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Fruit growth and chemical properties of *Ribes magellanicum* "parrilla"

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ABSTRACT

The aim of this work was to study the time course and pattern of fruit growth and the evolution of some of the chemical attributes such as soluble solids, acidity, and anthocyanin content in *Ribes magellanicum* plants growing in a natural environment near Ushuaia city, Tierra del Fuego (Argentina). Fruit growth and composition presented significant changes along the days from the full bloom phase. Fresh and dry fruit weight exhibited a typical double sigmoid curve. The first period of rapid fresh fruit growth was from full bloom phase until 28 days after, followed by a lag period until 42 days from the full bloom phase, and then by a second period of rapid increase until 56 days from the full bloom phase. Then, fresh fruit weight increased slowly (days 70–98 from the full bloom phase), reaching its maximum. Afterwards, fresh fruit weight decreased significantly until the end of the summer, and the fruiting period approximately ended 112 days from full bloom phase. On a dry weight basis the maximum fruit biomass was reached 98 days from the full bloom phase. Evolution of fruit growth was related with the compositional changes evaluated. By day 98 from the full bloom phase, soluble solids (17.5°Brix) and anthocyanin content (240.1 mg/100 g fruit fresh weight) were at their maximum, while at this time the total titratable acidity was at a minimum (0.4%). The results obtained not only contribute to the knowledge of the quantitative content of anthocyanin, a metabolite with nutraceutical value, but also give some tools for the definition of the optimal harvest time of *R. magellanicum* fruits, which it is important for fruit destination.

1. Introduction

The importance of wild flora as sources of food and medicinal substance is well known (Iriondo, 2001). However, more studies on diversity and agronomic and medicinal potential of the wild flora (Arena and Vater, 2005) are necessary as only a few species have been evaluated for these purposes (Iriondo, 2001). An attention is particularly focused on many of the small fruits that are now considered for their nutraceutical properties as functional foods, that is foods containing essential organic and inorganic nutrients and metabolic regulation factors and also specific metabolites that give additional health benefits (Henriques et al., 2004; Kuskoski et al., 2005). Soft fruits of *Ribes*, *Rubus*, and *Vaccinium* cultivated species are an excellent source of natural products such as pigments (Flores Cantillano, 2004; Henriques et al., 2004) with antioxidant properties (Deighton et al., 2002).

Ribes magellanicum Poiret, commonly named "parrilla", is found in the forest clearings and wood margins of *Nothofagus pumilio* in Tierra del Fuego (Moore, 1983). It is a deciduous, erect shrub up to 4 m high, with racemes with 20 or more yellow to reddish flowers and globose berries, purple at maturity (Moore, 1983), with an ornamental value. As a first step towards its domestication, shoot growth and fruiting were studied in plants growing naturally near Ushuaia city, Tierra del Fuego, Argentina (Arena et al., 2007). At present, this non-timber forest product has a local and regional market (Tacón Clavaín, 2004), because its purple berries can be consumed fresh, in marmalades and syrups (Correa, 1984). However, commercial orchards of this native *Ribes* species are being projected, as it represents an attractive potential crop such as the cultivated *Ribes* species in Argentinean Patagonia. Berries are currently of increasing importance for the local industry as for the fresh market, because of its diversity in soils and climates and, mainly, to the possibility of producing out of season, with regard to the north hemisphere. *Ribes* and *Rubus* species are important for diversification of production in mountainous and marginal areas such as Southern Apennines (Rotundo et al., 1998; Hummer and Barney, 2002), which present similar characteristics to Tierra del Fuego. The fruits, both for food and non-food (pharmaceutical, cosmetic industries) use, is a source of revenue for people living in marginal areas.

The aim of this work was to study the time course and pattern of fruit growth and the evolution of some of the chemical attributes such as soluble solids, acidity, and anthocyanin content along the

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days from the full bloom phase in *R. magellanicum* plants growing in a natural environment of Tierra del Fuego (Argentina). The results obtained could be of value in defining the optimal time for fruit harvesting according to the future use, as well as contribute to the establishment of the nutraceutical value at a particular stage of maturity (Gonzalez-San José et al., 1990).

