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Rootstock and harvest season affect the chemical composition and sensory analysis of grapes and wines of the Alicante Bouschet (*Vitis vinifera* L.) grown in a tropical semi-arid climate in Brazil

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ABSTRACT

Aim: In this study, we aimed to evaluate the composition of grapes and wines of the Alicante Bouschet variety from a tropical semi-arid area in Brazil, by studying two rootstocks and harvests in different semesters (climates) of the same year.

Methods, Results: Vines of *Vitis vinifera* L., Alicante Bouschet, a *teinturier* variety, were grown in a tropical semi-arid climate in Brazil. The phenolic composition of the grapes and wines was measured to assess how they had been affected by two rootstocks (IAC 572 and 1103P) and two harvest seasons, Semester 1 and Semester 2 (in this region, a grapevine produces two harvests per year, with an intra-annual climate variability). The grapes and wines were subjected to the usual physicochemical analyses, as well as to spectrophotometric and chromatographic analyses. Sensory analysis was carried out by professional tasters. The results show that there is a rootstock effect and a harvest season effect for several parameters on grape composition, as well as on the resulting wines. The grapes from vines grafted onto IAC 572 rootstock contained higher titratable acidity, tartaric acid, malic acid, total anthocyanins, total proanthocyanidins in skins and seeds and polymeric tannins in skins. The grapes from vines grafted onto 1103P rootstock contained higher concentrations of total phenols and non-flavonoids, total monomeric anthocyanins, monomeric and polymeric tannins in the seeds. The second semester wines were higher in total anthocyanins, total phenols, flavonoids and non-flavonoids, condensed tannins and low molecular weight flavanols, astringency potential, colour intensity, titratable acidity, total dry extract and alcohol content.

Conclusions: The results demonstrate an influence of rootstock and harvest season (semester/climate) on grape and wine composition. Some of the grape and wine phenolic compounds analysed individually (some monomeric anthocyanins and low molecular weight flavanols) were higher in the first semester than in the second. The overall phenolic parameters determined in grapes and wines from the second semester were higher than those from the first. According to the sensory analyses, the semester (climate) effect was stronger than the rootstock effect, and the wines from the second semester received the highest scoring than those from the first semester, mainly for olfactory and gustatory attributes.

Significance and impact of the study: This study uses a holistic methodology, mainly with respect to the phenolic compounds, to examine the combined effects of rootstocks and intra-annual climate variability on grape and wine composition. The results of this study may contribute to future research, as well as help the producers and wine companies of tropical regions to tailor viticultural and oenological parameters, in order to improve the quality and typicality of the products.

KEYWORDS

rootstock, phenolic composition, tropical semiarid climate, Alicante Bouschet, two harvest seasons per year.

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INTRODUCTION

In most winegrowing regions of the world, vineyards are established using grafted grapevines. This is mainly to avoid root damage from phylloxera (caused by the insect *Daktulosphaira vitifoliae*) and certain nematodes, which affect grape yield and quality and can be devastating (Miele and Rizzon, 2017). Rootstocks are also used to help vines cope with adverse soil conditions (salinity, alkalinity, acidity) and extremes in water availability (drought and flooding).

Wine quality largely depends on the quality of the grapes at harvest which, in turn, is attributable to the intrinsic characteristics of the grape cultivar, as well as to many environmental factors (including the climate and the soil, etc.) and viticultural practices (Reynolds, 2010; Liang *et al.*, 2012). The genotype of the scion is thought to have a predominating influence on the types of compounds synthesised in the fruit (Harbertson and Keller, 2012). However, some studies have found that rootstock can influence the quality of both the grape and the wine by affecting, for example, the concentration of volatile compounds (Ough *et al.*, 1986), minerals (Bavaresco *et al.*, 2003; Kodur, 2011), amino acids (Jogaiah *et al.*, 2013), phenolics, anthocyanins, total tannins and flavanols (Nedelkovskiet *et al.*, 2017), and wine pH and potassium (Kodur, 2011; Harbertson and Keller, 2012). All these combined effects determine the wine's sensory properties and quality (Mantilla *et al.*, 2017).

The São Francisco Valley is located in a semi-arid region of Brazil. It is considered a suitable region for tropical viticulture, being particularly distinguished by its soil and climatic conditions. These conditions, alongside appropriate irrigation techniques, allow growers to choose the harvest season by scheduling the production, and they can thus obtain two, or even three, annual harvests (Soares and Leão, 2009) during Semester 1 and Semester 2. Among the red varieties grown in Brazil, Alicante Bouschet has been widely used to make varietal-blend wine. However, in this tropical region, Alicante Bouschet can also exhibit good potential for the production of its own 'varietal' wine. Ideal rootstock for these semi-arid conditions needs to combine characteristics such as vigour, resistance to pests and diseases, good rooting properties and ease of grafting (good 'take'). Rootstocks must be suitable for high fruit yield and they must be of high winemaking quality. A single rootstock will not satisfy the needs of all growing conditions and scion cultivars; therefore, studies are needed to better understand the interactions between rootstock and scion under each set of regional conditions (Soares and Leão, 2009).

The Campinas Agronomic Institute (IAC) has developed new rootstocks in a crossbreeding programme which began over 50 years ago and uses *Vitis* species originating in tropical America. As a result, the IAC rootstocks typically exhibit high vegetative vigour, which makes them especially useful in this tropical climate. Their adaptability to different soil types, including those of high acidity or clay or sand, is also useful. In general, these rootstocks show high resistance to the major soil pests, such as phylloxera and nematodes, and their leaves are resistant to major fungal diseases. Lastly, their cuttings take and root very well. The IAC research has also produced rootstock propagation material that is free of viruses, ensuring high plant health and longevity in the production regions of Brazil (Instituto Agronomico de Campinas, 2020). The IAC 572 rootstock results from a cross between 101-14 MGT (*Vitis riparia* x *V. rupestris*) and *V. caribaea,* and shows high canopy vigour, productivity and rooting, compared to Paulsen 1103 (1103P). Therefore, IAC 572 is widely used in São Francisco Valley.

The aim of this study was to characterise the interactions between Alicante Bouschet (*Vitis vinifera* L.) and IAC 572 and 1103P rootstocks under tropical, semi-arid conditions in terms of the chemical characteristics of grapes at maturity and of wines from two harvest seasons.

MATERIALS AND METHODS

1. Vineyard characteristics and harvest seasons

The study area is located in the municipality of Lagoa Grande, in the state of Pernambuco, between the parallels of latitude 8-9 º in the Southern Hemisphere and at an altitude of 350 MSL. Soils are classified as yellow eutrophic argisol/ typical plintustalf (soil taxonomy alfisol), usually with medium natural fertility. The climate is classified for wine-growing as follows: (1) moderate drought with hot nights; (2) moderate drought, very hot days, and warm nights and (3) severe drought, very hot days and nights. Under these climate conditions and with multiple, intra-annual harvests, the wine style varies depending on the time of year in which the grapes are harvested (Tonietto *et al.*, 2012). The average weather data during the study period is shown in Figure 1.

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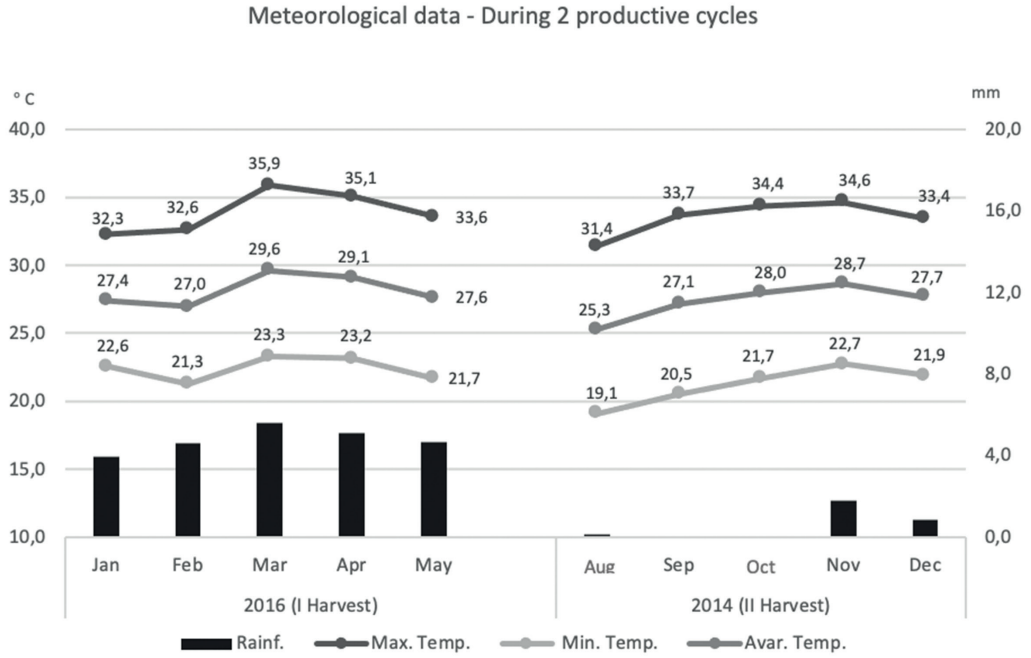


