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SO_2-generating pads reduce gray mold in clamshell-packaged 'Rubi' table grapes grown under a two-cropping per year system

Folhas geradoras de SO_2 reduzem a incidência de mofo cinzento em uvas de mesa 'Rubi' embaladas em cumbucas plásticas e produzidas sob o sistema de dupla safra anual

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Highlights

The dual release SO_2 pads are efficient in controlling the gray mold in 'Rubi' grape.
The disease can be controlled efficiently in both annual crops.
The slow release SO_2 pads resulted in intermediate efficiency of gray mold control.

Abstract

The aim of this work was to evaluate different SO_2-generating pads and liners to control gray mold in ventilated clamshell-packaged 'Rubi' table grapes grown under a two-cropping per year system. The treatments consisted of SO_2-generating pads (slow release or dual release) and plastic liners with different perforations (microperforated; 2.0; 4.0 or 5.0 mm in diameter) and a control, only with the standard microperforated plastic liner. The packaged grapes were stored in a cold chamber at 1.0 ± 1.0 °C and 95% relative humidity. After 45 days, the grapes were removed from cold storage and placed, without liners and SO_2-generating pads, for 3 days at room temperature (22.0 ± 1.0 °C). The evaluations occurred at 30 and 45 days after the beginning of cold storage, and the following variables were assessed: incidence of gray mold, mass loss, stem browning and shattered berries. At 3 days of shelf-life, the same variables were assessed, except mass loss. The completely randomized design was used as a statistical model with four replications, and

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each plot consisted of five bunches individually stored in ventilated clamshell-packaged. The dual release SO_2-generating pads are efficient in controlling the gray mold in ‘Rubi’ table grapes regardless of the type of perforation of the plastic liners, with low mass loss and shattered berries, with good conservation of the freshness of the rachis. The disease was efficiently controlled in both annual crops. The slow-release SO_2-generating pads, regardless of the type of perforation of the plastic liners, resulted in intermediate efficiency of gray mold control, with good physical quality of the bunches. Thus, the use of dual release SO_2-generating pads is recommended to control gray mold in ventilated clamshell-packaged ‘Rubi’ table grapes.
Key words: *Botrytis cinerea* Pers. Cold storage. Packing. SO_2. *Vitis vinifera* L.

Resumo

O objetivo deste trabalho foi avaliar diferentes folhas geradoras de SO_2 e filmes plásticos perfurados, no controle do mofo cinzento em uvas de mesa ‘Rubi’ armazenadas individualmente em cumbucas plásticas ventiladas cultivadas sob dupla safra anual. Os tratamentos foram constituídos por folhas geradoras de SO_2 (liberação lenta ou liberação dupla fase) e filmes plásticos com diferentes perfurações (microperfurado; 2,0; 4,0 ou 5,0 mm de diâmetro) e uma testemunha, somente com o filme plástico microperfurado. As uvas foram armazenadas em câmara refrigerada a 1,0 ± 1,0 °C e 95% de umidade relativa do ar. Após 45 dias, as uvas foram retiradas e mantidas sem os filmes plásticos e as folhas de SO_2 por 3 dias em temperatura ambiente (22,0 ± 1,0 °C). As avaliações ocorreram aos 30 e 45 dias após o início do armazenamento refrigerado, quando foram analisadas as seguintes variáveis: incidência de mofo cinzento, perda de massa, escurecimento da ráquis e degrana de bagas. Aos 3 dias em temperatura ambiente, as mesmas variáveis foram novamente avaliadas, com exceção da perda de massa. O delineamento experimental foi inteiramente casualizado, com quatro repetições por tratamento, sendo cada parcela composta por cinco cachos armazenados individualmente em cumbucas plásticas ventiladas. Verificou-se que a folha de liberação dupla fase de SO_2 é eficiente no controle do mofo cinzento nas uvas de mesa ‘Rubi’, independente do tipo de perfuração do filme plástico, com baixa perda de massa e degrana, e boa conservação do frescor da ráquis. A doença foi controlada com eficiência nas duas safras anuais. A folha de liberação lenta de SO_2, independente do tipo de perfuração do filme plástico, resultou em eficiência intermediária de controle do mofo cinzento, com boa qualidade física dos cachos. A folha de liberação dupla fase de SO_2 é recomendada para o controle do mofo cinzento em uvas de mesa ‘Rubi’ embaladas em cumbucas plásticas ventiladas.
Palavras-chave: *Botrytis cinerea* Pers. Armazenamento refrigerado. Embalagem. SO_2. *Vitis vinifera* L.

