Scientia Horticulturae 218 (2017) 63–71

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Changes in the phenolic compounds and antioxidant capacity of *Berberis microphylla* G. Forst. berries in relation to light intensity and fertilization

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ARTICLE INFO

Article history:
Received 31 October 2016
Received in revised form 30 January 2017
Accepted 1 February 2017

Keywords:
Total monomeric anthocyanins
Flavonoids
Total polyphenols
DPPH activity
Reducing power
Barberry
Patagonia

ABSTRACT

The objective of this study was to evaluate the qualitative and quantitative composition of the phenolic compounds and the antioxidant activity of *B. microphylla* fruit under different light intensities and levels of fertilization during two production years. Total monomeric anthocyanin concentration in high light intensity conditions was three times more than that obtained at medium light intensity, expressed for both fresh weight and dry weight terms (299.7 mg/100 g FFW and 9.5 mg/g DFW, respectively). However, in the case of total polyphenol concentration, minor increases of 30 and 11% appeared under high light intensity in comparison with medium light intensity (906.6 mg/100 g FFW and 30.7 mg/g DFW, respectively). Scavenging activity on DPPH was increased from 56.0 to 66.8% under the high light intensity treatment. This was also true for the reducing power which increased from 40 to 46.2%. Fertilizers reduced the flavonoid concentration, but increased the total polyphenol concentration at fertilization level 2. In the case of flavonoids, the maximum contents in fruits were 200.2 and 7.6 mg (+)-catechin equivalents/100 g FFW and DFW, respectively in the control treatment. The total polyphenol concentration was the highest (856.1 and 31.2 mg tannic acid equivalents/g FFW and DFW, respectively) with the level fertilization 2. The scavenging activity on DPPH was from 60.3 to 62.8% when raising the fertilization level from 0 to 2, while the reducing power varied between 40.5 and 44.3% at levels 1 and 2. The total monomeric anthocyanin concentration increased with the level of fertilization in the fruits of plants at high light intensity, whereas the trend was inversed in those under medium light intensity. Hence, these studies display the possibility for maximizing both the productivity and the antioxidant capacity of fruits by crop management.

1. Introduction

Phenolic compounds constitute one of most important groups of bioactive compounds in plants. In fruits and berries, the flavonoids are important determinants of quality and economic value because of their effect on color, aroma, astringency and antioxidant properties (He and Giusti, 2010). The major flavonoid compounds present in flowers and fruits are the flavonols, anthocyanins and proanthocyanins. Anthocyanins are the primary determinant of plant colors and they serve as visual signals for pollinator insects in flowers and as seed dispersers in ripe fruits. Flavonols have a role in photoprotection against ultraviolet radiation; they also act as free-radical scavengers. Proanthocyanins can offer protection against herbivory and pathogen attack during the early stages of fruit development because of their astringent nature (Zoratti et al., 2014). Knowledge of the chemical composition of the fruit, particularly the qualitative and quantitative content of phenolics as well as their antioxidant activity as influenced by environmental factors, is a relevant subject. They are valued due to the functional properties of secondary metabolites and also because their content constitutes a useful indication for determining the optimal harvest time (Vicente et al., 2009; Etienne et al., 2013).

Genetic and epigenetic factors, i.e. fruit ripening stages, environmental conditions during fruit growth and cultural practices all influence the synthesis of fruit phenolic compounds (Kähkönen et al., 2001; Ferreyra et al., 2007; Zoratti et al., 2014), and their antioxidant capacity (Roussos et al., 2009). For instance, in blueberries (*Vaccinium* spp.) the role of ontogeny is even more important,

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http://dx.doi.org/10.1016/j.scienta.2017.02.004
0304-4238/

e.g. when immature berries are to be used for the juice industry (Çelik et al., 2008). Also, the microclimate around the bushes must be taken into account when defining management practices of the aerial parts of the plants. Sun exposure of leaves determines the photosynthetic efficiency, while sun exposure of fruits can affect their quality as determined by several traits, such as the contents of total titratable acidity, soluble solids, color and anthocyanins, aroma and flavor (Vance et al., 2013). Reduction of light intensity and changes in its quality through the use of shade cloths provides a valid strategy for delaying fruit ripening in some species (Rylski and Spigelman, 1986a,b; Marini et al., 1991). It is also known that in different plant species, biosynthesis of anthocyanins is influenced by a number of environmental factors, such as exposure to sunlight, UV radiation, temperature and water availability, producing qualitative and quantitative changes in this group of phenolic compounds. Some fruits need light for anthocyanin synthesis, whereas anthocyanins can accumulate with or without light in others, e.g. fruits of *Vitis vinifera* (He et al., 2010).

Temperature is another factor affecting the synthesis of anthocyanins. Temperatures near to 25 °C promote the synthesis of anthocyanins in *Vitis*, whereas temperatures around 35 °C affect their accumulation and favor their degradation. However, high night temperatures inhibit the accumulation of anthocyanins (He et al., 2010), which highlights the role of the daily temperature range in the accumulation of these compounds.

On the other hand, nutritional status and biotic stress play a significant role in the accumulation of flavonoids in fruits (Zoratti et al., 2014). The effect of fertilizer application on several parameters of fruit quality has been cited, being very inconsistent and depending on the species and doses of nutrients provided. The contribution of fertilizers did not affect the antioxidant capacity of fruits of *Fragaria x ananassa* (World and Opstad, 2007) and *Vitis vinifera* (Abd El-Razek et al., 2011); however fertilizers affected the antioxidant capacity in *Fragaria x ananassa* according to the results of Wang and Lin (2003). Fertilizer applications also influenced the anthocyanin content of *Vitis vinifera* fruits (He et al., 2010).

