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Proposal of double-cropping system for 'BRS Isis' seedless grape grown in subtropical area

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ABSTRACT

'BRS Isis' is a new colored seedless table grape tolerant to downy mildew, the main vine disease in subtropical humid areas. This new seedless cultivar is an interspecific hybrid from the crossing of CNPUV 681-29 [Arkansas 1976 × CNPUV 147-3 ('White Niagara' × 'Venus')] × 'BRS Linda'. However, its performance under subtropical conditions hasn´t been assessed yet, especially under double annual cropping system. For this purpose, a research was designed with the objective to characterize the phenology and the main physicochemical properties of this new cultivar. The trial was carried out in a commercial vineyard of 'BRS Isis' seedless grape from 2-year-old vines grafted on 'IAC 766 Campinas' rootstock, located in Marialva, state of Paraná (PR), Brazil, in two consecutive crops, summer season 2016 and off-season 2017. The vines were trained on overhead trellises and spaced 2.0 × 5.0 m apart. The duration of the main phenological stages of 'BRS Isis' were evaluated from pruning until harvest, as well its thermal demand and main physicochemical characteristics. The summer and off-season crop cycles of 'BRS Isis' seedless grape grown under subtropical conditions last 144 and 125 days, with thermal demands of 1,931 and 1,815 degree days, respectively. This new seedless grape cultivar can be successfully grown under a double annual cropping system in subtropical conditions, however, as it is a very fruitful cultivar, it is necessary to perform a load crop adjustment after fruit set, equivalent to a density of 5 bunches.m^{-2}, to obtain a sustainable yield in each season. Under this growing system, the berries present in both seasons, appropriate physicochemical characteristics for table grape market, but with darker skin color in off-season. Based on these results, a detailed double annual cropping system, with some specific cultural practices, is proposed for this new hybrid seedless cultivar.

1. Introduction

Viticulture is an important economic activity in South America countries, such as Chile, Argentina, Uruguay, Peru and Brazil. In the recent years, it has also become important in generating employment in large enterprises for production of table grapes, mainly seedless cultivars. The worldwide trend for the consumption of seedless grapes has increased the competition between producers, which direct efforts to meet a more demanding consumer market (Nachtigal et al., 2005; Mello, 2016).

Seedless grapes have certain characteristics that make them a high quality fruit with better acceptance by consumers. Recently, the 'BRS Isis' seedless grape was released by Embrapa Wine and Grape (Brazilian Agricultural Research Corporation), Brazil. This new seedless cultivar is an interspecific hybrid from the crossing of CNPUV 681-29 [Arkansas 1976 × CNPUV 147-3 ('White Niagara' × 'Venus')] × 'BRS Linda'. 'BRS Isis' is tolerant to downy mildew, the main vine disease in subtropical humid areas. It is a vigorous and fruitful cultivar with strong shoot dominance, standing high bud fertility. The berries present pink to reddish color, with firm texture and neutral taste. Thermal requirements have been estimated to 1,800 degree-days from pruning to harvest when grown in tropical region. When subjected to cane pruning, it presents 2–3 great compact bunches per shoot, with natural weight of 375 g, without the use of growth regulators, making this cultivar a high yield grape. The bunch is medium-sized, predominantly cylindrical-winged, while the berries are large, with good adhesion.

The knowledge of phenological stages of grapevines is important in vineyard management because it provides valuable information to grape growers, as periods of higher demand for hand labor, pest and disease control and probable harvest dates, further indicating the

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regional climatic potential for grape cultivation and production. When a new grape cultivar is introduced to an area, phenology plays an important role as it allows the duration of developmental stages to be characterized in relation to climate, particularly in relation to seasonal variation, and it is used to interpret the impact of different climatic regimes on the crop (Fenner, 1998; Ribeiro et al., 2010).

Along the phenology, the degree-day (DD) is an expression of the amount of energy that a grapevine needs to satisfactorily complete its production cycle. It plays an important role in characterizing differences among species and in predicting plant development under different environmental conditions. The progression of in-season grapevine development is strongly influenced by air temperature. As such, DD is often used to compare regions and vine growing condition, as well to better understand the behavior of a new cultivar under certain weather conditions. It constitutes the accumulated difference between the mean environment temperature and the base-temperature, below this temperature, no development occurs (Miranda et al., 2013; Tecchio et al., 2013). Besides, grape qualitative characteristics, as sugar content, acidity, color, aroma, and flavor, as well bunch mass and yield are equally important when a new cultivar, like as 'BRS Isis' seedless grape, is desired to be grown in a new area (Ritschel et al., 2013; Sabbatini and Howell, 2013).

In most temperate regions, grapevines undergo dormancy from late fall to early spring, and a single pruning and harvest is the conventional grapevine practice, while in some subtropical regions, due the mild winter and the use of bud burst stimulators, a double annual cropping of grapes can be achieved (Fávero et al., 2011; Zhu et al., 2017; Chen et al., 2017), and this is the case of Brazilian subtropics, based on *Vitis vinifera* seeded cultivars (Roberto et al., 2015a). Thus, the cultivation of hybrid seedless grapes, such as 'BRS Isis', could be an alternative to diversify the current production system, opening the possibility of overseas market.

Considering the aspects above, the objective of this work was to evaluate the performance of 'BRS Isis' seedless grape, by means of its phenology, physicochemical and yield characteristics, grown under double-annual cropping system in subtropical area.

2. Material and methods

2.1. Phenology of 'BRS Isis' seedless grape

2.1.1. Experimental location

The study was conducted in a commercial vineyard of 'BRS Isis' seedless grape (*Vitis* spp.) from 2-year-old vines grafted on 'IAC 766 Campinas' rootstock, located in Marialva, state of Paraná (PR), Brazil (23° 29′52.8″ S, 51° 47′58.0″ W, elevation 570 m), in two consecutive crops, summer season 2016 and off-season 2017. The vines were trained on overhead trellis and spaced 2.0 × 5.0 m apart (1000 vines ha^{-1}). According to Köppen classification, the climate of the area is type Cfa, i.e., subtropical with an average temperature below 18 °C in the winter, and average temperature above 22 °C in summer. The average annual rainfall is 1596 mm, with most of the rainfalls occurring in summer (IAPAR, 2010).

The cane-pruning, consisted of 5–6 buds per cane, was performed on July 29th for summer crop 2016 and on January 18th for off-season crop 2017, with high bud fertility rates (> 95.0%). Subsequently, 3% hydrogen cyanamide solution was applied to the two terminal buds to induce bud burst in both seasons, since in subtropical areas, the use of this plant growth regulator is mandatory, especially to obtain an off-season crop. For the application, a sponge connected to a wood stick device was used to soak the terminal buds with the solution, and at this dose and way of application, the product is not dangerous.

As 'BRS Isis' is a hybrid seedless grape, berry thinning was performed as described by Roberto et al. (2015a). Other practices like fertilizer application, weed control, pest and diseases management were carried out according to the local practices used (Roberto et al., 2012a).

For assessments, 20 representative vines were selected in the area, which were used in both seasons. In each one, an average of 50 shoots per vine were adjusted. As 'BRS Isis' is a very fruitful grape, presenting up to 4 bunches per shoot, for the summer crop season, a load adjustment was performed after fruit set removing 50% of inflorescences per shoot, to leave two inflorescences per shoot, equivalent to a density of 10 bunches m^{-2}. Even removing 50% of inflorescences, the crop load observed was considered high, and for this reason, in the following season, just one inflorescence per shoot was left, equivalent to a density of 5 bunches m^{-2}.