FIGURE 1. Weather data during two production cycles of Alicante Bouschet, in the semi-arid region of Brazil. "Rainf" (Precipitation); "Max. Temp." (maximum Temperature); "Min. Temp." (minimum temperature); "Aver. Temp." (average temperature); "Jan" (January); "Feb" (February), "Mar" (March); "Apr" (April); "Aug" (August); "Sep" (September); "Oct" (October); "Nov" (November); "Dec" (December). Based on meteorological data from a weather station on the farm.

The experiment was carried out in a commercial vineyard planted with Alicante Bouschet (*Vitis vinifera* L.). Vines were grafted onto IAC 572 and 1103P rootstock, and all vines were ten years old at the beginning of the study. The rows were orientated north to south, with a vine spacing of 3 m between rows and 1 m within the row. The canopy was trained to a vertical trellis. Under the conditions of this region, grapes ripen twice a year (hereafter referred to as Semester 1 and Semester 2). The vineyard was drip irrigated, with the same quantity of water delivered to all rootstock treatments on the same day. Irrigation was applied according to ET x Kc, with evapotranspiration (ET) and the culture coefficient (Kc) being based on vine spacing and the developmental stage. The vines were all in the same general area and rootstock treatments were in a randomised complete block design. There were 30 vines in each of the two rootstock treatments (1103 P and IAC 572), replicated in three complete blocks with five marked vines per plot. Harvest times were determined using standard commercial criteria. Approximately 40 kg of grapes were collected per treatment. The harvests were in December 2014 (Semester 2) and in May 2016 (Semester 1). For the laboratory analyses, clusters were collected from marked vines. Grapes were mature and in a good state of health.

2. Extraction methods for grapes

For the analyses, individual berries were randomly selected from different parts of the cluster (proximal, middle and distal). Phenolic compounds were extracted by applying either one of two methods. In the first method, extracts were obtained from the macerated skins and seeds of samples of 200 berries, as described by Carbonneau and Champagnol (1993). Extraction was carried out for 24 h at 20 ºC using an ethanol (96 %) and tartaric acid solution (pH 3.2). The extract was centrifuged (3500 rpm for 10 to 15 min) before use. In the second method, skins, pulps and seeds were separated and weighed, and the phenolic compounds extracted from in each component, as described by Bourzeix *et al.* (1986). For the extraction, solvents of different polarities (methanol, water and acetone) were used, with different contact times for successive macerations: (1) overnight at -24 °C (methanol) and further macerations in a nitrogen atmosphere, (2) 4 h at room temperature in aqueous methanol (methanol: water; 80:20; v/v), (3) 4 h at room temperature in aqueous methanol (methanol: water; 50:50; v/v), (4) 15 h at -24 °C in distilled water, and (5) 1 h at room temperature in aqueous acetone (acetone: water; 75:25; v/v). Each separate extraction liquid was then mixed and stored at 4 ºC in a nitrogen atmosphere.

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3. Classical analyses

The must obtained from the pulp was analysed for the usual technological parameters, which were: pH, total soluble solids (TSS), titratable acidity (TA), tartaric acid and malic acid. All evaluations were according to the methods described by the International Organisation of Vine and Wine (OIV, 2014).

The spectrophotometry was carried out on the extracts obtained by applying the Carbonneau and Champagnol (1993) method. Total phenols (Ribéreau-Gayon, 1970), non-flavonoid and flavonoid phenols (Kramling and Singleton, 1969), total anthocyanins (Ribéreau-Gayon and Stonestreet, 1965), colour intensity and tonality (OIV, 2014). Astringency potential (De Freitas and Mateus, 2001) was evaluated using a turbidimeter.

4. Vinification of monovarietal wines

For each harvest, experimental wines were made in the Embrapa Semi-arid Oenology Laboratory using a microvinification process with composite samples of 40 kg of fruit per rootstock.

Winemaking followed traditional methods for red wines. Berries were removed from the rachises with semi-automatic equipment (Model DH150-DA, Recifer-Brazil). After pressing, 50 mg/L of sulphur dioxide and 20 g/hL of yeast (*Saccharomyces cerevisiae*, *bayanus*- Everintec, Italy) were added to the must for the alcoholic fermentation (22 to 25 ºC). Remontage was carried out once a day by rack and return. The contact between solid components and liquid ones (maceration time) was uniform (7 d) across treatments for a consistent extraction of phenolics. The end of alcoholic fermentation was identified by the stability of the density and alcohol content. Malolactic fermentation occurred spontaneously (16 to 18 ºC) with the end determined by paper chromatography (OIV, 2014). Cold stabilisation (0-5 °C) was for 30 days, the amount of free sulphur dioxide was corrected (40 mg/L) and the wine bottled and stored at 10 ± 2 ºC.

5. Physicochemical characterisation of wines

Wines were analysed six months after bottling for the following classical parameters: alcohol strength, residual sugar, free and total sulphur dioxide, volatile and TA, pH, dry extract, potassium and calcium. The analysis methodology of the International Organisation of Vine and Wine (OIV, 2014) was used.

The colorimetric parameters analysed were: total anthocyanins (Ribéreau-Gayon and Stonestreet, 1965); coloured anthocyanin (Somers and Evans, 1977); total phenols (Ribéreau-Gayon, 1970); flavonoids and non-flavonoids (Kramling and Singleton, 1969); anthocyanin copigmentation (Boulton, 2001); colour intensity and tonality (OIV, 2014); total and polymeric pigments (Somers and Evans, 1977); and astringency potential (De Freitas and Mateus, 2001).

6. Separation and quantification of individual monomeric anthocyanins by HPLC

To separate the monomeric anthocyanins, an HPLC (Perkin-Elmer, Waltham, MA, USA) comprising a pump (Series 200) and detector (LC95 UV/Visible) was used. Separation was carried out in a C18 column (250 x 4 mm) of reverse phase with 5 µm of compaction, and protected by a pre-column of the same material; both the column and pre-column were from LichroCart (Merck, Darmstadt, Germany). The solvents were 40 % formic acid and 60 % bidistilled water (A), acetonitrile PA (B) and bi-distilled water (C).

The initial conditions were 1) 25 % A, 6 % B and 69 % C for 15 min, followed by 2) a 25 % linear gradient of A, 25.5 % B and 49.5 % C for 70 min, and finishing with 3) 20 min of 25 % A, 25.5 % B and 49.5 % C. The flow was 0.7 mL/min, the detector wavelength 520 nm and the injected volume 20 µL. Analyses were carried out in triplicate with a column temperature of 30 °C. The identification of fourteen anthocyanin molecules followed the method of Roggero *et al.* (1986) and quantification was based on a standard curve with malvidin 3-*O*-glucoside.

7. Fractionation of low molecular weight flavanols by polyamide column chromatography and further quantification by HPLC

Extracts (5 mL) of seeds, skins and wines were fractionated on a polyamide column (Macherey-Nagel, Düren, Germany), as described by Ricardo-da-Silva *et al.* (1990). Phenolic acids were eluted with 80 mL of phosphate buffer (pH 7.0). Monomeric flavanols were eluted with 50 mL of ethyl acetate:water (30/70, v:v) and oligomeric procyanidins with 50 mL of acetone/water (75/25, v/v). Fractions were brought to dryness, dissolved in 1.2 mL of methanol/water (50/50, v/v), filtered through a 0.45 µm membrane and injected onto the HPLC column. A new polyamide column

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was used for each sample and analyses were carried out in triplicate.