The phenolic content in *Berberis* has been previously determined in leaves (Koncic et al., 2010), roots (Surveswaran et al., 2007; Tomosaka et al., 2008) and fruits (Fredes, 2009; Ruiz et al., 2010, 2013, 2014). However, the role of epigenetic factors on the secondary metabolism on *B. microphylla* fruits has not yet been studied. The objective of this study was to evaluate the qualitative and quantitative composition of the phenolic compounds and the antioxidant activity of *B. microphylla* fruit under different light intensities and levels of fertilization during two production years. The hypothesis to be tested is whether the qualitative-quantitative composition of the phenolic compounds and antioxidant activity of the fruits of *B. microphylla* were affected by light intensity together with the temperature, level of fertilization and production year.

2. Materials and methods

2.1. Plant material and growing conditions

Plants of *Berberis microphylla* G. Forst. were obtained by rhizome propagation (Arena and Martínez Pastur, 1995; Arena et al., 1998) from a natural population near to Ushuaia, Tierra del Fuego (54° 48′ LS, 68° 19′ LO, 30 m.a.s.l.).

In October 2006, two years old plants with one shoot of 8.5 ± 0.9 cm in length were put into plastic pots (12 L) containing substrate (7 kg) obtained from the site where the plants were collected. The soil had a loam sandy texture; with a pH of 5.2 ± 0.03, 13.9 ± 0.8% organic matter, 0.3% ± 0.03 total nitrogen, 35.8 ± 0.7 ppm available phosphorous, 117.0 ± 10.1 ppm available potassium and a water field capacity of 43.2 ± 3.4% ($n = 3$).

Three levels of light intensity were tested, i.e. 100%, 57% and 24% of the natural irradiance. The two latter were obtained using a micro tunnel covered with one or two layers of commercial black shade cloth, respectively. To set up this system, light intensities studied in previous years were considered. The shade of the plant reduced the light irradiation from 100% at the top of the plant, to 35–40% ($n = 15$) at half of the shrub height. Light intensity at a height of 0.80 m from the soil was measured at midday on days with a clear sky using a Luximeter (Tenmars Lux/FC Light meter TM 201). For the period between December and the middle of January the average value for the photon flux density (2197 ± 498 μmol m^{-2} s^{-2}) near to Ushuaia during the growing seasons studied was comparable with those of previous years reported by Martínez Pastur et al. (2007).

The air and soil (15 cm deep) temperatures and air relative humidity were recorded every hour by sensors (HOBO 8K). Soil moisture was determined using an Aquaterr Soil Moisture M-300 Instrument and the values varied between 70 and 87% of field capacity.

Fertilizers used were ammonium nitrate (NH_4NO_3), calcium superphosphate ($Ca(H_2PO_4)_2$) and potassium sulphate (K_2SO_4) in a N:P:K ratio of 1:0.6:1.2 at three levels: 0.00 (0), 3.36 (1) and 6.72 (2) g per plant. Fertilizers were divided into equal parts (0.00, 1.68 and 3.36 g) at the beginning of full flowering and during the fast growth of shoots, according to the previous experiments (Arena and Vater, 2003).

2.2. Sampling and determinations

All the fruits of the plants of each treatment ($n = 30$ plants) were harvested, with the exception of plants from low light intensity treatments which gave insignificant yields. Each sample ($n = 3$) consisted of all the fruits from 10 plants. A three factor analysis was performed considering the light level (high and medium), the fertilization level (0, 1 y 2) and the production year 1 and 2 (2008/09 and 2009/10, respectively). Fruit samples were frozen for later study of their phenolic profile and antioxidant activity, which was performed as described in the following section.

2.2.1. Total monomeric anthocyanins

Total monomeric anthocyanins were quantified by the pH differential method as described by Arena et al. (1998, 2012). Values were expressed in both terms of mg anthocyanin/100 g fresh fruit weight (FFW) and mg anthocyanin/g dry fruit weight (DFW).

2.2.2. Total polyphenols

Total polyphenols were quantified as reported by Makkar et al. (1993). Samples ($n = 3$, 3 g each one) were extracted for 24 h in 30 mL 80% MeOH-H_2O at 4 °C. Aliquots (15 μL) were adjusted to 500 μL with deionized water, and then 250 μL of 50% of the Folin-Ciocalteu reagent (Sigma-Aldrich) and 1.25 mL of 20% (w/v) aqueous sodium carbonate solution were added. After 40 min standing at 24 °C, the absorbance at 725 nm was measured. A calibration curve was prepared using tannic acid (Sigma) and the results were expressed as both, mg tannic acid equivalents/100 g FFW and mg tannic acid equivalents/g DFW.

2.2.3. Individual phenolic compounds

Individual phenolic compounds were extracted ($n = 3$, 3 g each one) by stirring the samples in methanol and hexane in a ratio of 0.2 g: 2 mL: 0.5 mL, at 200 rpm and 25 °C. The methanol: hexane mixture allows the solubilization of phenolic compounds to be determined, and avoids interference by non-phenolic non-polar molecules because they remain in the hexane phase. Then, extracts were centrifuged at 13,000 rpm for 10 min. The alcoholic fraction was taken to dryness in a rotary evaporator and resuspended in 200 μL of methanol (modified method Torres et al., 2005), and then

the analysis by HPLC on an Agilent 1100 system was performed. A ZORBAX Eclipse XDB -C18 with a pore size 80 Å, surface area 180 m^2/g, limit temperature 60 °C, range pH 2.0–9.0, end capped Double, Carbon Load 10%, USP designation L1 was used, with a flow of 1 mL/ min and 5 μL aliquot samples were injected. Detection was performed with MWD Agilent 1100 N° G1365 at different wavelengths (270, 280, 254 and 220 nm). Run solvents were a) water: acetic acid (99.9: 0.1) and b) acetonitrile: acetic acid (acid 99.9:0.1). The ramp was designed to start from 15.0% B to 36.5% B at 30 min. Patterns (Sigma) used for identification and calibration given in order of increasing retention time were: gallic acid, chlorogenic acid, catechin hydrate, rutine, feluric acid, quercetin, naringenin and kaempferol.

2.3. Methanolic extracts

Fruit methanolic extracts were obtained to study the flavonoids, the DPPH radical scavenging activity and the reducing power. Samples ($n = 3$, 3 g each one) were extracted in 30 mL methanol at 25 °C for 24 h with continuous stirring. The residue was extracted twice with additional volumes of 30 mL methanol for 24 h each time and the three extracts were combined. The extraction yield values were determined gravimetrically.

