Reproductive shoots of *Berberis microphylla* G. Forst. in relation with the floral bud development and the fruit set

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Abstract

The objective of this research was to study the reproductive shoots of *Berberis microphylla* G. Forst. in relation with the floral bud development and the fruit set among and within three populations of Tierra del Fuego during three consecutive years. Evolution of the different reproductive phenological phases of *B. microphylla* was in accordance with the climatic conditions of the sites and the years, in particular with the temperatures of the end of the winter and beginning of the spring. In fact, blooming period in *US* was advanced compared to *FL* and *CI* populations. Also, full bloom was shorter in *US* respect to *FL* and *CI* populations when the temperatures increased gradually as occurred in 2014 year. The development of reproductive shoots was significantly affected by the population, the shadow and the growing season. Mixed bud number/length was highest in *US* population; however fruit set was maximum in *FL* population. Shadow levels of 50% decreased total bud number, total bud number/node number, mixed bud number, mixed bud number/length and fruit number/length. Ultimately, mixed bud number, mixed bud number/length and aborted flowers were maxima in 2015–2016 growing season. The obtained results confirm the
presence of phenotypic plasticity of the reproductive shoots of *Berberis microphylla* G. Forst. in relation with the floral bud development and the fruit set.

**Keyword:** Plant biology

### 1. Introduction

Changes in factors like humidity, temperature, photoperiod, edaphic associations and topography strongly influence reproduction and population survival. Also, biotic factors, such as competition for pollinators, frugivory and herbivory, larvae foraging and seed dispersal may affect plant phenology. Plant phenology is defined as the study of the seasonal and recurrent timing of life cycle events like sprouting, leaf expansion, abscission, flowering, fertilization, seed set, fruiting, seed dispersal and germination. Thus, phenology patterns displayed by plants are adaptations to the surrounding abiotic and biotic environments (Martínez-Adriano et al., 2016). Particularly, floral induction responds to photoperiod and temperatures but interactions between environmental stimuli and endogenous developmental cues exert some control over floral initiation (Wilkie et al., 2008). Also, time of bloom is conditioned by species characteristics, ecological factors and cultivation technology (Soltesz, 1996).

Despite flowering phenology is under genetic control, biotic such as pollinators and abiotic factors like as photoperiod, temperature, precipitation and nutrients in the soil are also important forces triggering the expression of flowering phenology (Forrest and Miller-Rushing, 2010; Rodriguez-Perez and Traveset, 2016).

Evolution of plant phenophases studies in correlation with ambient temperatures have allowed considering the plant phenology as a biological indicator of anthropogenic climate change, being relevant for global carbon models, predictions of future climate change and in ecosystem services. However, a few antecedents consider how phenology correlates with several common plant traits (Wolkovide and Ettinger, 2014), i.e. morphological, physiological and biochemical traits. Information on reproductive phenology is basic to the understanding of tree biology, and of their interactions with other organisms and the dynamics and functioning of ecosystems, for example, the timing of flowering and fruiting controls the activities of many herbivores, flower visitors (pollinators), and frugivores. Thus, an understanding of reproductive phenology and pollination biology are basic elements that should be considered in the conservation, management and exploitation of plant species and when predicting the reproductive potential of vegetation at a landscape level (Kevede and Isotalo, 2016).

Phenotypic plasticity is the capability of a genotype to produce diverse phenotypic expressions under different environments. A wide range of genotypes in one area gives a broader base of phenotypes or phenotypic plasticity than a single genotype (Zunzunegui et al., 2009). Since environmental stresses, as well as ecological
differences among habitats, are more complex than a single factor, plant responses imply adjustments at various levels, from differentiation at the whole plant, to leaf phenology, morphology and to physiological processes, although such responses vary among species. It has been suggested that plants of each species present a trade-off among adjustment levels (such as stomatal control, chlorophyll content, type of leaves, shoot elongation) allowing a diverse patterns of adaptations (Zunzunegui et al., 2009). In fact, B. microphylla grown in environmental conditions of higher temperatures and lower irradiance showed modifications in the leaf morphology and structure (Radice and Arena, 2015).

Berberis microphylla G. Forst. (calafate), is a Patagonian evergreen shrub considered as a non-timber forest product whose little black—blue fruits are of nutraceutical value. They can be consumed fresh and processed in marmalades and jams, in non-alcoholic beverages and in ice creams (Arena et al., 2018). Some aspects of the phenological phases, shoot growth and development, flower anatomy, pollen structure and fertility, 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, 2012, 2013a, b, 2017, 2018; Arena and Radice, 2014; Radice and Arena, 2016a, 2017; Rodoni et al., 2014; Giordani et al., 2017). The phenotypic behavior in different environmental conditions was not studied, so it is also important to know its variability in the face of such changes. The objective of this research was to study the reproductive shoots of Berberis microphylla G. Forst. in relation with the floral bud development and the fruit set among and within three populations of Tierra del Fuego during three consecutive years.

2. Materials and methods

2.1. Plant material and growing conditions

Plants growing near Ushuaia city (US), bordering Fagnano lake (FL) and central area of the Tierra del Fuego island (CI) were selected and the height, diameter, shape, reproductive area, presence of old leaves, proximity to another plants, shading and geographical position were registered and described in a previous work (Arena et al., 2018). The mean air daily temperatures (T) and cumulative rainfall (R) were also registered for every population since August to March for the 2014—2015, 2015—2016 and 2016—2017 growing seasons (Table 1).

2.2. Sampling and determinations

Phenology was registered according to Arena et al. (2011), during the 2014—2015 and 2015—2016 growing seasons, and data were recorded thrice a week for every reproductive shoot selected. Button flowers, stigma receptive, anthesis, pollen shedding and fruit set were calculated according to the total flowers formed proportionally...
and expressed as phenograms. In this species anthesis and pollen shedding are coincident (Arena et al., 2011). Also, start, full and end of blooming were determined considering a 10%; 50% and 10% of flowers on anthesis phase respectively (Arena et al., 2013a). Ripe fruit were collected 98 days after full flower on February 2015, 2016 and 2017, according to Arena et al. (2011) and fruit set was calculated.