The HPLC equipment comprised a UV-Vis detector (Waters 2487) and a Merck L-7100 pump (Kenilworth, NJ, USA). Separation was carried out on a 250 x 4.6 mm x 5µm Lichrosphere C18 reverse phase column (Merck, Darmstadt, Germany) at room temperature. For the monomeric flavan-3-ols, a gradient consisting of solvent A (water/acetic acid, 97.5/2.5, v/v) and solvent B (acetonitrile/solvent A, 80/20, v/v) were used at 0.9 mL/min as follows: 7-25 % B linear from 0 to 31 min, followed by washing (methanol/water, 50/50, v/v) for 32 to 50 min. The column was re-balanced for 51 to 65 min under the initial gradient conditions. For oligomeric procyanidins, solvent gradient A (bi-distilled water) and solvent B (bi-distilled water/acetic acid, 90/10, v/v) were used at 1.0 mL/min as follows: 10-70 % linear B 0-45 min, 70-90 % linear B 45-70 min, 90 % B isocratic 70-82 min, 90100 % linear B 82-85/ min, 100 % B isocratic 85-90 min. Detection was carried out at 280 nm absorbance and injections in triplicate.

The identification of the compounds was undertaken according to Rigaud *et al.* (1991) and Ricardo-da-Silva *et al.* (1991) and later confirmed according to Monagas *et al.* (2003). The quantification of monomeric flavan-3-ol and small oligomeric procyanidins (dimers and trimers) was based on standard curves obtained with (+) catechin for the monomers and dimer B2 for the other compounds.

8. Isolation of flavanols in skins and seeds on a fractogel chromatographic column and further degradation by acid catalysed depolymerisation in the presence of toluene-α-thiol, followed by HPLC analysis

Skin or seed extracts (5 mL) were passed through a glass column (QuickFit CR 12/10 of (100 x 10mm) filled with fractogel (TSK HW-40FToyopearl®, Tokyo, Japan). Flow (0.7 mL/min) was maintained using a vacuum pump (VacuubrandMZ2C, Wertheim, Germany). A mixture of ethanol, water and trifluoroacetic acid (TFA) (55/45/0.05, v/v/v) was used for column conditioning before injecting 5 mL of extract. After the passage of the extract in the column, 30 ml of solvent containing TFA was then added to elute the simple and monomeric phenols (phenolic acids, anthocyanins, flavonols, stilbenes and catechins) and polymeric flavonoids in increasing order of polymerisation. The column was washed with 30 mL acetone/water (60/40, v/v) to collect the polymeric flavonoids still attached to the gel. This fraction was evaporated to dryness, dissolved in 1 mL methanol (seed samples) or 0.5 mL methanol (skin samples) and stored at -20 °C. The preparation of the fractogel column was prepared and the flavanols isolated according to Labarbe *et al.* (1999). The acid-catalysed degradation of the flavanols was carried out in the presence of toluene-α-thiol as described by Kennedy *et al.* (2000) and Monagas *et al.* (2003), but with some modifications. A 100 µL subsample was placed in a 1.0 mL screw-cap vial and mixed with 100 µL of toluene-R-thiol (5 %, seed samples; 12 %, skin samples) in methanol containing HCl (0.2 M). The mixture was held at 55 °C (water bath) for 10 min. The thiolysed sample was cooled under running water and immediately analysed by reverse-phase HPLC (Merck Hitachi L-7100 pump, HD, Germany; Waters 2487 detector, MA, USA). Separation was done on a reverse-phase HPLC (Gemini C18 110A, 150 x 3mm x 5 µm) at room temperature. A binary gradient consisting of solvent A (water/formic acid, 98/2, v/v) and solvent B (acetonitrile/water/formic acid, 80/18/2, v/v/v) at 1.0 mL/min was used as follows: 0 to 15 min with 10 % solution A and 90 % solution B, followed by washing (B) and re-equilibration of the column. DAD detection was at 280 nm.

The calculations of mean degree of polymerisation (mDP), percentage of galloylation and percentage of prodelphinidins, were based on the peak areas (kW) of the terminal units and extensions after HPLC analysis. The identification of the peaks was based on Monagas *et al.* (2003).

9. Separation of proanthocyanidins in Sep-Pak C18 cartridges and quantification of the obtained fractions by the vanillin assay

A Sep-Pak C18 cartridge (Waters, MA, USA) was used for the separation of flavanols (also known as proanthocyanidins or condensed tannins) in skin and seed extracts and in wine samples and was based on the degree of polymerisation into three fractions (monomeric, oligomeric and polymeric) according to Sun *et al.* (1998). The extracts and wines were de-alcoholised by rotary evaporation (BÜCHI Labortechnik, Switzerland) at < 30°C and adjusted to pH 7.0 with phosphate buffer (pH 7.0). The sample was then passed through two inseries, preconditioned, neutral Sep-Pak cartridges: first Sep-Pak tC18, then Sep-Pak C18. For the elution, 10 mL of H_2O was adjusted to pH 7.0

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to eliminate phenolic acids and the cartridges were then dried with N2. The elution was first carried out with 25 mL of ethyl acetate to elute catechins and oligomeric procyanidins, and some other small phenolic molecules (fractions FI and FII), and then with 10 mL of methanol to elute the polymeric procyanidins and anthocyanins (FIII). For the separation of catechins from oligomeric procyanidins (FI+II) was evaporated to dryness under vacuum at 25 °C, re-dissolved in distilled water and redeposited onto the same cartridges preconditioned with distilled water. Once again, the cartridges were dried with N2, separation of catechins and oligomeric procyanidins was done by sequential elution with 25 mL of diethyl ether (FI) and then with 10 mL of methanol (FII). For quantification, standard curves were produced for flavanol monomers, oligomers, and polymers of grape seed isolates, as described by Sun *et al.* (1998). The extractions on the C18 cartridge and the further readings after reaction with vanillin were in triplicate.

10. Characterisation of the sensory profile of wines

Sensory evaluation was carried out by a panel of twelve professional tasters in the sensory analysis laboratory of the Instituto Superior of Agronomia in Lisbon. A Quantitative Descriptive Analysis (QDA) was carried out to evaluate sixteen visual, olfactory and taste attributes of the wines: four visual (colour, intensity of colour, limpidity and fluidity), five aromatic (fruit, floral, herbaceous, spices and empyreumatic), and seven taste (sweetness, acidity, alcohol, bitterness, astringency, body, persistence). The evaluation wrapped up with a general assessment. The evaluation was quantified using a scale with an unstructured intensity of 10 points, with minimum anchorage on the left and maximum on the right.

Each taster evaluated two samples per session, with two sessions in 2015 and 2016. The test room had individual, white, illuminated booths, and samples were served individually and coded in tasting glasses (ISO) each containing 50 mL of wine at 18±2 ºC (ideal for red wines).

11. Statistical analyses

To confirm the significant differences between samples, based on the two rootstocks and two harvest seasons, we used analysis of variance (ANOVA). Analyses employed STATISTIX 9.0 software (FL, USA). All chemical analyses were carried out in triplicate.

RESULTS AND DISCUSSION

1. Characterisation of grapes

1.1. Classic analysis and organic acids

The results for classical chemical compositions are presented in Table 1. The highest acidities were for vines on IAC 572 in both semesters. In Semester 1, TA ranged from 5.2 (on 1103P) to 5.8 g/L (on IAC 572), and in Semester 2 from 3.9 (on 1103P) to 5.6 g/L (on IAC 572). The 1103P rootstock affected TSS in both Semester 1 and Semester 2. This agrees with a previous study showing higher TSS in grapes for juice (*Vitis labrusca*) harvested in the second semester as compared to the first semester (Padilha *et al.*, 2019). The second semester harvest, led to higher TSS in the grapes, because of higher temperatures.

The concentrations of tartaric and malic acid were higher in Semester 1 than in Semester 2. The highest contents were on IAC 572 with 6.5 g/kg of tartaric acid and 3.9 g/kg of malic acid in Semester 1, and 3.4 g/kg-1 (tartaric acid) and 1.8 g/kg (malic acid) in Semester 2. Tartaric acid does not usually suffer degradation due to high berry temperatures (Ruffner, 1982a; Ford, 2012). Its low concentration can also be due to precipitation with potassium, since the soils of this region are rather saline (Mantilla *et al.*, 2017), thus affecting the potassium contents in the wines (Table 4).

Low concentrations of malic acid in Semester 2 may be related to the high average temperatures during maturation. Malic acid concentrations generally decrease during maturation, being metabolised as an energy source. This is another reason why it can be degraded at high temperatures (Ruffner, 1982b; Ford, 2012). When evaluating Syrah grapes in this region, Oliveira *et al.* (2019) also found high concentrations of malic acid in Semester 1.