Introduction

Domestic and international table grape markets have demanded a high standard of fruit quality, and for this reason, cultivars that present excellent productive performance, prolonged postharvest conservation and desirable consumption characteristics have been preferred (Champa, 2015). Among the attributes that influence the purchase of the grape, flavor and appearance are very important and are related to the color of the berries, shape and size of the bunch, absence of defects, diseases, residues, among others, and even the coloring of the rachis, which goes through darkening after harvest (Silva-Sanzana et al., 2016). One of the most important table grapes grown in tropical and subtropical areas is 'Rubi' (*Vitis vinifera* L.), which originated from a spontaneous somatic mutation of the 'Italia' grape. This grape cultivar is large and resistant to shattered berries, differing from the original cultivar only in the pink color of the berries (Kishino, Marur, & Roberto, 2019).

In the postharvest stage, grapes are exposed to different risks of losses, including damage from handling, loss of water and attack of pathogens (Youssef, Oliveira, Tischer, Hussain, & Roberto, 2019). The fungus *Botrytis cinerea* Pers., the causal agent of gray mold disease, is primarily responsible for postharvest losses in table grapes, leading to severe losses (Tessmann, Vida, Genta, Roberto, & Kishino, 2019). This fungus can remain latent in the field and only express itself during the transport and storage of bunches and can also develop at low temperatures (± 0.5 °C) (Elad, Vivier, & Fillinger, 2015). In addition, it can spread during commercialization and even after consumer purchase, forming a cluster of rotten berries that quickly progress to healthy bunches, resulting in extensive damage to the production and quality of the grapes (Michailides & Elmer, 2000; Williamson, Tudzynski, Tudzynski, & Van Kan, 2007; Tessmann et al., 2019).

The control of gray mold in table grape berries is quite challenging since postharvest applications with synthetic fungicides are not allowed in many countries (Smilanick, Mansour, Gabler, Margosan, & Hashim-Buckey, 2010; Hashim, Youssef, & Abd-Elsalam, 2019). For this reason, the integration of management in the field with phytosanitary treatments and the use of appropriate packaging during cold storage are the commercial strategies that most contribute to reducing losses and controlling postharvest diseases (Palou et al., 2002; Droby & Lichter, 2004; Zutahy, Lichter, Kaplunov, & Lurie, 2008; Liguori, Sortino, Pasquale, & Inglese, 2015; Salem, Youssef, & Sanzani, 2016).

Among attempts to reduce the incidence of *B. cinerea* postharvest in different grape cultivars, the use of SO_2-generating pads during cold storage demonstrated good performance (Ahmed et al., 2018; Domingues et al., 2018; Chaves et al., 2019; Youssef, Chaves, Mühlbeier, & Roberto, 2020), mainly due to its efficiency, ease of use, affordable cost and low health risk when compared to fungicides (Melgarejo-Flores et al., 2013). This gas, in addition to inhibiting the development of microorganisms, has antioxidant action, influencing the physiological processes of the bunch itself, for example, maintaining the rachis or stem green and fresh (Muñoz, Benato, Sigrist, Oliveira, & Corrêa, 2000; Zoffoli & Latorre, 2011).

The choice of SO_2-generating pads must be made with discerning, as the gas in high concentration and/or residues can cause the stem to darken, affect the physicochemical and sensory characteristics of the grapes, in addition to being harmful to humans and the environment in high doses (Pires, Sousa, Pereira, Alvim-Ferraz, & Martins, 2008; Ngcobo, Opara, & Thiart, 2011). Thus, aiming at greater protection, the main markets for importing fresh grapes, such as the European Union and the United States, established a maximum tolerance level of 10 ppm in the postharvest handling of table grapes (Food and Drug Administration [FDA], 2003). Commercially, there are slow and dual release generating pads (fast and slow) of the gas, with different concentrations of the active ingredient (a.i.) sodium metabisulfite ($Na_2S_2O_5$). By absorbing the water vapor that originates from the respiration of the bunches and high relative humidity, the pads react with the a.i. releasing SO_2 gas (Mustonen, 1992; Fernández-Trujillo, Obando-Ulloa, Baró, & Martínez, 2008; Lichter, Zutahy, Kaplunov, & Lurie, 2008; Zoffoli & Latorre, 2011).

To further improve the efficiency of SO_2-generating pads, extend the shelf life and maintain postharvest quality, bunches of table grapes must be packaged with perforated LDPE films (low-density polyethylene) (Henríquez & Pinochet, 2016). The function of these plastic liners is to place a water vapor barrier to prevent dehydration of the bunches (Yamashita, Tonzar, Fernandes, Moriya, & Benassi, 2000). In addition to this material, an innovative alternative that has been practiced with great success in the packaging of table grapes is the use of ventilated plastic clamshells (Zoffoli & Latorre, 2011). Its main purpose is to store the bunches individually, avoiding their physical contact with the external environment. In this way, it not only provides greater attractiveness and practicality to the consumer but also enables better integrity of the bunches until their final destination is reached (Gabler, Mercier, Jiménez, & Smilanick, 2010; Karaca & Smilanick, 2011).