Eight year old shoots were selected in spring of 2014, 2015 and 2016 from the North, East, South and West orientations (two shoots from each sector) of each plant. The position of the branch respect to the height of the shrub (superior or inferior) was

| Site         | US | FL | CI |
|--------------|----|----|----|
| **Growing Season** | **T** | **R** | **T** | **R** | **T** | **R** |
| Aug 2014     | 2.80 | 90.7 | 2.40 | 28.20 | 1.50 | 23.90 |
| Sep 2014     | 4.00 | 31.9 | 4.00 | 78.10 | 3.40 | 47.30 |
| Oct 2014     | 6.08 | 58.10 | 5.88 | 9.91 | 5.54 | 13.89 |
| Nov 2014     | 6.82 | 38.80 | 7.64 | 3.30 | 7.19 | 14.18 |
| Dec 2014     | 8.03 | 61.80 | 9.20 | 82.29 | 9.06 | 19.33 |
| Jan 2015     | 9.52 | 29.80 | 10.02 | 20.32 | 9.75 | 11.40 |
| Feb 2015     | 8.92 | 44.20 | 9.61 | 26.92 | 9.36 | 13.80 |
| Mar 2015     | 9.19 | 48.00 | 9.11 | 29.97 | 7.09 | 2.80 |
| Aug 2015     | 2.00 | 119.9 | 1.90 | 2.50 | 0.50 | 27.00 |
| Sep 2015     | 2.50 | 145.5 | 3.20 | 27.4 | 2.30 | 4.60 |
| Oct 2015     | 6.41 | 37.00 | 5.55 | 0.00 | 4.98 | 4.80 |
| Nov 2015     | 7.96 | 63.10 | 7.71 | 14.48 | 7.75 | 18.80 |
| Dec 2015     | 8.15 | 48.70 | 7.81 | 44.71 | 7.82 | 40.40 |
| Jan 2016     | 9.63 | 30.20 | 10.42 | 14.40 | 9.46 | 8.40 |
| Feb 2016     | 9.23 | 60.40 | 9.28 | 20.40 | 9.21 | 45.00 |
| Mar 2016     | 5.87 | 1.50 | 7.93 | 10.40 | 8.61 | 10.20 |
| Aug 2016     | 2.80 | 54.9 | 0.18 | 18.80 | 1.60 | 20.80 |
| Sep 2016     | 6.80 | 17.9 | 4.24 | 6.60 | 4.98 | 4.00 |
| Oct 2016     | 8.01 | 11.20 | 5.97 | 15.20 | 6.61 | 10.80 |
| Nov 2016     | 8.23 | 52.40 | 7.60 | 20.00 | 8.29 | 14.40 |
| Dec 2016     | 8.41 | 65.20 | 8.05 | 31.00 | 8.71 | 23.80 |
| Jan 2017     | 9.58 | 36.40 | 9.65 | 27.60 | 9.36 | 19.80 |
| Feb 2017     | 10.75 | 42.20 | 11.20 | 11.60 | 9.83 | 26.40 |
| Mar 2017     | 8.78 | 28.00 | 9.25 | 25.00 | 8.94 | 27.00 |
considered as well as the shadow influence with a relative scale of five categories (0; 25; 50; 75 and 100). The following parameters were recorded and calculated per reproductive shoot: length (L), nodes number (NN), nodes number/length (NN/L), total buds number (TBN), total buds number/nodes number (TBN/NN), mixed buds number (MBN), mixed buds number/length (MBN/L), mixed buds number/total buds number (MBN/TBN), aborted flowers (AFl), fruits number (FrN), fruits number/length (FrN/L) and fruit set (fruit number/flower number).

2.3. Statistical analysis

Results obtained were analyzed for each population/site and each year by ANOVA and Tukey Test. Pearson correlations between pairs of variables were also made.

3. Results

3.1. Growing conditions

Mean temperatures of FL population among August to March of 2014–2015 (7.2 °C) and 2015–2016 (6.7 °C) were higher than CI (6.6 °C and 6.3 °C for 2014–2015 and 2015–2016 growing seasons, respectively) and US (6.9 and 6.5 °C for 2014–2015 and 2015–2016 growing seasons, respectively) (Table 1). However mean temperatures in the 2016–2017 growing season were higher in US (7.8 °C) than in CI and FL sites (7.3 and 7.0 °C, respectively). It is noticeable the differences in mean temperatures of August and September among the growing seasons for the three sites. The warmest month in the three sites was January, with mean air daily temperatures of 10.0, 10.4 and 11.2 °C in FL site. At the same time, accumulated rainfalls in US among August to March were higher (403.3, 502.5 and 313.2 mm for the 2014–2015, 2015–2016 and 2016–2017 growing seasons, respectively) than FL (279.0, 134.3 and 155.8 mm for the mentioned growing seasons, respectively) and CI (145.6, 159.2 and 147.0 mm for the mentioned growing seasons, respectively).

3.2. Phenology

Phenology observations started in October of 2014. In US population, 82.7% of flower buttons were recorded on day 20, and this proportion was in continuous decline until November 18 with 10.0%. On the other hand, 57% of flowers with receptive stigma and 17.3% of flowers with anthesis were also registered on day 20. Maximum values of stigmatic receptivity and anthesis were observed on November 4. The first fruits formed (10.7%) were observed on November 25 (Fig. 1A). Blooming period for the US population in 2014 began before October 20, probably on day 18, full bloom was between November 2 to 27 and the end of bloom was on December 2 (Fig. 2). The same year for FL population, 39.2% of flower buds were registered on October 20, and the proportion was increased.
Fig. 1. Phenograms of *Berberis microphylla* grown in Ushuaia (US), Fagnano Lake (FL) and Central Area of Tierra del Fuego (CI) during 2014 (A–C) and 2015 (D–F) years.

Fig. 2. Blooming period expressed as starting, full and end bloom time in *Berberis microphylla* grown in Ushuaia (US), Fagnano Lake (FL) and Central Area of Tierra del Fuego (CI) during 2014 and 2015 years.
to November 4 with 60.8% of flower buds and in the same date anthesis phase was started. Maximum proportion of anthesis flower (72.5%) was observed on December 2 in coincidence with the first fruits formed (5%). Previously, on November 25 the maximum stigmatic receptivity (89.2%) had been registered (Fig. 1B). Blooming period for the FL population in 2014 began November 6, full bloom was between November 18 to December 10 and the end of bloom was on December 20 (Fig. 2). On the other hand, CI population showed 23% of flower buds on October 20 of 2014 and this value increased between November 4 and 18–76%. On November 18, 24% of anthesis flowers and 78% of stigmatic receptivity was observed. Anthesis flower was maxima on November 25 and on December 16 there was still 24% of anthesis flowers (Fig. 1C). Blooming period for the CI population in 2014 began November 12, full bloom was between November 21 to December 5 and the end of bloom was on December 18 (Fig. 2).