1.2. Phenolic composition of grapes

Values for total flavonoid and non-flavonoid phenols are shown in Table 1. Total phenol concentrations ranged from 1139.7 mg/kg of gallic acid equivalents on IAC 572 to 1830.1 mg/kg on 1103P. The non-flavonoids ranged from 317.5 mg/kg of gallic acid equivalents on IAC 572 to 651.6 mg/kg on 1103P. The flavonoids were 801.0 mg/kg of gallic acid equivalents on 1103P in Semester 1 and 1168.8 mg kg in Semester 2. Fruit from vines on 1103P in Semester 2

TABLE 1. Effects of rootstock and semester on the grape composition, global phenolic and monomeric anthocyanins in Alicante Bouschet grapes of a tropical semi-arid region in Brazil.

Parameters	Semester 1		*sig.*	Semester 2		*sig.*	Rootstock	Semester
	IAC 572	1103P		IAC 572	1103P			
Classical analyses								
pH	3.56	3.66	*	3.78	3.82	*	*	*
Titratable acidity (g/L tartaric acid)	5.8	5.2	*	5.6	4.9	*	*	*ns*
TSS (°Brix)	17.2	18.7	*	21.9	23.4	*	*	*
Tartaric acid (g/kg)	6.5	5.3	*	3.4	2.9	*	*	*
Malic acid (g/kg)	3.9	2.5	*	1.8	1.1	*	*	*
Total phenols (mg/kg)	1139.7	1162.4	*	1456.9	1830.1	*	*	*
Non- flavonoids (mg/kg)	317.5	361.9	*	619.7	651.6	*	*	*
Flavonoids (mg/kg)	822.8	801.0	*	837.7	1168.8	*	*ns*	*
Total anthocyanins (mg/kg)	844.1	790.3	*	963.1	947.9	*	*	*
Colour Intensity (*a.u*)	11.67	11.38	*	15.42	24.87	*	*ns*	*
Tonality (*u.a*)	0.498	0.491	*ns*	1.983	1.810	*	*ns*	*
Anthocyanins monoglucosides (mg/kg)								
Cyanidin 3-*O*-glucoside	1.0	0.6	*	1.6	2.4	*	*ns*	*
Delphinidin 3-*O*-glucoside	2.4	1.7	*	3.5	5.3	*	*ns*	*
Peonidin 3-*O*-glucoside	31.5	45.3	*	30.3	31.2	*	*	*
Petunidin 3-*O*-glucoside	2.4	1.7	*	3.6	4.2	*	*ns*	*
Malvidin 3-*O*-glucoside	45.3	40.1	*	59.3	89.6	*	*ns*	*
Delphinidin 3-*O*-acetylglucoside	0.8	0.6	*ns*	0.4	0.6	*ns*	*ns*	*ns*
Petunidin 3-*O*-acetylglucoside	1.2	1.3	*ns*	0.8	1.0	ns	*ns*	*
Malvidin 3-*O*-acetylglucoside	4.3	2.5	*	4.0	4.8	*	*ns*	*ns*
Cyanidin 3-*O*-coumarylglucoside	0.6	0.4	*ns*	0.1	0.2	*ns*	*ns*	*ns*
Peonidin 3-*O*-coumarylglucoside	3.3	4.2	*	2.0	3.2	*	*	*
Petunidin 3-*O*-coumarylglucoside	1.6	1.8	*ns*	0.8	0.8	*ns*	*ns*	*
Malvidin 3-*O*-coumarylglucoside	4.5	5.2	*	6.3	9.1	*	*	*
Total monomeric anthocyanins	98.9	125.3	*	113.5	152.5	*	*	*

Rootstock (IAC 572 and Paulsen 1103P); Semester 1 (2016); Semester 2 (2014); TSS = total soluble solids; results in fresh weight; ANOVA ($p < 0.05$); ns (not significant); *(significant differences).

generally had higher concentrations of phenolic compounds (total and non-flavonoid). According to Cheng *et al.* (2017) rootstocks can have significant effects on the concentrations of total phenols and flavonoids in grapes.

The concentrations of total anthocyanins varied from 790.3 mg/kg on 1103P to 844.1 mg/kg on IAC 572 in Semester 1. In Semester 2, values were 947.9 mg/kg-1on 1103P and 963.1 mg/kg on IAC 572. There was a rootstock influence on anthocyanin composition with higher concentrations on the most vigorous rootstock, IAC 572. This demonstrates that rootstock has an influence on Alicante Bouschet. In semi-arid regions, anthocyanins can be degraded due to high temperatures and insolation; a rootstock with greater vigour in the canopy, provides greater shading of the bunches, which protects them from solar radiation and reduces the temperature in the vicinity of the bunch. Other authors do not identify rootstock influences on anthocyanin content for other grape cultivars (Keller *et al.*, 2012).

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TABLE 2. Effects of rootstock and semester on the condensed tannins in skin, pulp and seed of Alicante Bouschet grapes of a tropical semi-arid region in Brazil.

Parameters	Semester 1		*sig.*	Semester 2		*sig.*	Rootstock	Semester
	IAC 572	1103P		IAC 572	1103P			
Condensed tannins in seeds								
Monomeric	2.2	3.5	*	2.5	2.8	*	*	*ns*
Oligomeric	7.2	9.2	*	8.3	11.1	*	*	*
Polymeric	17.2	37.3	*	28.7	43.2	*	*	*
Total condensed tannins in seeds	26.6	50.0	*	39.5	57.1	*	*	*
mDP	12.7	10.3	*	6.7	7.1	*	*ns*	*
% galloylation	29.1	36.7	*	45.2	48.9	*	*	*
Condensed tannins in skins								
Monomeric	0.6	0.5	*ns*	0.4	0.5	*ns*	*ns*	*ns*
Oligomeric	1.6	1.1	*	1.8	1.8	*ns*	*ns*	*
Polymeric	3.4	2.2	*	4.2	3.2	*	*	*
Total condensed tannins in skins	5.6	3.7	*	6.5	5.5	*	*	*
mDP	17.3	18.1	*	22.7	27.5	*	*	*
% galloylation	2.9	3.5	*	3.8	4.3	*	*	*
% prodelphinidins	21.6	25.4	*	29.7	35.1	*	*	*
Condensed tannins in pulp								
Monomeric	0.1	0.0	*	0.0	0.0	*ns*	*ns*	*ns*
Oligomeric	0.0	0.0	*ns*	0.1	0.0	*	*ns*	*ns*
Polymeric	0.4	0.3	*	0.3	0.0	*	*	*ns*
Total condensed tannins in pulp	0.5	0.3	*	0.4	0.1	*	*	*

mDP (mean degree of polymerisation); Rootstocks (IAC 572 and Paulsen 1103 (1103P); Semester 1 (2016); Semester 2 (2014); concentrations in mg/g of seeds, skins or pulp; results as fresh weights; ANOVA ($p < 0.05$); ns (not significant); * (significantly different).

1.3. Monomeric anthocyanins in grapes

Twelve monomeric anthocyanins (Table 1) were evaluated. Most part of the glycosylated anthocyanins were at higher concentration in grapevines on 1103P in both the first and second semester. Concentrations were 5.3 mg/kg (delphinidin), 2.4 mg/kg^{-1} (cyanidin), 4.2 mg/kg (petunidin), 45.3 mg/kg (peonidin) and 89.6 mg/kg (malvidin). These values are lower than those found by Costa *et al.* (2015) for the same variety in the Douro and Dão r egions of Portugal.

The anthocyanins esterified with *p*-coumaric acid were higher in berries of vines on 1103P, with 4.2 mg/kg of peonidin and 1.8 mg/kg of petunidin. The highest concentration of malvidin was in the second semester on 1103P. Concentrations of total monomeric anthocyanins were highest in grapes of vines on 1103Pin the first (126.2 mg/kg) and second semesters (153.4 mg/kg). In studies on Cabernet-Sauvignon (Koundouras *et al.,* 2009) and Merlot and Syrah (Harbertson and Keller, 2012) no rootstock influences on anthocyanin composition were identified. Regarding the harvest season, the Alicante Bouschet grapes harvested in the second semester (summer), showed higher concentrations of total monomeric anthocyanins.