2.1.2. Phenology evaluation

To carry out the analysis of the phenological behavior of 'BRS Isis', two shoots were marked of each representative vine. Then, it was evaluated, through visual observations, the duration in days of each phenological stage from pruning (PR), according to the classification based on Baillod and Baggiolini (1993) and Baggiolini et al. (2008), as follows: bud swelling (BS): when 50% of the buds have reached the second stage of development of the vine, that is, when the scales get broken and the plumage is visible; sprouting (SP): when 50% of buds reach the fourth stage, i.e., the output of the leaves; emergence of the inflorescence (EI): when 50% of the shoots present the inflorescence, with clusters visible; flowering (FL): when 50% of flower are open, flowering itself with visible flowers; véraison (VE): when 50% of berries changed the color to red and when they started to softening; and harvest (HA): when 100% of the grapes show intense color, with the total soluble solids content as high as possible (Fig. 1).

It was then characterized the duration in days of each of the following sub-periods: pruning to bud swelling (PR-BS); pruning to sprouting (PR-SP); pruning to emergence of inflorence (PR-EI); pruning to flowering (PR-FL); pruning to véraison (PR-VE) and pruning to harvest (PR-HA) (Nunes et al., 2016). From these data, diagrams were created representing in scale the duration in days for each of the phenological stages of 'BRS Isis', as well as the duration of each subperiod (Borges et al., 2017).

To characterize the thermal demands of 'BRS Isis' seedless grape, the degrees-day (DD) from pruning to harvest was used, as well as for each of the phenological subperiod, using climate data from the INMET – National Institute of Meteorology, according to the following equations proposed by Villa-Nova (1972):

a) $DD = (Tm - Tb) + (TM - Tm)/2$, when $Tm > Tb$;

b) $DD = (TM - Tb)^2 / 2(TM - Tm)$, when $Tm < Tb$; and

c) $DD = 0$, when $Tb > TM$

where: TM = maximum daily temperature (ºC); Tm = Minimum daily temperature (ºC); and Tb = base temperature (10 °C).

2.1.3. Physicochemical analyses

The grapes were harvested at full ripe when total soluble solids were around 14°Brix. For physical characteristics evaluation of grapes, 5 bunches per vine were collected and the following variables were analyzed: mass (g) and diameter (mm) of berries, and mass (g) and width (cm) of bunches using digital calipers and scales. Berry color was analyzed using a colorimeter CR-10 Plus (Konica Minolta®, USA) to obtain the following variables from the equatorial portion of berries (n = 2 per berry): L^* (lightness), C^* (chroma) and $h°$ (hue). Lightness values range from 0 (black) to 100 (white). Chroma indicates the purity or intensity of color, the distance from gray (achromatic) toward a pure chromatic color and is calculated from the a^* and b^* values of the CIELab scale system, starts from zero for a completely neutral color, and does not have an arbitrary end, but intensity increases with magnitude. Hue refers to the color wheel and is measured in angles; green, yellow and red correspond to 180, 90 and 0°, respectively (Mcguire, 1992; Lancaster et al., 1997; Peppi et al., 2006). The color index for red grapes

Fig. 1. Representation of the main 'BRS Isis' phenological stages. A: bud swelling; B: sprouting; C: emergence of the inflorescence; D: flowering; E: véraison; F: harvest.

(CIRG) was calculated using the formula CIRG = $(180\text{-}h^{\circ})/(L^{*}+C^{*})$ (Carreño et al., 1995).

For total soluble solids (TSS), titratable acidity (TA) and maturation index (TSS/TA) evolution, samples of 50 berries collected from upper, middle and lower portion of each marked bunch, were evaluated weekly from the beginning of véraison up to 7 days after harvest. For this purpose, the samples were divided into 5 sub samples of 10 berries each. The samples were then crushed and the juice was used to determine TSS and TA contents. For determination of TSS, few drops from already obtained juice were subjected to reading in a digital refractometer with automatic temperature compensation (Model DR301-95, Kruss Optronic, Germany) at 20 °C, and the results were expressed in °Brix. The pH of the juice was recorded using a Jenway 3510 bench pH meter (Cole-Parmer, Staffordshire, UK) and then TA was determined by potentiometric titration with 0.1 N NaOH up to pH = 8.2, using 10 mL of diluted juice in 40 mL distilled H_2O, and the results were expressed in % of tartaric acid (Youssef and Roberto, 2014). For TSS, TA and TSS/AT evolution, regression analysis was done. The daily rate accumulation of these berry chemical properties was calculated by subtracting the final readings from the initial ones and divided by total number of days from véraison to harvest in both seasons.

The total anthocyanin concentration of berries was determined at harvest using 30 berries per plot, which were frozen and stored at −20 °C. The berry skins were removed using tweezers, taking care to remove only the skin, without pulp. The skins were washed once with water, followed by washing in deionized water and drying with absorbent paper. A 5g-skin sample was then placed in a polystyrene tube containing 50 mL of acidified methanol (1% HCl) and stored in the dark for 48 h at room temperature. The tubes were then removed from the dark and stirred manually for 5 s. Absorbance was determined using a spectrophotometer (Genesys 10S Spectrophotometer, UV-VIS®) at 520 nm with the solvent as the blank. The results were expressed in mg malvidin-3-glucoside per gram of skin ($mg.g^{-1}$) (Peppi et al., 2006).

The evaluation of total polyphenol concentration in the pulp and skin of berries at harvest was based on the Folin-Ciocauteau method. Ten berries per plot were macerated and then 5 g were homogenized with 50 mL of ethanol 50% in a blender during 2 min and centrifuged at 3500 rpm during 5 min. An aliquot of 0.2 mL of the extract was mixed with 1.8 mL of distilled water and 10 mL of 10-fold diluted Folin-Ciocalteau reagent. After 30 s to 8 min, 8 mL of 7.5% of Na_2CO_3 solution was added. All test tubes with the mixture were shaken for 10 s on the vortex and kept in darkness during 2 h. Absorbance of each sample was measured after 15 min using a spectrophotometer Genesys™ 10S UV-VIS® (Thermo Scientific, USA) at 765 nm against blank sample. Blank sample was prepared with water instead of the extract. Determination of total polyphenol was calculated from the calibration curve obtained with gallic acid at concentrations of 0.2; 0.4; 0.6; 0.8; 1.0 to 1.2. Readings were expressed as $mg.100g^{-1}$ of berries (gallic acid equivalents) (Bucic-Kojic et al., 2007; Borges et al., 2012).

The yield per vine (kg per vine) and yield (ton. ha^{-1}) estimates were obtained according to the average number of bunches per vine, the average mass of bunches and the number of vines per hectare (Roberto et al., 2012a).

2.1.4. Statistical analysis

The bunch and berry characteristics of 'BRS Isis' seedless grape assessed in both crops seasons were analyzed and compared using Student's test for independent samples with homogeneity of variances verification. Means and standard deviations were assessed with a significance level of 5%.

3. Results and discussion

The duration in days of 'BRS Isis' cycle during the summer season from pruning to harvest (PR-HA) was 144 days, while the duration of days from pruning to bud swelling (PR-BS), pruning to sprouting (PR-SP), pruning to emergence of inflorescence (PR-EI), pruning to flowering (PR-FL) and pruning to véraison (PR-VI) was 10, 15, 22, 50, and 116 days, respectively. During the off-season crop, the duration of the cycle was shorter, 125 days, while the duration of the PR-BS, PR-SP, PR-EI, PR-FL and PR-VI subperiods was 10, 13, 16, 39, and 85 days, respectively (Fig. 2).