By adopting the use of SO_2-generating pads in table grape packaging, the clamshells and perforated plastic liners can become a physical barrier to the circulation of SO_2 and negatively affect gas efficiency and residue levels. Thus, the study of the effect of packaging materials used in postharvest conservation in refrigerated storage of 'Rubi' table grapes regarding the incidence of gray mold must be further investigated, especially when vines are grown in subtropical areas under a two-cropping per year system.

Given the above, this work aimed to evaluate different SO_2-generating pads and perforated plastic liners to control the gray mold in 'Rubi' table grapes individually packaged in ventilated plastic clamshells.

Material and Methods

Location of the experiments

The 'Rubi' (*Vitis vinifera* L.) table grapes were harvested in a commercial vineyard located in Cambira, Paraná, Brazil (23°34'58''S, 51°34'40''W, elevation of 1,017 m a.s.l.). The 12-year-old vines were grafted into 'IAC 766 Campinas' rootstock, trained in an overhead trellis system covered by plastic black mash 18%. The bunches were harvested in two consecutive crops, the 2018 summer season crop and the 2019 off-season crop, when the soluble solids content reached 14 ºBrix and the titratable acidity reached 0.7% tartaric acid. The climate of this area, according to the classification proposed by Köppen, is subtropical Cfa type, with an average annual precipitation of 1,633.5 mm, minimum average temperature of 18 ºC and maximum of 22 ºC (Instituto das Águas do Paraná [IAP], 2017). The area was selected for having a recurrent history of gray mold (Ahmed et al., 2018; Chaves et al., 2019).

Origin of the materials, treatments and experimental design

The completely randomized design was used as a statistical model, with nine treatments, consisting of a control and SO_2 -generating pads (slow and dual release) associated with plastic liners with different perforations (microperforated; 2.0 mm; 4.0 mm and 5.0 mm in diameter) and four repetitions per treatment. Each plot was composed of five bunches stored individually in vented plastic clamshells. In the control, any SO_2-generating pad was used, only the microperforated plastic liner, standard in the growing area.

The evaluated SO_2-generating pads (Uvasys®, Tessara Fresh Science, Cape Town, South Africa) have 37.55% $Na_2S_2O_5$ as the active ingredient (a.i.), with more than 98% purity and dimensions of 35.0 × 53.0 cm. The slow release SO_2-generating pads (Uvasys Slow®) contain 3.85 g of the a.i. designed one layer of wax that contains the a.i. particles and release a continuous and slow dose of SO_2. The dual release SO_2-generating pads (fast and slow phases) (Uvasys® Green) containing 4.50 g of a.i. were designed to release 60% of the a.i. in the fast phase and 40% in the slow phase. This pad consists of a sequence of plastic membranes, each connected by a layer of wax that contains the particles of the a.i. The upper and medium plastic membranes cover the slow release layer that emits a low and continuous dose of SO_2. In the intermediate and lower membranes where the quick release layer is found, high SO_2 emission occurs in a short period of time (24 to 48 hours).

The evaluated plastic liners (Tessara Fresh Science, Cape Town, South Africa) had frontal dimensions of 65 × 65 cm and lateral dimensions of 65 × 40 cm. The microperforated plastic liners had approximately 1,240 holes per m^2, while the liners of 2.0, 4.0 and 5.0 mm in diameter had approximately 78, 88 and 72 holes per m^2, respectively.

Packaging of the grapes

After harvesting the grapes, the bunches were cleaned, eliminating damaged berries. The bunches were standardized to approximately 0.5 kg in mass and then individually packed in ventilated plastic clamshells, with 10 perforations of 2.0 × 0.5 cm each at the lid. The clamshells have a capacity of 0.5 kg and dimensions of 20 × 10 cm each.

The packaging process of the grapes followed the following steps: the corrugated carton boxes measuring 60 × 40 × 10 cm and storage capacity for 10 plastic clamshells were lined internally with plastic liners depending on the treatment. Above the plastic liners, a sheet of moisture-absorbing paper measuring 37 × 28 cm was placed at the bottom of the box. The plastic clamshells with the bunches were placed in the carton boxes, and above them, an SO_2-generating pad was arranged according to the treatment. Finally, the plastic film was sealed with adhesive tape (Figure 1).

Cooling system

The boxes with the grapes were stored for a period of 45 days in a refrigerated chamber at 1.0 ± 1.0 °C and 95% relative humidity. After 45 days, the boxes were removed from cold storage and kept for 3 days without plastic liners and SO_2-generating pads at room temperature (22.0 °C ± 1.0 °C) to simulate the commercial environment of a supermarket shelf.