1.4. Condensed tannins in skins, pulp and seeds

The results for condensed tannins in skins, pulps and seeds are shown in Table 2. In the seeds, the highest concentrations of condensed tannins, monomeric flavanols, were on 1103P with values of 3.5 mg/g (first semester) and 2.8 mg/g (second semester). In the second semester, the highest concentrations in the seeds were of oligomeric flavanols (11.1 mg/g) and polymeric flavanols (43.2 mg/g). The concentrations of monomeric flavanols in seeds were similar to those reported by Sun *et al.* (2001) for the Castelão and Vinhão grapes; although the oligomeric and polymeric tannins were smaller, they found concentrations ranging from 2.1 to 3.5 mg/g (monomers), 23.7 to 27.4 mg/g (oligomers) and 58.0 to 73.5 mg/g (polymers).

The higher concentrations of monomers, oligomers and polymers compounds in 1103P may be related to the lower vigour of this rootstock compared with IAC 572 in tropical regions. This could result in a reduced canopy, promoting earlier fruit maturation, and thus lower tannin degradation in the seeds during the shorter maturation period, causing a divergence of technological and phenolic maturation. The seed is the component of the berry containing the greatest concentrations of the oligomeric and polymeric fraction, as already reported by other authors (Sun *et al.*, 2001; Monagas *et al.*, 2003; Ó-Marques *et al.*, 2005). In addition, in all parts of the berry (seeds, skins and pulps), the polymeric fraction is always the dominant fraction.

The concentration of total condensed tannins in seeds were higher for 1103P, with 50.0 mg/g of seeds (Semester 1) and 57.1 mg/g of seeds (Semester 2). For both rootstocks there was a trend of higher concentrations in Semester 2. This may be related to the high daily temperatures and low thermal amplitude during ripening that promote a distinction between technological (pH, TSS, and TA) and phenolic maturation (total phenols, anthocyanins, and tannins).

For the condensed tannins in the skins, we can see that the values for oligomeric tannins were higher in Semester 2 with 1.8 mg/g of skins on both rootstocks. The polymeric fraction was higher in the skins of grapes on IAC 572, with 3.4mg/g and 4.2 mg/g in Semester 1 and Semester 2 respectively.

The highest concentrations of total condensed tannins in skins were in Semester 2 with 6.5 mg/g in skins (IAC 572). The high concentrations of tannins in Semester 2 may be related to the slightly early harvest due to occasional rainfall (Figure 1). The concentration of total condensed tannins in the skins were higher than those reported by Cosme *et al.* (2009) in the skins of Trincadeira, Cabernet-Sauvignon, Touriga Nacional and Castelão, ranging from 1.09 to 5.84 mg/g of skin.

Condensed tannins in the pulp were below 0.5 mg/g. Being a *teinturier* cultivar, Alicante Bouschet has low concentrations of condensed tannins in the pulp, perhaps because most phenolic compounds are in the seeds and skins. However, the pulp condensed tannins values for *teinturier* grapes are usually higher than those encountered in the pulps of most part of the red and white grapevine varieties (Ricardo-da-Silva *et al.*, 1992).

The values of mDP, indicative of the degree of maturation (Table 2), were highest (12.7) for seeds on IAC 572 (Semester 1) and for skins (27.5) on 1103P (Semester 2). This difference may be more related to degree of maturation of the grapes than to an influence of rootstock. In both harvests, the grapes (IAC 572) contained lower technological maturation (pH, TSS, and higher TA) when compared to 1103P. With increasing maturity, mDP generally decreases in the seeds and increases in the skins (Kyraleou *et al.*, 2016).

The percentage of galloylation in the seeds and skins was affected by rootstock and semester. The highest values were for 1103P in Semester 2 with 48.9 % in the seeds and 4.3 % in the skins. The percentage of prodelphinidins in skins was affected by rootstock and semester. The highest values were in skins of grapes on the 1103P: 25.4 % in Semester 1 and 35.1 % in Semester 2. Briefly, statistical differences were observed in the detailed condensed tannin chemical analysis on skins and seeds, showing that their mDP values for the condensed tannins are higher in 1103P rootstock plants than in IAC 572, especially in Semester 2. The percentage of galloylation is higher in the condensed tannins of 1103P seeds and skins in both Semesters 1 and 2. Finally, the highest percentage of skin prodelphinidins was found on 1103P grapevines, in both harvest seasons (Semester 1 and 2).

1.5. Low molecular weight for flavanols in seeds and skins

Concentrations of flavanols in skins and seeds are shown in Table 3. For the seeds (with only one exception of procyanidin B4) higher flavanol values were found on IAC 572 than on 1103P, indicating a rootstock effect. In Semester 1, the highest concentrations were: catechin (514.9 mg/kg), epicatechin (824.4 mg/kg), dimer B1 (198.5 mg/kg) and dimer B2 (547. 4 mg/kg). The other compounds were higher in Semester 2. Total flavanol concentration in seeds was highest in Semester 1 (2453.8 mg/kg), followed by Semester 2 (2363.9 mg/kg), both on IAC 572.

The concentrations of flavanols in skins are presented in Table 3. Of the fourteen compounds analysed, six are shown to have highest concentrations in skins on 1103P in Semester 2. Only epicatechin and B2 3'-*O*-gallate were higher on IAC 572, with concentrations of 204.4 mg/kg (Semester 1), and 3.4 mg/kg (Semester 2) respectively. The concentrations of proanthocyanidins were lower than those

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TABLE 3. Effects of rootstock and semester on the flavanols in skins and seeds of Alicante Bouschet grapes of a tropical semi-arid region in Brazil.

Parameters		Semester 1		*sig.*	Semester 2		*sig.*	Rootstock	Semester
		IAC 572	1103P		IAC 572	1103P			
Flavanol monomers									
(+) Catechin	Skins	62.0	86.2	*	53.1	34.9	*	*ns*	*
	Seeds	514.9	409.0	*	413.0	336.7	*	*	*
(-) Epicatechin	Skins	204.4	128.5	*	183.2	174.3	*	*	*ns*
	Seeds	824.4	403.3	*	628.2	533.0	*	*	*ns*
(-) Epicatechin 3-*O*-gallate	Skins	0.4	0.6	*ns*	0.1	1.7	*	*ns*	*ns*
	Seeds	78.6	23.6	*	123.3	78.3	*	*	*
Procyanidin dimers									
B1	Skins	6.5	8.5	*	7.2	10.4	*	*	*
	Seeds	198.5	112.0	*	251.3	146.1	*	*	*
B2	Skins	3.9	2.4	*	1.1	3.2	*	*ns*	*ns*
	Seeds	457.4	187.6	*	423.0	359.2	*	*	*ns*
B3	Skins	2.4	4.2	*	4.0	6.2	*	*	*
	Seeds	98.6	41.9	*	142.0	70.7	*	*	*
B4	Skins	0.5	0.7	*ns*	1.0	1.6	*	*ns*	*
	Seeds	0.6	12.9	*	0.1	31.4	*	*	*ns*
Procyanidin dimers gallate									
B1 3-*O*-gallate	Skins	0.2	1.2	*	0.7	3.0	*	*	*ns*
	Seeds	80.4	56.1	*	104.9	78.3	*	*	*
B2 3-*O*-gallate	Skins	1.2	1.9	*	2.6	3.8	*	*	*
	Seeds	27.3	11.7	*	44.4	34.5	*	*	*
B2 3'-*O*-gallate	Skins	1.0	0.4	*	3.4	0.5	*	*	*ns*
	Seeds	87.9	50.5	*	107.0	69.6	*	*	*
Procyanidin trimers									
C1	Skins	0.1	2.3	*	0.3	5.2	*	*	*
	Seeds	13.4	3.7	*	21.7	6.5	*	*	*
Trimer 2	Skins	3.1	4.2	*	4.5	5.5	*	*	*
	Seeds	34.8	12.7	*	53.1	34.3	*	*	*
Total small flavanols									
	Skins	261.0	192.0	*	223.0	194.5	*	*	*ns*
	Seeds	2436.1	1328.9	*	2329.3	1773.1	*	*	*ns*

Rootstock (IAC 572 and Paulsen 1103 (1103P); Semester 1 (2016); Semester 2 (2014); results in mg/kg of fresh weight; concentrations in mg/kg; ANOVA ($p < 0.05$); ns (not significant); * (significantly different).

reported by Ricardo-da-Silva *et al.* (1992) for seeds and skins of Alicante Bouschet in a temperate region.

