Fruit growth and composition of two Ribes rubrum varieties growing in Tierra del Fuego, Argentina

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Fruit growth and composition of two *Ribes rubrum* varieties growing in Tierra del Fuego, Argentina

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Abstract

The small fruits or berries are products of a great interest due to the increasing world demand, and particularly attention is focused on many of the small fruits which are now considered for their nutraceutical properties. Over the past years, an increasing interest has been shown to the intensive agriculture in Argentinean Patagonia, mainly due to its diversity in soils and climates and to the possibility of producing out of season, with regard to the north hemisphere. The aim of this work was to study the fruit growth and composition along the ripening period and among different years of two varieties of *Ribes rubrum* L. (Jonkheer van Tets and Rovada), growing in Ushuaia, Tierra del Fuego, Argentina. Rovada variety presented higher fresh and dry fruit weight and equatorial and polar diameters than Jonkheer van Tets. These variables, as well as the fruit firmness decreased with the ripening period, except for the fresh and dry fruit weight for the 2005 year. However, the soluble solids, pH and anthocyanin content increased significantly along the ripening period, attaining 13.2°Brix, 3.2 and 14.9 mg anthocyanin/100 g fresh fruit weight respectively. The higher mean daily temperatures in 2004/2005 compared with the other growing seasons may be related with the higher *R. rubrum* fruit weight and fruit diameters, as well as with the higher soluble solids and soluble solids/total titratable acidity ratio in the fruits in the 2005 year. It is expected that the information obtained throughout this study could be of value in defining the optimal time for harvesting of *Ribes rubrum* fruits according to their future use, while contributing to the knowledge of their nutraceutical properties through the quantitative content of soluble solids, total titratable acidity and anthocyanin.

Key words: Berries, ripening, anthocyanin, Tierra del Fuego, South Patagonia.

Introduction

Over the past years, an increasing interest has been shown to the intensive agriculture in Argentinean Patagonia [1-4]. This is due to its diversity in soils and climates and, mainly, to the possibility of producing out of season, with regard to the north hemisphere. The industrial demand for berries, worldwide, is well known. Likewise, in Argentina, berries are currently of increasing importance for the local industry as for the fresh market. Andean Patagonia is the most important zone for producing *Rubus* and *Ribes* berries in Argentina, particularly the Andean region of the 42nd parallel. The *Ribes* culture, however, is still incipient, with a cultivated area of about 10 ha in the cited region. This leads to a lack of information on the growth and fruiting of *Ribes* species in Argentinean Patagonia. Nevertheless, cold hardiness and early ripening make of *Ribes* a viable alternative in such regions as Tierra del Fuego, where winters are too cold or growing seasons too short and cool for the culture of grapes, other berries or tree fruit crops [5].

Particularly, attention is focused on many of the small fruits which are now considered not only as source of minerals and vitamins, but also considered for their nutraceutical properties, as functional foods, i.e., foods containing some specific metabolites which give additional benefits for health [6, 7]. Soft fruits of *Ribes*, *Rubus* and *Vaccinium* species are an excellent source of natural products as pigments [8, 6] with antioxidant properties [9].

Hence, the aim of this work was to study the fruit growth and composition along the ripening period and among different years of two varieties of *Ribes rubrum* L. (Jonkheer van Tets and Rovada), growing in Ushuaia, Tierra del Fuego, Argentina. It is expected that the information obtained throughout this study could be of value in defining the optimal time for harvesting of *Ribes rubrum* fruits according to their future use, while contributing to the knowledge of their nutraceutical properties through the quantitative content of soluble solids, total titratable acidity and anthocyanins.

Material and Methods

The studied area is located near Ushuaia city, 54º48´SL, 68º19'WL (Tierra del Fuego, Argentina). Climatic data on maximal, minimal and mean air daily temperatures (ºC); mean ambient relative humidity (%) and accumulated rainfall (mm) were recorded by the Meteorological Station at Centro Austral de Investigaciones Científicas from October to March for the 2004/2005, 2005/2006 and 2006/2007 growing seasons.