2.3.1. Flavonoids in methanolic extracts

Total flavonoids were measured by the aluminum chloride colorimetric assay as described by Arena et al. (2012). A calibration curve was prepared with (+)-catechin (Sigma-Aldrich). Results were expressed as both mg (+)-catechin equivalents/100 g FFW and mg (+)-catechin equivalents/g DFW.

2.3.2. DPPH radical scavenging activity in methanolic extracts

Scavenging activity on DPPH radicals was measured following the method used by Arena et al. (2012). A low absorbance indicates a high antioxidant activity. Scavenging activity on DPPH radicals was calculated as follows:

$$\mathrm{DPPH scavenging activity (RS\%)} = [(A_{blank} - A_{sample}/A_{blank}) \times 100]$$

Where A_{blank} is the absorbance of the control reaction (containing all reagents except for the test compound) and A_{sample} is the absorbance of the reaction mixture for the compound scavenging effect. Anthocyanin interference was corrected by subtracting the absorbance of the fruit extracts (in absence of DPPH) (Sigma-Aldrich) of all A_{sample} values. The EC_{50} value (mg/mL) was obtained by interpolation from linear regression analysis.

2.3.3. Reducing power in methanolic extracts

Reducing power was determined according to Arena et al. (2012). A high absorbance indicates a high reducing power. Reducing power values were obtained with the following equation:

$$\mathrm{Reducing power (\%)} = [100 - (A_{maximum} - A_{sample}/A_{maximum}) \times 100]$$

Where $A_{maximum}$ is the maximum absorbance value obtained with the color solution developed by the reaction of ferricyanide salt with the compound under testing, and A_{sample} is the absorbance value obtained with the color solution developed by the reaction of ferricyanide salt with the compound under testing for each concentration.

2.4. Statistical analysis

Data were analyzed by two and three-way ANOVA and the means were separated with the Tukey test ($\alpha = 0.05$). Linear regression analysis was performed using Statgraphics Plus 5.1 Software.

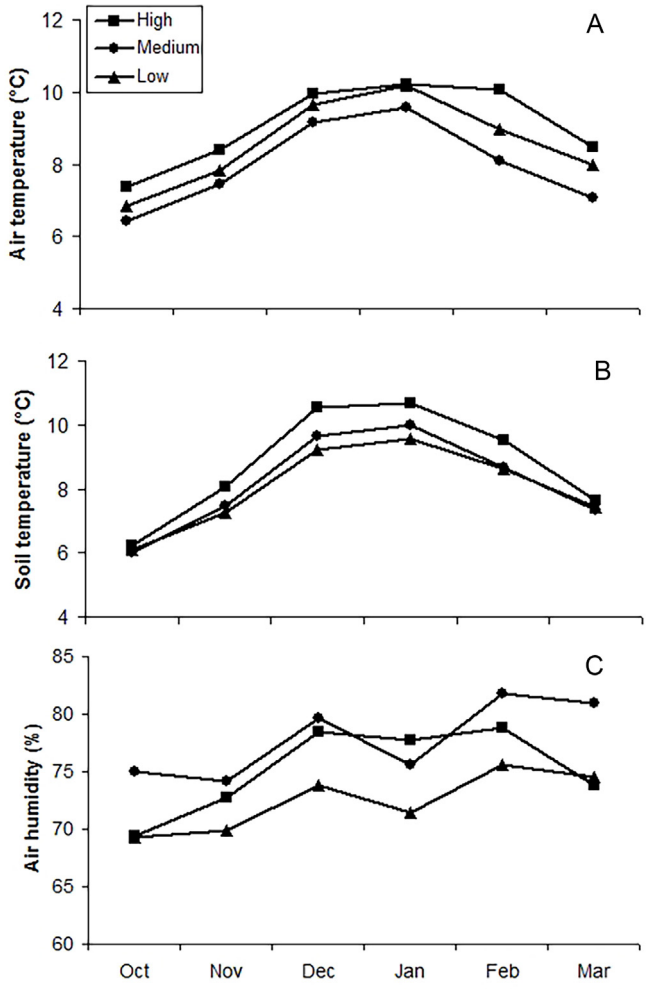

Fig. 1. Mean air temperature (°C) (A), mean soil temperature (°C) (B) and mean ambient relative humidity (%) (C) during the 2008/09 growing season (October–March) under high, medium and low light intensity treatments.

3. Results

3.1. Environmental parameters

In both production years, the air and soil temperatures showed maximum values in December and January, whereas minimum values were observed in October in the 1st production year, and in October and November in the 2nd production year. In accordance with light intensity treatments, the air and soil temperatures increased (Figs. 1 and 2).

Relative humidity remained steady between 70 and 80% in the 1st production year, whereas a minimum of 60–65% occurred in December in the 2nd production year. In both production years the minimum relative humidity coincided with a lower precipitation and/or the combination of temperature and precipitation events. Interestingly, at medium light intensity, the relative humidity presented higher values than at low or high intensity conditions (Figs. 1 and 2).

3.2. Total monomeric anthocyanins, flavonoids and total polyphenols

Total monomeric anthocyanin and total polyphenol concentrations varied significantly with the light intensity (Table 1), the values being at a maximum with high light intensity. Total monomeric anthocyanin concentration in high light intensity con-

Table 1
Berberis microphylla fruit composition. Mean values of ANOVA considering light intensity, fertilization level and production year as main factors, and the concentration of total monomeric anthocyanins (ANT), flavonoids (mg (+)- catechin equivalents) (FLA) and total polyphenols (mg tannic acid equivalents) (PHE) (mg 100 g^{-1} FFW and mg g^{-1} DFW) as dependent variables.