2. Materials and methods

2.1. Geographic data and climatic parameters

R. magellanicum plants were studied from an area located near Ushuaia city, 54°48′S, 68°19′W (Tierra del Fuego, Argentina). Values of maximal, minimal and mean daily air temperatures (°C), mean ambient relative humidity (%), and rainfall (mm) were collected using a meteorological station located at the Centro Austral de Investigaciones Científicas (CONICET, Argentina) from October to March for the 2004/05 growing season. Mean daily air temperature was 9.0 °C, with the highest mean daily air temperature in February (11.2 °C). Minimal and maximal daily air temperatures were 4.6 and 13.5 °C respectively. Mean ambient relative humidity of the registered months was 74.9%. Cumulative rainfall was 295.6 mm, while December had the highest rainfall (83.8 mm).

2.2. Plant material, samplings, and measurements

Fruits (200 g) were manually collected from *R. magellanicum* plants ($n = 20$, with a mean height of 1.85 ± 0.30 m), growing naturally in *Nothofagus* forest clearings, from November (14 days from full bloom phenological phase) to February (112 days from full bloom phenological phase) (Figs. 1 and 2). For chemical properties, fruits were analyzed from 14 days from full bloom to 98 days from full bloom, due to the fruits were overripened at 112 days from full bloom, being difficult to obtain the juice and therefore to perform the analysis of the chemical variables.

Fig. 1. *Ribes magellanicum* racemes at full bloom.

Fig. 2. *Ribes magellanicum* ripened fruits.

2.2.1. Morphological characterization

The following parameters were recorded and evaluated: fresh fruit weight, dry fruit weight, dry fruit weight as percentage of fresh weight, equatorial and polar fruit diameters (using a digital calliper Mitutoyo Model 500-196, 150 mm × 6″ − 0.01 mm × 0.0005″), fresh seed weight, dry seed weight, dry seed weight as percentage of fresh weight, seed number, and dry seed weight/dry fruit weight ratio.

2.2.2. Soluble solids and total titratable acidity

Soluble solids were determined in fruit juice using an ATAGO N1-α refractometer with 0–32°Brix measurement range with 0.2°Brix increments, and no temperature compensation. Total titratable acidity was measured by titration with 0.1 N NaOH solution. Total titratable acidity was expressed as citric acid. Soluble solids/total titratable acidity ratio and initial pH were also recorded.

2.2.3. Anthocyanin content

Anthocyanin quantification was performed by the pH differential method of Giusti and Wrolstad (2001). Samples (5 g) of initially frozen fruits were extracted for 24 h in 50 mL 0.1% HCl–MeOH solution at 4 °C. Then, aliquots were diluted from 1:5 to 1:80 with either a 0.025 M KCl (pH 1) or 0.4 M sodium acetate (pH 4.5) buffer. Absorbance measurements were made at 510 and 700 nm with a Shimadzu 1203 UV-Visible spectrophotometer. Anthocyanin fruit tissue content was determined on the basis of a molar extinction coefficient of 26,900 and a molecular weight of 449.2 for cyanidin 3-glucoside. Values were expressed in terms of milligrams of anthocyanin/100 g of fresh-frozen fruit.

Anthocyanin fruit content (mg/100 g fruits) = (A × molecular weight × dilution factor × initial volume/ε × sample weight) × 100

$$\text{with } A \text{ (absorbance)}$$

$$= (A_{510\,\text{nm}} - A_{700\text{nm}})_{\text{pH}\ 1.0} - (A_{510\,\text{nm}} - A_{700\,\text{nm}})_{\text{pH}\ 4.5}$$

2.3. Statistical analysis

Data were subjected to an analysis of variance, and means were then separated using the Tukey multiple range test at $p \le 0.05$ through the Statgraphics Plus (version 5.1) program.