Plants of *Ribes rubrum* L. varieties Jonkheer van Tets and Rovada, of eight years old at the beginning of the experiment, growing at the experimental field of the Centro Austral de Investigaciones Científicas were used for the experiment. The irrigation, fertilization and weed control were described previously[10]. Fruit samples (200 g) were collected during February along 2005, 2006 and 2007 years. The following variables were recorded: fresh fruit weight, dry fruit weight, dry fruit weight concentration, equatorial and polar fruit diameters (using a digital caliper Mitutoyo Model 500-196 (150 mm x 6" – 0.01 mm x 0.0005"). Fruit firmness was evaluated using a digital penetrometer Wagner Instruments Model FDI 2 (0.001 to 1 kgf), with tips of 1 mm diameter in February 2007.

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

Anthocyanin quantification was performed by the pH differential method of Giusti and Wrolstad [11]. Samples (5 g) of initially frozen fruits were extracted during 24 h in 50 ml 0.1% HCl-MeOH solution at 4°C. Then, aliquots were diluted from 1:2 to 1:5 with either a 0.025 M KCl (pH 1) or a 0.4 M sodium acetate (pH 4.5) buffers. Absorbance measurements were made at 510 and 700 nm in a Shimadzu 1203 UV-Visible spectrophotometer. Anthocyanin fruit tissue content was determined on a cyanidin 3-glucoside molar extinction coefficient of 26,900 and a molecular weight of 449.2. Resultant values were expressed in terms of mg of anthocyanin/ 100 g of fresh-frozen fruit. Anthocyanin (mg/100 g fruits) = (A x molecular weight x dilution factor x initial volume/ε x sample weight) x 100, where A (absorbance) = $(A_{510\,nm} - A_{700\,nm})_{pH\,1.0}$ - $(A_{510\,nm} - A_{700\,nm})_{pH\,4.5}$.

Data were subjected to an analysis of variance, where means were separated through a Tukey multiple range test at $p \leq 0.05$.

Results

Mean daily temperature (9.0°C) was higher during 2004/2005 than in 2005/2006 and 2006/2007 growing seasons (8.3 and 8.5°C respectively). The greatest differences in mean daily temperatures (near 3.0°C) were found in November and February among the studied growing seasons (Fig. 1A). Maximal daily temperatures (13.5°C) were higher during 2004/2005 than in the following growing seasons (12.8 and 12.9°C respectively), as well as minimal daily temperatures (4.6, 4.4 and 4.4°C) for the 2004/2005, 2005/ 2006 and 2006/2007 growing seasons, respectively. Mean ambient relative humidity was 74.9, 90.6 and 70.2% for the 2004/2005, 2005/ 2006 and 2006/2007 growing seasons, respectively. Total rainfall reached the maximum value (324.4 mm) in 2005/2006, being of 295.6 and 300.0 mm in 2004/2005 and 2006/2007 growing seasons respectively (Fig. 1B). The maximum rainfall occurred in December for the 2004/2005 growing season, while in January for the 2005/ 2006 growing season and later (February and March) for the 2006/ 2007 growing season.

The fresh fruit weight varied significantly with the variety, along the ripening period and among the years (Table 1). Rovada variety presented the highest fresh fruit weight (538.7 mg), this parameter having a maximum (543.9 mg) at February 1st. The higher fresh fruit weight (585.5 mg) was reached in 2005 in comparison with the other years (459.6 and 460.6 mg for 2006 and 2007 years respectively). The dry fruit weight significantly changed with the variety and among years (Table 1). Rovada variety presented the highest dry fruit weight (87.8 mg), while this parameter was higher (99.3 mg) in 2005 than in 2006 and 2007 years (70.0 and 74.0 mg