Main effects	ANT FFW	ANT DFW	FLA FFW	FLA DFW	PHE FFW	PHE DFW
A = light intensity						
Medium	103.8b	3.3b	156.5	6.1	693.8b	27.5b
High	299.7a	9.5a	142.3	5.0	906.6a	30.7a
p	*<0.001*	*<0.001*	*0.090*	*0.090*	*<0.001*	*<0.001*
B = fertilization level						
0	195.7	6.3	200.2a	7.6a	714.2c	25.7c
1	197.9	6.2	138.1ab	5.0ab	830.3b	30.3b
2	211.7	6.7	110.0b	4.1b	856.1a	31.2a
p	*0.221*	*0.194*	*0.010*	*0.010*	*<0.001*	*<0.001*
C = production year						
1st	350.0a	10.8a	140.5b	4.9b	890.0a	30.3a
2nd	53.6b	1.9b	157.8a	6.2a	710.4b	27.8b
p	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
Interactions A × B × C(p)	*<0.001*	*<0.001*	*0.105*	*0.155*	*<0.001*	*<0.001*

Values followed by different letters in each column and for each factor are significantly different according to the Tukey test at a $p \leq 0.05$.

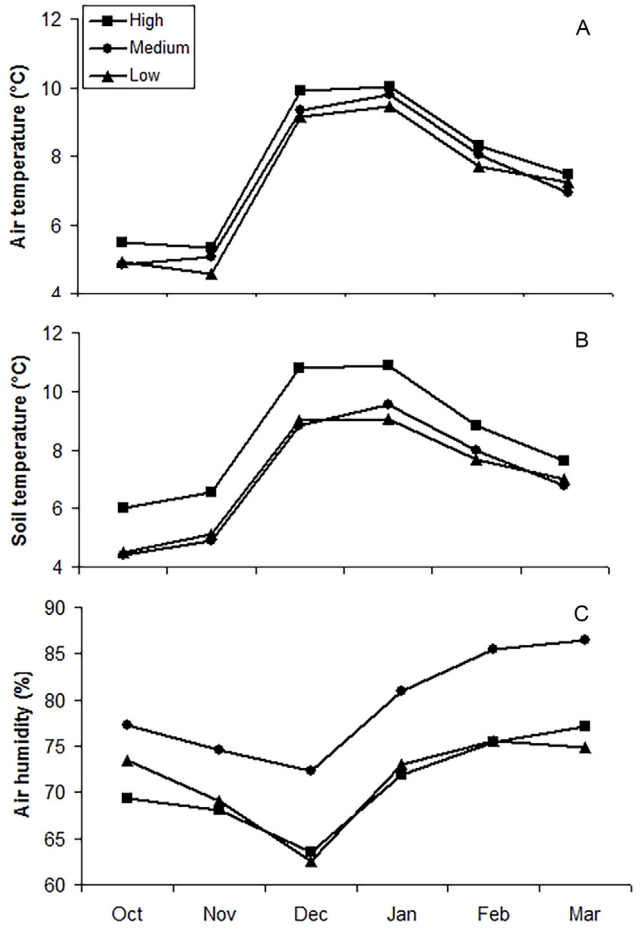

Fig. 2. Mean air temperature (°C) (A), mean soil temperature (°C) (B) and mean ambient relative humidity (%) (C) during the 2009/10 growing season (October–March) under high, medium and low light intensity treatments.

ditions was three times more than that obtained at medium light intensity, for both fresh weight and dry weight (299.7 mg/100 g FFW and 9.5 mg/g DFW, respectively). However, in the case of total polyphenol concentration, minor increases of 30 and 11% appeared under high light intensity in comparison with medium light intensity (906.6 mg/100 g FFW and 30.7 mg/g DFW, respectively).

Fertilizers reduced the flavonoid concentration, but increased the total polyphenol concentration at fertilization level 2 (Table 1). In the case of flavonoids, the maximum concentrations in fruits were 200.2 and 7.6 mg (+)-catechin equivalents/100 g FFW and DFW, respectively. The polyphenol concentration was 856.1 and 31.2 mg tannic acid equivalents/g FFW and DFW, respectively.

Total monomeric anthocyanin, flavonoid and total polyphenol concentrations varied with the production year (Table 1), the total monomeric anthocyanin concentration (350.0 mg/100 g FFW and 10.8 mg/g DFW) and total polyphenol concentration (890.0 mg tannic acid equivalents/100 g FFW and 30.3 mg tannic acid equivalents/g DFW) were highest in the 1st production year, whereas the flavonoid content was at a maximum in the 2nd production year (157.8 mg (+) – catechin equivalents/100 g FFW and 6.2 mg catechin equivalents/g DFW).

Some significant interactions between light intensity, fertilization level and production year were found for the total monomeric anthocyanin and polyphenol concentrations (Table 1). Indeed, differential increments were verified in the values between the main factors and combinations (Fig. 3). The total monomeric anthocyanin concentration increased with the level of fertilization in the fruits of plants at high light intensity, whereas the trend was inversed in those under medium light intensity (Fig. 3A). The total monomeric anthocyanin concentration did not vary with the level of fertilization in the 1st production year, but it did increase with the level of fertilization in the 2nd production year (Fig. 3B). However, the total polyphenol concentration varied with the level of fertilization in both the 1st and 2nd production years (Fig. 3C).

Regarding the fruits formed in the 1st production year, total monomeric anthocyanin and total polyphenol concentrations were positive and significantly correlated with the yield of methanolic extracts ($r = 0.891$; $p = <0.001$) and ($r = 0.948$; $p = <0.001$), respectively.

3.3. Individual phenolic compounds

The concentration of catechin, rutin, quercitin, ferulic and gallic acid did not show any significant variations in response to either light intensity or fertilization level (Tables 2 and 3). However when considering phenolic acids, their highest concentration (157.7 mg/100 g FFW) was found under high light intensity (Table 4).

3.4. Yield of methanolic extracts

The highest yield of methanolic extraction was under high light intensity ($p < 0.001$) and fertilization level 2 ($p = 0.008$), with no significant interactions ($p = 0.410$). The value resulting from high

Table 2
Berberis microphylla fruit composition during the 1st production year. Mean values of ANOVA considering light intensity and fertilization as main factors, and the content of catechin (CAT), rutin (RUT) and quercetin (QUE) (mg 100 g^{-1} FFW and mg g^{-1} DFW) as dependent variables.