3. Results

3.1. Fruit growth

As expected, the fresh ($F = 30.98$, $p = 0.000$) and dry ($F = 38.56$, $p = 0.000$) weight of fruits significantly varied along the days from

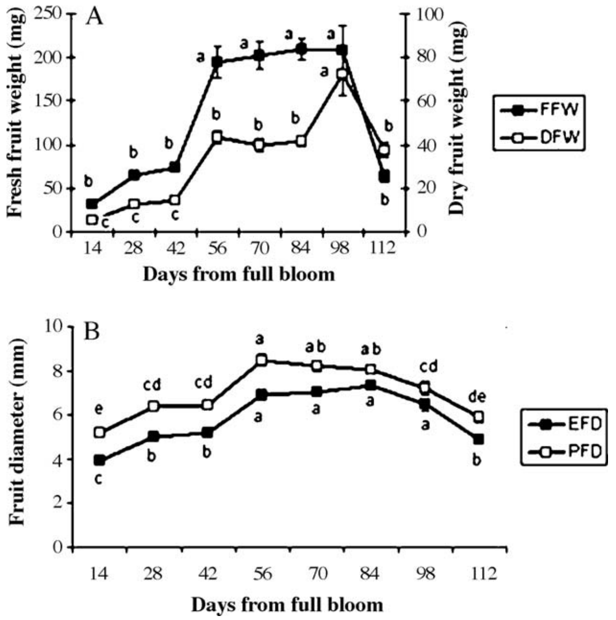

Fig. 3. *Ribes magellanicum* fruit growth along the days from full bloom. (A) Fresh fruit weight (mg) (*FFW*) and dry fruit weight (mg) (*DFW*). (B) Equatorial fruit diameter (mm) (*EFD*) and polar fruit diameter (mm) (*PFD*) ($n = 20$). Values followed by different letters in each variable are significantly different with Tukey multiple range test at $p < 0.05$.

the full bloom (Fig. 3A). The fresh fruit weight increased with an average relative growth rate of 8.0% per day (g FW/100 g FW per day) from day 14 after full bloom phase, to obtain the highest biomass of 209.0–208.0 mg by days 84–98 from full bloom phase. Then, fresh biomass decreased averaging 2.5% per day till 112 days from full bloom phase. The dry fruit weight increased with an average relative growth rate of 14.4% per day (g DW/100 g DW per day) from day 14 after full bloom phase, to obtain the highest biomass of 72.3 mg by day 98 from full bloom phase. Then dry biomass decreased averaging 3.4% per day till 112 days from full bloom phase. The dry fruit weight as percentage of fresh weight significantly changed during the fruiting period ($F = 82.35$, $p = 0.000$), from 14 days from full bloom (17.6%), reaching a maximum of 61.3% by day 112. The equatorial ($F = 54.38$, $p = 0.000$) and polar ($F = 37.44$, $p = 0.000$) fruit diameters also varied with the fruiting period (Fig. 3B). These parameters increased from day 14 after the full bloom phase to attain the maximum of 7.3 and 8.5 mm for equatorial and polar fruit diameters, respectively, on days 84 and 56, and then decreased towards the end of the growing season. Fresh weight of fruits had a visible separation into two subsequent periods of rapid increase. The first period of rapid fresh weight increase ended at 28 days from the full bloom phase, followed by a lag period until 42 days from the full bloom phase and then by a second period of rapid increase until 56 days from the full bloom phase. Then fresh fruit weight increased slowly until days 84–98 when it reached its maximum. Afterwards, fresh fruit weight decreased significantly until the end of the fruiting period. The dry fruit weight evolution closely followed the fresh weight pattern until the maximum fruit biomass was reached. The dry fruit weight as percentage of fresh weight had significant increments from day 84 after the full bloom phase until day 112 after full bloom phase. As a whole, the equatorial and polar fruit diameters followed the same pattern as the fruit weight did, particularly until attaining the maximum values.

3.2. Seed growth

The fresh and dry seed weight significantly varied during the fruiting period (Table 1). The fresh seed weight did not differ statistically from day 56 till day 98 after full bloom phase, when highest values (84.7 and 66.2 mg for the fresh and dry weight, respectively) were obtained, decreasing significantly towards the end of the fruiting period. The dry seed weight as percentage of fresh weight significantly varied during the fruiting period (Table 1), reaching its maximum (79.5%) by day 112 from the full bloom phase. Seed number significantly varied during the fruiting period (Table 1). After 56 days from the full bloom phase, seed number was maximum (23.3), then decreased during the remaining growing season, because not all the initial seeds grew during the fruiting period. Dry seed weight/dry fruit weight ratio also significantly varied during the fruiting period (Table 1). The dry seed weight/dry fruit weight ratio did not differ statistically from day 56 till day 98 after full bloom phase, when it was maximum (78.8), decreasing significantly towards the end of the growing season.