Phenology observations in the next year (2015) started in October too. In US population, 100.0% of flower buds were observed on October 20, followed by a continuous decrease until November 26 with 3.2%. Stigmatic receptivity and anthesis flowers were maxima on November 18 with 62.0% and 80.3%, respectively. On the same date 2.1% of started fruits were recorded (Fig. 1D). Blooming period for US population in 2015 began November 5, full bloom was between November 11 to 20 and the end of bloom was on December 10 (Fig. 2). In FL population, 58.33% of flower buttons were observed on October 20, followed by a continuous decrease until November 27 with 25.3%. There were observed maxima values for stigmatic receptivity on November 19 with 71.0% and anthesis flower on November 27 with 71.5%. Fruit started on December 7 with 17.3% (Fig. 1E). Blooming period for FL population in 2015 began November 10, full bloom was between November 22 to December 13 and the end of bloom was on December 22 (Fig. 2). In CI population registered were made in started blooming stages, nevertheless, on November 19, flower with stigmatic receptivity was 81.10%. Between November 27 and December 7 it was observed maxima value of anthesis flowers with 71.2%–68.2% and fruit started on November 27 with 6.3% (Fig. 1F). The beginning of blooming time could not been established but full bloom was calculated between November 12 to December 13 and the end of bloom was on December 22 (Fig. 2).

3.3. Reproductive shoots

Population significantly affected the length, node number, node number/length, total bud number/node number, mixed bud number, mixed bud number/length, mixed bud number/total bud number, aborted flowers, fruit number and fruit set (Table 2). Length and node number were maxima in FL population (18.8 cm and 17.5 nodes, respectively), while node number/length values were highest for US and CI populations (1.1 nodes/cm for both sites). Total bud number/node number was maxima for
Table 2. Reproductive shoots of *Berberis microphylla* plants taken from Ushuaia (US), Fagnano Lake (FL) and Central area of Tierra del Fuego (CI) populations along three consecutive growing seasons. I.- Effect of the population (Po), shoot orientation (Or), shoot position (Ps), shoot shadow (Sh) and growing season (GS) on the different variables studied: length (L), node number (NN), node number/length (NN/L), total bud number (TBN), total bud number/node number (TBN/NN), mixed bud number (MBN), mixed bud number/length (MBN/L), mixed bud number/total bud number (MBN/TBN), aborted flowers (AbFw), fruit number (FrN), fruit number/length (FrN/L) and fruit number/flower number (Fruit set).

| Population (Po) | L (cm) | NN (N°) | NN/L | TBN | TBN/NN | MBN | MBN/L | MBN/TBN | AbFw | FrN | FrN/L | Fruit set |
|----------------|-------|---------|------|-----|--------|-----|-------|---------|------|-----|-------|-----------|
| US             | 13.98b| 14.37b  | 1.10a| 22.73| 1.54b  | 10.44a| 0.85a| 0.45a   | 9.71a| 0.49b| 0.40a  | 4.79c     |
| FL             | 18.84a| 17.51a  | 0.98b| 22.01| 1.25c  | 8.35b | 0.46c| 0.36b   | 5.41c| 1.44a| 0.74a  | 15.06a    |
| CI             | 14.26b| 14.71b  | 1.08a| 24.01| 1.67a  | 9.73ab| 0.70b| 0.47a   | 6.85b| 1.07a| 0.79a  | 10.97b    |
| F              | 16.278| 11.109  | 4.305| 0.630| 12.933 | 5.166| 20.439| 3.097   | 15.705| 6.212| 2.631  | 8.316     |

| p | <0.001 | <0.001 | 0.014 | 0.533 | <0.001 | 0.005 | <0.001 | 0.046   | <0.001| 0.020 | 0.073  | <0.001   |
|---|--------|--------|-------|-------|--------|-------|--------|---------|--------|-------|--------|----------|

| Orientation (Or) | L (cm) | NN (N°) | NN/L | TBN | TBN/NN | MBN | MBN/L | MBN/TBN | AbFw | FrN | FrN/L | Fruit set |
|------------------|-------|---------|------|-----|--------|-----|-------|---------|------|-----|-------|-----------|
| East             | 15.62 | 15.61   | 1.05 | 22.97| 1.51   | 10.53| 0.71  | 0.43    | 7.94 | 1.11 | 0.72a | 10.67     |
| North            | 15.76 | 15.84   | 1.06 | 24.13| 1.53   | 10.54| 0.73  | 0.43    | 8.33 | 0.91 | 0.49a | 9.05      |
| West             | 15.10 | 15.44   | 1.07 | 22.67| 1.44   | 9.77 | 0.69  | 0.43    | 8.13 | 0.89 | 0.07a | 8.98      |
| South            | 15.36 | 15.07   | 1.05 | 22.08| 1.46   | 8.78 | 0.62  | 0.41    | 6.83 | 0.98 | 0.07a | 10.39     |
| F               | 1.174 | 0.753   | 0.727| 0.452| 0.557  | 0.295| 0.354 | 0.067   | 0.092| 1.015| 0.703  | 0.692     |

| p | 0.319 | 0.521 | 0.536 | 0.716 | 0.644 | 0.829 | 0.786 | 0.997 | 0.965 | 0.385 | 0.550 | 0.557 |

| Position (Ps) | L (cm) | NN (N°) | NN/L | TBN | TBN/NN | MBN | MBN/L | MBN/TBN | AbFw | FrN | FrN/L | Fruit set |
|---------------|-------|---------|------|-----|--------|-----|-------|---------|------|-----|-------|-----------|
| 0             | 15.21 | 15.20   | 1.06 | 20.81| 1.39   | 8.38 | 0.55b | 0.42b   | 7.33 | 0.95 | 0.064a | 10.55     |
| 1             | 15.52 | 15.66   | 1.06 | 23.88| 1.53   | 10.15| 0.65a | 0.43a   | 9.21 | 0.88 | 0.058a | 7.96      |
| F             | 0.817 | 0.575   | 0.005| 3.609| 2.337  | 6.777| 5.480 | 0.156   | 3.327| 2.553| 3.226  |

| p | 0.367 | 0.449 | 0.943 | 0.058| 0.127 | 0.411| 0.040 | 0.693   | 0.069| 0.111| 0.073  |

| Shadow (Sh) | L (cm) | NN (N°) | NN/L | TBN | TBN/NN | MBN | MBN/L | MBN/TBN | AbFw | FrN | FrN/L | Fruit set |
|-------------|-------|---------|------|-----|--------|-----|-------|---------|------|-----|-------|-----------|
| 0           | 14.30 | 14.80   | 1.07 | 26.04a| 1.75a  | 10.06a| 0.72a | 0.37    | 9.19a| 0.69ab| 0.073a | 7.50      |
| 25          | 15.11 | 15.97   | 1.11 | 24.18a| 1.54ab | 11.41a| 0.81a | 0.49    | 10.17a| 1.22a | 0.095a | 9.95      |