Despite some variation among rootstocks, the concentrations of flavanols in the skins were generally higher on IAC 572 than on 1103P, with 288.0 mg/kg in Semester 1 and 262.7 mg/kg in Semester 2. This result may be related to the vigour of the canopy on this rootstock promoting the formation of more leaves, and affecting the ripening of the grapes at harvest. According to the classical analyses (Table 1), the fruit on IAC 572 was less ripe than that on 1103P. Some authors suggest that the highest levels of tannins are at the beginning of ripening and that they decrease with time (Ó-Marques *et al.*, 2005). Briefly, when considering all the low molecular weights of

flavanols obtained in the analysis, no significant differences were observed between semesters. However, a significant rootstock effect was found across semesters, as well as a significant semester effect.

2. Alicante Bouschet wines

2.1. Classical analysis of the wines

The results for the classical composition and statistical analysis of wines are shown in Table 4. Higher levels of TA were observed for Semester 2, regardless of rootstock. Values were 10.2 g/L on IAC 572 and 10.5 g/L on 1103P. Nevertheless, no significant differences were observed for pH related to rootstock effects. Only a significant semester effect was detected, the pH being lower in the wines of the second harvest (Semester 2). The high pH values found in wines from this region may be related to the mineral characteristics of the soil. Excess available soil potassium can possibly increase pH in must and wines (Soyer and Molot, 1993; Van Leeuwen *et al.*, 2018). Alcohol strength ranged from 11.4 to 12.2 % v/v in wines from Semester 1, and from 15.5 to 17.6 in wines from Semester 2. These high amounts are related to factors controlling ethanol production, sugar content, fermentation temperature and yeast strain (Jackson, 2008). The highest concentrations of alcohol and total dry extract were found in wines from Semester 2 in 2014; this may be related to berry shrivelling, as can be deduced from the values for two hundred berries ranging from 208 g (IAC 572) to 169 g (1103P), while, in 2016, the values were 279 and 203 g for IAC 572 and 1103P respectively. The weather conditions during this summer period generate a higher concentration of tartaric acid and sugars, increasing wine acid levels and alcohol strength.

The highest concentrations of potassium and calcium were in wines from grapes on 1103P in both semesters: potassium contents were 1812.4 mg/L (Semester 1) and 1777.2 mg/L (Semester 2). This rootstock has greater ease of absorption of potassium (Kodur *et al.*, 2010). Concentrations of calcium were 62.7 mg/L (Semester 1) and 58.3 mg/L (Semester 2). In both treatments, the potassium values are high, being above 1.5 g/L; wines from the São Francisco Valley are commonly high in potassium, mainly due to high soil potassium levels in the region, which are characteristic of the terroir. Coli *et al.* (2015) point out that the mineral composition of wine reflects its origin and development, making the wine unique and identifiable. Another factor linked to the high potassium and calcium concentrations in the wine may be the rootstock effect. A semester effect for calcium and potassium contents of the wines was not observed. Additionally, the alcohol content and total dry extract were statistically higher in the wines of Semester 2. According to some authors, rootstocks may differ in their ability to take up potassium, and they can influence grape and wine mineral composition (Gautier *et al.*, 2020; Miele and Rizzon, 2017; Kodur, 2011).

2.2. Total phenols, flavonoids and non-flavonoids

The concentrations of total phenols (4805.3 mg/L), and flavonoids (4607.5 mg/L) (Table 4) were highest in Semester 2 wines from 1103P rootstocks. In Semester 1, the highest concentrations were found in wines from IAC 572. Nedelkovskiet *et al.* (2017) found that the phenolic composition of cv. Vranec grapes depends on rootstock. The differences between studies on the influences of rootstock on wine phenolic composition can be explained by the complex interactive effects of weather, soil and rootstock; Jackson (2008) noted the influence of weather and soil conditions, which can vary from year to year and/or from location to location.

Concentrations of total phenols, flavonoids, oligomeric, polymeric and total tannins were higher in the harvest of the second semester, in both grapes and wines (Table 1 and Table 4). The phenolic profiles of the wines largely reflect those of the grapes, with the methods for extraction and winemaking affecting the chemical reactions taking place during fermentation (Fang *et al.*, 2008).

2.3. Total anthocyanins, pigments and colour in wines

The highest concentrations of total (756.4 mg/L) and coloured (272.4 mg/L) anthocyanins were found in wines from Semester 2 on 1103P rootstocks. In the Semester 1 wines, the highest levels of total anthocyanins (334.1 mg/L) were found on IAC 572, impling that the rootstock effect on total anthocyanins depends on semester. Similarly, when evaluating the effects of irrigation and rootstock on Syrah grapes and wines in southern Australia, Stevens *et al.* (2016) concluded that the strength of the rootstock effect depends on the season. The concentrations of total and coloured anthocyanins for Alicante Bouschet wines in this tropical region are close to the lowest levels reported by Revilla *et al.* (2016)

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TABLE 4. Effects of rootstock and semester on the classical composition, colour, global phenolic compounds and condensed tannins in Alicante Bouschet wines of a tropical semi-arid region in Brazil.

Parameters	Semester 1		*sig.*	Semester 2		*sig.*	Rootstock	Semester
	IAC 572	1103P		IAC 572	1103P			
Classic analyses								
pH	4.00	4.25	*ns*	3.94	3.81	ns	*ns*	*
Titratable (g L tartaric acid)	8.2	6.5	*	10.2	10.5	ns	*ns*	*
Volatile acidity (g/L acetic acid)	0.23	0.12	*	0.31	0.42	*	*ns*	*
Alcohol content (v/v %)	11.4	12.2	*	15.5	17.6	*	*	*
Total dry extract (g/L)	29.9	26.3	*	45.1	54.9	*	*ns*	*
Reducing substances (g/L)	1.3	0.6	*	1.5	1.0	*	*	*
Free sulphur dioxide (mg/L)	36	32	*	28	30	*	*ns*	*
Total sulphur dioxide (mg/L)	88	80	*	35	41	*	*ns*	*
Potassium (mg/L)	1680.7	1777.2	*	1590.2	1812.4	*	*	*ns*
Calcium (mg/L)	41.5	58.3	*	38.8	62.7	*	*	*ns*
Colour and global phenolic compounds								
Total phenols (mg/L)	2262.7	1460.1	*	3784.8	4805.3	*	*ns*	*
Non-flavonoids (mg/L)	165.6	202.7	*	164.1	198.4	*	*	*ns*
Flavonoids (mg/L)	2097.6	1257.9	*	3621.3	4607.5	*	*ns*	*
Total anthocyanins (mg/L)	334.1	266.6	*	527.2	756.4	*	*ns*	*
Coloured anthocyanins (mg/L)	67.7	21.9	*	204.5	272.4	*	*ns*	*
Ionisation index (%)	20.3	8.2	*	38.8	36.0	*	*	*
Total pigments (a.u.)	23.5	8.7	*	33.2	44.1	*	*ns*	*
Polymerised pigment (a.u.)	4.0	3.2	*	4.1	3.8	*	*	*ns*
Polymerisation index (%)	17.0	17.3	*ns*	12.4	8.5	*	*ns*	*
Colour Intensity (*u.a*)	14.410	8.731	*	26.967	34.128	*	*ns*	*
Tonality (*a.u.*)	0.636	0.641	*ns*	0.616	0.642	*ns*	*ns*	*ns*
Co-pigmentation (%)	31.3	25.2	*	22.4	24.1	*	*ns*	*
Turbidity (NTU)	18.6	11.2	*	9.2	6.9	*	*	*
Condensed tannins (mg/L)								
Monomeric	40.4	44.7	*	42.2	58.6	*	*	*
Oligomeric	154.7	119.8	*	188.9	133.7	*	*	*ns*
Polymeric	780.7	1039.3	*	1009.3	1219.5	*	*	*
Total condensed tannins	975.8	1203.7	*	1240.5	1411.7	*	*	*
Astringency potential (NTU/mL)	135.0	177.6	*	215.0	238.5	*	*	*

Rootstock (IAC 572 and Paulsen 1103 (1103P); Semester 1 (2016); Semester 2 (2014); ANOVA ($p < 0.05$); ns (not significant); * (significantly different).

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for commercial wines of the same variety in the region of Galicia, Spain, where they ranged from 334 mg/L to 1433 mg/L (total anthocyanins) and from 71 to 287 mg/L (coloured anthocyanins).

Low concentrations of phenolic compounds in wines from Semester 1 in 2016 may be related to the early harvest due to rain, as can be observed in Figure 1. The combination of rain and high temperature during berry ripening causes rain-cracking and fungal disease.