Table 1
Mean values of ANOVA analyzing *Ribes magellanicum* seed growth during the fruiting period. Fresh seed weight (mg) (*FSW*), dry seed weight (mg) (*DSW*), dry seed weight as percentage of fresh weight (%) (*DSWP*), seed number (*SN*), and dry seed weight/dry fruit weight ratio (*DSW/DFW*) were considered as dependent variables ($n = 20$).

Days from full bloom	FSW	DSW	DSWP	SN	DSW/DFW
56	72.00a	23.00b	33.27b	23.30a	60.65b
70	72.15a	22.88b	32.15b	21.15ab	61.13b
84	74.85a	22.42b	31.04b	21.50ab	60.59b
98	84.70a	66.21a	78.37a	16.40b	78.77a
112	22.23b	17.42b	79.46a	16.30b	51.29c
F(*p*)	11.62(0.000)	52.06(0.000)	151.80(0.000)	4.31(0.003)	24.11(0.000)

$F(p) = F$ statistical and probability at $p = 0.05$. Values followed by different letters in each column are significantly different with Tukey multiple range test at $p < 0.05$.

Table 2
Mean values of ANOVA analyzing *Ribes magellanicum* composition during the fruiting period. Soluble solids (°Brix) (*SS*), total titratable acidity (%) (*TTA*), soluble solids/total titratable acidity relation (*RATIO*), initial pH (*pH*), and anthocyanin content (mg anthocyanin/100 g fresh fruit) (ANTH) were considered as dependent variables ($n = 6$).

Days from full bloom	SS	TTA	RATIO	pH	ANTH
70	13.42c	1.57a	8.52d	3.65c	9.52e
77	14.12b	1.50a	9.33d	3.58c	29.64d
84	14.24b	0.99b	14.24c	3.79c	42.89c
91	14.11b	0.38c	36.78b	4.68b	146.49b
98	17.49a	0.41c	42.02a	4.97a	240.15a
F(*p*)	223.81(0.000)	721.97(0.000)	508.58(0.000)	184.29(0.000)	606.20(0.000)

$F(p) = F$ statistical and probability at $p = 0.05$. Values followed by different letters in each column are significantly different with Tukey multiple range test at $p < 0.05$.

3.3. Fruit chemical properties

Chemical properties significantly varied during the fruiting period (Table 2). Soluble solids increased during the fruiting period becoming maximum (17.5°Brix) by day 98 from the full bloom phase. Total titratable acidity reached maximum values 70–77 days from the full bloom phase, and then decreased towards the end of ripening (0.4%). As expected, the soluble solids/total titratable acidity ratio increased along the fruiting period to a maximum (42.0) by day 98 from full bloom phase. The initial pH as well as anthocyanin fruit tissue content increased during the fruiting period to maximums of 5.0 and 240.1 mg/100 g fresh fruit weight respectively by day 98 from the full bloom phase.

4. Discussion

4.1. Fruit growth pattern

The growth rates and composition of fruits greatly vary among species, cultural practices, and fruit positions in the same crop, seasons, and environmental conditions (Biale, 1950; Boynton and Wilde, 1959; Kramer and Kozlowski, 1979; Predieri and Dris, 2005). In *R. magellanicum* the fruiting period is about 16 weeks, while the maximum fresh fruit weight is attained between 8 and 14 weeks after the full bloom phase.

It is well known that the kinetics of fruit growth exhibit two general patterns, the first being a simple sigmoid curve in which an exponential increase in size is followed by a deceleration of growth, all this resulting in a sigmoidal shape, a typical growth behaviour of non-stone fruits. A second type of fruit growth consists of two successive sigmoidal growing periods separated by a lag phase of growth (Coombe, 1976; Gil-Albert Velarde, 2006), as was found to be the case for *Vitis* (Coombe and McCarthy, 2000) and *Ribes* (Toldam-Andersen and Hansen, 1997). In *R. magellanicum* the fresh and dry weight of the fruits nearly followed the double sigmoid growth pattern, and the same was observed when the growth was evaluated through the evolution of both the equatorial and polar fruit diameters, although less distinctly.