(continued on next page)
| Growing Season (GS) | L  | NN | NN/L | TBN | TBN/NN | MBN | MBN/L | MBN/TBN | AbFw | FrN | FrN/L | Fruit set |
|---------------------|----|----|------|-----|--------|-----|-------|---------|------|-----|-------|-----------|
|                     | cm | N° | N°/cm | N°/N° | N° | N°/cm | N°/N° | N° | N° | N°/cm | N°/N° | N° | N°/cm | N°/N° |
| 50                  |    |    |       |      |    |       |       |    |    |       |       |    |       |       |
|                     | 16.72 | 15.72 | 1.01 | 20.90b | 1.34ab | 7.88b | 0.51b | 0.39 | 7.09b | 0.72ab | 0.041b | 7.73 |
| 75                  |    |    |       |      |    |       |       |    |    |       |       |    |       |       |
|                     | 16.08 | 14.79 | 0.99 | 15.79c | 1.07b | 5.31c | 0.40b | 0.38 | 4.77c | 0.52b | 0.045b | 9.22 |
| F                  | 1.102 | 0.805 | 1.261 | 9.200 | 8.961 | 7.326 | 6.477 | 1.652 | 6.469 | 4.662 | 3.797 | 0.972 |
| p                  | 0.348 | 0.492 | 0.287 | <0.001 | <0.001 | <0.001 | <0.001 | 0.177 | <0.001 | 0.003 | 0.010 | 0.405 |
| Interactions        |    |    |       |      |    |       |       |    |    |       |       |    |       |       |
| PoxOrxPssSh(F)      | 0.102 | 0.356 | 0.391 | 0.121 | 0.061 | 0.292 | 0.330 | 1.900 | 0.176 | 3.715 | 2.924 | 4.236 |
| PoxOrxPssSh(p)      | 0.903 | 0.701 | 0.677 | 0.886 | 0.941 | 0.747 | 0.648 | 0.151 | 0.838 | 0.025 | 0.054 | 0.015 |
| PoxOrxPssGS(F)      | 1.356 | 0.140 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| PoxOrxPssGS(p)      | 0.256 | 0.936 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| OrxPxxShxGS(F)      | 0.391 | 0.577 | 1.015 | 0.215 | 0.061 | 0.044 | 0.276 | 0.158 | 0.142 | 1.178 | 0.822 | 0.674 |
| OrxPxxShxGS(p)      | 0.676 | 0.562 | 0.363 | 0.807 | 0.941 | 0.947 | 0.898 | 0.846 | 0.868 | 0.379 | 0.553 | 0.510 |

Values followed by different letters in each column are significant different according to the Tukey test at p < 0.05.
CI population (1.7), while mixed bud number for US population (10.4 mixed buds) was significantly higher than for FL population (8.3 mixed buds). However, mixed bud number/length was the highest in US population (0.8 mixed buds/cm), while mixed bud number/total bud number were maxima in US and CI populations (0.5). The highest aborted flower values were found on US population (9.7), in correspondence with the lowest fruit number (0.5 fruit). Fruit set was highest in FL population (15.1%) (Table 2).

Orientation did not affect the shoot variables studied, while the shoot position only significantly affected the mixed bud number/length and the mixed bud number/total bud number (Table 2). The reproductive shoots formed on the superior sector the highest mixed bud number/length (0.65 mixed buds/cm) and mixed bud number/total bud number (0.43).

Shadow significantly affected the total bud number, total bud number/node number, mixed bud number, mixed bud number/length, aborted flowers, fruit number and fruit number/length (Table 2). The total bud number was maximum under shadow of 0 and 25% (26.0 and 24.2 buds, respectively), while total bud number/node number was significantly higher with 0% shadow (1.7), respect to 75% shadow (1.1). The mixed bud number and mixed bud number/length were also maxima for 0 and 25% of shadow (10.1—11.4, and 0.7 to 0.8 mixed buds/cm, respectively), as well as aborted flowers (9.2 and 10.2, respectively). Fruit number was higher with 25% of shadow (1.2) than in 75% (0.5), while fruit number/length was maxima for 0 and 256% shadow (0.07 and 0.09 fruits/cm, respectively).

Growing season significantly affected the total bud number, total bud number/node number, mixed bud number, mixed bud number/length, mixed bud number/total bud number, aborted flowers, fruit number and fruit set (Table 2). Total bud number, total bud number/node number, mixed bud number, mixed bud number/length, mixed bud number/total bud number, aborted flowers and fruits were maxima during 2015—2016 growing season (24.7 buds, 1.5 buds/node, 11.2 mixed buds, 0.8 mixed buds/cm, 0.5 mixed buds/total bud, 10.3 aborted flowers and 1.4 fruits, respectively). However, maximum fruit set was observed on 2014—2015 growing season (14.9%), which was only significantly higher than in 2016—2017 (10.1%).

Significant interactions were found between factors for some of the studied variables. Indeed, differential increments were verified in the values between the main factors and combinations (Table 2). Total bud number was maxima in CI population in 2015, although was minima in 2016. Fruit number in FL population was maximum in 2015 and 2016; however, in 2017 this variable was maximum in CI population. Fruit set was the highest in FL population in 2015 and 2016 but in 2017 year, this variable was maximum in CI population (data no showed).
3.4. Reproductive shoots in relation to the plant

The plant significantly affected most of the studied variables, except the mixed bud number/total bud number, fruit number, fruit number/length and fruit set in US population and node number in FL population (Tables 3, 4, and 5).

US population showed maximum shoot length in plant 200 (19.9 cm), without significant differences with plant 123 (17.7 cm) (Table 3). Plants 122, 125, 126 and 202 showed significant shorter branches respect plants 200 and 123 (12.1–10.3 cm). Shoots of plants 109, 123 and 200 presented the highest node number (15.7, 16.8 and 16.22, respectively) and the plants 111 and 202 the minimum values. However, plant 126 presented the maxima node number/length (1.4 nodes/cm). While total bud number was highest in plant 109 (31.6), the maximum relation with the length were observed in plants 109 and 200 (1.8 and 1.9, total bud/cm respectively). Plant 122 presented the maximum mixed bud number (18.9), without significant differences with plants 109 and 200 (17.74 and 13.91 respectively) as well as the maximum mixed bud number/length (1.6), as well as plant 109 (1.25) respect to the others. On the other hand, mixed bud number/total bud number only were different between plants 122 and 125 (0.31 and 0.44 mixed bud number/total bud number, respectively). While aborted flower number was maximum in plant 122 (17.2) without significant differences with plants 109, 123 and 200; plants 111, 125 and 126 showed only 5.36, 5.71 and 5.43 aborted flower, respectively.