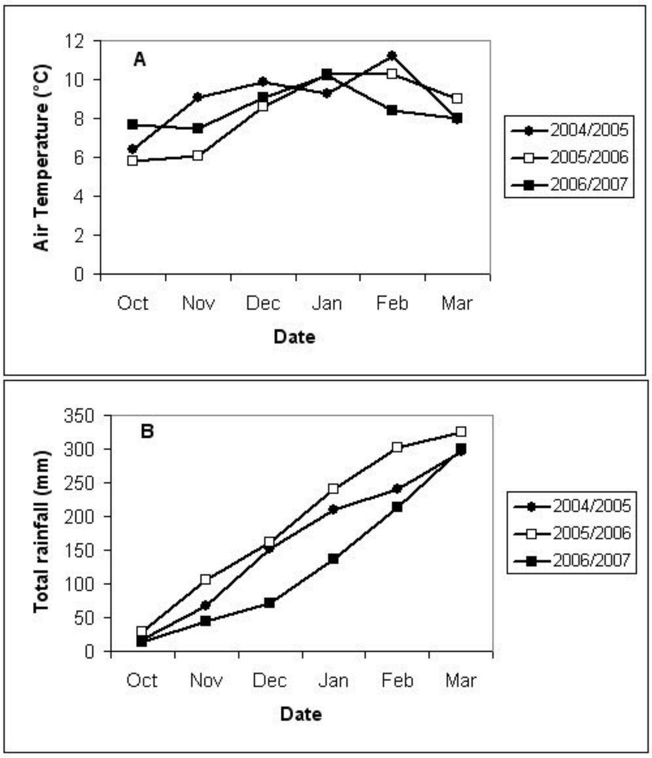

Figure 1. Mean air daily temperatures and accumulated rainfall and accumulative growing degree days recorded from October to March for the 2004/2005, 2005/2006 and 2006/2007 seasons.

respectively). The dry fruit weight concentration significantly varied along the ripening period (Table 1), having a maximum (17.4%) at February 15th. The equatorial and polar fruit diameters were affected by the variety and varied along the ripening period. The equatorial fruit diameter was higher (9.9 mm) in Rovada variety than in Jonkheer van Tets (9.3 mm). This variable was higher (10.1 mm) at February 1st than 15 days later (9.2 mm). The polar fruit diameter followed the same tendency as the equatorial fruit diameter, having a maximum in Rovada variety at the beginning of February (9.5 mm). Firmness varied significantly with the ripening period, having a maximum at the beginning of February (0.09 kgf) and decreasing at mid February (0.05 kgf) (data not shown).

Significant interactions were found in the described variables among several of the studied factors, mainly due to different value increments between the main factors (Table 1) and differences between combinations (Fig. 2A-D), e.g., fresh and dry fruit weight in Rovada variety had different behavior in 2005 in comparison with 2006 and 2007 years, while fresh and dry fruit weight in Jonkheer van Tets variety had different behavior in 2005 in comparison with 2006 year."

. The soluble solids significantly varied along the ripening period and among years (Table 2). This variable had a maximum (13.2°Brix) at February 15th. The soluble solids were significantly higher (13.2°Brix) in 2005 than in the other years (11.2 and 11.5°Brix for the 2006 and 2007 years respectively). The total titratable acidity significantly varied among the years (Table 2), presenting the maximum values (2.4%) in the 2005 year. However, the soluble solids/total titratable acidity ratio was only affected by the date along the ripening period (Table 2). The initial pH significantly varied along the ripening period and among the years (Table 2). This variable had a maximum (3.2) at February 15 and presented

Table 1. Mean values of ANOVA analyzing fruit growth of *Ribes rubrum* considering variety, date along the ripening period and year as main factors and fresh fruit weight (mg) (FFW), dry fruit weight (mg) (DFW), dry fruit weight concentration (%) (DFWC), equatorial fruit diameter (mm) (EFD) and polar fruit diameter (mm) (PFD) as dependent variables (n = 20).

Main effects	FFW	DFW	DFWC	EFD	PFD
A = Variety					
Rovada	538.7a	87.8a	16.52	9.94a	9.40a
Jonkheer van Tets	457.8b	74.3b	16.09	9.31b	8.84
F(p)	9.14(0.003)	7.00(0.009)	1.01(0.316)	12.93(0.000)	8.78(0.003)
B = Date along the ripening period					
Feb 1st	543.9a	83.5	15.17b	10.06a	9.47a
Feb 15th	453.3b	78.7	17.43a	9.20b	8.76
F(p)	11.66(0.000)	0.94(0.335)	28.91(0.000)	23.86(0.000)	14.49(0.000)
C = Year					
2005	585.5a	99.3a	16.81	9.83	9.21
2006	459.6b	70.0b	15.59	9.52	9.03
2007	460.6b	74.0b	16.50	9.54	9.11
F(p)	10.73(0.000)	13.04(0.000)	3.06(0.051)	1.33(0.269)	0.31(0.737)
Interactions F(p)					
A X B	0.33(0.118)	0.99(0.852)	1.43(0.113)	0.02(0.281)	0.01(0.826)
A x C	12.52(0.008)	9.60(0.002)	0.96(0.121)	14.09(0.000)	11.55(0.000)
B x C	5.68(0.004)	1.29(0.280)	9.47(0.000)	3.23(0.043)	3.18(0.045)
A x B x C	2.42(0.093)	3.89(0.023)	3.37(0.038)	0.99(0.374)	2.10(0.127)