Main effects	CAT FFW	CAT DFW	RUT FFW	RUT DFW	QUE FFW	QUE DFW
A = light intensity						
Medium	112.9	4.6	17.5	0.7	2.6	0.1
High	94.1	3.3	24.2	0.8	1.9	0.1
p	*0.380*	*0.107*	*0.202*	*0.619*	*0.462*	*0.236*
B = fertilization level						
0	125.0	4.9	12.9	0.5	2.6	0.1
1	100.1	3.8	20.6	0.7	1.5	0.1
2	85.4	3.2	29.1	1.0	2.6	0.1
p	*0.288*	*0.179*	*0.081*	*0.085*	*0.447*	*0.445*
Interactions A × B (p)	*0.279*	*0.194*	*0.727*	*0.574*	*0.602*	*0.712*

Table 3
Berberis microphylla fruit composition during the 1st production year. Mean values of ANOVA considering light intensity and fertilization as main factors, and the content of chlorogenic acid (CLO), ferulic acid (FER) and gallic acid (GAL) (mg 100 g^{-1} FFW and mg g^{-1} DFW) as dependent variables.

Main effects	CLO FFW	CLO DFW	FER FFW	FER DFW	GAL FFW	GAL DFW
A = light intensity						
Medium	113.9	4.3	0.9	0.03	18.0	0.7
High	130.3	4.9	1.4	0.05	26.0	0.9
p	*0.104*	*0.104*	*0.202*	*0.202*	*0.071*	*0.353*
B = fertilization level						
0	116.5	4.4	0.8	0.03	19.7	0.8
1	125.2	4.7	1.3	0.05	25.4	0.9
2	124.5	4.7	1.3	0.05	20.9	0.7
p	*0.675*	*0.675*	*0.396*	*0.396*	*0.453*	*0.509*
Interactions A × B (p)	*0.513*	*0.513*	*0.098*	*0.098*	*0.257*	*0.352*

Table 4
Berberis microphylla fruit composition during the 1st production year. Mean values of ANOVA considering light intensity and fertilization as main factors, and the content of the sum of flavonoids (SFL), sum of phenolic acids (SPA) and sum of total phenols (SPH) (mg 100 g^{-1} FFW and mg g^{-1} DFW) as dependent variables.

Main effects	SFL FFW	SFL DFW	SPA FFW	SPA DFW	SPH FFW	SPH DFW
A = light intensity						
Medium	95.2	3.8	132.7b	5.0	225.9	8.8
High	95.9	3.3	157.7a	5.8	253.6	9.1
p	*0.923*	*0.649*	*0.039*	*0.077*	*0.408*	*0.819*
B = fertilization level						
0	26.2	4.9	137.1	5.2	263.3	10.1
1	69.7	2.6	151.8	5.6	221.5	8.2
2	87.7	3.2	146.7	5.4	234.4	8.7
p	*0.290*	*0.231*	*0.483*	*0.580*	*0.564*	*0.455*
Interactions A × B (p)	*0.689*	*0.542*	*0.278*	*0.352*	*0.915*	*0.778*

$p \leq 005$. Values followed by different letters in each column and for each factor are significant different according to the Tukey test at a $p \leq 0.05$.

light intensity and fertilization level 2 was 420.4 mg/g DFW. Moreover, yields of methanolic extraction were correlated with total monomeric anthocyanin concentration ($r = 0.891$; $p = <0.001$) and total polyphenol concentration ($r = 0.948$; $p = <0.001$).

3.5. Antioxidant activity

High light intensity was related to the higher antioxidant capacity as revealed by the scavenging activity on DPPH radicals and reducing power assays (Table 5). Indeed, scavenging activity on DPPH was increased from 56.0 to 66.8% under the high light intensity treatment. This was also true for the reducing power which increased from 40 to 46.2%.

Moreover, both the scavenging activity on DPPH and the reducing power were slightly increased by the increment in the use of fertilizers (Table 5). The scavenging activity on DPPH was from 58.2 to 63.8% when raising the fertilization from 0 to 1, while the reducing power varied between 39.9 and 44.9% at level 0 and 1.

The maximum antioxidant activities were found at 0.75 mg/mL for scavenging activity on DPPH and at 5.0 mg/mL in the case of reducing power. Moreover, significant interactions were found on both antioxidant attributes in relation to production year/high light/fertilization (Figs. 4 and 5).

Values of the standard antioxidants from natural (ascorbic acid, tocopherol) and synthetic (BHA) sources showed a very high scavenging activity which was highest at c.a. 0.10 mg/mL (data not shown). As mentioned, the values of methanolic extracts of *B. microphylla* also showed very low active concentrations reaching maximum activity (0.25 and 0.50 mg/mL). This result is in accordance with the values of the effective concentration at which the DPPH radicals and the reducing power were scavenged by 50% (EC50) (0.20 and 1.44 mg/mL, respectively). This trait was reduced in response to the lower light intensity achieved when using shade cloths for the plant cultures (Table 6).

4. Discussion

4.1. Changes in the phenolic compounds and antioxidant capacity with light intensity

The biosynthesis of the anthocyanins in *B. microphylla* occurs in the fruits and their skin, where they also accumulate; such pro-

