Universidade Estadual de Londrina

SAEED AHMED

GROWTH PERFORMANCE AND POST-HARVEST BEHAVIOR OF 'BRS ISIS' SEEDLESS GRAPE IN SUBTROPICAL CONDITIONS

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Thesis Presented for Doctorate, Graduate Program in Agronomy, Londrina State University, Paraná, Brazil.

Advisor: Prof. Dr. Sergio Ruffo Roberto

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DEDICATION

To my loving parents, who have helped me throughout my life.

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ABSTRACT

'BRS Isis' is a new hybrid red seedless table grape, whose characteristics come to meet some of the main demands of the grape industry, as tolerance to downy mildew and good adaptation to the tropical climate. It also stands the high bud fertility and neutral taste, large berries, good adhesion and crunchy texture. However, the performance and post-harvest behavior of 'BRS Isis' under subtropical conditions has not been studied yet, especially under two crops per year. For this purpose, a research was designed with the objective to evaluate the growth performance and post-harvest behavior of 'BRS Isis' seedless grape by means of phenology and physicochemical characteristics determination. The trial was conducted in an established commercial vineyard of 'BRS Isis' table grape (*Vitis* spp.), located at Marialva city, state of Paraná (PR), Brazil. The experiment was conducted in two consecutive seasons, in summer crop of 2016 and off-season crop of 2017. For assessments, 20 representative vines were selected in the area. The duration of the main phenological stages of grapevines were evaluated from pruning until harvest, as well its thermal demand in degree-days. Summer and off-season crops lasted 144 and 125 days, with thermal demands of 1,931 and 1,815, respectively. The soluble solids and titratable acidity contents were quite similar in both seasons, with an average of 14.3 °Brix and 0.7%, respectively. Based on these results, a double annual cropping system with some specific cultural practices is proposed for this mid-season cultivar grown under subtropical conditions that allows an accumulative yield of 50 $tons.ha^{-1}.year^{-1}$, what is considered profitable for this agricultural activity. Post-harvest trials under cold storage conditions were also performed, and grapes were subjected to following treatments in cold chamber at 1 °C: (i) Control; (ii) SO_2-generating pad; (iii) Control with bunches inoculated with *B. cinerea* suspension; (iv) SO_2-generating pad with bunches inoculated with *B. cinerea suspension*. The randomized complete design was used as statistical model with four treatments and five replicates, with 4 bunches per plot. The incidence of gray mold on grapes was evaluated at 50 days after the beginning of cold storage and at 7 days at room temperature (25 °C) after the end of cold storage. Grape physicochemical characteristics, such as bunch mass, bunch mass loss, skin color, SS, TA and SS/TA were evaluated at 50 days after the period of cold storage and at 7 days at room temperature after the end of cold storage. The new hybrid 'BRS Isis' seedless grape, packaged with SO_2-generating pads and plastic liners, has a high potential to be preserved for long periods under cold storage at 1 ºC, at least for 50 days, keeping very low natural incidence of gray mold, mass loss and shattered berries.

Keywords: Table grape. Phenology. Anthocyanin. Polyphenols. cold storage. *Botrytis cinerea.*

AHMED, Saeed. **Desempenho de crescimento e comportamento pós-colheita da uva sem sementes 'BRS Isis' em condições subtropicais**. 2019. 76f. Tese de Doutorado em Agronomia – Universidade Estadual de Londrina, Londrina, 2019.

RESUMO

A 'BRS Isis' é uma nova uva híbrida de mesa vermelha sem sementes, cujas características atendem a algumas das principais demandas do sistema de produção de uvas por ser tolerante ao míldio, e adapta-se bem ao clima tropical. Além disso, apresenta alta fertilidade e sabor neutro, bagas grandes, boa aderência e textura crocante. No entanto, o desempenho e o comportamento pós-colheita da 'BRS Isis' sob condições subtropicais ainda não foram estudados, especialmente sob duas safras por ano. Desta forma, a pesquisa foi desenvolvida com o objetivo de avaliar o desempenho do crescimento e o comportamento pós-colheita da uva sem sementes 'BRS Isis' por meio da determinação de características fenológicas e físico-químicas. O experimento foi realizado em um vinhedo comercial da uva de mesa 'BRS Isis' (*Vitis* spp.), em Marialva, PR, Brasil. O experimento foi conduzido em duas safras consecutivas, na safra de verão 2016 e na safra fora de época 2017. Para as avaliações, foram selecionadas 20 videiras representativas na área. A duração dos seus principais estádios fenológicos foi avaliada desde a poda até a colheita, bem como a sua exigência térmica em graus-dia. As bagas foram submetidas à análise dos sólidos solúveis totais (SST), acidez titulável (AT), índice de maturação (SS/AT), pH, polifenóis totais, antocianinas totais, massa, diâmetro e cor das bagas, e massa e largura de cachos. As safras de verão e temporã duraram 144 e 125 dias, com demandas térmicas de 1.931 e 1.815, respectivamente. os teores de sólidos solúveis e acidez titulável foram semelhantes em ambas as estações, com média de 14,3 °Brix e 0,7%, respectivamente. Com base nesses resultados, propõe-se um sistema de duplo cultivo anual com algumas práticas culturais específicas para esta cultivar de meia estação cultivada em condições subtropicais, permitindo uma produtividade de 50 $t.ha^{-1}.ano^{-1}$, o que é considerado rentável para esta atividade agrícola. Foram também realizadas análises pós-colheita sob condições de armazenamento refrigerado, e as uvas foram submetidas aos seguintes tratamentos em câmara fria (1°C): (i) Controle; (ii) Folha geradora de SO_2; (iii) Controle com cachos inoculados com suspensão de *Botrytis cinerea*; (iv) Folha geradora de SO_2 com cachos inoculados com suspensão de *B. cinerea*. O delineamento inteiramente casualizado foi utilizado como modelo estatístico com quatro tratamentos e cinco repetições, com 4 cachos por parcela. A incidência de mofo cinzento nas uvas foi avaliada aos 50 dias após o início do armazenamento em câmara fria e aos 7 dias à temperatura ambiente após o fim do armazenamento refrigerado. As variáveis fisico-químicas da uva, tais como massa da cacho, perda de massa do cacho, cor, SS, AT e SS/AT foram avaliadas aos 50 dias após o período de armazenamento refrigerado e aos 7 dias à temperatura ambiente após a fim do armazenamento frio. O nova uva sem sementes 'BRS Isis', embaladas com folhas geradoras de SO_2 e filmes plástico, apresentaram alto potencial de preservação das uvas por longos periodos sob armazenamento rerfigerado a 1 ºC durante um período de 50 dias, apresentando incidência de mofo cinzento, degrana e perda de massa em níveis muito baixos.

Palavra-chave: Uva de mesa. Fenologia. Antocianina. Polifenóis. armazenamento refrigerado. *Botrytis cinerea.*

LIST OF FIGURES

LIST OF TABLES

SUMMARY

1 INTRODUCTION

Grapes are widely grown and consumed all over the world, due to its economic importance and advantageous effects on human health. It is known that previously the production and export of grapes was exclusively controlled by traditional European countries, nevertheless, South American countries, such as Chile, Peru and Brazil, have shown a significant increase in growth and production of grapes in recent years, and in some cases, with two crops per year (FAOSTAT, 2016).

Viticulture is a traditional activity in temperate climates, but along with the current developments of grape production technology in subtropical and tropical conditions, this culture, especially table grapes, has been under constant development in several states of Brazil, covering an area of approximately 75,510 hectares (FAOSTAT, 2018). In the last decades, it has become important in generating employment in large enterprises for production of table grapes, mainly seedless cultivars for export (NACHTIGAL et al., 2005; MOURA et al., 2009; MELLO, 2010).

In this context, concerned about the future of the national viticulture, Embrapa Grape and Wine, has been conducting a Table Grape Breeding Program since 1997 aimed at creating hybrid seedless table grapes well adapted to tropical and subtropical conditions. In 2003, the first cultivars of seedless grapes such as 'BRS Clara', 'BRS Linda' and 'BRS Morena' were released (CAPOBIANCO, 2002; NACHTIGAL; CAMARGO, 2004). Few years later, in 2013, the 'BRS Isis' seedless grape was released. It is the result of crossing CNPUV 681-29 [Arkansas 1976 x CNPUV 147-3 ('Niagara White' x 'Venus')] x 'BRS Linda', and presents large and red berries, good adhesion and crunchy texture. It is tolerant to downy mildew and adapts well to the tropical climate. Besides, it also stands the high bud fertility and presents firm texture and neutral taste. In tropical regions, it can reach yields of around 26 t ha^{-1} (RITSCHEL et al., 2013).

The introduction of new cultivars, however, requires the development of indexes of identity and quality, which permits the identification of standards of these new materials in the market (CHITARRA; CHITARRA, 2005). These references are important in defining specific features, such as the harvest point and sensory characteristics to minimize the physiological disorders and postharvest losses (KAYS, 1997; KADER, 2002; MASCARENHAS et al., 2010).

The subtropical region of Paraná State is a consolidated area in table grape production, but it is based on seeded grapes such as 'Benitaka', 'Italia', 'Rubi' and 'Brasil'

(*Vitis vinifera* L.). In this area, due the mild winter and subtropical conditions, two crops of grapes per year are obtained per year. Thus, seedless grape cultivation, such as 'BRS Isis', could be an alternative to diversify the current production system, opening the possibility of international market. However, there is no information about the performance of 'BRS Isis' grape grown in subtropical areas, and the results cannot always be extrapolated from one region to another, because these characteristics vary depending on the genotype and climatic conditions of each region, influencing also on the fruit quality (BOLIANI; PEREIRA, 1996; LEÃO; SILVA., 2003).

Considering the aspects above, the objective of this work was to evaluate the growth performance and the post-harvest behavior of 'BRS Isis' seedless grape, by means of its phenology and physicochemical characteristics grown in subtropical area under two crops a year.

2 REVIEW OF LITERATURE

2.1 THE GRAPEVINE AND THE VITICULTURE

Grapevine has been among the first fruit species to be domesticated and it is the world's most economically important fruit crop. The grapevine belongs to the family Vitaceae, which comprises about 60-70 species distributed in Asia, North America and Europe under subtropical, Mediterranean and continental–temperate climatic conditions. It is the single *Vitis vinifera* L. that acquired significant economic interest over time, while other species, such as *Vitis labrusca*, *Vitis rupestris*, *Vitis riparia* or *Vitis berlandieri*, are used in breeding programs due to their resistance against some pathogens. Indeed, a great majority of cultivars widely cultivated for fresh fruit, juice, dried grapes (raisins) and mainly for wine, classified as *V. vinifera* L. The family contains approximately 1000 species assigned to 17 genera that are typically shrubs or woody lianas that climb by means of their leaf-opposed tendrils, hence the name *Vitaceae* (Latin *viere* = to attach) (CRESPAN, 2004; JEAN-FREDERIC et al., 2010).

The cultivation and domestication of the grapevine appears to have occurred between the seventh and the fourth millennia BC, in a geographical area between the Black Sea and Iran. From this area, cultivated forms would have been spread by humans in the Near East, Middle East and Central Europe. As a result, these areas may have constituted secondary domestication centres (GRASSI et al., 2003; ARROYO-GARCIA et al., 2006).