In viticulture, the knowledge of phenology can be used for selection of cultivars, vineyard planning, skilled labour and equipment requirements, and a schedule of cultural practices as part of grapevine management. Off-season crop develops mostly in the summer, which implies in the reduction of number of days to be completed. It happened because at this season, the vines are pruned in early January (summer), showing intense metabolic activity, and the air temperature is high. Thus, the response of the hydrogen cyanamide application is faster, because at this period the vines are not dormant like in late winter. As a result, the PR-VE period is quite reduced (Fig. 2). On the other hand, the ripening develops during autumn, where the average air temperature is lower, and the VE-HA period is longer. It is known that in warm

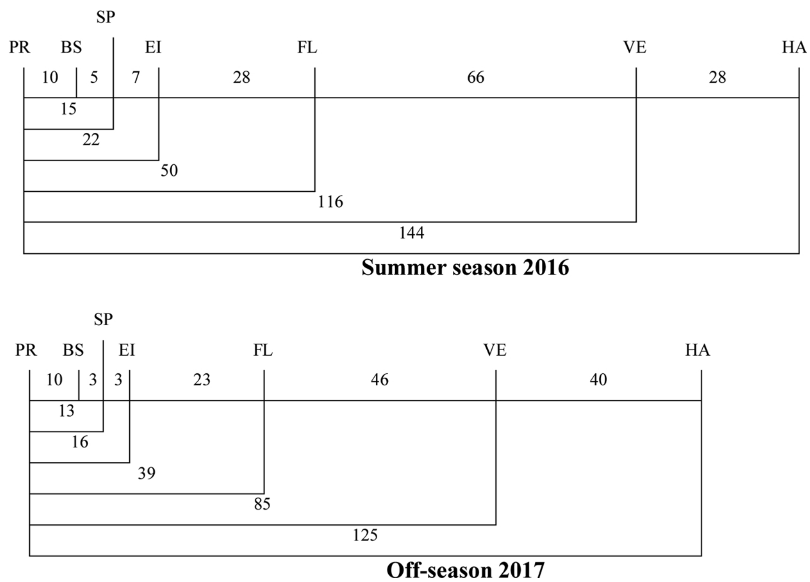

Fig. 2. Duration in days of the main phenological stages of 'BRS Isis' seedless grape. Summer season 2016 and off-season 2017. PR: pruning; BS: bud swelling; SP: sprouting; EI: emergence of inflorescence; FL: flowering; VE: véraison; HA: harvest.

locations with high temperatures, vegetative growth of vines tends to be higher and the cycle is reduced in relation to cooler regions (Pedro-Júnior and Sentelhas, 2003).

Since 'BRS Isis' seedless grape has been recently released, information about its behavior, such as the phenological characteristics in subtropical areas are scarce. However, it was possible to verify that the cycle of 'BRS Isis', in both seasons, is similar to the main seeded grapes grown in subtropics, such as 'Benitaka' and 'Black Star' (Ribeiro et al., 2010; Roberto et al., 2015a), i.e., this grape is considered a mid-season cultivar. As a result, the double annual cropping system can be easily achieved for this cultivar by performing the pruning in late winter, and the harvest is achieved in December (summer season crop), leaving enough time for the realization of a summer pruning (January) in order to obtain a harvest in the early fall (off-season crop). Besides, it was also verified that 'BRS Isis' is a vigorous cultivar, which contributed to the good vine development, especially during the training period.

The duration of the productive cycle of vines also varies according to the canopy/rootstock combination, the vigor of the plant in a given edaphic and climatic condition and the yield (Kishino et al., 2019a). The knowledge of the duration of the phenological phases is a requirement of modern viticulture, since it makes possible the rationalization and optimization of the cultural practices, which are indispensable for the cultivation of the vine, where the correct choice of rootstock play an important role (Mandelli et al., 2004). The 'IAC 766 Campinas' rootstock used in this trial showed to be very suitable for its purpose, allowing a good development for 'BRS Isis'.

The thermal demand required by 'BRS Isis' during the subperiod PR-HA in the summer crop was 1931.48 DD. For the evaluated phenological subperiods PR-BS, BS-SP, SP-EI, EI-FL, FL-VE and VE-HA, the demand was of 134.15, 42.50, 82.35, 299.48, 918.95 and 454.05 DD, respectively. Regarding the off-season crop, the thermal demand requirement was 1815.7 DD from PR to HA, and for the evaluated subperiods, the demand was of 152.3, 42.25, 42.55, 401.14, 715.4 and 462.05 DD respectively (Table 1). The DD required for 'BRS Isis' seedless grape to complete the cycle was similar to other grape mid-season cultivars grown in subtropics, such as 'Benitaka', 'Black star' and 'Brasil' table grapes (Nagata et al., 2000; Ribeiro et al., 2010; Roberto et al., 2015a).

Evaluation of the thermal demand in different regions showed that the amount needed to complete the cycle may vary considerably (Pedro-Júnior et al., 1993). Therefore, studies to establish crop thermal demand *in loco* are essential for the adoption of this prediction model (Busato et al., 2013). In addition to environmental factors, the vine cycle duration can also be altered, in the same region, due to the combination of scion and rootstock cultivars (Sato et al., 2008). Subtropical areas are colder than the tropics and warmer than temperate zones, and winter usually is mild to cool with unpredictable air temperatures. Thus, unpredictable winter conditions may result in a different accumulation of DD over several years at the same site, which may accelerate or delay the onset of plant development (Scariotto et al., 2013; Hussain et al., 2016). The DD concept is a good indicator of heat accumulation on vine behavior within a given locality. However, other factors, such as altitude, latitude, precipitation, thermal amplitude, solar radiation, among others, also have a direct influence on vine development, and can vary among regions even for a same cultivar (Leão and Silva, 2004; Kishino et al., 2019b; Santos et al., 2009).

Table 1
Thermal requirement in degrees-day (DD) and standard deviation for each phenological subperiod of 'BRS Isis' seedless grape grown under double annual cropping system in subtropical area. Summer season of 2016 and off-season of 2017.

Phenological subperiods	Degree days (DD)*	
	Summer season 2016	Off-season 2017
PR-BS	134.15 ± 1.54	152.3 ± 0.98
BS-SP	42.50 ± 1.83	42.25 ± 0.75
SP-EI	82.35 ± 1.97	42.55 ± 0.49
EI-FL	299.48 ± 4.23	401.14 ± 1.79
FL-VE	918.95 ± 3.1	715.4 ± 2.08
VE-HA	454.05 ± 1.8	462.05 ± 1.98
PR-HA	1931.48 ± 3.50	1815.7 ± 2.99

PR-BS: pruning – bud swelling; BS-SP: bud swelling – sprouting; SP-EI: sprouting – emergence of inflorescence; EI-FL: emergence of inflorescence – flowering; FL-VE: flowering – véraison; VE-HA: véraison – harvest. *Base-temperature = 10 °C.

For mass, diameter and length of the 'BRS Isis' berries no significant differences were observed between seasons (Table 2). The minimum diameter required for national market is 12 mm, with averages between 14 and 17 mm recommended for a good commercialization of table grapes (Brasil, 2002; Santos et al., 2015), and it is also considered in the market for the export of table grapes (Santos et al., 2013).

It was possible to verify that 'BRS Isis' has medium sized berries,

Table 2
Bunch characteristics of 'BRS Isis' seedless table grape grown under double annual cropping system in subtropical area. Summer season 2016 and off-season 2017.