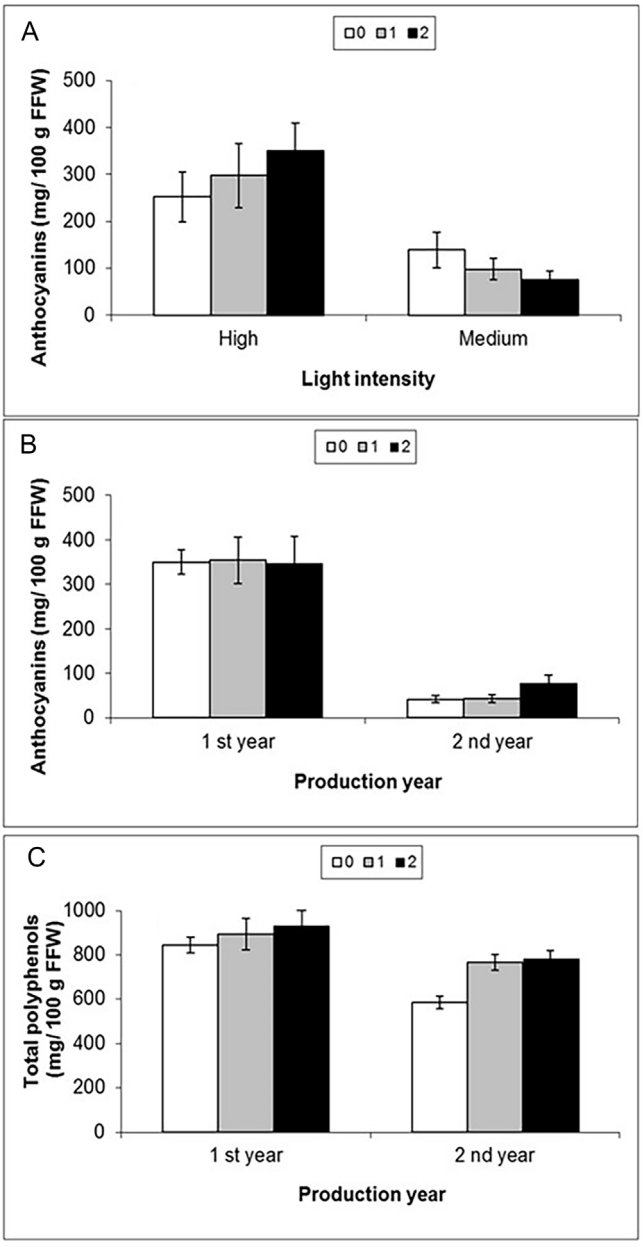

Fig. 3. Total monomeric anthocyanin and total polyphenol concentration (mg 100 g^{-1} fresh fruit weight) of *B. microphylla*, A: total monomeric anthocyanin concentration interactionbetweenthe light intensity and fertilization level; B: total monomeric anthocyanin concentration interactionbetween theproduction year and fertilization level and C: total polyphenol concentration interactionbetween theproduction year and fertilization level.Bars represent ± standard error of the mean.

cesses are affected during the progression of fruit development by environmental factors such as light and available nutrients (Zoratti et al., 2014). In fact, present research demonstrated in field conditions that the anthocyanin contents were related to the level of light intensity, since they increased 2.9 times from medium to high light intensities. This fact was associated with a higher photosynthetic rate and a concomitant increase in soluble solids and sugar content (Arena, 2016) measured under the same situation. The precise conditions that trigger the onset of anthocyanin synthesis have still not been established, but it is known that sugars derived from photosynthesis are the substrate needed for anthocyanin synthesis (Jackson, 2008). The accumulation of flavonoids in fruits is sensitive to the quality of the spectrum of light that radiates to the plant. Blue light and UV significantly increase the expression of the

Table 5
Berberis microphylla fruit antioxidant activity during the 1st production year. Mean values of ANOVA considering light intensity, fertilization level and the concentration of the methanolic extracts (mg mL^{-1}) as main factors, and the values of DPPH radical scavenging activity (DPPH) (%) and reducing power (*RP*) (%) as dependent variables.

Main effects	DPPH	RP
A = light intensity		
Medium	56.0b	39.4b
High	66.8a	46.2a
p	*<0.001*	*<0.001*
B = fertilization level		
0	58.2b	39.9b
1	63.2a	44.9a
2	62.8a	43.6a
p	*0.011*	*<0.001*
C = concentration (mg mL^{-1})		
0	0.00e	0.7h
0.10	26.9d	7.9g
0.25	64.6c	17.9f
0.50	85.8b	29.7e
0.75	94.6a	43.4d
1.00	96.5a	57.1c
2.5		86.4b
5.0		99.4a
p	*<0.001*	*<0.001*
Interactions A × B × C (p)	*0.602*	*<0.001*

Values followed by different letters in each column and for each factor are significant different according to the Tukey test at a $p \leq 0.05$.

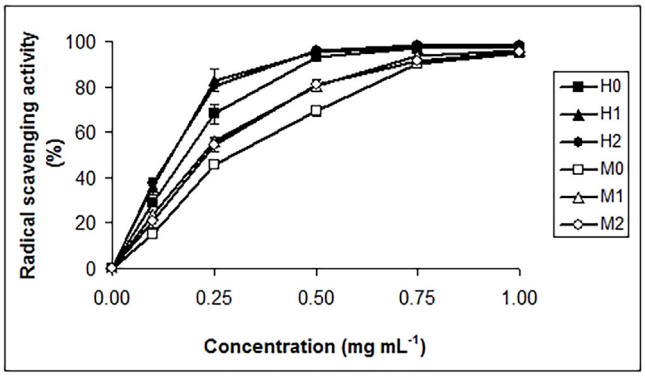

Fig. 4. DPPH radical scavenging activity of *B. microphylla* fruits under light intensity and fertilization level treatments and at different concentrations of the methanolic extracts. Bars represent ± standard error of the mean. H0: high light intensity-level 0 fertilization; H1: high light intensity-level 1 fertilization; H2: high light intensity-level 2 fertilization; M0: medium light intensity-level 0 fertilization; M1: medium light intensity-level 1 fertilization; M2: medium light intensity-level 2 fertilization.