F(p) = F statistic and probability at p=0.05. Values followed by different letters in each column and for each factor are significantly different with Tukey Multiple Range test at p<0.05.

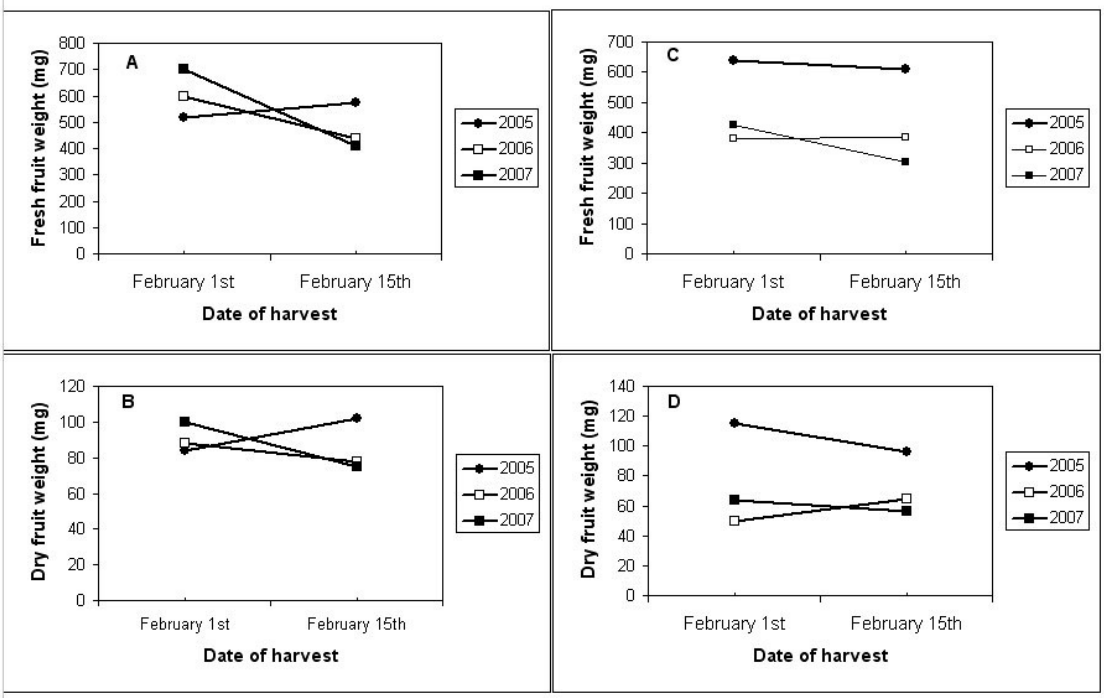

Figure 2. Fresh fruit weight and dry fruit weight analyzed in *R. rubrum* fruits (A and B Rovada variety; C and D Jonkheer van Tets variety) along February of 2005, 2006 and 2007 years.

the highest values (3.2) in the 2007 year. Anthocyanin concentration varied between varieties and along the ripening period (Table 2). This variable was higher in Jonkheer van Tets variety (13.4 mg anthocyanin/100g fresh fruit weight) than in Rovada variety (11.7 mg anthocyanin/100 g fresh fruit). Also, it was higher (14.9 mg anthocyanin/100 g fresh fruit) at February 15 than earlier (10.25 mg anthocyanin/100 g fresh fruit).

Significant interactions were found in the described variables among several of the studied factors, mainly due to different value increments between the main factors (Table 2) and differences between combinations (Fig. 3A-F), e.g., anthocyanin concentration and total titratable acidity in Rovada and Jonkheer van Tets varieties had different behavior in 2006 in comparison with 2007 year.