The grapevine is considered one of the major fruit trees grown in the world, and the annual grape production in 2015 was 76.8 millions tons, of which 13.7 millions were produced by China, representing 17.8% of the world total production. Other countries, such as Italy, USA, France and Spain accounted for 10.3; 9.1; 8.1 and 7.5% of the total respectively, and along China, contributed 52.8% of the world total grapes production. Brazil is the 15th largest producer of grapes, with 1,497,302 tons harvested in 2015 (FAOSTAT, 2016; MELLO et al., 2017).

The world production concerning table grapes reached almost 27 million tonnes in 2014, an increase of 71% since 2000. China is the main producer of table grapes with the production of 9.2 millions tons followed by India (2.1), Turkey (2.1), Eygpt (1.4), USA (1.2), Iran (1.1), Uzbekistan (1.1), Italy (1.0) and Brazil (0.8) millions tons, respectively (FAO-OIV, 2016).

2.2 VITICULTURE IN BRAZIL

In Brazil, viticulture began with the arrival of Portuguese settlers in the 16th century and remained a domestic practice until the mid-19th century when italian immigrants started to produce 'Isabel' grape (*Vitis labrusca*) (SOUSA, 1996). In the 20th century, fine table grapes returned for fresh consumption. High scale production of commercial table grapes in the northeastern semi-arid area started originated the tropical viticulture in Brazil. Tropical/subtropical viticulture gave a start to new production centers of table grapes in the northern regions of Paraná, Northwest of São Paulo and northern of Minas Gerais states. Viticulture has been continuously developing over the past years and proved a high potential for the development of the country (LEÃO; POSSÍDIO, 2000; PROTAS et al., 2006).

Among the grape producing States in Brazil, Rio Grande do Sul (49,739 ha) is the main producer with the production of 876,215 tons, followed by Pernambuco (6,814 ha), São Paulo (7,803 ha), Bahia (2,861 ha), Santa Catarina (4,846 ha), Paraná (4,465 ha) and Minas Gerais (856 ha) with the production of 237,367; 142,631; 77,408; 69,118; 69,035 and 12.615 tons respectively. The production of grapes for processing (wine, juice and derivatives) corresponds to 52% of national production, while the remaining amount of 48% is used for fresh consumption (MELLO, 2017; IBGE, 2017).

In Paraná state, there are two important major areas of viticulture, the region of Curitiba metropolitan located in the south of the State, traditionally known for the elaboration of table wines, mainly with 'Bordô' (*Vitis labrusca*), and the region of Maringá, located in the Northern region of the state. The northern region includes Londrina, Marialva, Uraí, Maringa, Rolândia and other adjacent areas. This region dominates the production of fine table grapes (*Vitis vinifera*). The viticulture in northern Paraná was developed by japanese colony in the 1970s, with the production of 'Italia' grape. The diversification in the production took place with the introduction of mutant clones derived from 'Italia', such as 'Rubi', 'Benitaka' and 'Brasil' grapes (PROTAS; CAMARGO, 2011; MELLO, 2015; IBGE, 2016).

In some subtropical areas, the obtainment of two crops of table grape per year (summer crop and off-season crop) is possible due the mild winter and the use of budburst stimulators. The summer crop starts from the end of grapevine dormancy in late winter, and the harvest occurs during summer. Right after that, a new cycle is forced, the grapevines are pruned and forced to sprout again by using budburst stimulators, and an off-season crop is obtained during autumn. The main difference between these two crops is that in the first one, the incidence of some fungal disease, such as downy mildew is low, whereas during the out of season crop, the incidence is higher due the most favorability for fungal infection (RICCE et al., 2013). Different times of pruning influence the phenological behavior of the vines. Therefore, the phenological and productive characterization of the new cultivars is needed during summer and off-season crops to allow the planning of agricultural activities and to estimate pruning and harvesting dates, under each one of these conditions (SANTOS et al., 2013; ROBERTO et al., 2015).

In the beginning of the 1990s, some seedless table grapes were introduced in Brazil, such as ‘Thompson Seedless’, ‘Crimson Seedless’ and ‘Centeniall Seedless’, but these grapes did not extant great success, since it had an inconsistency yield, pathogen sensitivity and cracking of berries (NACHTIGAL, 2005; MASCARENHAS et al., 2012; RITSCHEL et al., 2013). Besides, it was verified that these cultivars had difficulties in adaptation to regions of tropical/subtropical climate, not reaching good production performances (CAMARGO et al., 2003a,b,c).

In order to overcome this difficulty, in 1997, the Table Grape Breeding Program held by the Brazilian Agricultural Research Corporation - EMBRAPA was started, in order to solve the limiting factors of earlier valid seedless cultivars. Then, new hybrid materials adapted to the Brazilian conditions were developed, possessing characteristics desirable for the viticulture such as high yield, production consistency, desirable bunch characteristics and tolerance to diseases. Since then, several new hybrids seedless table grape have been released, such as ‘BRS Morena’, ‘BRS Clara’, ‘BRS Linda’, ‘BRS Vitoria’ and ‘BRS Isis’ (NACHTIGAL, 2005; COLOMBO et al., 2011; MAIA et al., 2012; RITSCHEL et al., 2013).

2.3 ‘BRS ISIS’ SEEDLESS GRAPE

‘BRS Isis’ is a hybrid table grape obtained by the crossing of CNPUV 681-29 [Arkansas 1976 x CNPUV 147-3 (‘Niagara White’ x ‘Venus’)] x ‘BRS Linda’ (Figure 2.3.1). It is tolerant to downy mildew, the main vine disease in subtropical humid areas. It is a vigorous late cultivar with strong shoot dominance. Thermal requirements were estimated in 1,800 degree-days from pruning to harvest, and 1,675 degree-days from bud sprouting to the end of maturation. 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 berry is medium size, red, elliptical shape having firm and colorless pulp, neutral flavor with traces of rudimentary seeds large fleshy (Figure 2.3.2) (RITSCHEL et al., 2013).

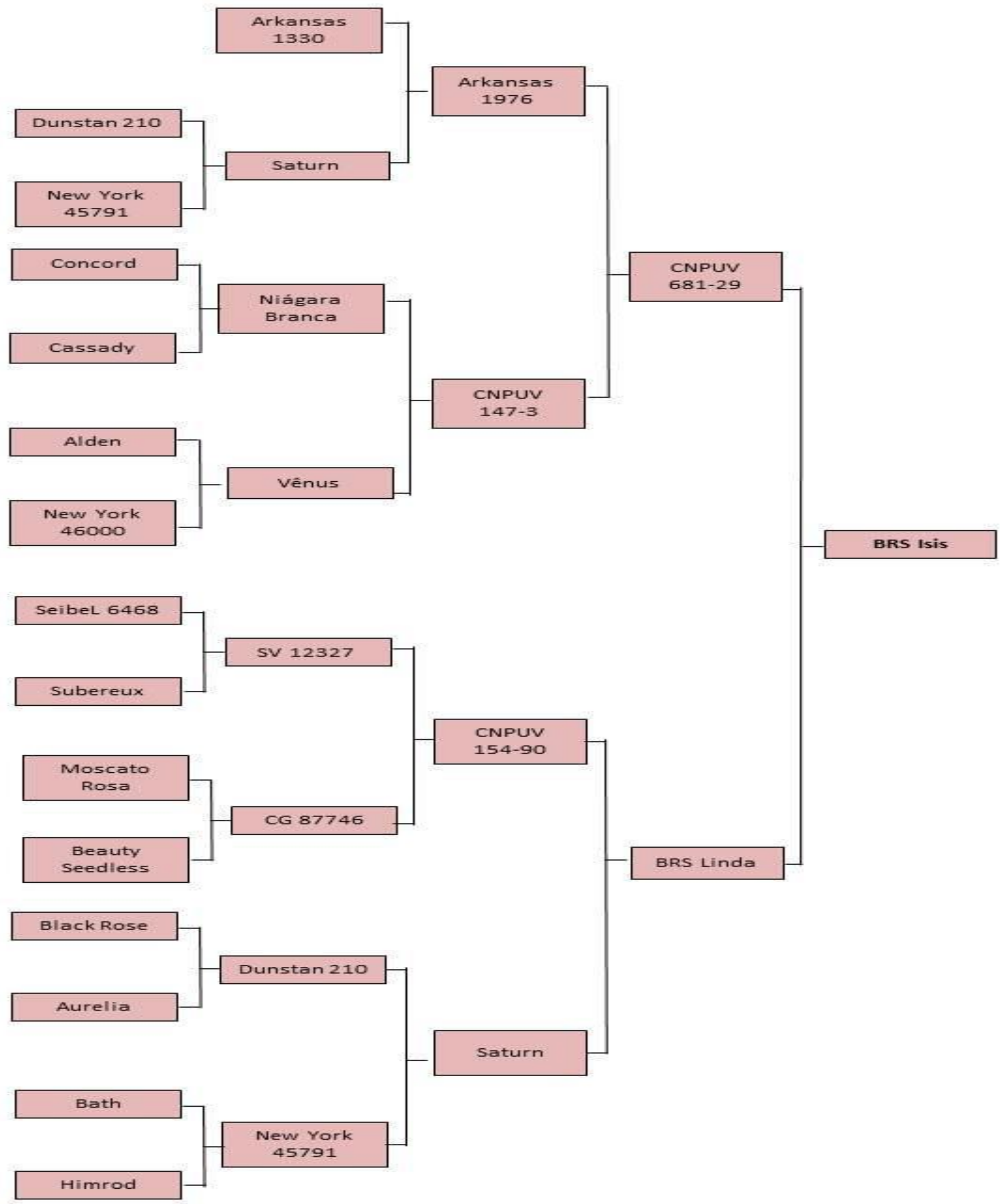

Figure 2.3.1. Genealogy of ‘BRS Isis’ seedless grape (RITSCHEL et al., 2013).

Figure 2.3.2. Bunches of 'BRS Isis' seedless grape (RITSCHEL et al., 2013).

2.4 Phenology And Thermal Requirements Of Grapevines

Phenology is the study of cyclic and seasonal natural phenomena, which depends on genetic factors and environmental and climatic conditions. The phenological characterization describes the details of the plant growth cycle, allowing determination of the optimal time to carry out cultivation practices or verification of the occurrence of an important event such as frost or drought associated with well-defined stages (TADEU et al., 2015; HUSSAIN et al., 2016).

Phenology is the most important attribute involved in the adaptation of grapevine, as other crops, to its growing environment and to climatic changes. Its aim is to describe the causes of variation in timing of developmental events by seeking correlations between weather indices and the dates of particular growth events and the intervals between them. 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 (DUCHÊNE; SCHNEIDER, 2005; JONES, 2008; ANZANELLO et al., 2012).

The duration and the date of occurrence of the different phenological stages of the vine vary according to the variety, climate and geographical location of the vineyard. Usually, winegrowers use this information to choose the variety that is more suitable to their vineyard and to adapt their practices (i.e. fertilization, pruning, application of plant and agrochemical regulators, thinning and harvesting) to variations in climatic conditions in space (among vineyards) and in time (among vintages) (COOMBE, 1995; SCHWARTZ, 2013; IÑAKI et al., 2017).