Bunch characteristics	Summer season 2016	Off-season 2017
Berry mass (g)	6.7 ± 1.0^{ns}	6.4 ± 0.3
Berry diameter (mm)	19.0 ± 1.7^{ns}	19.3 ± 1.1
Berry length (mm)	27.3 ± 2.5^{ns}	27.7 ± 1.6
Bunch mass (g)	512.7 ± 0.04^{ns}	529.0 ± 0.1
Bunch length (cm)	21.2 ± 2.3^{ns}	21.7 ± 1.7
Lightness (L*)	22.8 ± 2.4^{ns}	22.4 ± 1.0
Saturation (C*)	5.9 ± 2.3*	3.3 ± 1.1
Hue angle (h°)	58.0 ± 35.7^{ns}	54.0 ± 12.7
Color index (CIRG)	4.3 ± 1.3*	4.9 ± 0.4

Student's test (within lines) for independent samples with homogeneity of variances verification with means ± standard deviations.*: significant ($P < 0.05$); ^{ns}: not significant.

wide elliptical, firm with colorless flesh and neutral flavor. It has big seed traces that can eventually develop normal seeds, and berries format is similar to that of 'Black Star' and 'Redimeire' grapes (Roberto et al., 2012b, 2015b), but with color coverage. The bunch presented weight of around 500 g and length of 21.0 cm, with medium size and cylindrical shape. The bunch presented moderate compactness and demanded similar berry thinning to 'BRS Vitoria' seedless grape.

Color is one of the most important visual features in table grapes, and this attribute is commonly associated with bunch quality and is directly related to consumer´s acceptance, being one of the main determining factors in the commercial value of grapes. Regarding skin color attributes of 'BRS Isis', there was no significant difference regarding $L*$ and h° (22.8 and 22.4) and (58.0 and 54.0) for summer and off-season crops, only for $C*$ (5.9 and 3.3), respectively (Table 2). In addition, the CIRG observed in the off-season crop was higher than summer season (4.9 and 4.3, respectively), which displays that berries have red and red-dark violet color, respectively (Carreño et al., 1998), and it can be related with the crop load adjustment done in off-season, since greater number of bunches increases competition for photosynthates in vines. Besides, it may also have happened because during off-season crop, the ripening develops under cooler temperatures (autumn) and mainly sunny days. As previously reported, the subperiod VE-HA is longer than in summer season, which allows a higher accumulation of anthocyanins (Mori et al., 2005, 2007), what was confirmed when the concentration of these compounds have been assessed (Table 3).

For some colored grape cultivars, the climatic conditions, especially the diurnal temperature variation at ripening, have a great influence on the berry color. When cultivated under high temperatures in a semi-arid region, 'BRS Isis' bunches do not express well the red color, but allows high yield levels (Ritschel et al., 2013).

Table 3
Chemical characteristics of 'BRS Isis' seedless table grape grown under double annual cropping system in subtropical area. Summer season 2016 and off-season 2017.

Chemical characteristics	Summer season 2016	Off-season 2017
Total soluble solids - TSS (°Brix)	14.2 ± 0.3^{ns}	14.4 ± 0.2
Titratable acidity - TA (%)	0.6 ± 0.04*	0.8 ± 0.04
Maturation index (TSS/TA)	24.1 ± 1.5*	17.9 ± 0.9
pH	4.3 ± 0.02^{ns}	4.4 ± 0.1
Total anthocyanins (mg. g^{-1})	0.3 ± 0.1*	1.0 ± 0.5
Total polyphenols (mg.100 g^{-1})	23.8 ± 6.1^{ns}	28.4 ± 4.3

Student's test (within lines) for independent samples with homogeneity of variances verification with means ± standard deviations.*: significant ($P < 0.05$); ^{ns}: not significant.

The accumulation of anthocyanins in colored grapes begins at véraison and their biosynthesis is controlled by the MYB transcriptor factors, which modulate the expression of the structural genes and are responsive to abscisic acid (ABA) concentrations in the tissues (Koyama et al., 2018). It has been already demonstrated the benefits of exogenous application of the enantiomer *S*-ABA to promote color of table grapes grown in warm conditions (Peppi et al., 2006; Roberto et al., 2012a, 2013). Thus, this plant growth regulator could be used to promote color of 'BRS Isis' seedless grape at the summer season, which temperatures during the subperiod VE-HA are high, since it occurs during spring-summer seasons.

The TSS evolution of 'BRS Isis' berries were best fitted to linear regression for both seasons (Fig. 3). The early ripening period of 'BRS Isis' table grape, considered when 50% of berries changed the color, occurred 66 and 46 days after flowering in the summer season and off-season respectively, with no significant difference in both seasons reaching at harvest 14.2 and 14.4° Brix in summer and off-seasons, respectively (Table 3). The daily rate of TSS observed for summer and off-seasons were quite similar, 0.10 and 0.09° Brix, respectively (Table 4).

TA evolution was also best fitted to linear regression for both seasons (Fig. 3). 'BRS Isis' presented significant differences at harvest 0.6 and 0.8% for tartaric acid during both seasons, respectively (Table 3), similar to those observed by Leão et al. (2016) when this cultivar was grown under semi-arid conditions. Even the pH was quite similar in both seasons; the variation in acidity could be because of climatic differences among seasons and because of the load adjustment (bunch thinning) of grapes (Pastore et al., 2011). The decreasing evolution of TA (Fig. 3) can be due to several factors, such as dilution of the organic acids by increasing the volume of the berry; activation of the breakdown of organic acids; and inhibition of synthesis and transformation of organic acids into sugar. The decrease of TA content in this phase can be mainly due to the malic acid respiratory process and to the organic acid reduction in relation to the berry size increase. The daily decrease rate of TA observed was -0.03% for both crop seasons, respectively (Table 4) (Mullins et al., 1992; Mota et al., 2006).

Similar behavior was observed for TSS/TA evolution for both seasons (Fig. 3), showing significant differences for TSS/TA reaching 24.1 in summer season and 17.9 in off-season, respectively (Table 3). The difference values of TSS/TA between the seasons may have occurred due to the higher TA observed in off-season crop. The TSS/TA is one of the indexes used for determination of grape quality, but it has been used carefully, since an increase in TSS does not always correspond to equal reduction of TA. However, this index may indicate the ideal balance between sugar and acidity of a cultivar for a given region, in most of the cases higher than 18. The daily evolution rate of maturation index was 0.57 for summer crop and 0.35 for off-season crop, respectively (Table 4).

The means of total anthocyanins observed during summer and off-season crop were 0.3 and 1.0 mg.g^{-1}, respectively, representing a significant difference behavior between both seasons (Table 3), as previously described, whereas no significant difference was observed in case of total polyphenols, and the means recorded were 23.8 and 28.4 mg.100g^{-1}, respectively. The variation of the anthocyanin content of 'BRS Isis' grapes between both seasons can be related to the fact that the synthesis of this compound is directly influenced by the climatic conditions, such as temperature, light intensity and precipitation (Bevilaqua, 1995), and it explains the lower CIRG observed in the summer season (Table 2). The constant presence of light intensity stimulates the synthesis of these compounds in colored grapes (Lima, 2009). It should be considered that temperature also influences the synthesis of anthocyanins, and the vines subjected to conditions of higher temperatures during the ripening phase tend to reduce the synthesis of this compound, while under conditions of higher diurnal temperature variation, its synthesis is favored (Champagnol, 1984). Beside this, it is known that bunch thinning (load adjustment) may