2.4. Condensed tannins in Alicante Bouschet wines

The concentrations of condensed tannins in wines are shown in Table 4, where an influence of rootstock on tannin composition can be seen. Highest contents for monomeric, polymeric and total monomers were found in Semester 2 in wines on 1103P, with 58.6 mg/L, 1219.5 mg/L and 1411.7 mg/L respectively. In wines from Semester 1 grapes on 1103P, the concentrations were 44.7 mg/L (monomeric), 1039.2 mg/L (polymeric) and 1203.7 mg/L (total). Oligomeric tannins were higher in wines on IAC 572, with 154.7 mg/L in Semester 1 and 188.9 mg/L in Semester 2.

Finally, total pigments and colour intensity showed higher values in the wines of the second semester, which is in agreement with the values obtained for total anthocyanins or even total polyphenolic compounds (Table 4).

High values for astringency potential were found in the Semester 2 wines, with 215 NTU.mL-1 on IAC 572 and 238.5 NTU.mL-1 on 1103P. Briefly, significantly higher values for condensed tannins (proanthocyanidins) in all of their fractions were found in the wines from the second semester than in the wines from the first semester (Table 4), which is again consistent with the values reached for flavonoids and astringency potential.

TABLE 5. Effects of rootstock and semester on the concentrations of monomeric anthocyanins in Alicante Bouschet wines in northeast Brazil.

Parameters	Semester 1		*sig.*	Semester 2		*sig.*	Rootstock	Semester
	IAC 572	1103P		IAC 572	1103P			
Monoglucosylated anthocyanins								
Cyanidin 3-*O*-glucoside	1.4	0.2	*	0.5	0.6	*ns*	*ns*	*ns*
Delphinidin 3-*O*-glucoside	5.7	3.9	*	2.6	3.6	*	*ns*	*
Peonidin 3-*O*-glucoside	8.7	6.1	*	10.7	11.4	*	*ns*	*
Petunidin 3-*O*-glucoside	2.7	5.3	*	1.9	2.2	*ns*	*	*
Malvidin 3-*O*-glucoside	35.3	29.1	*	21.2	21.3	*ns*	*ns*	*
Esterified with acetic acid								
Delphinidin 3-*O*-acetylglucoside	s0.6	0.7	*ns*	3.7	1.4	*	*ns*	*
Cyanidin 3-*O*-acetylglucoside	0.4	0.5	*ns*	0.0	0.0	*ns*	*ns*	*
Petunidin 3-*O*-acetylglucoside	0.2	0.8	*	0.6	2.0	*	*	*
Peonidin 3-*O*-acetylglucoside	0.3	0.4	*ns*	0.1	0.1	*ns*	*ns*	*
Malvidin 3-*O*-acetylglucoside	1.1	0.7	*	1.6	1.1	*	*	*
Esterified with p-coumaric acid								
Delphinidin 3-*O*-coumarylglucoside	0.8	0.9	*ns*	1.1	1.5	*	*ns*	*
Cyanidin 3-*O*-coumarylglucoside	0.0	0.7	*	0.4	1.0	*	*	*ns*
Petunidin 3-*O*-coumarylglucoside	0.9	1.5	*	1.3	1.2	*ns*	*ns*	*ns*
Malvidin 3-*O*-coumarylglucoside	3.5	1.9	*	2.5	2.3	*ns*	*ns*	*ns*
Total monomeric anthocyanins								
	61.8	52.5	*	48.4	49.6	*ns*	*ns*	*

Means followed by the same letter in the row did not differ according to the Tukey test ($p < 0.05$). IAC 572 Rootstock and Paulsen 1103 (1103P); Semester 1 (2016); Semester 2 (2014); concentrations in mg/L; ANOVA ($p < 0.05$); ns (not significant); * (significantly different).

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2.5. Monomeric anthocyanins in wines

The results of the analyses of monomeric anthocyanins in the wines are shown in Table 5. Among the five glycosylated anthocyanins, three reached their highest concentrations on IAC 572 in Semester 1: 5.73 mg/L of delphinidin, 1.42 mg/L of cyanidin, and 35.29 mg/L of malvidin. Higher anthocyanin concentrations can be seen on 1103P: 5.29 mg/L of Petunidinand 11.37 mg/L of peonidin. Wines from vines on IAC 572 contained mostly acetylglucosylated anthocyanins, while in those from vines on 1103P most of the anthocyanins were esterified with *p*-coumaric acid.

The individual profile of monomeric anthocyanins in grapes and wines did not follow the same trend. The categories and concentration of the monomeric anthocyanins in young red wines depend mainly on the grape cultivar, other factors, including viticultural practices, the ripening state, weather conditions and winemaking procedures, can all have significant effects on the anthocyanin profiles of red wines (He *et al.*, 2012). Furthermore, the monomeric anthocyanins in red wines are not particularly stable and are easily oxidised (Lopes *et al.*, 2007).

The grapes of vines grafted onto 1103P contained higher concentrations of monomeric anthocyanins in the grapes, but the wines from IAC 572 showed the highest concentration, especially in Semester 1. In addition to reactions such as self-association, the presence of high concentrations of total phenols and tannins in the wines (1103P) can accelerate the co-pigmentation processes of anthocyanins, preventing its detection (Suriano *et al.*, 2015).

The wines from Semester 2 generally showed lower concentrations of molecular monomeric anthocyanins, which is likely related to the greater reactivity of free anthocyanins in the presence of high concentrations of condensed tannins and flavonoid compounds in the wines.

TABLE 6. Effects of rootstock and semester on the concentrations of flavanol monomers and procyanidins in Alicante Bouschet wines of a tropical semi-arid region in Brazil.

Parameters	Semester 1		*sig*	Semester 2		*sig*	Rootstock	Semester
	IAC 572	1103P		IAC 572	1103P			
Flavanols monomers								
(+) Catechin	1.1	1.9	*	2.0	2.9	*	*	*
(-) Epicatechin	2.6	2.6	*ns*	2.1	5.4	*	*ns*	*ns*
(-) Epicatechin 3-*O*-gallate	1.4	0.8	*	1.5	0.0	*	*	*ns*
Procyanidin dimers								
B1	15.9	20.9	*	26.7	29.0	*	*	*
B2	17.7	7.4	*	21.2	9.6	*	*	*
B3	1.5	2.2	*	10.0	8.5	*	*ns*	*
B4	0.7	1.9	*	1.5	4.3	*	*	*
Procyanidin dimers gallato								
B1 3-*O*-gallate	1.7	0.9	*	0.2	0.3	*ns*	*ns*	*ns*
B2 3-*O*-gallate	1.4	1.2	*	0.9	1.3	*	*ns*	*ns*
B2 3'-*O*-gallate	3.5	2.9	*	3.8	1.4	*	*	*ns*
Procyanidin trimers								
C1	2.6	0.5	*	5.2	0.0	*	*	*ns*
Trimer 2	0.1	6.7	*	0.0	13.7	*	*	*ns*
Total flavanols								
	35.7	31.1	*	75.2	76.4	*	*ns*	*

Means followed by the same letter in the row did not differ significantly according to the Tukey test ($p < 0.05$). Rootstock (IAC 572 and Paulsen 1103P); Semester 1 (2016); Semester 2 (2014); concentrations in mg L; ANOVA ($p < 0.05$); ns (not significant); * (significantly different).

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2.6. Low molecular weight flavanols in wines

Table 6 shows the results for the low molecular weight flavanol compositions of the wines. Wines on IAC 572 contained higher levels of gallocatechin (1.4 mg/L), epicatechin 3 gallate (1.5 mg/L), dimers B2 (21.2 mg/L), B3 (10.0 mg/L), B2 3' gallate (3.8 mg/L) and the trimer C1 (5.2 mg/L). The wines on 1103P had higher concentrations of catechin (2.9 mg/L), epicatechin (mg/L), epigallocatechin (27.6 mg/L), B1 (29.0 mg/L), B4 (4.3 mg/L) and trimer T2 (13.7 mg/L). These differences in procyanidin profile may be related to a rootstock effect. Wines from Semester 2 had higher levels of most of the low-molecular-weight flavanols with 104.9 mg/L (1103P), followed by 85. mg/L (IAC 572). This result may be related to a higher tannin extraction from the seeds, due to a higher alcohol strength in these wines (Table 4). Gambuti *et al.* (2009) found flavanols in grape seeds easier to extract as the alcohol concentration increased, due to the lipidic layer that protects seeds being disrupted.