The modified Eichhorn–Lorenz is a widely used scale which describes the vegetative and reproductive stages of grapevine, and identifies seven major phenological stages: budburst, shoot development, flowering, fruit set, berries pea-sized, veraison, and harvest (EICHHORN; LORENZ, 1977). Baggiolini and Baggiolini (1993), on the other hand, described various developmental stages in order to allow better timing of grapevine protective measures. This scale covered the period from winter rest to fruit set which was divided into ten stages. The increasing importance of handling experimental data electronically demanded a more detailed scale which had to incorporate a numerical description of the various growth stages, and this resulted in the new scale for the phenological stages of the development of the grapevine. In order to unify growth stage schemes, Coombe (1995) proposed a combination of both scales, which has been used to characterize the phenological development of grapevines.

The main factor that affects the occurrence of phenological stages is air temperature, which is expressed primarily as heat accumulation or growing degree days index (DD). The growing degree days is an expression of the amount of energy that a cultivated plant species 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. In addition, it has been used to study the physiological processes that control the duration of phenological stages in grapevines. The growth cycle can be significantly different, particularly under subtropical conditions, and it can also vary annually due to the accumulation of growing degree-days. It constitutes the accumulated difference between the mean environment temperature and the base-temperature, below this temperature, no development occurs (DUCHÊNE et al., 2012; MIRANDA et al., 2013; TECCHIO et al., 2013).

The DD is the most used parameter in tropical viticulture because of its easy application and reliability. When phenological and climatic data are combined, it is possible to understand the relationship between the duration of plant developmental phases and seasonal variations. Additionally, it is possible to understand how a specific plant species interact with the different climatic regions. Therefore, phenological analysis and DD measurement may be used as a tool to evaluate the climatic potential of a region for the development of a crop species and contribute for the knowledge about the periods of harvest, improving agricultural practices (BRIGHENTI et al., 2013; BORGES et al., 2017).

2.5 GRAPE QUALITY

Grape is a non-climacteric fruit and does not ripen further after harvest, so harvesting at the proper stage of maturity is essential for optimum fruit quality. Grape ripening is a physiological period that starts at the moment of veraison and lasts until the fruit is harvested. This is a very important period that influences the composition of the grapes and determines varietal characteristics. Grapes undergo many changes during the ripening process which involve a number of physical and biochemical modifications, including mass, soluble solids, acidity, color and aroma (PIAZZOLLA et al., 2016).

Sugars and organic acid compositions, which are measured through total soluble solids (TSS) and titratable acidity (TA), are most commonly associated with the taste of fruits, including table grapes. From the consumers' perspective, the organoleptic quality of table grapes depends mainly on the sugar content, organic acid content and the balance between them. The color and aroma of fruit are the most important factors that attract consumers and are essential for the highly competitive market and food industry (SHIRAISHI et al., 2010; YANG et al., 2011).

In addition to visual characteristics, physico-chemical properties are involved in the sensory and quality evaluation. Degree of ripeness, type of soil, climatic conditions, cultural practices and growing location are important factors affecting the physico-chemical properties of table grapes, but the phenolic composition strongly depends on the table grapes cultivar. (BAIANO; TERRACONE, 2011; ROLLE et al., 2013).

Several studies have shown that consumption of fruits and vegetables decreases the risk of chronic diseases, such as cardiovascular diseases and cancer. This beneficial effect has been attributed to the presence of fibers, minerals, vitamins and phytochemical compounds including phenolic acids, flavonoids, and anthocyanins (SHAHIDI; AMBIGAIPALAN, 2015; RODRIGUEZ-CASADO, 2016; VIEIRA et al., 2016).

Grapes contain a wide range of vitamins, carotenoids and phenolic compounds. Phenolic compounds play one of the most important roles in grape quality because of their contribution to the taste and color. Grapes also contain carotenoids, mainly β-carotene and lutein. Carotenoids are well-known natural pigments, responsible for the red, orange, and yellow hues in fruit and vegetable, but they are also important to human health since they are precursors of vitamin A. Vitamin A is essential for normal growth, reproduction, and resistance to infection. It also plays a role in vision, and a severe deficiency can lead to blindness. Grapes are also sources of vitamin C and E (FRASER; BRAMLEY, 2004; WADA et al., 2007).

Grapes accumulate a wide range of phenolic compounds, especially polyphenols. The basic composition of phenols produced in different varieties and different environment is almost the same. However, biosynthesis of these compounds is directly influenced by the cultivars and their genetic characteristics, temperature, humidity, sun exposure, soil type and fertilization, among other factors (PINTO et al., 2011; CASTELLARIN et al., 2012).

Total phenolic contents in ‘BRS Morena’ and ‘BRS Clara’ table grapes were recorded as 1,008 and 577 $mg.kg^{-1}$ of fresh fruit, respectively. For ‘BRS Morena’, 86.2% of the contents were distributed in skin while 13.85% in flesh, while for ‘BRS Clara’, the skin contained 76.5% and the flesh had 23.5% of the total. With regard to total antioxidant capacity, ‘BRS Morena’ and ‘BRS Clara’ grapes exhibited high values (39.62±1.11 and 15.93±0.24 $mmol.kg^{-1}$ as Trolox equivalents, respectively), that were mainly located at the skins (92.0% in ‘BRS Morena’ and 86.8% in ‘BRS Clara’) (LAGO-VANZELA et al., 2011).

The composition of grape seeds is basically (w/w) 40% fiber, 16% essential oil, 11% protein, 7% complex phenolic compounds like tannins, sugars, minerals, and other substances. Grape skin is a source of anthocyanidins and anthocyanins, natural pigments with antioxidant properties acting through inhibition of lipoperoxidation and which also have antimutagenic activities (KOTHAWADE et al., 2013).

The accumulation of most of phenolic compounds in grapes takes place in seeds and skins. They can be divided into: non-flavonoids and flavonoid compounds. Non-flavonoids, except hydroxycinnamic acids, are found in grapes in low concentrations, while flavonoids constitute the largest class of plant phenolic compounds. Different types of flavonoids play different roles in plants, including pigmentation and defense. The most common group of flavonoids pigment consists of the anthocyanins, which are responsible for red, pink and blue color of plants. The grape is rich in these compounds, which are synthesized in the herbaceous parts throughout the production cycle, being stored mainly in berries (CONDE et al., 2007; GUERRA, 2012).

2.6 FACTORS THAT DETERMINE GRAPE QUALITY

Grapes can be grown commercially in climates ranging from temperate to tropical. It is therefore one of the world's most widely distributed fruit crops. There are many factors in grape growing which define quality, such as pruning, thinning, crop load and the use of plant growth regulators. However, increasing grape quality of regional vineyards is dependent on carrying out specific practices (KAMILOĞLU, 2011; HARINDRA, 2015).

Pruning is a cultural technique which drives vine vigour to ensure fruit quality and plant vegetative balance. It helps to improve the microclimate in the canopy, promotes good ripening of the grapes and creates less suitable conditions for the development of pathogens. Good results depend on the vegetative-productive behavior of the vineyard, intensity and age of cultural operation. Pruning defines the final productivity of plants by modifying the number of shoots per plant with, the number of clusters per shoot with cluster thinning, and the number of berries per bunch with berry thinning (CRESCIMANNO et al., 2011).

Cluster thinning is usually performed after fruit set in order to adjust the crop load, to distribute clusters evenly on the vine and canes, to select the best clusters (shape, size and position) and to eliminate those that are misshaped and weak. Generally the aim is to have an equal number of clusters and shoots on the plant.

Berry thinning is a widely performed technique and involves the removal of a few berries from the cluster. This operation is necessary to decrease the compactness of bunches and to give them a more attractive shape with large, uniform-size berries (DI LORENZO et al., 2011).

2.7 COLD STORAGE OF TABLE GRAPES

The quality of grape berries is the prime consideration in both domestic and export markets. The maintenance of post-harvest quality of table grapes is becoming increasingly significant as the supply of high quality commodities constantly exceed demand. The consumer expectation in the supply of fresh produce is partly matched by long-term storage. Cold storage is one of the most used methods to prolong postharvest quality and extend the shelf life of a broad range of horticultural commodities. Grapes experience many ripening changes during storage, including physico-chemical modifications and different sensorial attributes (PIAZZOLLA et al., 2016).

The important quality characteristics comprise both bunch as well as berry properties, and these include sanity conditions, uniform bunch color, bunch mass, size and shape, stem quality, berry size and shape, firmness, total soluble solids (TSS), and titratable acidity (TA) (HARINDRA, 2015).

Grapes are non-climacteric fruits with a relatively low physiological activity, and are subject to serious water loss softening during postharvest handling and cold storage, which can result in stem browning, berry shatter, wilting and shriveling of berries. Quality and shelf-life are important in grapes intended for table use. Decreased quality during postharvest handling of table grapes is usually associated with water loss, softening, and decay. Berry softening, shattering, stem browning and decay development are the main barriers for long term storage of grapes (DAUDT; FOGACA, 2013; SILVA-SANZANA, et al. 2016; SEN et al., 2016).

Grapes are highly perishable commodities, and thus length of storage is limited by certain factors such as fruit maturity at harvest, pre-cooling and storage conditions, such as temperature and relative humidity (SEN et al., 2012). Cold storage, where only temperature and relative humidity are controlled in the storage chamber, is one of the main methods for conservation of fruit quality. Thus, the reduction of temperature, up to a certain limit, increases the quality maintenance and extends the period of fruit supply to the consumer market. Once harvested, most fruits need to be pre-cooled as quickly as possible to remove field heat, to decrease respiration and water loss, as well to keep harvest quality. This practice is particularly important when air temperature at harvest is relatively high, and can lead to enhanced loss of water, resulting in drying that starts from stems or pedicels and enhanced senescence processes. Loss of even relatively small amounts of water from table grapes has a large negative impact on their quality (BRACKMANN et al., 2010; ROMANAZZI et al., 2016).

High relative humidity and low temperature storage environments play an important role in maintaining the quality of produce. Optimum storage temperatures for most grape cultivars are between -1.0-3.0 ºC. The relative humidity should be 90-95% and an air velocity of approximately 0.1-0.2 $m.s^{-1}$ is suggested during storage to minimize moisture loss of berries and to maintain stems in good condition (KADER, 2002; HUNG et al., 2011). In addition, the temperature during cold storage needs to be optimal and constant, especially for long distance shipment, because any interruption of the cold chain can allow the development of a pathogen from quiescent infections, mainly *Botrytis cinerea*, known as gray mold. This condition favors rapid disease development, particularly under the high humidity within packages (LIGUORI et al., 2015).

B. cinerea is the primary cause of bunch rot in grapes, and the pathogen attack may take place during harvest and subsequent handling, storage, marketing, and after consumer purchase. *B. cinerea* is the asexual stage (anomorph) of the teleomorph *Botryotinia fuckeliana*, and the genus *Botrytis*, classified within the family Sclerotiniaceae (Inoperculate Discomycetes), comprises over 20 recognized species and one hybrid all of which are plant pathogens. It is the only species in the genus with a broad host range, whereas all other *Botrytis* species are considered to be specialized on a single plant species. The major postharvest losses due to *B. cinerea* occur in a long list of fresh fruits such as apple, blackberry, blueberry, pear, strawberry, grapes and many others (DEAN et al., 2012; LEROCH et al., 2013; ELAD et al., 2015).