Plants in LF population showed maximum length in plant 183 (23.9 cm) but only significant longer than plants 81, 83 and 148 (15.12, 16.21 and 16.8 cm, respectively), while the reproductive shoots of plant 81 presented the highest node number/length (1.2 nodes/cm) (Table 4). If total bud number was maximum in plant 172 (29.2), the maximum relation with its node number was observed in plant 146 (1.6). This plant also presented the maximum mixed bud number (15.7), as well as the maxima mixed bud number/length (0.9 mixed buds/cm), mixed bud number/total bud number (0.6 mixed bud number/length) and aborted flower number (9.9). While the plant 172 formed 3.3 fruits and 0.2 fruits/cm, plant 83 presented a fruit set significantly higher (32.2) than plants 81, 82 and 183 (1.7, 6.4 and 3.9, respectively).

In CI population, the maximum length was observed in plant 78 (18.6 cm), while the reproductive shoots of plant 182 presented the highest node number and node number/length (17.1 nodes and 1.4 nodes/cm) (Table 5). Plant 78 had the maximum total bud number (33.5 buds), while plants 74 and 78 presented the maximum relation with respect the node number (2.1 and 2.3, respectively). Plants 78 and 180 presented the maximum mixed bud number (15.6 and 15.1, respectively), and then again plant 180 with the highest mixed bud number/length (1.0 mixed buds/cm), while the plant 171 the maximum mixed bud number/total bud number, fruit.
Table 3. Shoot characteristics of Berberis microphylla plants taken from Ushuaia (US) population along three consecutive growing seasons. II- Effect of the plant on the different variables studied: length (L), node number (NN), node number/length (BNN/L), total bud number (TBN), total bud number/node number (TBN/NN), mixed bud number (MBN), mixed bud number/length (MBN/L), mixed bud number/total bud number (MBN/TBN), aborted flowers (AbFw), fruit number (FrN), fruit number/lenth (FrN/L) and fruit number/flower number (Fruit set).

| Plants | US L cm | NN N°  | NN/L N°/cm | TBN N° | TBN/NN N°/N° | MBN N° | MBN/L N°/N° | MBN/TBN N° | AbFw N° | FrN N° | FrN/L N°/cm | Fruit set |
|--------|---------|--------|------------|--------|--------------|--------|-------------|------------|--------|-------|-------------|-----------|
| 109    | 13.74bc | 15.74a | 1.20b      | 31.56a | 1.77a        | 1.25a | 0.60ab      | 16.39ab    | 1.22   | 0.09  | 4.75        |
| 110    | 14.29bc | 15.38abc | 1.19b | 22.69abc | 2.01ab | 9.92cd | 0.68b | 0.57ab | 9.46bcd | 0.63 | 0.05 | 5.33        |
| 111    | 13.59bc | 11.68c | 1.11c      | 14.00d | 1.49b        | 5.77d | 0.44b       | 0.40ab     | 5.36d  | 0.09  | 0.01        | 0.95      |
| 121    | 13.94bc | 14.50abc | 0.87bc | 23.45abc | 1.13ab | 9.11cd | 0.66b | 0.38ab | 8.22cd  | 0.56 | 0.04 | 4.72        |
| 122    | 12.07c  | 14.57abc | 1.07b  | 25.92abc | 1.55ab | 18.86a | 1.58a | 0.31a  | 17.18a  | 0.36 | 0.03 | 1.80        |
| 123    | 17.68ab | 16.77a | 1.20b      | 26.14abc | 1.64ab | 11.45bcd | 0.68b | 0.66ab | 11.00abcd | 0.23 | 0.01 | 3.80        |
| 124    | 13.75bc | 13.58abc | 0.97bc | 21.55abcd | 1.51ab | 9.05cd | 0.66b | 0.46ab | 8.55cd  | 0.45 | 0.03 | 5.32        |
| 125    | 11.17c  | 12.12bc | 1.02b      | 16.89cd | 1.44ab | 6.00d  | 0.56b | 0.44b  | 5.71d   | 0.24 | 0.02 | 2.14        |
| 126    | 10.28c  | 13.90abc | 1.44a  | 16.77cd | .120b | 5.95d  | 0.65b | 0.38ab | 5.43d   | 0.48 | 0.04 | 12.47       |
| 149    | 14.37bc | 14.61abc | 1.23bc | 18.60cd | 1.51b | 8.00cd | 0.58b | 0.55ab | 7.25cd  | 0.58 | 0.05 | 5.24        |
| 200    | 19.87a  | 16.22a  | 0.86c      | 30.60ab | 1.89a | 13.91abc | 0.74b | 0.41ab | 13.22abc | 0.57 | 0.03 | 2.98        |
| 202    | 11.12c  | 11.75c  | 1.12b      | 20.58bcd | 1.72ab | 6.63cd | 0.58b | 0.33ab | 6.13cd  | 0.31 | 0.03 | 8.86        |
| F      | 7.009   | 4.392   | 8.486      | 6.220  | 4.642        | 8.486 | 12.553      | 7.777     | 1.568  | 1.710  | 1.290       |
| p      | <0.001  | <0.001  | <0.001     | <0.001 | <0.001       | <0.001 | <0.001      | 0.063     | 0.001  | 0.109  | 0.058       | 0.231     |

Values followed by different letters in each column are significant different according to the Tukey test at p < 0.05.
Table 4. Shoot characteristics of *Berberis microphylla* plants taken from Fagnano Lake (FL) population along three consecutive growing seasons. Effect of the plant on the different variables studied: length (L), node number (NN), node number/length (BNN/BL), total bud number (TBN), total bud number/node number (TBN/NN), mixed bud number (MBN), mixed bud number/length (MBN/L), mixed bud number/total bud number (MBN/TBN), aborted flowers (AbFw), fruit number (FrN), fruit number/length (FrN/L) and fruit number/flower number (Fruit set).