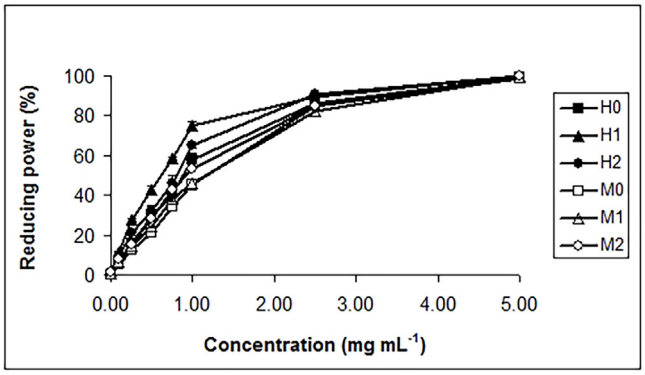

Fig. 5. Reducing powerof *B. microphylla* fruits underlight intensity and fertilization level treatments and at different concentrations of the methanolic extracts.Bars represent ± standard error of the mean. H0: high light intensity-level 0 fertilization; H1: high light intensity-level 1 fertilization; H2: high light intensity-level 2 fertilization; M0: medium light intensity-level 0 fertilization; M1: medium light intensity-level 1 fertilization; M2: medium light intensity-level 2 fertilization.

Table 6
Concentration of methanolic extracts (mg mL^{-1}) (EC_{50}) at which the DPPH radicals were scavenged by 50% (mg mL^{-1}) and the reducing power (mg mL^{-1}) was 50% in *B. microphylla* fruits, considering light intensity and fertilizations level as main effects during the 1st production year.

Main effects	EC_{50}DPPH	EC_{50}RP
A = light intensity		
Medium	0.3a	1.8a
High	0.2b	1.4b
p	*<0.001*	*0.003*
B = fertilization level		
0	0.3	1.7
1	0.2	1.5
2	0.2	1.6
p	*0.109*	*0.082*
Interactions A × B (p)	*0.879*	*0.078*

The EC_{50} values were obtained by interpolation from linear regression analysis between the sample concentration (mg mL^{-1}) and DPPH and RP. Values followed by different letters in each column are significantly different with the Tukey test at a $p \leq 0.05$.

flavonoid biosynthesis genes. Some flavonoids, especially flavonols, are reported to be highly effective as ROS scavengers, as well as for absorption of UV-B radiation. The rapid induction of flavonoid biosynthesis that is generally observed under high light conditions reflects the important role of flavonoids in photoprotection (Zoratti et al., 2014). A decrease in anthocyanin content was observed in fruits of *Ribes nigrum* (Šavikin et al., 2009) and *Vitis vinifera* (Cortell and Kennedy, 2006) when decreasing the light intensity. However, in some fruit species flavonoid biosynthesis is less affected by light, such as in *Garcinia mangostana* in which anthocyanin is unaffected by light, and in *Pyrus* where high light intensity may even decrease the anthocyanin (Zoratti et al., 2014). In berries of *B. microphylla* exposed to high light irradiations with no addition of fertilizer, the anthocyanin content during the first year of production (435 mg/100 g FFW) is comparable with that obtained in previous years in a natural population and they match the fruit ripening stage (Arena and Curvetto, 2008; Arena et al., 2012). In turn, the anthocyanin content of fruit exposed to high light conditions without fertilization during the first year of production is similar to the values cited for *B. microphylla* in the southern region of Chile (Ruiz et al., 2010); moreover they were lower than the obtained in *Vaccinium myrtillus* fruits (3666 mg/100 g dry weight) (Ancillotti et al., 2016), and higher than those determined for other berries, such as *Ribes nigrum* (350 mg/100 g fresh weight), *Rubus idaeus* (55–60 mg/100 g PF), *Fragaria x anannassa* (40 mg/100 g fresh weight) (Lister et al., 2002) and *Vaccinium* sp. (1.2 mg/100 g fresh weight) (Zheng and Wang, 2003).

Contents of flavonoids in fruits exposed to high light conditions without fertilization during the first year of production coincides with the fruit ripening stage (Arena et al., 2012), and are similar to those cited for *Fragaria* sp. (123.2 and 30.0 mg quercetin equivalents/100 g of FFW) (Cheel et al., 2007). Interestingly, the flavonoid content in fruits of *B. microphylla* decreased as the soluble solids increased (Arena, 2016), which was the opposite to that observed with anthocyanins.

The concentrations of total polyphenols could also be related to the intensity of light irradiation as it was increased 1.3–1.2 times from medium to high light irradiation, in terms of fresh and dry weight, respectively. This fact could be explained by considering the significant positive correlation between this variable and total monomeric anthocyaninconcentrations ($r = 0.776$; $p = <0.001$). In this case, the polyphenol concentration was correlated with the total monomeric anthocyanin and sugar concentrations and therefore also with the photosynthetic rate (Arena, 2016). The total polyphenol concentration in ripe fruits exposed to high light irradiation with no fertilization treatment was in agreement with that previously reported (Arena et al., 2012), and it was even higher than in fruits of this species grown in Chile (13.44 mg/g DFW) (Reyes-Farias et al., 2014), although similar to those cited by Mariangel et al. (2013). Furthermore, the total polyphenol concentration of *B. microphylla* in this study was considerably higher than that reported in other reddish-purple colored berries, such as *Ribes rubrum* (14 mg/g dry weight), *Fragaria x ananassa* (16–24 mg/g dry weight) (Kähkönen et al., 2001), *Rubus idaeus* (300 mg/100g fresh weight) (Lister et al., 2002), and other native South American fruit, such as *Fragaria* sp. (106–268 mg/100 g fresh weight) (Cheel et al., 2007). On the other hand, the total polyphenol concentrationwas found to be lower than the obtained in *V. myrtillus* fruits (4768 mg/100 g dry weight) (Ancillotti et al., 2016) and similar to the ones reported in other recognized berry species, such as *B. vulgaris* (Gundogdu, 2013), *V.myrtillus* (33–38 mg/ g dry weight) (Kähkönen et al., 2001) and *Ribes nigrum* (1000 mg/100 g fresh weight) (Deighton et al., 2002). The effect of light intensity on the flavonoid and polyphenol contents determined by colorimetric methods is also seen on the profile of these secondary metabolites, although with no significant differences between them.