In the field, it can survive under a wide range of conditions as a saprophyte, where it colonizes flower residues, fruit juice drops, dead leaves, or other non-living plant tissue. The colonization of flower residues by *B. cinerea* is considered to be an important mode of infection in grapes (Figure 2.7.1).

Figure 2.7.1. Botrytis symptoms in table grape (LATORRE et al., 2015).

The pathogen can remain into the cluster and start additional infections of the berries when environmental conditions are favorable to the development of the disease, but the fungus develops mostly in autumn on ripe grape berries. At the beginning of the infections, a darker circular area is visible where the fruit tissues are softer than the other fruit parts, subsequently with an abundant sporification, whose color ranges from white to gray, can develop from the site of infection. Infection starts from natural openings or mechanical wounds that occur on fruit. Often, *B. cinerea* can develop from rotted fruit next to healthy one, causing extensive breakdown, and sometimes spoiling entire lots. After harvest, *B. cinerea* is capable of infecting fruits and vegetables through the damaged tissue in the stem end, which is rich in nutrient exudates. Stem end infections can develop and spread to the entire fruit (MICHAILIDES; ELMER, 2000; ROMANAZZI et al., 2012; TELES et al., 2014).

Efforts to minimize gray mold infections and the subsequent development of decay have focused on a better understanding of its biology and etiology on grapes to develop pre and postharvest control strategies. Among these approaches, the use of sulfur dioxide (SO_2) generator pads is found to be relatively successful worldwide due to their efficiency, easy to use and affordable cost (MELGAREJO-FLORES et al., 2013). Different types of pads, where the rate of SO_2 is controlled, have been developed accoding to the industry needs, with one or two different release phases, quick and/or slow, and they are available in many different sizes, e.g.13 cm × 23 cm, 26 cm × 36 cm, 26 cm × 46 cm etc. depending on the carton size used (Figure 2.7.2).

The slow release pads contain sodium metabisulfite enclosed in a sheet of plastic and paper. This product is designed to be used in packing facilities that use SO_2 gas to treat and sterilize table grapes, by releasing a large enough dose of SO_2 to kill and eliminate any actively growing *B. cinerea* fungal spores. These pads emit a low, continual dose of SO_2 gas, concentrated enough to inhibit any latent or inherent *B. cinerea* spores from growing. The dual release pads contain sodium metabisulfite enclosed between paper sheets of differing permeability. Moisture within the package of grapes is absorbed by the pads and reacts with the sulfite, releasing SO_2. The quick release part of the pad gives a flush of SO_2, which peaks after 24 h and then diminishes in 1 week. The slow-release part of the pad emits a low concentration of SO_2 over a long period (HARINDRA, 2015).

The SO_2 pads are positioned over, but not touching the grapes during transportation in order to prevent or limit damage. The use of dual release SO_2 generating pads in combination with a plastic liner bag is advised when grapes are stored for periods longer than 10 days and during long periods of retail handling. The amount of SO_2 released is affected by the temperature, therefore, the effectiveness of these pads depends on good cold chain management (ZUTAHY et al., 2008; CHERVIN et al., 2012).

Figure 2.7.2. Sulfur dioxide (SO_2) pad. Source: The author.

Excessive levels of $SO_{2,}$ however can damage table grapes by bleaching or causing sunken areas on the berry surface or contributing to premature browning of the stems. Other studies have shown that grape hairline splits, commonly associated with significant water loss, are also induced by excessive SO_2 doses. However, high levels can also result in fruit damage, unpleasant aftertaste, and allergies. The conditions that favoured higher concentrations of SO_2 (in practice commercial conditions), such as the use of two SO_2 generating pads (one on top and one on the bottom of the packaged table grapes), promoted hairline cracking. Based on these findings, it is recommended to use a minimal dose of SO_2 that allows adequate protection from decay without reducing the berry quality, in order to reduce incidence of hairline split (LURIE et al., 2006; ZUTAHY et al., 2008).

A small segment of the population may experience severe allergic reactions to sulfites, and because of it, a 10 ppm tolerance for sulfite residues in table grapes has been proposed. Fruits with residues exceeding the tolerance are not allowed to be marketed.

Vinifera grapes tolerate SO_2 gas in concentrations that would damage other fruits, vegetables, eggs, meat, or poultry. It has been demonstrated that the amount of SO_2 gas needed to kill *Botrytis* spores, or to inactivate exposed mycelium, is dependent on the concentration and the length of time the fungus is exposed to the fumigant (CARLOS, 2008).

SO_2 generators pads inside boxes have been widely used for table grape storage and transport for periods of up to 2 months. For longer period of storage it is important to find the best method of packaging, that will minimize water loss on the one hand, and prevent both decay which would occur if SO_2 is too low, or SO_2 damage which would occur if SO_2 is too high. There are two main methods in use for packing grapes for extended storage and shipping. One is to pack the grapes with a SO_2 generator pad inside a carton box with a perforated plastic liner, and then to cool them. The second method is to place SO_2 generator pad on the grapes, cool the grapes and wrap the package or pallet with stretch polyethylene on all sides and the top, leaving the bottom of the pallet unwrapped (LICHTER et al., 2008).

In a long term (120 days) cold storage study, the effect of SO_2 generating pads lasted for over 45 days and effectively reduced the decay caused by *Penicillium. expansum* (blue mold) and *B. cinerea* in ‘Red Globe’ and ‘Thompson Seedless’ table grapes (FRANCK et al., 2005).

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3 ARTICLE A - PROPOSAL OF DOUBLE-CROPPING SYSTEM FOR 'BRS ISIS' SEEDLESS GRAPE GROWN IN SUBTROPICAL AREA

3.1.1 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. Summer and off-season crops lasted 144 and 125 days, with thermal demands of 1,931 and 1,815, respectively. The soluble solids and titratable acidity contents were quite similar in both seasons, with an average of 14.3 °Brix and 0.7%, respectively. As ‘BRS Isis’ seedless grape showed to be a fruitful and over cropped cultivar, reaching an yield of 49.0 tons.ha^{-1}, the crop load ajustment is required after fruit set to achieve a sustainable yield of 25.0 tons.ha^{-1}. Based on these results, a double annual cropping system with some specific cultural practices was proposed for this mid-season cultivar grown under subtropical conditions. In late winter, when the frost risk is low, the pruning is performed after a 60-day rest period, and the harvest of summer season occurs in mid-December, summer. After a 30-day rest period, the pruning is again performed in late-January, and the off-season harvest occurs in mid-May, autumn. As a result, this system allows an accumulative yield of 50 tons.ha^{-1}.year^{-1} of table grapes, what is considered profitable for this agricultural activity.

Keywords: Table grape, phenological stages, quality attributes.

3.2 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, 2010).

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 Grape and Wine, 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 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 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 g 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, such 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; 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., 2018), 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 in subtropical area under double annual cropping system.

3.3 Material And Methods

3.3.1 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 (800 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 1,596 mm, with most of the rainfalls occurring in summer (IAPAR, 2010).

The cane-pruning was performed on July 29th for summer crop 2016 and on January 18th for off-season crop 2017, and subsequently, 3% hydrogen cyanamide was applied to terminal buds to induce and standardize sprouting in both seasons. 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}.

3.3.2 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 (Figure 3.3.2.1).

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).

Figure 3.3.2.1. Representation of the 'BRS Isis' phenological stages. A: bud swelling; B: sprouting; C: emergence of the inflorescence; D: flowering; E: véraison; F: harvest.

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 et al. (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 (10ºC).

3.3.3 Physicochemical analysis

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 (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 (CIRG) was calculated using the formula CIRG = $(180-h°)/(L^*+C^*)$ (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 0.1N 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; ROBERTO, 2014). For TSS, TA and TSS/AT evolution, linear 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 hrs at room temperature. The tubes were then removed from the dark and stirred manually for 5 seconds. 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 3,500 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 hrs. 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 plant, the average mass of bunches and the number of vines per hectare (ROBERTO et al., 2012a).

3.4 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 (Figure 3.4.1).

In viticulture, the knowledge of phenology can be used for selection of cultivars, vineyard planning, man power 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 (Figure 3.4.1). 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 locations with high temperatures, vegetative growth of vines tends to be higher and the cycle is reduced in relation to cooler regions (PEDRO JÚNIOR; SENTELHAS, 2003).

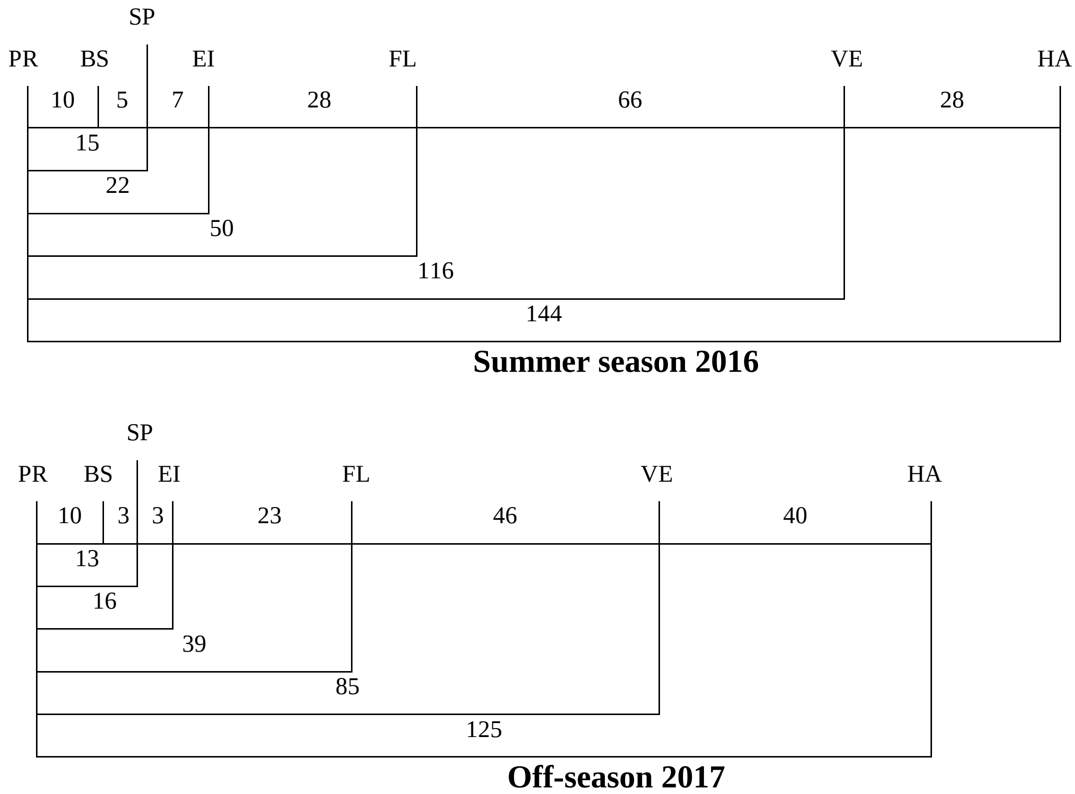


Figure 3.4.1. Duration in days of the main phenological stages of ‘BRS Isis’ seedless grape. Summer season 2016 and off-season 2017. PR: pruning; BS: bud swelling; SP: sprouting; EI: emergence of inflorescence; FL: flowering; VE: véraison; HA: harvest.