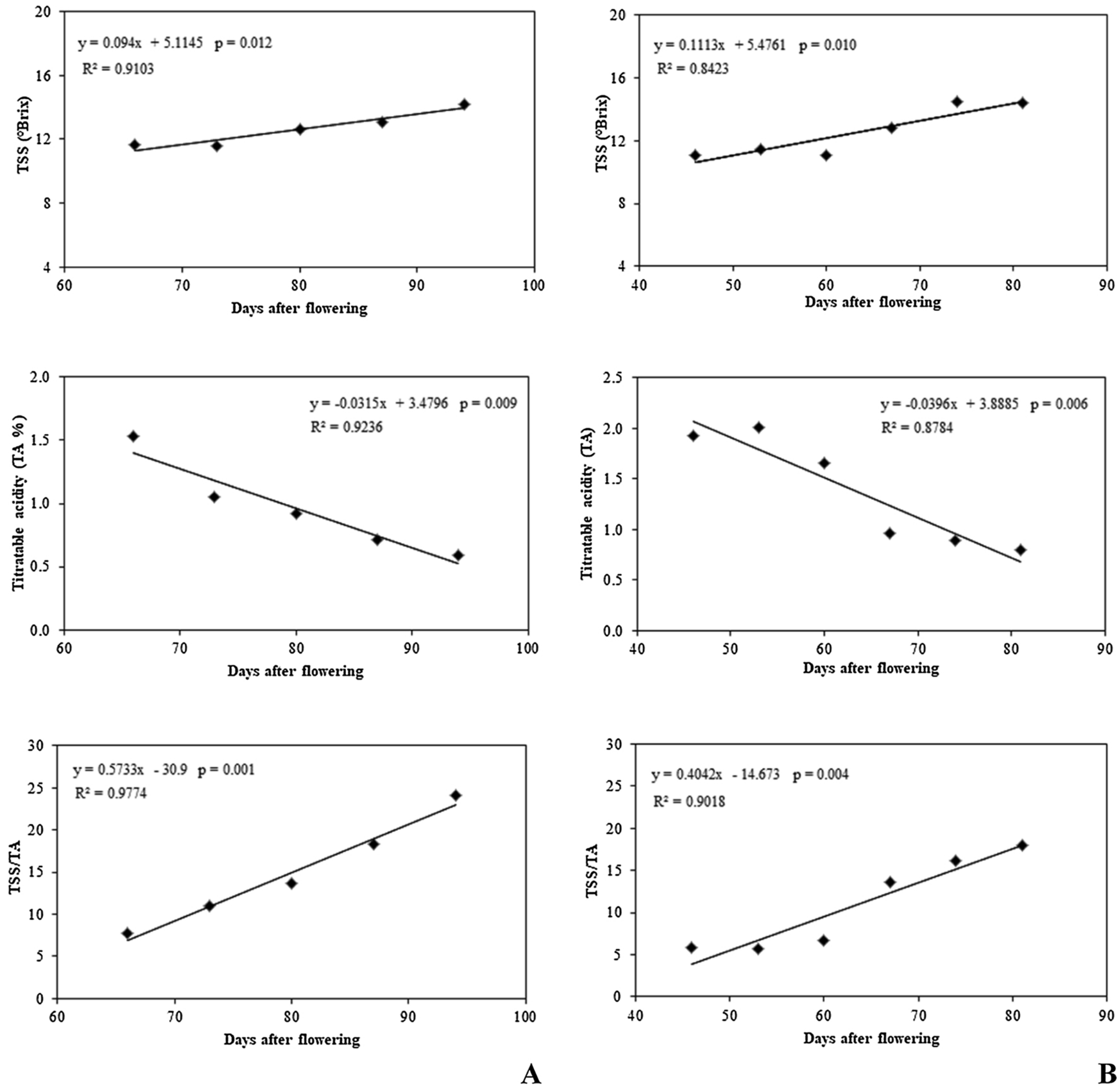

Fig. 3. Evolution of total soluble solids (TSS), titratable acidity (TA) and maturation index (TSS/TA) of 'BRS Isis' seedless table grape grown under double annual cropping system in subtropical area. A: summer season 2016; B: off-season 2017.

Table 4
Total soluble solids (TSS), titratable acidity (TA) and TSS/TA daily rate accumulation of 'BRS Isis' seedless grape grown under double annual cropping system in subtropical area. Summer season of 2016 and off-season of 2017.

	Daily rate accumulation	
	Summer season 2016	Off-season 2017
TSS (°Brix)	0.10 ± 0.04	0.09 ± 0.01
TA (%)	—0.03 ± 0.001	—0.03 ± 0.01
TSS/TA	0.57 ± 0.04	0.35 ± 0.03

promote the biosynthesis of some phenolic compounds (Fanzone et al., 2011), as observed in 'Sugraone' table grape subjected to different levels of bunch densities (Leão et al., 2016).

Regarding the productive characteristics of 'BRS Isis' seedless grape (Table 5), for summer crop the production per vine was 49.0 kg, and estimated yield per hectare was 49.0 ton.ha^{-1}. However, as previously reported, the 'BRS Isis' showed to be a very fruitful grapevine, and a load adjustment was necessary to be applied in the off-season crop, leaving 5 bunches. m^{-2}. As a result, the production of this season was more balanced, 24.0 kg per vine with an estimated yield of 24.0 ton.ha^{-1}, almost the half compared to the summer season.

Table 5
Productive characteristics of 'BRS Isis' seedless grape grown under double annual cropping system in subtropical area. Summer crop of 2016 and off-season crop of 2017.

Productive characteristics	Summer season 2016	Off-season 2017
Number of bunches per vine	94.9 ± 11.2	45.4 ± 8.1
Yield per vine (kg per vine)	49.0 ± 5.8	24.0 ± 4.3
Yield per hectare (ton. ha^{-1})	49.0 ± 5.8	24.0 ± 4.3

Considering this average yield level in just one season, as this new cultivar can be grown under double annual cropping system in subtropical areas, as demonstrated in this work, an annual yield of 49 ton.ha^{-1} could be achieved, what is considered highly profitable. In addition, it is important to note that the productive characteristics of the 'BRS Isis' seedless grape are compatible with those observed for other table grapes under the same intensive cultivation system in subtropical region (Kishino et al., 2019a; Colombo et al., 2011).

The response of the load adjustment is the result of the changes in photosynthates uptake to grapes, as reported for 'Thompson Seedless' grapes when the bunch density was reduced from 6 to 4 bunches. m^{-2} (Leão and Lima, 2017) and for other cultivars (Keller et al., 2005, 2008;

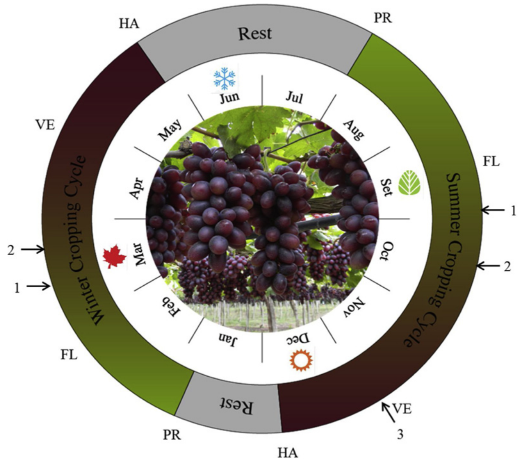

Fig. 4. Proposal of a double annual cropping system for 'BRS Isis' seedless grape grown in subtropical region with some specific cultural practices. PR: pruning followed by hydrogen cyanamide application for bud burst; FL: flowering; VE: véraison; HA: harvest; 1: crop load adjustment followed by shoot trimming; 2: berry-cluster thinning; 3: exogenous *S*-ABA application for color improvement. The onset of winter, spring, summer and autumn seasons is indicated by symbols above the respective month.

Dami et al., 2006; King et al., 2012; Sun et al., 2012; Ozer et al., 2012; Avizcuri-Inac et al., 2013; Gil et al., 2013). These authors exemplified that deficiencies in fruiting, as a consequence of restrictive climatic conditions, may limit the expected benefits to the quality of the grapes and / or their derivatives when bunch thinning is adopted. This technique has great influence on the source-sink association and the supply of photosynthates between fruits and leaves (Dry, 2000; Mota et al., 2010), turning into a mandatory technique required to grow 'BRS Isis' seedless grape successfully.