Discussion

Fruit growth and composition of the two R. rubrum varieties along the ripening period: The fruit ripening phase is characterized by a variation in physical fruit characteristics and is correlated with a number of changes in the plant metabolism, a

Table 2. Mean values of ANOVA analyzing fruit composition of *Ribes rubrum* considering date along the ripening period, maturity and year as main factors and soluble solids (ºBrix) (SS), total titratable acidity (%) (TTA), soluble solids/total titratable acidity relation (RATIO), initial pH (pH) and anthocyanin concentration (mg anthocyanin/100 g fresh fruit) (ANTH) as dependent variables (n = 6).

Main effects	SS	TTA	RATIO	pH	ANTH
A = Variety					
Rovada	12.11	2.18	5.56	3.09	11.73b
Jonkheer van Tets	12.15	2.16	5.69	3.09	13.41a
F(p)	0.50(0.487)	0.06(0.817)	0.45(0.516)	0.09(0.772)	4.38(0.043)
B = Date along the ripening period					
Feb 1st	11.09b	2.11	5.27	3.00b	10.27b
Feb 15th	13.17a	2.23	5.98	3.19a	14.87a
F(p)	1098.1(0.00)	2.48(0.146)	12.5(0.005)	174.9(0.00)	32.9(0.00)
C = Year					
2005	13.16a	2.40a	5.69	2.95c	-----
2006	11.16c	2.08b	5.37	3.13b	11.94
2007	11.48b	2.01b	5.82	3.20a	13.20
F(p)	837.6(0.000)	10.4(0.003)	1.62(0.246)	118.2(0.00)	2.47(0.124)
Interactions F(p)					
A X B	15.75(0.000)	8.79(0.297)	6.02(0.056)	0.29(0.605)	43.1(0.000)
A x C	2.05(0.151)	1.04(0.386)	0.87(0.510)	3.35((0.076)	2.91(0.096)
B x C	16.95(0.000)	4.18(0.047)	4.44(0.041)	24.82(0.000)	0.00(0.969)
A x B x C	2.75(0.085)	6.62(0.014)	6.19(0.017)	6.75(0.014)	3.94(0.054)

F(p) = F statistic and probability at p=0.05. Values followed by different letters in each column and for each factor are significantly different with Tukey Multiple Range test at p<0.05.