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., 2007). 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 3.4.1). The DD required for 'BRS Isis' seedless grape to complete the cycle was similar to other grape midseason 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; SILVA, 2004; PEZZOPANE et al., 2005; KISHINO; MARUR, 2007; SANTOS et al., 2009).

Table 3.4.1. 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, the averages observed were 6.5 g, 19.2 mm and 27.5 mm, respectively (Table 3.4.2). 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, 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 515.2 g and length of 21.4 cm, with medium size and cylindrical shape. The bunch presented moderate compactness and demanded similar berry thinning to 'BRS Vitoria' seedless grape.

Table 3.4.2. 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	6.4 ± 0.3
Berry diameter (mm)	19.0 ± 1.7	19.3 ± 1.1
Berry length (mm)	27.3 ± 2.5	27.7 ± 1.6
Bunch mass (g)	512.7 ± 0.04	529.0 ± 0.1
Bunch length (cm)	21.2 ± 2.3	21.7 ± 1.7
Lightness ($L*$)	22.8 ± 2.4	22.4 ± 1.0
Saturation ($C*$)	5.9 ± 2.3	3.3 ± 1.1
Hue angle ($h°$)	58.0 ± 35.7	54.0 ± 12.7
Color index (CIRG)	4.3 ± 1.3	4.9 ± 0.4

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 color attributes of 'BRS Isis' berries, the averages of $L*$, $C*$ and $h°$, observed were 22.6; 4.6 and 56.0, respectively, while the average color index (CIRG) found was 4.6 (Table 3.4.2). The CIRG observed in the off-season crop was a little bit 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., 1996), 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 as previously reported, the subperiod VE-HA is longer than in summer season, allowing a higher accumulation of anthocyanins (MORI et al., 2005; 2007). 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). 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, and 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 (PEPPI et al., 2006; ROBERTO et al., 2012a; 2013).

The TSS evolution of 'BRS Isis' berries were best fitted to linear regression for both seasons (Figure 3.4.2). 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, reaching at harvest 14.2 and 14.4 °Brix in summer and off-seasons, respectively (Table 3.4.3). The daily rate of TSS observed for summer and off-seasons were quite similar, 0.10 and 0.09 °Brix, respectively (Table 3.4.4).

Table 3.4.3. Chemical characteristics of 'BRS Isis' seedless table grape grown under double annual cropping system in subtropical area. Summer season 2016 and off-season 2017.

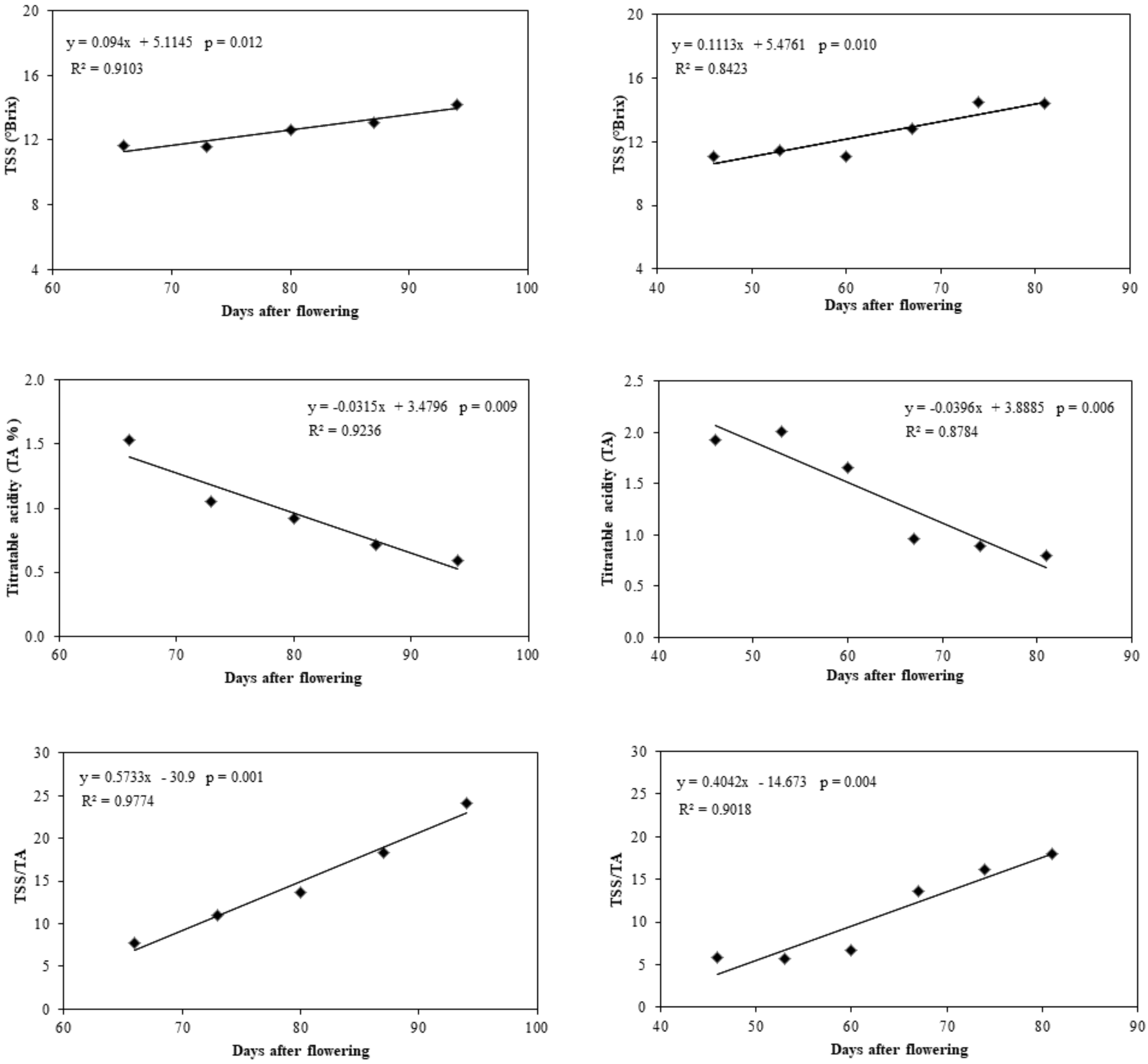
Chemical characteristics	Summer season 2016	Off-season 2017
Total soluble solids - TSS (°Brix)	14.2 ± 0.3	14.4 ± 0.2
Titratable acidity - TA (%)	0.6 ± 0.04	0.8 ± 0.04
Maturation index (TSS/TA)	24.1 ± 1.5	17.9 ± 0.9
pH	4.3 ± 0.02	4.4 ± 0.1
Total anthocyanins ($mg.g^{-1}$)	0.3 ± 0.1	1.0 ± 0.5
Total polyphenols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

TA evolution was also best fitted to linear regression for both seasons (Figure 3.4.2). 'BRS Isis' presented at harvest 0.6 and 0.8% of tartaric acid, respectively (Table 3.4.3), 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 (Figure 3.4.2) 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 3.4.4) (MULLINS et al., 1992; MOTA et al., 2006).

Table 3.4.4. 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

Similar behavior was observed for TSS/TA evolution for both seasons (Figure 3.4.2), reaching 24.1 in summer season and 17.9 in off-season, respectively (Table 3.4.3). 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. The daily evolution rate of maturation index was 0.57 for summer crop and 0.35 for off-season crop, respectively (Table 3.4.4).



A **B**

Figure 3.4.2. 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.

The means of total anthocyanins observed during summer and off-season crop were 0.3 and 1.0 mg.g^{-1} respectively (Table 3.4.3), whereas the means total polyphenols 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 3.4.2). 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 promote the biosynthesis of some phenolic compounds (FANZONE et al., 2011), as observed 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 3.4.5), 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. 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., 2007; 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 et al., 2017) and for other cultivars (KELLER et al., 2005, 2008; 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.

Table 3.4.5. Productive characteristics of 'BRS Isis' seedless grape grown under double annual cropping system in subtropical area. Summer season 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

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 (Figure 3.4.3). In this model, we considered the vines trained on overhead trellis system with bilateral arms, spaced 2.0 × 5.0 m apart (800 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 7-18mm, 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 0.5kg, the estimate yield at harvest would be 25 $tons.ha^{-1}$. This summer crop cycle lasts 144 days, from winter pruning to harvest.

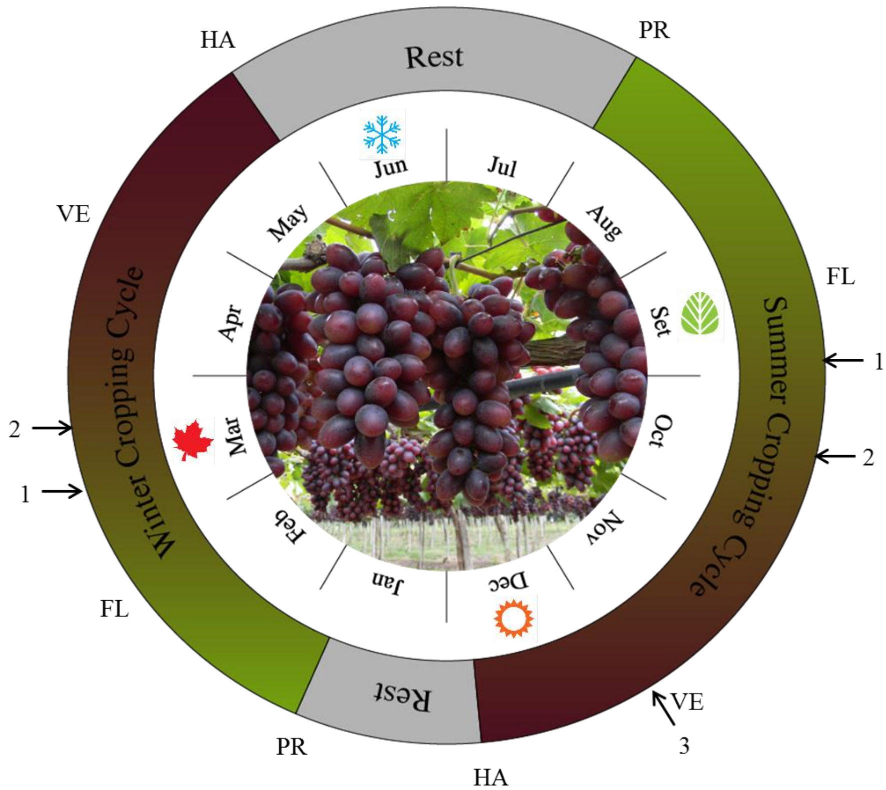


Figure 3.4.3. Proposal of a double annual cropping system for ‘BRS Isis’ seedless grape grown in subtropical region with some specific cultural practices. PR: pruning followed by hydrogen cyanamide application for bud burst; FL: flowering; VE: véraison; HA: harvest; 1: crop load adjustment followed by 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.

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 cyle 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.