Antioxidant activity, measured by the DPPH scavenging activity and reducing power, resulted 1.2 times higher in fruits when obtained at medium and high light intensity irradiations. This antioxidant activity was associated with the anthocyanin concentration ($r = 0.697$; $p = <0.001$ and $r = 0.533$; $p = <0.001$, for DPPH radical scavenging activity and reducing power, respectively) and the total polyphenol concentration ($r = 0.822$; $p = <0.001$ and $r = 0.646$; $p = <0.001$, respectively) rather than the flavonoid concentration. Previous reports on the relationship between total polyphenol concentration and antioxidant activity were also found in *Diospyros kaki cv. mopan* (Chen et al., 2008), *B. vulgaris* and in *B. croatica* (Koncic et al., 2010) and in *V. myrtillus* (Ancillotti et al., 2016). Regarding DPPH radical scavenging activity, values obtained with *B. microphylla* were comparable to that reported for the fruits of *B. vulgaris* (Motalleb et al., 2005) and *B. koreana bark* (Qadir et al., 2009). Results reporting significant positive correlations between the total polyphenol concentrationand antioxidant activity in fruits of *B. microphylla* have been also reported by Ruiz et al. (2010). With regard to EC50DPPH and EC50RP, their values decreased 1.5 and 1.2 times in fruits under high and medium light intensities, respectively. Previously, it had been mentioned that higher antioxidant activity was obtained under these light conditions; however with this outcome the lower EC50 values mean that those antioxidants were also more efficient.

4.2. Changes in the phenolic compounds and antioxidant capacity with fertilization level

Anthocyanin concentration in fruits under high light irradiation increased with the level of fertilization. This response was not significant under medium light irradiation, which should be associated with the rate of net photosynthesis and photoassimilate (Arena, 2016). Nutrient quality and fertilization levels affect fruit maturation, pigment content and antioxidant activity. When considering effects on pigmentation, if nitrogen is present at high levels it can cause a poor coloration and delayed fruit maturation (Kéller and Hrazdina, 1998; Kliewer, 1977). Furthermore, an increase in vegetative growth competes strongly with the translocation of sugars and pigment accumulation in the fruits (Abd El-Razek et al., 2011). An adequate potassium level contributes to an increase in both the color and the total polyphenol content in fruits (Sommers, 1977). Davenport (1996) found no correlation between the level of fertilization and anthocyanin content in *Vaccinium*, as in the case of *Rubus* (Alleyne and Clark, 1997). In *Vitis vinifera* the increase in nitrogen levels increased the anthocyanin content, in comparison to treatments without nitrogen (Martin et al., 2004).

Flavonoid and total polyphenol concentration were related to the level of fertilization. Flavonoids increased 1.8 times by changing the fertilization level from 2 to 0 level, in terms of fresh and dry weight, whereas total polyphenols increased 1.2 times between the level of fertilization 0 and 2 in terms of fresh and dry weight. These results are consistent with those found in *Fragaria x ananassa* Duch., where the contents of quercetin, kaempherol and ellagic acid were higher at low levels of fertilization (Anttonen et al., 2006). The influence of fertilization level on flavonoids and total polyphenols determined by colorimetric methods is also seen on the profile of these secondary metabolites, although with no significant differences between them. Thus, for example, catechin content showed a clear tendency to increase without fertilization, but the opposite occurs in the case of the rutin.

A relationship between DPPH radical scavenging activity and reducing power was found with the level of fertilization. Both antioxidant properties increased 1.1 and 1.3 times between fertilization levels 0 and 1, respectively, a fact that could be explained by the close relationship between these variables and the total polyphenol concentration, as described above. No relationship between either EC50DPPH or EC50RP activities and the fertilization level was found. So the previous consideration about light intensity as the cause of those trait variations is reinforced.

4.3. Changes in the phenolic compounds and antioxidant capacity with production year

Anthocyanin concentration could be related to the production year, as it was higher by 6.5 and 5.7 times in the first production year than in the second one, in terms of both fresh and dry weight respectively. Also, the total polyphenol concentration was higher in the first production year as well with respect the second one, but in a lower extent (1.2 and 1.1 times in terms of both fresh and dry weight). However, flavonoid concentration was 1.1 and 1.3 times higher in the second production year than in the first one, in terms of both fresh and dry weight. The higher anthocyanin and total polyphenol concentrations found in the first production year could be associated with the higher soluble solids, the soluble solids/acidity ratio and sugar content (Arena, 2016) measured under the same situation, Indeed, the higher content of soluble solids and the soluble solids/acidity ratio in the first production year could be related to the higher temperatures of that period compared with the second production year. It is also known that temperature is a limiting factor of the photosynthetic rate, so that at higher temperatures the photo-assimilates are produced at a faster rate, which makes an increase in the translocation from leaves to different destinations possible, including fruits (Madore and Lucas, 1995; Zamski and Schaffer, 1996). For example, fruit composition of *Ribes nigrum* and *Prunus avium* presented some correlations between different growing seasons and their climatic conditions (Toldam-Andersen and Hansen, 1993; Predieri and Dris, 2005).

5. Conclusions

Differences in the phonological stages showing different foliar nutrient content, carbon/nitrogen ratio and rate of photosynthesis can account for the differences in the phenolic total polyphenol content of fruit and hence in their antioxidant activity. These traits should be considered for future studies involving the agronomic management of commercial plantations. The qualitative and quantitative composition of phenolic compounds and antioxidant activity of *B. microphylla* fruit showed changes with the light intensity and the level of fertilization, and phenolic compounds also showed changes with the growing season. Fruits of cultivated plants under high light intensity, and with higher air and soil temperatures and fertilizer levels 1 and 2, had the highest concentrations of total monomeric anthocyanins and total polyphenols. Fruits had the highest concentration of total monomeric anthocyanins and total polyphenols in the 1st year of production. The DPPH radical scavenger activity of fruit methanolic extracts was highest in plants grown under high light irradiation. Hence, these studies display the possibility for maximizing both the productivity and the antioxidant capacity of fruits by crop management.

Acknowledgments

The authors thank Mr Juan Peralta for his technical assistance and to Rosemary Scoffield M.Sc. for her correction of the English language. This research was supported by grants PIP 263 by CONICET.

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