According to the results observed in this work, we propose here a model to grow 'BRS Isis' seedless grape under double annual cropping system, considering some specific cultural practices required to obtain a sustainable cultivation in a subtropical area (Fig. 4). In this model, we considered the vines trained on overhead trellis system with bilateral arms, spaced 2.0 × 5.0 m apart (1000 vines ha^{-1}), grafted onto 'IAC 766 Campinas', a tropical and vigorous rootstock. Under these conditions, the number of canes is adjusted to 40–50 per vine, evenly distributed along the arms.

Under this system, the summer crop starts from the end of grapevine dormancy in late winter, and the harvest occurs in mid-December. In late July, where the risk of frost is low, the vines are cane-pruned and the hydrogen cyanamide 2.5% is applied to the two apical buds aiming to induce and standardize sprouting, resulting in 80–100 new shoots per vine. Flowering begins around 50 days after pruning, and about 20 days later, after the fruit set stage, the crop load can be adjusted followed by shoot trimming.

As the 'BRS Isis' seedless grape is a very fruitful and over cropped cultivar, the shoot thinning is performed leaving 50 shoots evenly distributed along the vine arms with just 1 bunch each, equivalent to a density of 5 bunches. m^{-2}. The bunches are selected according to their size, shape and position along the vine, and those misshape and weak are removed. By removing the excess of bunches, the number of berries that receive nutrients and photosynthates from the vine is decreased, which ends up improving the overall quality of the remaining crop.

Around 35 days after flowering, berry-cluster thinning is necessary to avoid bunch compactness. Flower-cluster thinning or brushing prior to flowering, usually used for seeded cultivars, should not be used for 'BRS Isis' seedless grape because some flowers may abscise before flowering, and still others may abort prior to flowering. Thus, berry-cluster thinning can be performed over a longer period of time, i.e., when berries are between 7–18 mm, as it is a time consuming activity (Roberto et al., 2015a).

Sixty-six days after flowering, the onset of grape ripening occurs, also known as véraison, which can be identified from berry softening and increase in TSS content, followed by a sudden increase in the color of the berry skin. In summer crop, as this stage occurs under high temperatures in subtropical conditions, the anthocyanin accumulation usually is not sufficient to provide a good berry color coverage. In situations like this, the plant growth regulator *S*-ABA at 400 mg.L^{-1} can be applied around 7 days after véraison to promote anthocyanin accumulation and improve berry color. A second application, 14 days after the first one, might be necessary.

Ninety-four days after flowering, by mid-December, the bunches reach full ripe and can be harvested. Considering a density of 5 bunches. m^{-2} and an average bunch weight of 0.5 kg, the estimate yield at harvest would be around 25 tons.ha^{-1}. This summer crop cycle lasts 144 days, from winter pruning to harvest.

In between seasons, the grapevines should rest at least for 30 days. The off-season crop starts in late January and the harvest occurs in mid-May, autumn. At the rest period, the air temperature is high, and combined to the rainy days, the vine metabolism allows a second cycle. In late January, the vines are cane-pruned and the hydrogen cyanamide 2.5% is applied to the two apical buds to force a new sprouting, resulting in 80–100 new shoots per vine.

Flowering begins around 39 days after pruning, and about 20 days later, after the fruit set stage, the crop load can be adjusted, followed by shoot trimming, leaving 50 shoots evenly distributed along the vine arms with just 1 bunch each, equivalent to a density of 5 bunches. m^{-2}, as previously described for the summer crop. At this period, as the air humidity is higher, the incidence of downy mildew, the main vine disease in the humid subtropics, is higher than the previous season, and a strict fungicide program is needed (Ricce et al., 2013), even though 'BRS Isis' is considered tolerant to this disease.

Around 30 days after flowering, berry-cluster thinning is needed to prevent bunch compactness. The off-season crop develops under lower air temperature than summer crop, with higher diurnal temperature variation, which allows a higher accumulation of anthocyanins. For this reason, exogenous applications of *S*-ABA at véraison, which starts 46 days after flowering, is not necessary for berry color improvement. Eighty-six days after flowering, by mid-May, the bunches reach full ripe and can be harvested. Because of the load crop adjustment, the estimate yield for autumn crop is also 25 tons.ha^{-1}, and the off-season cycle is 125 days, from summer pruning to autumn harvest, 19 days shorter than the summer crop. After harvesting, the vines are left to rest for a period of about 60 days, before a new cycle is initiated in late winter.

Additionally, as the risk of frosts is low in subtropical areas, using this model, the winter and the summer prunings, followed by the hydrogen cyanamide application for bud burst, can be performed according to a scheduling in order to provide a better distribution of man power labor for the demanded cultural practices and harvest. In other words, the vineyard can be, for example, split into 2 or 3 sections, and the prunings can be performed in a 7-day interval from each other.

4. Conclusion

The summer and off-season crop cycles of 'BRS Isis' seedless grape grown under subtropical conditions last 144 and 125 days, with thermal demands of 1,931 and 1,815 degree days, respectively. This new seedless grape cultivar can be successfully grown under a double annual cropping system in subtropical conditions, however, as it is a very fruitful cultivar, it is necessary to perform a load crop adjustment after fruit set, equivalent to a density of 5 bunches. m^{-2}, to obtain a sustainable yield in each season. Under this growing system, the berries present in both seasons, appropriate physicochemical characteristics for table grape market, but with darker skin color in off-season.

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References

Avizcuri-Inac, J.M., Gonzalo-Diago, A., Sanz-Asensio, J., Martínez-Soria, M.T., López-Alonso, M., Dizy-Soto, M., Echávarri-Granado, J.F., Vaquero-Fernández, L., Fernández-Zurbano, P., 2013. Effect of cluster thinning and prohexadione calcium applications on phenolic composition and sensory properties of red wines. J. Agric. Food Chem. 61 (5), 1124–1137.

Baggiolini, M., Lorenz, H., Bleiholder, H., T, V.D.B, Stauss, R., Weber, E., Witzenberger, A., Hack, H., Buhr, L., Meier, U., Baillod, M., Bloesch, B., Viret, O., 2008. Stades phénologiques repères de la vigne. Revue Suisse de Viticulture. Arboric. Hortic. 44 (12), 7–9.

Baillod, M., Baggiolini, M., 1993. Les stades repères de la vigne. Revue Suisse Viticulture Horticulture 25 (1), 7–9.

Bevilaqua, B.A.P., 1995. Avaliações físico-químicas durante a maturação de videiras cultivadas no Rio Grande do Sul. Rev. Bras. Agrociência 1 (3), 151–156.

Borges, R.S., Roberto, S.R., Yamashita, F., Bonametti, J.B.O., Assis, A.M., 2012. Sensibilidade ao rachamento de bagas das videiras 'Concord', 'Isabel' e 'BRS Rúbea'. Rev. Bras. Fruticultura 34, 814–822.

Borges, W.F.S., Koyama, R., Silva, G.B., SHAHAB, M., Souza, R.T., Roberto, S.R., 2017. Phenological characterization and thermal demand of 'BRS Vitoria' seedless grape grown in subtropical area. Agron. Sci. Biotech 3, 25–28.

Brasil, 2002. Instrução Normativa 1/2002. Disponível em: < http://sistemasweb.agricultura.gov.br/sislegis/action/detalhaAto.do?method=visualiz arAtoPortalMapa &chave=661183307 > . .

Bucic-Kojic, A., Planinić, M., Tomas, S., Bilić, M., Velić, D., 2007. Study of solid liquid extraction kinetics of total polyphenols from grapes seeds. J. Food Eng. 8 (1), 236–242.

Busato, C.C.M., Soares, A.A., Motoike, S.Y., Busato, C., 2013. Fenologia e exigência térmica da cultivar de videira 'Niágara Rosada' produzida no Noroeste do Espírito Santo. Revista Trópica: Ciências Agrárias e Biológicas 7 (2), 135–148.