3.5 CONCLUSION

Based on these results, a double annual cropping system with some specific cultural practices is feasible for this mid-season cultivar grown under subtropical conditions that allows an accumulative yield of 50 tons.ha^{-1}.year^{-1}, what is considered profitable for this agricultural activity. Besides, the off-season crop occurs in a period where the supply of fresh table grapes is low in most of the markets, and because of it, profits are usually higher in this season, what justifies the grower´s efforts to obtain two crops along the year.

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4 ARTICLE B – POST-HARVEST PRESERVATION OF THE NEW HYBRID SEEDLESS GRAPE 'BRS ISIS' GROWN IN SUBTROPICAL AREA

4.1 ABSTRACT

'BRS Isis' is a new hybrid seedless table grape tolerant to downy mildew, with good adaptation to the tropical and subtropical climates. Gray mold, caused by *Botrytis cinerea* Pers. Fr., is known as the main cause of postharvest decay in table grapes resulting in extensive losses worldwide. As the postharvest behavior of 'BRS Isis' grown in subtropical conditions is still unknown, the objective of this work was to evaluate the post-harvest preservation and Botrytis mold control of this new grape cultivar, grown under double cropping a year system. Grape bunches were obtained from a commercial field of 'BRS Isis' seedless table grape trained on overhead trellis located at Marialva, state of Parana (PR) (South Brazil). Samples were collected from two consecutive crops, summer season, 2016 and off-season, 2017. Grapes were subjected to following treatments in cold chamber at 1 °C: (i) Control; (ii) SO_2-generating pad; (iii) Control with bunches inoculated with *B. cinerea* suspension; (iv) SO_2-generating pad with bunches inoculated with *B. cinerea* suspension. The completely randomized experimental design was used as statistical model with four treatments and five replications. The incidence of gray mold and other physicochemical variables that include bunch mass loss, shattered berries, skin color index, soluble solids (SS), titratable acidity (TA) and SS/TA ratio of grapes were evaluated at 50 days after the beginning of cold storage and at 7 days at room temperature (25 °C). The 'BRS Isis' seedless grape, packaged with SO_2-generating pads and plastic liners, has a high potential to be preserved for long periods under cold storage, at least for 50 days, keeping very low natural incidence of gray mold, mass loss and shattered berries.

Keywords: *Vitis vinifera* (L.); sulfur dioxide pad; postharvest decay; table grape quality attributes.

4.2 INTRODUCTION

'BRS Isis' is a new hybrid seedless table grape obtained by the crossing of CNPUV 681-29 [Arkansas 1976 × CNPUV 147-3 ('Niagara White' × 'Venus')] × 'BRS Linda'. This cultivar was released in 2013 and is tolerant to downy mildew (*Plasmopara viticola*), the main vine disease in subtropical humid areas. It presents high bud fertility with 2-3 great inflorescences per shoot, with natural weight of 375 g, without the use of growth regulators, making this cultivar a high yielding grape. The bunch is medium-sized, predominantly cylindrical-winged, while the berry is medium size, reddish, elliptical, firm and colorless flesh, and neutral flavor with traces of rudimentary seeds large fleshy (RITSCHEL et al., 2013). This new seedless and early season cultivar has the ability to gain the attention of consumers from domestic and international markets, as there has been a significant demand of table grape supply for extended periods throughout the year worldwide. For this reason, in subtropical regions, where the double cropping system is possible due the mild winter and the use of bud burst stimulators, the 'BRS Isis seedless grape has a high potential to be grown.

Grapes are non-climacteric fruits with a relatively low physiological activity, and are subject to serious postharvest problems during cold storage, such as decay, mass loss stem browning, shattered berries, wilting and shriveling of berries. Thus, these factors are the main barriers for long-term storage of table grapes (DAUDT; FOGACA, 2013; SILVA-SANZANA, et al. 2016; SEN et al., 2016).

Gray mold, caused by *Botrytis cinerea* Pers. Fr., is known as the main cause of postharvest decay of table grapes. Infection caused by this fungus remains inactive in the field unless it gets favorable environmental conditions, i.e., fruit injuries that assist pathogen propagation (CARISSE; VAN DER HEYDEN, 2015; FELIZIANI; ROMANAZZI, 2016). Even a small infection on a single berry can damage the whole lot of grapes, and if it is not noticed at pre-harvest stage, during packaging or during shipment, it may progress and spread the infection in postharvest or during cold storage of table grapes, even at low temperatures (CRISOSTO et al., 2002; CELIK et al., 2009; ROMANAZZI et al., 2016).

The infection first starts as small, round and necrotic mark on the surface of softer berries, which then changes to gray color. As the infection proceeds, different symptoms, which include loose of berry skin, berry splitting and softness can be observed, that not only leads to the establishment of *B. cinerea*, but also spreads the infection to the neighboring berries, making a nest of infected berries resulting in extensive loss (ZOFFOLI et al., 2009).

Cold storage, where only temperature and relative humidity are controlled in the chamber, is one of the main methods for conservation of fruit quality. Thus, the reduction of temperature, up to a certain limit, increases the quality maintenance and extends the period of fruit supply to the consumer market (SEN et al., 2012). After harvesting, bunches are pre-cooled as soon as possible to remove field heat and to reduce water loss (BRACKMANN et al., 2010; ROMANAZZI et al., 2016). For extended export and shipment purposes, the cold storage temperature must be kept optimum and constant because any disturbance can initiate the growth of fungi, mainly *B. cinerea* (LIGUORI et al., 2015).

The postharvest control of this pathogen is not easy, as most of the countries no longer allow the application of synthetic fungicides on bunches. Combined to cold storage, different pre- and postharvest techniques can be used to control gray mod, such as the use of sulfur dioxide (SO_2) generating pads, which is the most common method worldwide (LICHTER et al., 2008; MELGAREJO-FLORES et al., 2013; DOMINGUES et al., 2018). The slow release SO_2-generating pads contain sodium metabisulfite ($Na_2S_2O_5$) as active ingredient enclosed in a sheet of plastic and paper, which are designed to be used in packing materials by releasing a low and continual dose of SO_2 with contact to humidity to kill and eliminate any actively growing *B. cinerea* fungal spores.

The SO_2-generating pads are highly effective in controlling and killing the spores of *B. cinerea*, but also can result in unwanted situations, such as bleaching and shattered berries. Other studies have also shown that grape hairline splits, commonly associated with significant water loss, are also induced by excessive SO_2 doses. However, high levels of SO_2 can also result in fruit damage, unpleasant aftertaste and allergies. Based on these findings, it is recommended to use a minimal dose of SO_2 that allows adequate protection from decay without reducing the berry quality, in order to avoid these situations (LURIE et al., 2006; ZUTAHY et al., 2008).

As there is lack of information regarding the cold storage of ‘BRS Isis’ seedless grape, it is very important to know the behavior of this new hybrids cultivar grown under double cropping a year system, especially for long-distance and international markets. Under this system, two crops per year are achieved (summer and off-season crops). Summer crop starts from the end of grapevine dormancy in late winter and harvest is obtained in summer, while for off-season crop, vines are pruned after summer crop and forced to sprout again using budburst stimulators and harvest occurs during autumn. The main difference between both crops is that in summer crop, the rate of some fungal infection is quite low, while on the other hand, in off-season crop, the incidence of fungus diseases is high because of favorable environmental conditions that promote the infection and can restraint long-distance transportation of table grapes (RICCE et al., 2013; YOUSSEF et al., 2015).

The objective of this work was to evaluate the post-harvest preservation and control of gray mold of ‘BRS Isis’ seedless grape grown under double cropping a year system in subtropical conditions.

4.3 Material And Methods

4.3.1 Experimental location

Grape bunches were obtained from a commercial field of ‘BRS Isis’ seedless table grape grafted on ‘IAC 766’ rootstock, from 2-year-old vines trained on overhead trellis located at Marialva, state of Parana (PR) (South Brazil) (23°29 S, 51°47 W, elevation 570 m), with history of gray mold. The vines were grown under double cropping a year system, and samples were collected from two consecutive crops, summer season 2016 and off-season 2017.

4.3.2 Treatments and storage

Grapes were harvested at full ripe when berry soluble solids content reached around 14 °Brix. Bunches were subjected to the following treatments into a cold chamber at 1 °C: (i) Control; (ii) SO_2-generating pad; (iii) Control with bunches inoculated with *B. cinerea* suspension; (iv) SO_2-generating pad with bunches inoculated with *B. cinerea* suspension. The slow release SO_2-generating pad used in treatments (ii) and (iv) (Osku Hellas®, Grapeguard, Santiago, Chile) contain 73.5% of the active ingredient ($Na_2S_2O_5$), with 26 cm × 36 cm of dimensions.

As the incidence of gray mold can be low depending of the season, the grapes from treatments (iii) and (iv) were inoculated with a *B. cinerea* suspension isolated from infected grapes with typical symptoms of the disease, purified, and identified morphologically and molecularly, according to Youssef and Roberto (2014a). The isolates were maintained on potato dextrose agar (PDA) slants and stored at 4 °C for further use.

Fungal conidia were harvested from 2-week-old PDA cultures of *B. cinerea* grown at 23±1 °C. A volume of 5 mL of distilled water, containing 0.05% (v/v) Tween 80, was added to a Petri plate culture. The conidia were gently dislodged from the surface with a distilled glass rod, and suspensions were filtered through three layers of cheesecloth to remove any adhering mycelia. The suspensions were diluted with sterile water and the concentration of 10^6 conidia mL^{-1} was determined with a hemacytometer.

The inoculation was carried out approximately 6 h after grape harvest by spraying the conidial suspension. The control treatment received a water-only 'inoculation' application. After 3 h, the inoculation treatments were completely dry before packaging.

The grapes of all treatments were packaged as follows: a micro-perforated plastic liner (1% of the ventilated area) was placed inside the carton box measuring 23 cm × 16 cm × 9 cm (4-kg capacity); grapes were placed inside the box; an SO_2-releasing pad was placed on top only for treatments (ii) and (iv); and the liner was sealed.

The boxes were stored in a cold chamber at 1 °C and high relative humidity (>95%). After 30 days of cold storage, the boxes were opened for inspection, and as the bunches of all treatments were intact, with fresh and green stems, free of any mold or injuries, it was decided to keep the boxes in the chamber for an extended period, i.e., 50 days, followed by 7 days of shelf-life at room temperature (25 °C). The completely randomized experimental design was used as statistical model with four treatments and five replications, and each plot consisted of one carton box.

4.3.3 Gray mold incidence analysis

The incidence of gray mold on grapes was evaluated at 50 days after the beginning of cold storage and at 7 days at room temperature after the end of cold storage. The disease incidence was then obtained: disease incidence (% of diseased berries) = (number of infected berries/total number of berries) × 100 (YOUSSEF; ROBERTO, 2014a).

4.3.4 Physicochemical analysis

The grape physicochemical analysis were evaluated at 50 days after the beginning of cold storage and at 7 days at room temperature after the end of cold storage. The bunch mass loss (%) during postharvest storage was determined by periodical weighing, and calculated by dividing the mass change during storage by the original mass: mass loss (%) =

[(m_i− m_s)/m_i] × 100, where m_i = initial mass and m_s = mass at examined time (MATTIUZ et al., 2009). Shattered berries were evaluated by counting the separated berries from the bunch stem and were expressed as a percentage of the total number of berries: shattered berries (% of diseased berries) = (number of shattered berries/total number of berries) × 100.