| Plants | L     | NN    | NN/L  | TBN   | TBN/NN | MBN   | MBN/L  | MBN/TBN | AbFw  | FrN   | FrN/L  | Fruit set |
|--------|-------|-------|-------|-------|--------|-------|--------|---------|-------|-------|--------|-----------|
| 81     | 15.12c| 17.12 | 1.19a | 17.54bc| 1.01c  | 5.47d | 0.41bcd| 0.28bc  | 4.00bc| 0.12c | 0.01c  | 1.68b     |
| 82     | 18.09abc| 17.14| 0.97bc| 22.47abc| 1.27abc| 5.48d | 0.31cd | 0.26bc  | 3.95c | 0.38bc| 0.03bc | 6.38b     |
| 83     | 16.21bc| 16.58| 1.09ab| 19.69abc| 1.19abc| 7.32bcd| 0.40bcd| 0.38abc | 4.42abc| 2.74ab| 0.14abc| 32.19a    |
| 84     | 20.10abc| 18.74| 0.98bc| 22.38abc| 1.23abc| 5.84cd| 0.30cd | 0.25bc  | 3.89c | 0.95abc| 0.04bc | 14.34ab   |
| 85     | 17.33abc| 17.89| 1.09ab| 19.00abc| 1.01c  | 4.89d | 0.30cd | 0.32bc  | 3.33c | 0.78bc| 0.04abc| 16.22ab   |
| 86     | 19.27abc| 15.89| 0.86cd| 19.67abc| 1.25abc| 4.17d | 0.25d  | 0.22c   | 2.67c | 0.67bc| 0.03bc | 12.97ab   |
| 87     | 22.12ab| 19.63| 0.92bde| 22.69abc| 1.17abc| 6.17cd| 0.30cd | 0.27bc  | 4.96abc| 1.21abc| 0.06abc| 22.01ab   |
| 146    | 17.83abc| 16.83| 0.96bc| 27.63abc| 1.63a  | 15.75a| 0.87a  | 0.60a   | 9.88a | 2.08abc| 0.11abc| 11.69ab   |
| 148    | 16.79bc| 16.58| 1.04abc| 17.44abc| 1.10bc | 9.58abcd| 0.60abc| 0.50ab  | 4.96abc| 2.38abc| 0.15abc| 22.40ab   |
| 172    | 20.40abc| 18.60| 0.95bc| 29.25a  | 1.55ab | 12.90ab| 0.65ab | 0.44abc | 9.55ab | 3.35a  | 0.18a  | 22.19ab   |
| 183    | 23.95a | 17.05| 0.73d  | 15.00c  | 0.87c  | 5.86cd | 0.26d  | 0.33abc | 4.00bc | 0.29c  | 0.02c  | 3.86b     |
| 184    | 17.43abc| 17.96| 1.07ab| 27.87abc| 1.55ab | 12.22abc| 0.71ab | 0.34abc | 6.74abc| 1.52abc| 0.10abc| 13.12ab   |
| F      | 3.422  | 1.210| 7.682  | 4.220  | 6.587  | 8.043  | 11.151 | 4.627   | 4.189 | 4.235 | 4.405 | 3.113     |
| p      | <0.001 | 0.281| <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001  | <0.001 | <0.001 | <0.001 | <0.001     |

Values followed by different letters in each column are significant different according to the Tukey test at p < 0.05.
Table 5. Shoot characteristics of *Berberis microphylla* plants taken from Central area of Tierra del Fuego (CI population) along three consecutive growing seasons. II- Effect of the plant on the different variables studied: length (L), node number (NN), node number/length (BNN/L), total bud number (TBN), total bud number/node number (TBN/NN), mixed bud number (MBN), mixed bud number/length (MBN/L), mixed bud number/total bud number (MBN/TBN), aborted flowers (AbFw), fruit number (FrN), fruit number/length (FrN/L) and fruit number/flower number (Fruit set).

| CI   | L     | NN   | NN/L | TBN   | TBN/NN | MBN  | MBN/L | MBN/TBN | AbFw | FrN | FrN/L | Fruit set |
|------|-------|------|------|-------|--------|------|-------|---------|------|-----|-------|-----------|
| 72   | 11.62bc | 13.63cde | 1.19ab | 19.63bc | 1.57ab | 8.29b | 0.74ab | 0.52abc | 6.29abc | 0.71bc | 0.07b | 8.12bc |
| 73   | 15.79ab | 16.88ab | 1.12b  | 19.80bc | 1.18b  | 10.71ab | 0.75ab | 0.58ab  | 7.13abc | 0.92abc | 0.07b | 7.03abc |
| 74   | 12.12bc | 13.39de | 1.11b  | 27.33ab | 2.12a  | 7.21b  | 0.63bc | 0.32c   | 4.54bc  | 1.13abc | 0.10ab | 17.32abc |
| 77   | 11.25c  | 12.10e  | 1.06bc | 22.00bc | 1.69ab | 6.73b  | 0.56bc | 0.42bc  | 5.36abc | 0.82bc  | 0.07b | 10.76bc |
| 78   | 18.65a  | 14.09bcde | 0.78d  | 33.47a  | 2.28a  | 15.61a | 0.82ab | 0.46abc | 10.78a  | 1.39abc | 0.08b | 9.95abc |
| 150  | 13.35bc | 14.3abcde | 1.09bc | 23.80abc | 1.73ab | 9.15b  | 0.73ab | 0.43bc  | 8.10abc | 0.95abc | 0.08b | 10.21abc |
| 171  | 12.50bc | 12.07e  | 1.04bc | 12.71c  | 1.19b  | 7.31b  | 0.65bc | 0.70a   | 3.44c   | 2.31a   | 0.20a | 27.15a  |
| 180  | 15.12abcd | 15.67ab | 1.09bc | 26.50ab | 1.72ab | 15.13a | 1.01a  | 0.58ab  | 9.58ab  | 1.67ab  | 0.11ab | 11.39b  |
| 181  | 18.52abc | 16.38ab | 0.91cd | 19.21bc | 1.17b  | 6.61b  | 0.38c  | 0.42bc  | 5.00bc  | 0.22c   | 0.02b | 4.67c   |
| 182  | 13.04bc | 17.13a  | 1.37a  | 29.00ab | 1.77ab | 9.54b  | 0.74ab | 0.40bc  | 7.29abc | 0.92abc | 0.07b | 8.02abc |
| F    | 8.852   | 8.494   | 13.401 | 5.139   | 5.499  | 9.037 | 5.348  | 3.305   | 3.248   | 3.046   | 2.876 | 3.300   |
| p    | <0.001  | <0.001  | <0.001 | <0.001  | <0.001 | <0.001 | <0.001 | <0.001  | <0.001  | <0.001  | <0.001 | <0.001  |

Values followed by different letters in each column are significant different according to the Tukey test at p < 0.05.
number, fruit number/length and fruit set were observed (0.7 mixed buds, 2.3 fruits, 0.2 fruits/cm and 27.1 fruits/flowers, respectively).