Carreño, J., Martínez, A., Almela, L., Fernández-López, J.A., 1995. Proposal of an index for the objective evaluation of the color of red table grapes. Food. Res. Int. Monticello 28 (4), 373–377.

Carreño, J., Martínez, A., Almela, L., Fernández-López, J.A., 1998. Measuring the color of table grapes. Color Res. Appl. 21, 50–54.

Champagnol, F., 1984. Elements de physiologie de la vigne et de viticulture generale. Saint-Gely-du-Fesc: Champagnol 351p.

Chen, W.K., Bai, X.J., Cao, M.M., Cheng, G., Cao, X.J., Guo, R.R., Wang, Y., He, L., Yang, X.H., He, F., Duan, C.Q., Wang, J., 2017. Dissecting the variations of ripening progression and flavonoid metabolism in grape berries grown under double cropping system. Front. Plant Sci. 8, 1912.

Colombo, L.A., Assis, A.M.D., Sato, A.J., Tessmann, D.J., Genta, W., Roberto, S.R., 2011. Produção fora de época da videira 'BRS Clara' sob cultivo protegido. Ciã¯â¿â½ncia Rural. 41 (2), 798–808.

Dami, I., Ferree, D., Prajitna, A., Scurlock, D., 2006. A five-year study on the effect of cluster thinning on yield and fruit composition of Chambourcin grapevines. HortScience 41, 586–588.

Dry, P.R., 2000. Canopy management for fruitfulness. Aus. J. Grape Wine Res 6, 109–115.

Fanzone, M., Zamora, F., Jofré, V., Assof, M., Peña-Neira, A., 2011. Phenolic composition of Malbec grape skin and seeds from Valle de Uco (Mendoza, Argentina) during ripening. Effect of cluster thinning. J. Agric. Food Chem. 59 (11), 6120–6136.

Fávero, A.C., Amorim, D.A., Mota, R., Soares, A.M., Souza, C., Regina, M.A., 2011. Double-pruning of 'Syrah' grapevines: a management strategy to harvest wine grapes during the winter in the Brazilian Southeast. Vitis – J. Grapevine Res. 50, 151–158.

Fenner, M., 1998. The phenology of growth and reproduction in plants. Perspect. Plant Ecol. Evol. Syst. 1, 78–91.

Gil, M., Esteruelas, M., Gonzáles, E., Kontoudakis, N., Jiménes, J., Fort, F., Canals, J.M., Hermosín-Gutiérrez, I., Zamora, F., 2013. Effect of two different treatments for reducing grape yield in Vitis vinifera cv. Syrah on wine composition and quality: berry thinning versus cluster thinning. J. Agric. Food Chem. 61 (22), 4968–4978.

Hussain, I., Roberto, S.R., Fonseca, I.C.B., Assis, A.M., Koyama, R., Antunes, L.E.C., 2016. Phenology of 'Tupy' and 'Xavante' blackberries grown in a subtropical area. Sci. Hort. 201, 78–83.

Keller, M., Mills, L.J., Wample, R.L., Spayd, S.E., 2005. Cluster thinning effects on three deficit-irrigated *Vitis vinifera* cultivars. Am. J. Enol. Vitic. 56, 91–103.

Keller, M., Smithyman, R.P., Mills, L.J., 2008. Interactive effects of deficit irrigation and crop load on Cabernet Sauvignon in an arid climate. Am. J. Enol. Vitic. 59, 221–234.

King, P.D., Mcclellan, D.J., Smart, R.E., 2012. Effect of severity of leaf and crop removal on grape and wine composition of Merlot vines in Hawke's Bay vineyards. Am. J. Enol. Vitic. 63, 500–507.

Kishino, A.Y., Carvalho, S.L.C., Roberto, S.R., 2019a. Viticultura Tropical: O Sistema De Produção De Uvas De Mesa Do Paraná. pp. 709.

Kishino, A.Y., Marur, C.J., Roberto, S.R., 2019b. Características da planta. In: Kishino, A.Y., Carvalho, S.L.C., Roberto, S.R. (Eds.), Viticultura Tropical: o sistema de produção de uvas de mesa no Paraná. pp. 155–249.

Koyama, R., Roberto, S.R., de Souza, R.T., Borges, W.F.S., Anderson, M., Waterhouse, A.L., Cantu, D., Fidelibus, M.W., Blanco-Ulate, B., 2018. Exogenous Abscisic Acid Promotes Anthocyanin Biosynthesis and Increased Expression of Flavonoid Synthesis Genes in*Vitis vinifera* × *Vitis labrusca* Table Grapes in a Subtropical Region. Front. Plant Sci. 9, 323.

Lancaster, J.E., Lister, C., Reay, P.F., Triggs, C.M., 1997. Influence of pigment composition on skin color in a wide range of fruits and vegetables. J. Am. Soc. Hortic. Sci. 122, 594–598.

Leão, P.C.S., Lima, M.A.C., 2017. Effect of shoot and bunch density on yield and quality of 'Sugraone' and 'Thompson Seedless' table grapes. Rev. Bras. Frutic. 39 (4), 1–10.

Leão, P.C.S., Silva, E.E.G., 2004. Fenologia e fertilidade de gemas de variedes de uvas sem sementes no Vale do São Francisco. Anais do IX Seminário Novas Perspectivas para o Cultivo da Uva Sem Sementes, Petrolina. Embrapa Semi-Árido Petrolina.

Leão, P.C., de, S., Nunes, B.T.G., Souza, E.M.C., Rego, J.I.S., Nascimento, J.H.B., 2016. . BRS Isis: New Seedless Grape Cultivar for the Tropical Viticulture in Northeastern of Brazil. BIO Web of Conferences, 39th World Congress of Vine and Wine, Bento Gonçalves, vol.7, 4.

Lima, M.A.C., 2009. Fisiologia, Tecnologia e Manejo Pós-Colheita. Soares, J.M., Leão, P.C.S. A Vitivinicultura no Semiárido Brasileiro. Brasília: Embrapa Informação Tecnológica; Petrolina: Embrapa Semiárido. pp. 599–656.

Mandelli, F., Tonietto, J., Camargo, U.A., Czermainski, A.B.C., 2004. Phenology and thermal needs of the vine in Serra Gaucha. Brazilian Congress of Fruits. 2004, Florianopolis. p.18.

Mcguire, R.G., 1992. Reporting of objective color measurements. HortScience, Alexandria, v. 27, 1254–1255.

Mello, L.M.Rde, 2016. O Brasil no contexto do mercado vitivinícola mundial: panorama 2015. Bento Gonçalves, RS: Embrapa Uva e Vinho. (Embrapa Uva e Vinho. Comunicado Técnico, 192).

Miranda, C., Santesteban, L.G., Royo, J.B., 2013. Evaluation and fitting of models for determining peach phenological stages at a regional scale. Agric. For. Meteorol. 178, 129–139.

Mori, K., Saito, H., Goto-Yamamoto, N., Kitayama, M., Kobayashi, S., Sugaya, S., Gemma, H., Hashizume, K., 2005. Effects of abscisic acid treatment and night temperatures on anthocyanin composition in 'Pinot Noir' grapes. Vitis – J. Grapevine Res. 44 (4), 161–165.

Mori, K., Goto-Yamamoto, N., Kitayama, M., Hashizume, K., 2007. Loss of anthocyanins in red-wine under hight temperature. J. Exp. Bot. 58 (8), 1935–1945.

Mota, R.V., Regina, M.A., Amorim, D.A., Fávero, A.C., 2006. Fatores que afetam a maturação e a qualidade da uva para vinificação. Informe Agropecuário, Embrapa Semiarid; Embrapa Grape and Wine. 27 (234), 56–64.