The highest concentrations of procyanidins were in the seeds and skins of grapes from vines on IAC 572, in Semester 1 (Table 3). The highest levels were in the wines on 1103P in Semester 2. The highest values for the sum of the concentrations of all low-molecular-weight flavanols analysed, were obtained in Semester 2 with statistical significance.

3. Wine sensory analyses

Figure 2 (A, B, C and D) shows the sensory profile of the wines on different rootstocks and semesters. Wines produced from vines on both rootstocks in Semester 1 (harvested in May) were found to be similar. Regarding the sensory profile in the second semester, there were differences between the rootstocks, the wines from IAC 572 having higher scores for seven attributes: colour, colour intensity, clarity, floral, herbaceous, bitterness and astringency. The wines from 1103P, had high scores for ten attributes: fluidity, fruity aroma, spices, empyreumatic, sweetness, acidity, body, persistence and global appreciation (Figure 2A).

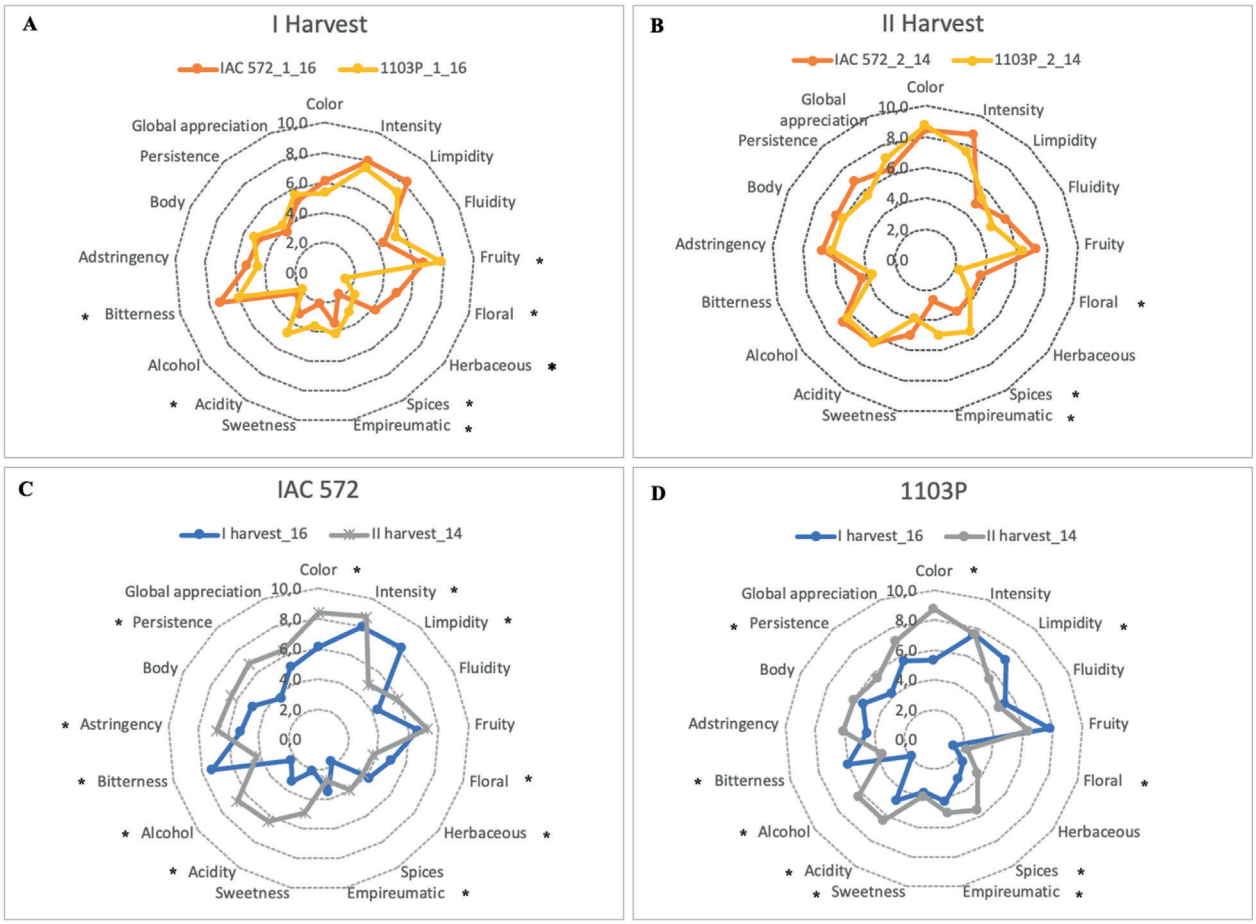


FIGURE 2. Sensory profile of tropical wines Alicante Bouschet, influenced by different harvest times and rootstocks. * (significantly different ($p < 0.05$)).

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Colour scored highest in wines from 1103P in Semester 2 (score 8.7) followed by those from IAC 572, also in Semester 2, (score 8.4). This agrees with the chemical analyses, as these wines contain higher concentrations of total anthocyanins and colour (Table 4).

Regarding the olfactory attributes, high notes of fruity aroma were found in all samples and in both semesters. Wines from IAC 572 received higher scores for floral and herbaceous aroma. Spicy and empyreumatic notes were found in the aromatic profiles of wines from Semester 2 from 1103P.

Wines from Semester 2 received higher scores for the gustatory attributes of sweetness, alcohol and acidity. The results of the chemical analyses showed that wines from Semester 2 had higher concentrations of TA and alcohol, but lower concentrations of residual sugar (Table 4). Sweetness in wines may be related to high alcohol strength (above 15 % v/v). According to Jackson (2008), perceptible sweetness is markedly influenced by other wine constituents, notably ethanol, acids and tannins, as well as by individual sensitivity.

Semester 2 wines from both rootstocks received the highest scores for astringency: 6.7 for IAC 572 and 6.1 for 1103P. This may be related to the high astringency potential and procyanidins in these samples (Tables 4 and 6). The grapes used to make these wines were high in total phenols and condensed tannins in both the seeds and skins (Tables 1 and 2), thus possibly contributing to a higher extraction rate. According to Ren *et al.* (2017) astringent wines generally show high percentages of polymeric fragments. Specifically, polymeric proanthocyanidins have a strong binding affinity for proteins and they have high astringency.

The semester had a strong influence on the sensorial composition of the wines. The next strongest effect was the rootstock. A rootstock effect has also been found by other authors in the evaluation of Cabernet-Sauvignon on seven rootstocks over three years in Italy (Sivilotti *et al.,* 2007).

CONCLUSIONS

Given the viticultural conditions of the São Francisco Valley, the harvest date has an important role in directing the styles of the wines to be produced. Red grapes are not only harvested to make red wines, but they are also used for sparkling wines, which represent around 65 % of the total wine produced per year in the region (Pereira *et al.*, 2016). Depending on the harvest semester, the Alicante Bouschet grape variety has the potential to be used in this region to produce varietal or blend red wines and sparkling wine, as it improves their colour and structure, adding acidity and contributing to the different styles of wines produced. The results of this research indicate that in tropical conditions the chemical composition of Alicante Bouschet grapes and wines (*Vitis vinifera* L.) was influenced by both rootstock and harvest season (climate), the rootstock effect being more significant in terms of grape composition. Both evaluated rootstocks are considered to be vigorous, but under the tropical conditions of the area, the IAC 572 was proven superior to 1103P. Grapes from grapevines grafted onto IAC 572 showed higher concentrations of acids (tartaric and malic) and proanthocyanidins, while those grafted onto 1103P presented high levels of total phenols, non-flavonoids and total monomeric anthocyanins. The semester (intra-annual climate variability) influenced the composition of grapes and wines, with higher concentrations for most of the phenolic indices evaluated (total phenols, flavonoids, total and colored anthocyanins, total pigments, condensed tannins and proanthocyanidins), as well as for colour intensity, alcohol strength and total dry extract in Semester 2 (warmest season). The sensory analysis showed that wines from the second semester promoted greater differences between rootstocks. Wines from Semester 2 (2014) obtained higher scores for most of the sensory attributes, when compared to the wines from Semester 1. Wines from grapes of vines grafted onto IAC 572 scored higher than those from 1103P for most of the examined attributes. Both evaluated rootstocks can be used by producers in this region, but with a focus on different kinds of products, such as young wines or wines for aging.

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