Although some correlations are predictable as length with node number, it is noteworthy the negative and significant correlation between total bud number, mixed bud number/length and aborted flowers with shadow (Table 6). The same variables were positive and significantly correlated with the shoot position, showing that those reproductive shoots formed in the superior part of the shrubs presented the highest values of the mentioned variables.

4. Discussion

It is well known that flowering is a characteristic of the species, but the vigor of the bushes, the formation of buds and different management applied to the garden are decisive in the production of flowers and fruits. Nevertheless, with native species that grow spontaneously, it is discarded the effect of crop management; therefore, the flowering phenology can be considered as an expression closely linked to the genotype when studying plants grown in similar sites.

Intensity of flowering increase with the duration of the cold treatment but in this case, low temperature effects could be dismissed by the large amount of cold hours accumulated in the three populations. Starting time and blooming period is determined by the weather conditions occurred before and during the flowering time (Soltész, 1996). In fact, starting time and blooming time period was significant different between the three populations and two years studied due the particular temperature, humidity and cumulative rainfall registered from every specific site selected (Arena et al., 2018). It is worth noting the differences in temperatures between the sites and the years at the end of winter and beginning of the spring and its relation with the reproductive phenology, i.e. the highest mean temperatures among August and October on 2014 in US site probably advanced the button flower and anthesis phases respect to FL and CI sites. The same tendency was observed in US and FL sites at the end of winter and beginning of the spring in 2015 and its relation with the reproductive phenology, although the reproductive phenology in CI site presented an unexpected behavior. Full bloom was shorter in US respect to FL and CI populations when the temperatures increased gradually as occurred in 2014 year.

On the other hand, rainfall patterns affect the phenology (Fischer et al., 2016) and reproductive behavior of many fruits species. Specially, in those species pollinated by insects such as B. microphylla (Radice et al., 2016c), as well as snowmelt and early high spring temperatures (Forrest and Miller-Rushing, 2010), which could be responsible for the advance of the floral phenology, in particular the bottom flower and anthesis phases how it was observed in US population.
Table 6. Pearson correlations between different pairs of variables.

|       | L   | NN  | NN/L | TBN  | TBN/NN | MBN  | MBN/L | MBN/TBN | Position | Shadow | AbFw | FrN  | FrN/L | Fruit set |
|-------|-----|-----|------|------|--------|------|-------|---------|----------|--------|------|------|------|-------|-----------|
| L     | –   | 0.806** | –0.620** | 0.423** | –0.101* | 0.219** | –0.257** | –0.088* | 0.023 | 0.129** | 0.130** | 0.151** | –0.038 | 0.028 |
|       | ≤0.001 | ≤0.001 | ≤0.001 | ≤0.001 | ≤0.001 | 0.048 | 0.607 | 0.003 | ≤0.001 | ≤0.001 | 0.297 | 0.440 |
| NN    | 0.806** | –   | 0.145** | 0.518** | –0.124** | 0.282** | –0.117** | –0.030 | 0.048 | 0.019 | 0.189** | 0.183** | 0.008 | 0.042 |
|       | ≤0.001 | ≤0.001 | ≤0.001 | ≤0.001 | ≤0.001 | 0.511 | 0.285 | 0.675 | ≤0.001 | ≤0.001 | 0.825 | 0.255 |
| NN/L  | –0.620** | –0.145** | –   | –0.115 | –0.015 | –0.071 | 0.231 | 0.047 | –0.002 | –0.113 | –0.032 | –0.053 | 0.059 | 0.005 |
|       | ≤0.001 | ≤0.001 | 0.010 | 0.741 | 0.052 | ≤0.001 | 0.300 | 0.959 | 0.011 | 0.384 | 0.384 | 0.105 | 0.895 |
| TBN   | 0.423** | 0.518** | –0.115 | –   | 0.746** | 0.541** | 0.278** | –0.055 | 0.145** | –0.297** | 0.544** | 0.154** | 0.028 | –0.070 |
|       | ≤0.001 | ≤0.001 | 0.010 | ≤0.001 | ≤0.001 | ≤0.001 | 0.221 | 0.001 | ≤0.001 | ≤0.001 | 0.001 | 0.528 | 0.119 |
| TBN/NN| –0.101* | –0.124** | –0.015 | 0.746** | –   | 0.334** | 0.337** | –0.081 | 0.121** | –0.347 | 0.350** | 0.038 | 0.032 | –0.102* |
|       | 0.024 | 0.005 | 0.741 | ≤0.001 | ≤0.001 | 0.072 | 0.007 | ≤0.001 | ≤0.001 | 0.001 | 0.395 | 0.476 | 0.023 |
| MBN   | 0.219** | 0.282** | –0.071 | 0.541** | 0.334** | –   | 0.820** | 0.729** | 0.023 | –0.028 | 0.683** | 0.406** | 0.292** | 0.112* |
|       | ≤0.001 | ≤0.001 | 0.052 | ≤0.001 | ≤0.001 | ≤0.001 | 0.513 | 0.528 | ≤0.001 | ≤0.001 | 0.001 | 0.013 |
| MBN/L | –0.257** | –0.117** | 0.231 | 0.278** | 0.337** | 0.820** | 0.783** | 0.095** | –0.230* | 0.752** | 0.251** | 0.295** | –0.030 |
|       | ≤0.001 | 0.001 | 0.001 | ≤0.001 | ≤0.001 | ≤0.000 | 0.032 | ≤0.001 | ≤0.001 | ≤0.001 | 0.001 | 0.401 |
| MBN/TBN| –0.088* | –0.030 | 0.047 | –0.055 | –0.081 | 0.729** | 0.783** | –   | 0.023 | –0.028 | 0.683** | 0.406** | 0.422** | 0.112* |
|       | 0.048 | 0.511 | 0.300 | 0.221 | 0.072 | ≤0.001 | ≤0.001 | 0.613 | 0.528 | ≤0.001 | ≤0.001 | 0.001 | 0.013 |
| Position| 0.023 | 0.048 | –0.002 | 0.145** | 0.121** | 0.023 | 0.095* | 0.023 | 0.023 | –0.344** | 0.126 | –0.200 | –0.026 | 0.008 |
|       | 0.607 | 0.285 | 0.959 | 0.001 | 0.007 | 0.613 | 0.032 | 0.613 | 0.001 | 0.004 | 0.645 | 0.554 | 0.854 |
| Shadow| 0.129** | 0.019 | –0.113 | –0.297** | –0.347 | –0.028 | –0.230* | –0.028 | –0.344** | –   | –0.201 | –0.043 | –0.057 | –0.184 |
|       | 0.003 | 0.675 | 0.111 | ≤0.001 | ≤0.001 | 0.528 | ≤0.001 | 0.528 | ≤0.001 | ≤0.001 | 0.327 | 0.200 | ≤0.001 |
| AbFw  | 0.130** | 0.189** | –0.032 | 0.544** | 0.350** | 0.683** | 0.752* | 0.683** | 0.126 | –0.201 | –   | 0.094** | 0.042 | 0.008 |
|       | ≤0.001 | ≤0.001 | 0.384 | ≤0.001 | ≤0.001 | ≤0.001 | ≤0.001 | 0.004 | ≤0.001 | ≤0.001 | 0.009 | 0.247 | 0.854 |
| FrN   | 0.151** | 0.183** | –0.053 | 0.154** | 0.038 | 0.406** | 0.251** | 0.406** | –0.200 | –0.043 | 0.094** | –   | 0.920** | 0.712** | 0.743** |
|       | ≤0.001 | ≤0.001 | 0.384 | 0.001 | 0.395 | 0.001 | 0.001 | 0.645 | 0.327 | 0.009 | ≤0.001 | ≤0.001 |
| FrN/L | –0.038 | 0.008 | 0.059 | 0.028 | 0.032 | 0.292** | 0.295** | 0.422** | –0.026 | –0.057 | 0.042 | 0.920** | 0.743** |
|       | 0.297 | 0.825 | 0.105 | 0.528 | 0.476 | ≤0.001 | ≤0.001 | ≤0.001 | 0.554 | 0.200 | 0.247 | ≤0.001 | ≤0.001 |
| Fruit set | 0.028 | 0.042 | 0.005 | –0.070 | –0.102* | 0.112* | –0.030 | 0.112* | 0.008 | 0.008 | –0.184 | 0.712** | 0.743** |
|       | 0.440 | 0.255 | 0.895 | 0.119 | 0.023 | 0.401 | 0.013 | 0.854 | 0.854 | ≤0.001 | ≤0.001 | ≤0.001 |

