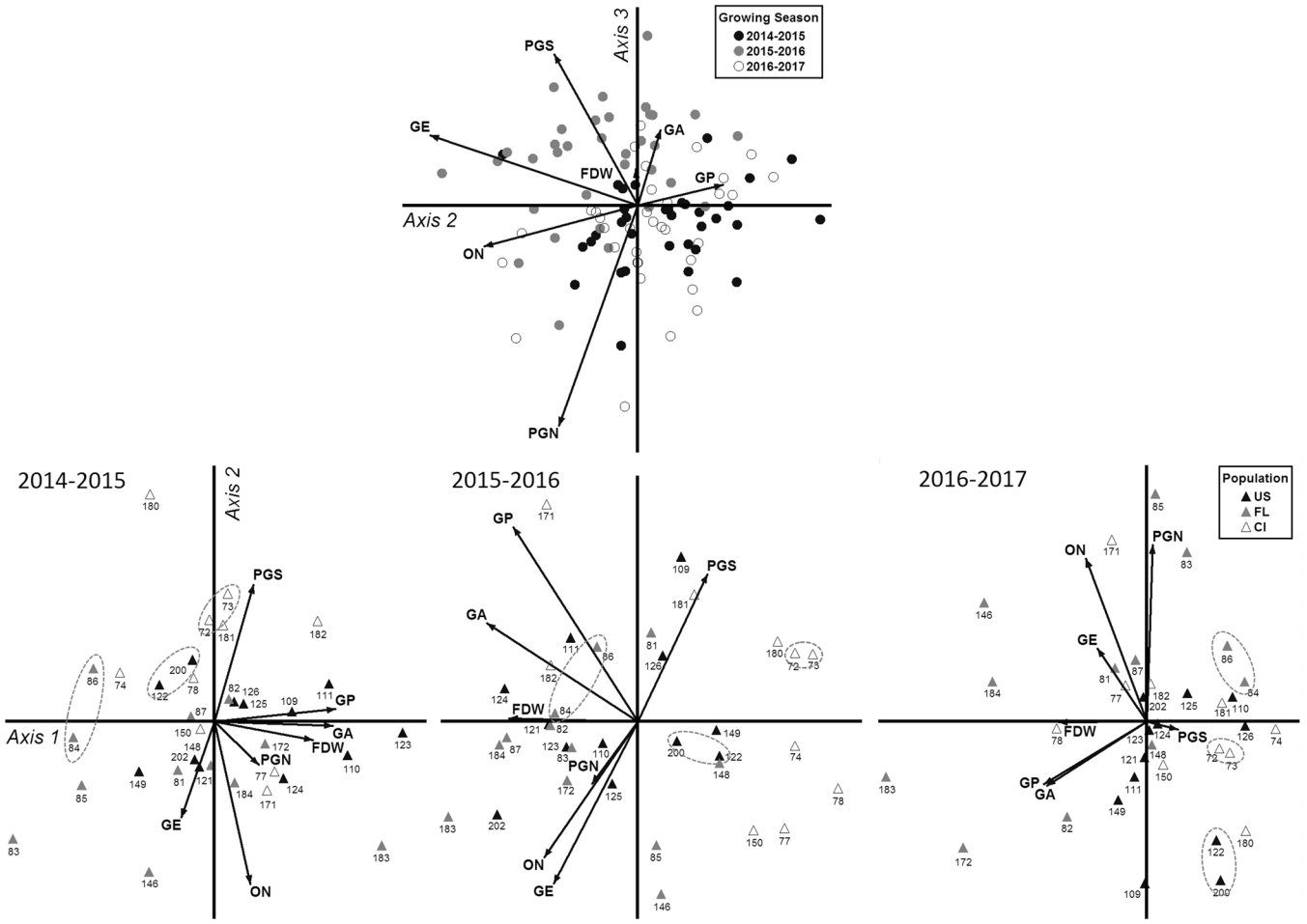

Fig. 1. Principal Component Analysis (PCA) graphic results for the three studied growing seasons, together (up) and one by one (below). Vectors represent the influence of variables (flower dry weight-FDW, gynoecium area-GA, gynoecium perimeter-GP, gynoecium elongation-GE, ovule number-ON, pollen grain size-PGS and pollen grain number-PGN) in each analysis. Populations are Ushuaia-US, Fagnano Lake-FL and central area of the Tierra del Fuego island-CI. Numbers represent selected plants. Dotted line remark plants with similar responses along time at each population.

1.30 MW h/m^{2}upper the Andes (Righini and Grossi Gallegos, 2011), as was referenced by Ferreira et al. (2014). Also, the activity of pollinators is dependent of such climatic conditions. Finally, it is important to highlight the behavior of some plants for each of the populations studied. In *US* population, plant 124 (with 18.75% of shadow and 30% of productive area) presented the maxima flower dry weight, while plant 111 (with a 25% of shadow and 50% of productive area) the maxima gynoecium area and plant 202 (with a 25.0% of shadow and 50% of productive area) the maxima ovule and pollen grain numbers. However, the plant 126 (with 50% of shadow and 50% of productive area) was highlighted by its highest pollen grain number/ovule number, seed number/ovule number and fecundity index.

In the *FL* population, the plant 183 (with 75% of shadow and 35% of productive area) presented the maxima values for flower dry weight, gynoecium area and perimeter and ovule number, while plant 81 (with 75% of shadow and 30% of productive area) showed the maxima pollen grain size. The highest pollen grain number was observed in plant 83 with 12.5% of shadow and 60% of productive area, which also presented the maxima pollen grain number/ ovule number and fruit/ flower values. Plant 172 (with 0% of shadow and 60% of productive area) showed the highest fecundity index.

In *CI* population, plant 182 (with a 12.5% of shadow and 70% of productive area) presented the maxima flower dry weight and gynoecium area, and the plant 171 (with a 87.5% of shadow and 40% of productive area) the highest gynoecium perimeter and ovule number. Plant 180 (with a 18.75% of shadow and 70% of productive area) presented the maxima pollen grain number/ovule number, while plant 171 the highest fruit/flower and fecundity ratios.

5. Conclusions

Results obtained confirm the presence of variability in some floral traits as well as reproductive success of *B. microphylla* among and within three Tierra del Fuego populations. Phenotypic plasticity was observed in agreement with the environmental conditions i.e. mean daily temperatures and accumulated rainfall for the three populations and the three growing seasons. However, a clear relationship between plant floral traits and reproductive success with its particularly growing conditions i.e. plant shadow and reproductive area, was not observed. The intrinsic plant nutritional and hormonal status could be responsible in part of the observed plant variability. Also, the particular reproductive shoot architecture like vegetative and mixed bud relation could correlate with the observed variability in floral traits and reproductive success. Flower dry weight and gynoecium area are good indicators of flower quality (i.e. ovule number), while pollen/ovule, seed/ovule, fruit/flower and fecundity indices are good indicators not only of flower quality but also of the reproductive success. The multivariate analysis allowed to analyze jointly the whole measured variables, and explored the influence of annual climatic variability in the response of plants and populations. The variables with great changes among years were those representing quantities (the numbers of ovules and pollen grains) as well as some of the related with size (gynoecium

elongation and pollen grain size). Likewise, the influence of each variable in the population split was highlighted at each growing season, which helps to understand the drivers of the differences among them. Several plants with a highlight performance along the studied period were detected and could be selected for their clonal propagation and ex-situ evaluation for the beginning of a breeding program of *B. microphylla*.

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