It is possible to detect some growth variations caused by lag periods of fruit growth when seed develop, and of course influences of environmental and/or cultural conditions cannot be discarded. In *R. magellanicum* the first period of growth lasted from the full bloom phase till approximately 28 days afterwards, and was similar to *Ribes nigrum* varieties (Toldam-Andersen and Hansen, 1997). It is well known that during this first period of growth the berry is formed and the seed embryos are produced. A rapid cell division occurs during the first few weeks, and by the end of the period, the total number of cells within the berry has been settled (Harris et al., 1968). The extent of cell division will at last be reflected on the definitive size of the berry. In the following lag or transition period, the fleshy tissue grows slowly but the seeds continue to grow rapidly, as has also been cited for *Ribes* fruits (Toldam-Andersen and Hansen, 1997). During the second phase of *R. magellanicum* fruit growth, berries nearly double in size (in terms of dry fruit weight) during the span of fruit growth needed to reach the end of the second period of growth, presumably due to both cell division and enlargement of the fleshy tissue with the arrival of carbohydrates, nitrogen compounds, and other substances including minerals translocated from source tissues. A decrease in both the fresh and dry weight of fruits begins after 98 days from the full bloom phase, due to the fruits were overripened, fact that must be considered to determine the optimal time for harvest (Bisson, 2001). The seed growth performed as the berry growth did, as was observed through the dry seed weight/dry fruit weight ratio.

4.2. Changes in chemical properties during fruit ripening

The beginning of the second phase of *R. magellanicum* berry growth, time when the fruits ripen, is correlated with a number of changes in the plant metabolism, a process driven by energy derived from respiration. All *R. magellanicum* fruit surfaces attain their final and characteristic purple colour during the second phase of berry growth. Thus, as a whole, the observed changes in the fruit growth are associated with changes in fruit metabolism which include other physiological phenomena at the whole plant level, and which can be partially reflected in compositional changes during the fruit ontogeny, as is shown in this case changes through soluble solids, acidity, and anthocyanin content.

During *R. magellanicum* ripening, an initial rapid phase of soluble solid accumulation took place, and then, at some point of berry development and aging, an increase in soluble solid concentration can arise from fruit dehydration, as has been previously reported for *Vitis* (Bisson, 2001). The soluble solids found in ripened *R. magellanicum* fruits averaging near 17.5°Brix were higher than the cited for *R. rubrum* and *R. nigrum* fruits grown at Ushuaia (Arena, 2008, 2010). Anthocyanins are the major phenolic components of soft berry fruits, and their antioxidant activity was closely related to total phenolic content (Deighton et al., 2002). Its production began during the second phase of fruit growth, just when the fresh fruit weight was closed to the maximum. Anthocyanin content evolution during fruiting of *R. magellanicum* underwent a similar process to the evolution of soluble solids, reaching its maximum content when the total titratable acidity was at a minimum. Lack of production during the early stages can be explained by carbon allocation mainly used to sustain biomass increase from primary metabolism when growth is very active. On the other hand, when growth ends, carbon is no longer needed in large quantities for primary metabolism and secondary compounds are more actively synthesized (Bourgaud et al., 2001). The anthocyanin content found in *R. magellanicum* fruits at maturity was higher than the ones reported for *R. rubrum* (Arena, 2008; Kähkönen et al., 2001) and even higher than that corresponding to other reddish-purple berries, such as *Vaccinium* spp., *Rubus* spp. and *Fragaria* spp. (Burrows and Moore, 2002; Lister et al., 2002). However, while anthocyanin content in *R. magellanicum* fruits was higher than *R. nigrum* grown at the same ambient conditions (Arena, 2010), it was lower compared to *R. nigrum* grown elsewhere in the world (Kähkönen et al., 2001; Lister et al., 2002).

5. Conclusions

The fruit growth and chemical properties studied in *R. magellanicum* showed significant changes along the days from the full bloom, so they could be considered as good markers for defining the fruiting phases. However, it is necessary to study other physiological and chemical markers, such as fruit respiration, secondary metabolites and antioxidant activity, in order to design adequate tools for defining the optimal time of harvest according to fruit utilisation (fresh fruit, industrial uses), as well as to keep its nutraceutical properties, findings that together with the previous ones on shoot growth and fruit production are valuable for the commercial use of this wild fruit species.

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