Berry color was analyzed using a colorimeter CR-10 (Konica Minolta®, Tokyo, Japan) to obtain the following variables from the equatorial portion of berries (n=2 per berry): *L** (lightness), *C** (chroma) and *h*° (hue). The color index for red grapes (CIRG) was then calculated using the formula CIRG = (180-*h*°)/(*L**+*C**) (CARREÑO et al., 1995). 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).

For the chemical analysis, 10 berries were collected from each plot. The samples were crushed, and the juice was then used to determine soluble solid (SS) content and titratable acidity (TA). For determination of SS, a few drops of the juice were analyzed with a digital refractometer (Krüss DR301-95; A. Krüss Optronic, Hamburg, Germany) with automatic temperature compensation at 20 °C, and the results were expressed in °Brix. TA was determined using a semi-automatic titrator with 0.1 N NaOH by using 10 mL of the juice diluted in 40 mL of distilled H_2O, and pH = 8.2 was considered as the endpoint. The results were expressed in % of tartaric acid (YOUSSEF; ROBERTO, 2014b). The maturation index of the berries was then obtained from the TSS/TA value.

4.3.5 Statistical Analysis

All data was subjected to analysis of variance (ANOVA) using Statistica 8.0 software. Mean values of treatments were compared by using Fisher's protected LSD test and judged at $P \leq 0.05$ levels.

4.4 RESULTS AND DISCUSSION

4.4.1 Incidence of gray mold

Although the commercial area where the grapes were harvested has a history of gray mold occurrence, the disease incidence found at 50 days of cold storage was considered low in both seasons, and no significant differences were observed when grapes were subjected to control and SO_2-generating pads treatments only (Figures 4.4.1 and 4.4.2). On the other hand, when grapes were inoculated with Botrytis suspension, the SO_2-generating pads significantly reduced the incidence of gray mold of grapes harvested in summer crop season, as compared to the control with bunches inoculated with Botrytis. In case of off-season crop, although the incidence of gray mold was higher in grapes of control and SO_2-generating pads, both inoculated with Botrytis suspension, no significant differences were observed between them.

It was observed that the incidence of gray mold was higher in off-season crop (~30%) when grapes were inoculated with *B. cinerea* suspension, and it could be explained by the occurrence of some invisible minor cracks or spots on berry skin caused by powdery mildew (*Uncinula necator*), which is more prevalent in this season (TESSMANN et al., 2018). Besides, this situation was also found after the 7-day period at room temperature, where the gray mold incidence (~50%) was higher as compared to the 50-day period of cold storage (Figure 4.4.3). These results are related with the fact that when grapes are subjected to room temperature, the disease incidence increases because of the more favorable environmental conditions for fungi development, especially the higher air temperature. After 7 days of room temperature at 25 ºC, no significant differences were found among treatments with SO_2-generating pads in comparison to the control (with no botrytis inoculation) in both crop seasons. However when grapes were inoculated with Botrytis suspension, significant differences were observed in summer season crop, where the SO_2-generating pads resulted in lower gray mold incidence (4.2%) in comparison to the control with grapes inoculated (7.8%), while in case of off-season crop, no differences were found (Figures 4.4.3 and 4.4.4). Our findings also confirms that SO_2-generating pads showed better results in controlling Botrytis mold of 'BRS Isis' seedless grape at 50 days of cold storage and at 7 days of room temperature. Besides, this new hybrid seedless cultivar showed to be non-sensitive to the amount of SO_2 gas released by the evaluated pads, as at high concentrations, this compound can cause bleaching or premature stem browning, and may also damage the fruits, resulting in unwanted conditions. Considering these aspects, combined to cold storage, the SO_2-generating pads can be used as tool to control the gray mold of 'BRS Isis' seedless grape, at least for a period of 50 days. A similar performance has also been observed in maximum reduction of the disease incidence of 'BRS Vitoria' and 'Italia' table grapes at 50 days of cold storage and at 7 days of room temperature, respectively (DOMINGUES et al., 2018; AHMED et al., 2018).

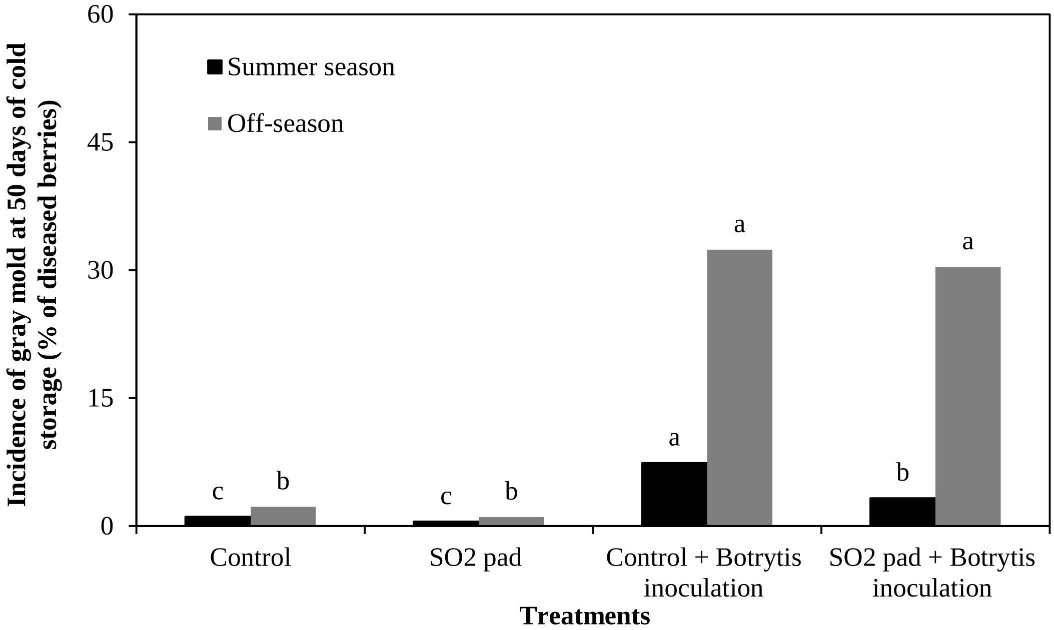

Figure 4.4.1. Incidence of gray mold (% of diseased berries) at 50 days of cold storage of 'BRS Isis' seedless table grape, during summer season 2016 and off-season 2017. Columns followed by unlike letters, in relation to the treatments within each individual crop, are statistically different according to Fisher's protected LSD test ($P \leq 0.05$).

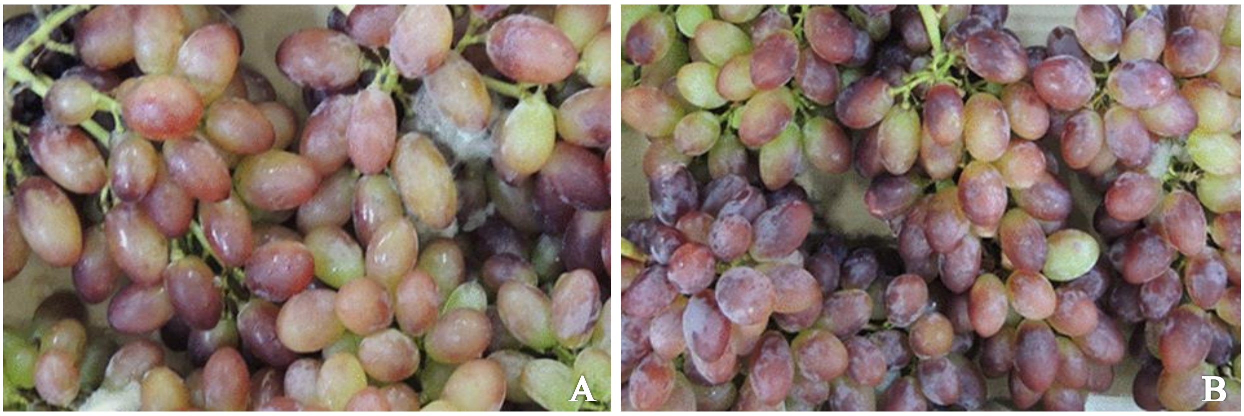

Figure 4.4.2. Bunches of 'BRS Isis' seedless table grape at 50 days of cold storage. A: Control; B: SO_2-generating pads.

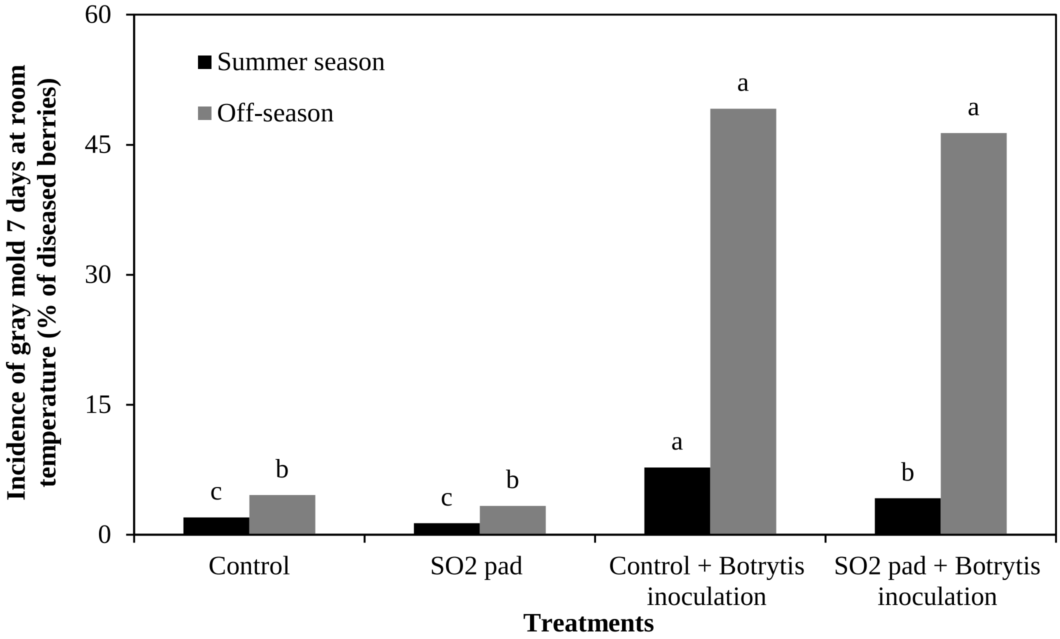

Figure 4.4.3. Incidence of gray mold (% of diseased berries) at 7 days of room temperature of 'BRS Isis' seedless table grape during summer season 2016 and off-season 2017. Columns followed by unlike letters, in relation to the treatments within each individual crop, are statistically different according to Fisher's protected LSD test *($P \leq 0.05$)*.