* and ** of Pearson correlations are significant at p < 0.05 and p < 0.01, respectively.
According to these results it is relatively easy to detect a correlation between some climate variable and a particular phenological response; but it does not demonstrate that the climate variable in question is the proximate cue regulating phenology (Forrest and Miller-Rushing, 2010). Phenotypic plasticity was greater in physiological than in morphological traits in species from areas with high irradiation levels (Zununegui et al., 2009). In addition, it has also been shown that this species is able to flower and fructify in warm temperate climates (Radice et al., 2018a). Precedent studies confirmed that it was not noticeable a clear relationship between plant floral traits and reproductive success with its particularly environmental conditions (Arena et al., 2018). In fact, mixed bud number did not seem to be affected by the climatic conditions of the site, i.e. the rainfall, due to the mixed bud number/shoot length was the highest in US population where the rainfall was maximum, followed by the CI population where the rainfall was minimal. However, warm and dry conditions during bloom have a positive effect in pollination and fertilization of different fruit species (Nyéki and Soltész, 1996), and could be responsible in part of the high fruit set obtained in B. microphylla in FL and CI population where spring and summer are dryer with a slightly higher temperature than US site. Then, those reproductive shoots with a high fruit number will be a strong sink in competence with the new shoots in active grow where the flower buds must be differentiated for the next year’s fruit production (Arena and Radice, 2014). However, if the competence for carbohydrates and growth regulators between reproductive shoots and the new ones is noticeable, it is expected that the level of bud induction will be poor and the number of differentiated mixed buds low, as was found in the FL population. There is evidence that the inhibitory effect of fruit load on flowering is due to GA export from the seeds as occurs in Malus domestica (Wilkie et al., 2008), as well as the high demand of fruits for carbohydrates affect the flower buds induction (Reig et al., 2006). 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). In B. microphylla, fruit set varied from 4.8 to 15.3% among sites, nonetheless, when hand cross pollination was useful in plants grown in US site, value of fruit set was double indicating that from anthesis to start fruit growth there are many factors that prevent pollination and fertilization of flowers (Radice and Arena, 2016b).

Allograpta, Carposcalis (ex Platycheirus) and Syrphus genus were recognized by Radice et al. (2018b) as specific syrphids pollinators of B. microphylla. Previous studied showed that Carposcalis was more efficient as pollinator because it had a longer proboscis that allowed it to reach the base of the flower and for being less demanding with temperatures (Suárez, 2015). The proportion of insects per species was variable from year to year. In particular, Carposcalis was abundant in 2014 year but very scarce in 2015 year so this fact could be another factor that conditioned the fruit set.
Analyzing all the variables studied highlights that plant 122 of US population grown with 31.25% of shadow and 40% of productive area presented maxima values of mixed buds number/total buds (0.31), mixed buds number/length (1.6 mixed buds/cm), with 0.36 fruits but one of the lowest fruit set (1.8) observed.

Plant 146 of FL population, a shrub grown with 50% of shadow and 40% of productive area, presented the maxima values of mixed buds number/total buds (0.60), mixed buds number/length (0.87 mixed buds/cm), with 2.1 fruits and a medium fruit set relation (11.7).

Finally, in CI population, plant 171 grown with 87.5% of shadow and 40% of productive area, presented maximum mixed buds number/total buds (0.70), although the highest mixed buds number/length (1.0 mixed bud/cm) was observed in plant 180 (with a 18.7% of shadow and 70% of productive area). The maximum fruit number and fruit set were observed again in plant 171 (2.3 and 27.1%, respectively).

5. Conclusions

Evolution of the different reproductive phenological phases of *Berberis microphylla* was in accordance with the climatic conditions of the sites and the years, in particular with the temperatures of the end of the winter and beginning of the spring.

Previous studied confirmed that a clear relationship between plant floral traits and reproductive success with its particularly environmental conditions was not observed, but results obtained in this work clearly showed that shadow despite promoting fewer total bud number, total bud number/node number, mixed bud number, mixed bud number/length, aborted flowers, fruit number and fruit number/length. However, fruit set was not affected by the different levels of shadow tested.

Adaptive plasticity of *B. microphylla* respect to the adjustment of its morphological and physiological characteristics to the environmental conditions of growth are reinforced with the presented results.

These results contribute to the *in situ* conservation of this species in its natural environment where other biotic factors interact with it, as well as to its breeding and subsequent use in commercial plantations.

Declarations

Author contribution statement

Silvia Radice, Miream Arena: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.
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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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