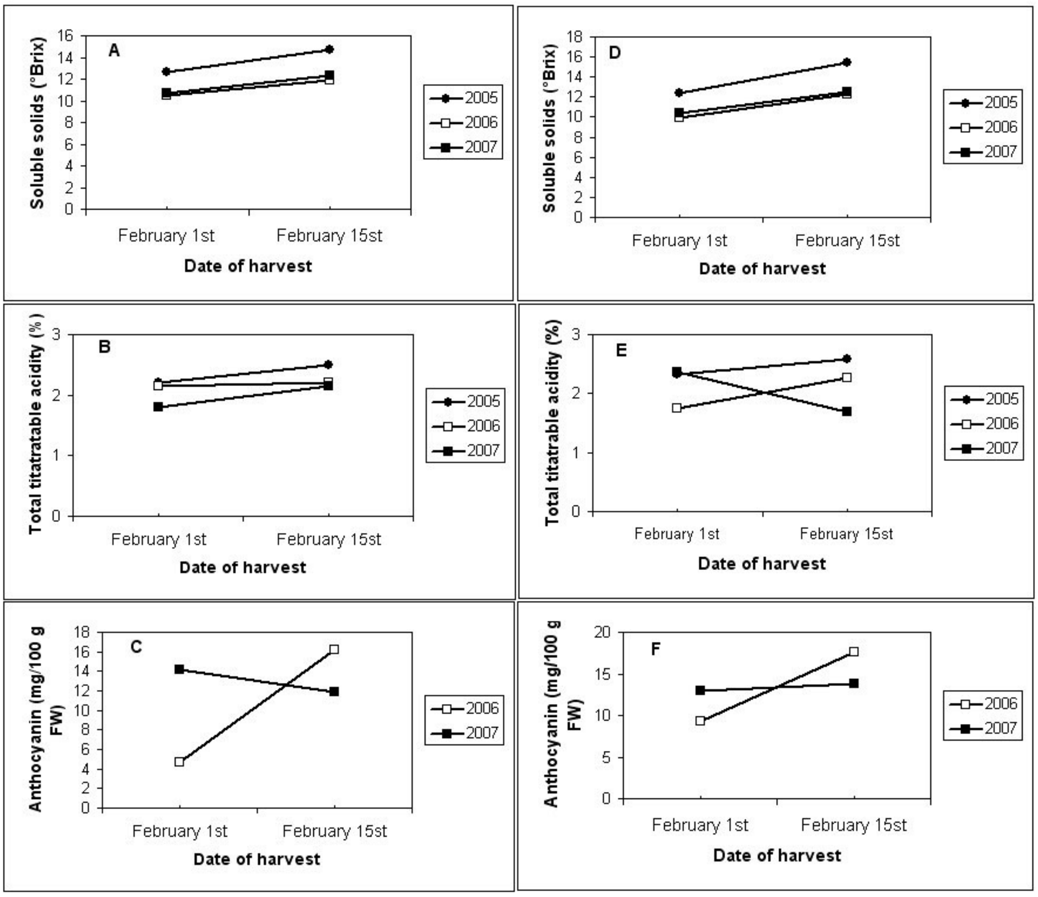

Figure 3. Soluble solids, soluble solid/total titratable acidity ratio and anthocyanin concentration analyzed in *R. rubrum* fruits (A, B and C Rovada variety; D, E and F Jonkheer van Tets variety) along February of 2005, 2006 and 2007 years.

process driven by energy derived from respiration. It has been previously reported that changes associated with fruit ripening include the loss of chlorophyll, which reveals other pigments loss (degradation), softening of the fruit flesh, development of odor and flavor, and a decrease in dry weight mainly due to respiration[12]. Soluble solids and acidity are relatively easy to assay, and are useful chemical traits to define the optimal time for harvest. Acidity can be evaluated as either pH or titratable acidity or both [13]. Working with *R. rubrum* fruits, the differences between both varieties were found only in the fruit weight and diameters, Rovada being the variety which presented the highest values. These variables, as well as the fruit firmness, decreased with the ripening period, except for the fresh and dry fruit weight for the 2005 year. However, the soluble solids, pH and anthocyanin content increased significantly along the ripening period. The soluble solids found in ripen *R. rubrum* fruits grown at Ushuaia averaging close to 11 to 13°Brix were comparable to the ones cited for other *Ribes* species, as *R. grossularia* growing in Norway [14]. However, the ratio values were higher than those found for *R. nigrum* [15]. Anthocyanins are the major phenolic components of soft berry fruits [9], and their concentration in *R. rubrum* fruits at maturity was close to the cited for *R. rubrum* var. Red Dutch [16] and lower than corresponding values of other reddish-purple berries as *Ribes nigrum*, *Vaccinium* spp., *Rubus* spp. and *Fragaria* spp. [15, 17, 18].

Fruit growth and composition of the two R. rubrum varieties along the years: The growth rate and composition of fruits greatly vary among seasons and environmental conditions [12, 19-21]. Dramatic effects of environmental and cultural conditions on both sugar and anthocyanin contents have been reported for grape berries [22, 23]. The higher mean daily temperatures in 2004/2005 compared with the other growing seasons may of course be related with the higher *R. rubrum* fruit weight and fruit diameters, as well as with the higher soluble solids and soluble solids/total titratable acidity ratio in the fruits in 2005. It is already well established that temperature is a limiting factor of photosynthesis rate in plants, thus at those higher temperatures, photoassimilates are produced at a higher rate, which in turn will make possible to increase the translocation from leaves to different sinks, including the fruits [24, 25]. Among small fruit berries, it was found for *Ribes nigrum,* that fruit growth is also correlated with climate conditions [26].

The results obtained in this work highlight the importance of considering all the variables for determine the optimal time of harvest due to the particular behaviour of each of them. Indeed, these results are the first antecedents on the knowledge of *Ribes rubrum* fruit quality at Ushuaia, Tierra del Fuego.

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