Mota, R.V., Souza, C.R., Silva, C.P.C., Freitas, G., de, F., Shiga, T.M., Purgatto, E., Lajolo, F.M., Regina, M., de, A., 2010. Biochemical and agronomical responses of grapevines to alteration of source-sink ratio by cluster thinning and shoot trimming. Bragantia 69, 17–25.

Mullins, F., Bouquet, A., Williams, L.E., 1992. Biology of the Grapevine. University Press, Cambridge, pp. 239.

Nachtigal, J.C., Camargo, U.A., Maia, J.D.G., 2005. Efeito de reguladores de crescimento em uva apirênica, cv. BRS Clara. Rev. Bras. Frutic. 27 (2), 304–307.

Nagata, R.K., Scarpare-Filho, J.A., Kluge, R.A., Nova, N.A.V., 2000. Temperatura-base e soma térmica (graus-dia) para videiras 'Brasil' e 'Benitaka'. Rev. Bras. Frutic. 2 (3), 329–333 2000.

Nunes, N.A., Leite, A.V., Castro, C.C., 2016. Phenology, reproductive biology and growing degree days of the grapevine 'Isabel' (*Vitis labrusca*, Vitaceae) cultivated in northeastern Brazil. Bras J. Bio 76 (4), 975–982.

Ozer, C., Yasasin, A.S., Ergonul, O., Aydin, S., 2012. The effects of berry thinning and gibberelin on Recel Uzumu table grapes. Pak. J. Agr. Sci. 49, 105–112.

Pastore, C., Zenoni, S., Tornielli, G.B., Allegro, G., Santo, Sdal., Valentine, G., Intrieri, C., Pezzotti, M., Filippetti, I., 2011. Increasing the source/sink ratio in *Vitis vinifera* (cv. Sangiovese) induces extensive transcriptome reprogramming and modifies berry ripening. BMC Genomics 12, 631.

Pedro-Júnior, M.J., Sentelhas, P.C., 2003. Clima e produção. POMMER, C. V. Uva: tecnologia de produção, pós-colheita, mercado. Porto Alegre: Cinco Continentes. pp. 63–107.

Pedro-Júnior, M.J., Sentelhas, P.C., Pommer, C.V., Martins, F.P., Gallo, P.B., Santos, R.B., Bovi, V., Sabino, J.C., 1993. Caracterização fenológica da videira 'Niagara Rosada' em diferentes regiões paulistas. Bragantia 52 (2), 153–160.

Peppi, M.C., Fidelibus, M.W., Dokoozlian, N., 2006. Abscisic acid application timing and concentration affect firmness, pigmentation and color of 'Flame Seedless' grapes. HortScience 41, 1440–1445.

Ribeiro, D.P., Corsato, C.E., Franco, A.A.N., Lemos, J.P., Pimentel, R.M.A., 2010. Fenologia e exigência térmica da videira 'Benitaka' cultivada no norte de Minas Gerais. Rev. Bras. Frutic. 32 (1), 296–302.

Ricce, W.S., Caramori, P.H., Roberto, S.R., 2013. Potencial climático para a produc¸ ãode uvas em sistema de dupla poda anual no estado do Paraná. Bragantia 72 (4), 408–415.

Ritschel, P.S., Maia, J.D.G., Camargo, U.A., Souza, R.T., Fajardo, T.V.M., Naves, R.L., Girardi, C.L., 2013. BRS Isis: nova cultivar de uva de mesa vermelha, sem sementes e tolerante ao míldio. Bento Gonçalves: Embrapa Uva e Vinho. Comunicado técnico 143 20p.

Roberto, S.R., Assis, A.M., Yamamoto, L.Y., Miotto, L.C.V., Sato, A.J., Koyama, R., Genta, W., 2012a. Application timing and concentration of abscisic acid improve color of 'Benitaka' table grape. Sci. Hort. 142, 44–48.

Roberto, S.R., Assis, A.M., Genta, W., Yamamoto, L.Y., Sato, A.J., 2012b. 'Black Star': Uma mutação somática natural da uva na de mesa cv. Brasil. Rev. Bras. Frutic. 34 (3), 947–950.

Roberto, S.R., De Assis, A.M., Yamamoto, L.Y., Miotto, L.C.V., Koyama, R., Sato, A.J., BORGES, R.S., 2013. Ethephon use and application timing of abscisic acid for

improving color of ‘Rubi’ table grape. Pesq. Agropec. Bras. 48 (7), 797–800.
Roberto, S.R., Borges, W.F.S., Colombo, R.C., Koyama, R., Hussain, I., Souza, R.T., 2015a. Berry-cluster thinning to prevent bunch compactness of ‘BRS Vitoria’, a new black seedless grape. Sci. Hort. 197, 297–303.
Roberto, S.R., Mashima, C.H., Colombo, R.C., 2015b. Phenological characterization and quality of fine’ Black Star’ table grape. Agron. Sci. Biotech. 1 (2), 77–82.
Sabbatini, P., Howell, G.S., 2013. Rootstock scion interaction and effects on vine vigor, phenology, and cold hardiness of interspecific hybrid grape cultivars (*Vitis* spp.). Int. J. Fruit Sci. 13 (4), 466–477.
Santos, A.O., Rolim, G.S., Hernandes, J.L., Pedro-júnior, M.J., 2009. Maturação fisiológica da videira em São Paulo: comentários sobre as safras de verão e inverno na média altitude paulista.
Santos, A.E.O., Silva, E.O., Oster, A.H., Mistura, C., Dos-Santos, M.O., 2013. Resposta fenológica e exigência térmica de uvas apirenas cultivadas no Submédio do São Francisco. Rev. Bras. Cienc. Agrar. 8 (3), 364–369.
Santos, S.D.L., Ribeiro, V.G., De Lima, M.A.C., Souza, E.R., Shishido, W.K., 2015. Influência do ácido giberélico na fisiologia e qualidade da videira cv. Sweet Celebration® no Submédio São Francisco. Rev. Bras. Frutic 37 (4), 827–834.
Sato, A.J., Jubileu, B.S., dos Santos, C.E., Bertolucci, R., Silva, R.A.L., Carielo, M., Guiraud, M.C., Fonseca, I.C.B., Roberto, S.R., 2008. Phenology and thermal demand of ‘Isabel’ and ‘Rubea’ grapevines on different rootstocks in North of Parana. Semina 29, 283–292.
Scariotto, S., Citadin, I., Raseira, M.C.B., Sachet, M.R., Penso, G.A., 2013. Adaptability and stability of 34 peach genotypes for leafing under Brazilian subtropical conditions. Sci. Hortic. 155, 111–117.
Sun, Q., Sacks, G.L., Lerch, S.D., Vanden–Heuvel, J.E., 2012. Impact of shoot and cluster thinning on yield, fruit composition and wine quality of Corot noir. Am. J. Enol. Vitic. 63, 49–56.
Tecchio, M.A., Moura, M.F., Paioli-Pires, E.J., Terra, M.M., 2013. Effect of rootstock and pruning season on the duration of the phenological phases and accumulation of degree-days by ‘Niagara Rosada’ vine. Rev. Bras. Frutic. 35 (4), 1073–1080.
Villa-Nova, N.A., 1972. Estimativa de graus-dia acumulados acima de qualquer temperatura base em função das temperaturas máxima e mínima. Ciência da Terra 30, 1–8.
Youssef, K., Roberto, S.R., 2014. Applications of salt solutions before and after harvest affect the quality and incidence of postharvest gray mold of ‘Italia’ table grapes. Postharv. Biol. Technol. 87, 95–102.
Zhu, L., Huang, Y., Zhang, Y., Xu, C., Lu, J., Wang, Y., 2017. The growing season impacts the accumulation and composition of flavonoids in grape skins in two-crop-a-year viticulture. J. Food Sci. Technol. 54 (9), 2861–2870.