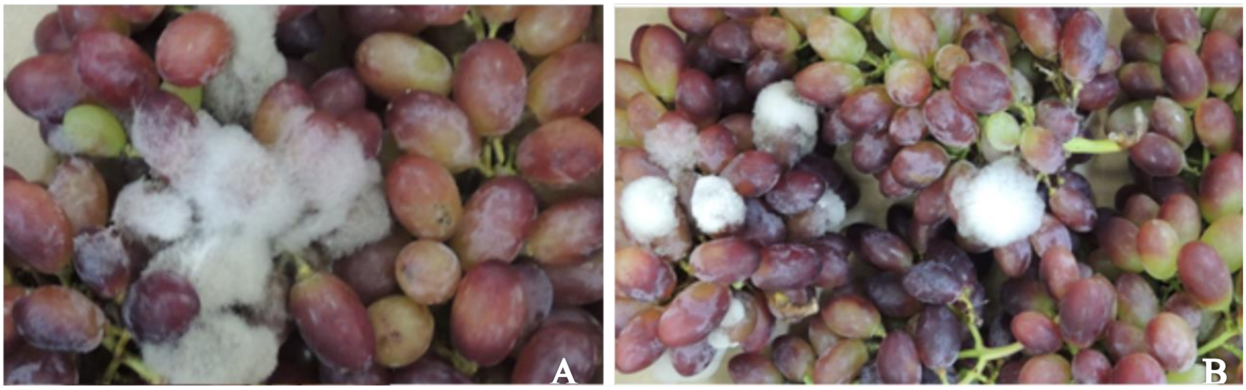

Figure 4.4.4. Bunches of 'BRS Isis' seedless table grape at 7 days of room temperature; A: control with Botrytis inoculation; B: SO_2-generating pads with Botrytis inoculation.

4.4.2 Physical characteristics of grapes

There were no significant differences among treatments for mass loss at 50 days of cold storage in summer crop, and means varied from 5.4 to 7.0%, while in case of off-season crop, significant differences were observed, where both Botrytis inoculated treatments (control and SO_2-generating pad) showed higher mass loss as compared to non-inoculated treatments. This behavior can be related with the fact that the incidence of gray mold was higher in off-season crop that may have caused higher mass loss in inoculated treatments. As mass loss is concerned as one of the key factors that determine excellence and quality of table grapes, the more water is lost from the produce, the more it gets quality deterioration problems. Even though during the cold storage period, temperature and relative humidity are controlled to reduce mass loss and to extend shelf-life of table grapes, sometimes mass loss can vary depending upon different aspects, i.e., grape cultivar, harvesting conditions, storage period and packing materials used. Among other factors, fungal infection, absence or delay in pre-cooling, and high temperature with low humidity are also the main causes of mass loss (VALVERDE et al., 2005; NELSON, 2007). For mostly fresh produces, mass loss percentage should be low that do not affect quality attributes (wilting or wrinkling), and the same behavior was observed in the current study of 'BRS Isis' table grapes. Besides, the SO_2-generating pads were also found to reduce mass loss in both evaluated situations, i.e., grapes inoculated or not with Botrytis suspension.

In case at 7 days of room temperature period, significant differences were observed among treatments and the control treatment showed the lowest mass loss in both seasons (5.8% and 1.2% for summer and off-season crops, respectively). In summer season, a higher mass loss (7.6%) was observed in control with grapes inoculated with Botrytis, and in case of off-season, a higher mass loss (1.8%) was recorded in SO_2-generating pad with grapes inoculated with *B. cinerea* suspension (Table 4.1.1). When grapes are subjected to room temperature, water loss increases because of the favorable environmental conditions, especially the higher air temperature that reduces the fruit quality.

No significant differences were found in terms of shattered berries for both seasons, and at 50 days of cold storage, the means ranged from 0.0 to 0.7% and from 0.7 to 3.1% for summer season and off-season, respectively (Table 4.1.1). However, statistically differences were found in both seasons at 7 days of room temperature, where the control with grapes inoculated with Botrytis suspension showed higher shattered berries (1.5%). On the other hand, in off-season crop, when grapes were inoculated with Botrytis suspension, combined or not with SO_2-generating pads, a higher percentage of shattered berries was found (~12%), what can be explained by the higher incidence of gray mold in this season, as previously discussed (Figure 4.1.1). However, the percentage of shattered berries was high at 7 days of room temperature, especially for off-season crop, what may have occurred due to the water loss, as during room temperature period, more water was lost, negatively affecting grape bunch quality (WRIGHT et al., 2009).

Table 4.4.1. Mass loss (%), shattered berries (%) and color index (CIRG) of 'BRS Isis' seedless table grape at 50 days of cold storage and at 7 days of room temperature during summer season 2016 and off-season 2017.

Treatments	Mass loss (%)		Shattered berries (%)		CIRG	
	At 50 days of cold storage					
	Summer season	Off-season	Summer season	Off-season	Summer season	Off-season
Control	6.3	3.1 b	0.0	1.2	3.8	5.1
SO_2 pad	5.6	3.0 b	0.3	0.7	4.4	5.4
Control + Botrytis	5.4	3.7 a	0.7	3.1	4.5	5.4
SO_2 pad + Botrytis	7.0	3.6 a	0.2	2.1	4.6	5.5
F value	0.7^{NS}	5.4*	2.5^{NS}	2.6^{NS}	3.4^{NS}	1.5^{NS}
CV (%)	32.2	9.8	14.8	25.4	9.6	5.3
	At 7 days of room temperature					
	Summer season	Off-season	Summer season	Off-season	Summer season	Off-season
Control	5.8 c	1.2 c	0.1 b	1.8 b	4.0	4.8
SO_2 pad	7.2 ab	1.6 ab	0.7 b	1.5 b	3.8	4.6
Control + Botrytis	7.6 a	1.3 bc	1.5 a	12.1 a	4.4	4.8
SO_2 pad + Botrytis	6.8 b	1.8 a	0.6 b	11.9 a	4.2	4.8
F value	10.1*	3.8*	6.3*	6.4*	2.0^{NS}	0.3^{NS}
CV (%)	7.9	19.9	15.2	28.3	10.5	6.7

Means within columns followed by the same letters are not statistically different by Fisher's protected LSD test ($P \leq 0.05$). ^{NS}: non-significant, *: significant at 5% level of significance.

There was no change in berry color index among treatments in both evaluated seasons (Table 4.1.1). In summer season, the berry color ranged from 3.8 to 4.6 (red), while in off-season crop, the means ranged from 5.1 to 5.5 (red-violet) (CARREÑO et al., 1998). During the off-season crop, the anthocyanin accumulation develops under a higher diurnal temperature variation in subtropics, what intensifies berry color, what explains these variations. Nevertheless, the original color of 'BRS Isis' seedless grape was well preserved in both storage periods.

4.4.3 Chemical characteristics of grapes

Regarding berry TSS content, even though differences among treatments have been only observed at 50 days of cold storage for the summer season, the observed means are in an acceptable range (~14 ºBrix), which is a standard for local and international market of some table grape cultivars (UNECE, 2010). There was no difference in terms of TA and SS/TA of berries among treatments in both evaluated seasons. As grape ripening develops under different weather conditions in summer and off-season crops, a slight change between them usually occurs in terms of the main berry chemical properties (Table 4.4.2), but not decreasing grape quality. During cold storage, the recommended temperature for grapes is around 0 °C because most of the variables like TSS, TA and TSS/TA remain stable in different grape cultivars at this temperature with controlled atmosphere (ARTÉS-HERNÁNDEZ et al., 2006; ROSALES et al., 2016).

Table grapes intended for long period of storage are kept in cold chambers, but each cultivar has a different behavior, i.e., each one has a different storage performance that may comprises from few days to few weeks, what is determined by its susceptibility to quality defects under low temperatures. Regarding the performance of the new hybrid seedless grape, 'BRS Isis', this cultivar showed to have a high potential to be stored for long periods under cold chambers, since after 50 days under this conditions, the bunches packaged with SO_2-generating pads and liners were virtually intacts. Also, the natural incidence of gray mold was found very low, what indicates that the natural incidence of Botrytis in this hybrid grape, unlike in some of *Vitis vinifera* table grape cultivars, is not a major concern. Besides, even with the use of SO_{2}-generation pads, unwanted situations like bleaching, hairline cracking and berry softening were not found on the surface of berries, as these are the main barriers for grape post-harvest quality and maintenance. Shattered berries were also noticed in low levels, what contributes to a better storability and marketability of this grape cultivar in markets.

Table 4.4.2. Soluble solids – SS (ºBrix), titratable acidity – TA (%) and SS/TA of 'BRS Isis' seedless table grape at 50 days of cold storage and at 7 days of room temperature during summer season 2016 and off-season 2017.

Treatments	Soluble solids - SS (°Brix)		Titratable acidity - TA (%)		SS/TA	
	At 50 days of cold storage					
	Summer season	Off-season	Summer season	Off-season	Summer season	Off-season
Control	14.5 a	14.3	0.6	0.9	23.1	16. 8
SO_2 pad	13.9 b	14.2	0.6	0.8	23.2	17.7
Control + Botrytis	13.6 b	14.1	0.6	0.9	23.5	16.6
SO_2 pad + Botrytis	14.0 ab	14.1	0.6	0.8	23.3	18.3
F value	3.8*	0.3^{NS}	0.8^{NS}	1.8^{NS}	0.04^{NS}	2.1^{NS}
CV (%)	2.9	2.6	9.1	8.3	9.3	6.9
	At 7 days of room temperature					
	Summer season	Off-season	Summer season	Off-season	Summer season	Off-season
Control	15.0	13.9	0.7	0.9	22.9	15.9
SO_2 pad	14.4	14.1	0.7	0.7	22.1	19.1
Control + Botrytis	14.3	13.8	0.6	0.7	22.2	19.0
SO_2 pad + Botrytis	14.6	14.0	0.7	0.8	21.8	18.8
F value	2.1^{NS}	0.1^{NS}	1.1^{NS}	2.6^{NS}	0.7^{NS}	2.8^{NS}
CV (%)	3.4	4.7	7.6	11.2	5.9	11.2

Means within columns followed by the same letters are not statistically different by Fisher's protected LSD test ($P \leq 0.05$). ^{NS}: non-significant, *: significant at 5% level of significance.

The time period from harvest till marketing of table grape has a significant importance regarding maintenance of fruit quality. The results obtained herein showed that 'BRS Isis' seedless grape has a large potential for domestic, long distance and international markets, because a high quality of bunches can be achieved under cold storage at least for 50 days. For domestic markets, including when a long distance transportation is required, the 'BRS Isis' grapes, after being properly packaged, can be transported in refrigerated trucks and easily kept in cold chamber of the market chains, and gradually exposed to the consumers with a minimum loss quality. The same could also be applied when the intention is to export this grape overseas, to the European Community or even to North America countries. As long as the cold chain is retained, and considering that it takes up to 3 weeks to transport a refrigerated container by ship from South America to these regions, ‘BRS Isis’ seems to fit well for this type of international trade due its high capacity of storage in cold chamber during long periods, up to 50 days or longer. However, since large proportion of table grapes can be traded overseas, more attention is required for better shipment and quality management.

Finally, for long-term storage and transportation, packaging materials, such as SO_2-generating pads and proper liners, also play a crucial role to preserve ‘BRS Isis’ grapes under cold storage, reducing some unwanted situations and providing a higher efficiency of the SO_2 gas for controlling the incidence of gray mold.

4.5 CONCLUSION

The new hybrid ‘BRS Isis’ seedless grape, packaged with SO_2-generating pads and plastic liners, has a high potential to be preserved for long periods under cold storage at 1 ºC, at least for 50 days, keeping very low natural incidence of gray mold, mass loss and shattered berries.

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