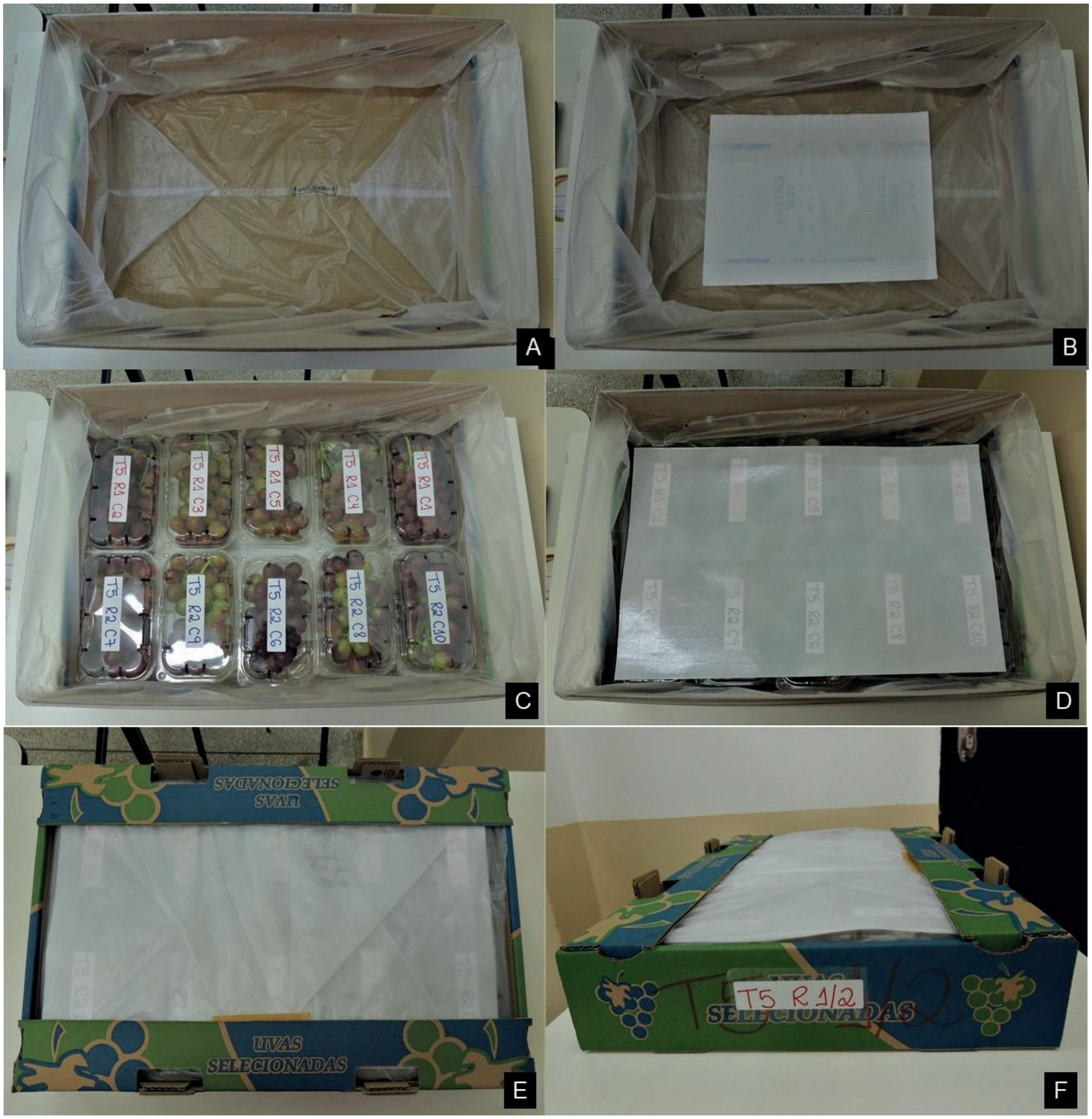

Figure 1. Packaging steps of 'Rubi' table grape. A: carton box and perforated plastic liners. B: sheet of moisture-absorbing paper on the bottom of the box. C: placement of vented plastic clamshells with grapes. D: SO_2-generating pad on top of clamshells. E: sealing of the liners with adhesive tapes. F: carton box ready to be placed in cold storage.

Assessments

The evaluations occurred at 30 and 45 days after the beginning of cold storage, when the following variables were analyzed: incidence of gray mold in berries, bunch mass loss, stem browning and shattered berries. After 3 days of storage at room temperature (22.0 °C ± 1.0 °C), the described variables were evaluated again, except bunch mass loss.

The incidence of gray mold was calculated by the following formula: incidence (%) = (number of affected berries/total of berries) x 100 (Youssef & Roberto, 2014).

The bunch mass loss was obtained by weighing the bunches at the initial time of storage and at the time of each evaluation: mass loss (%) = [(initial mass - mass at examined date)/initial mass] x 100 (Mattiuz, Miguel, Galati, & Nachtigal, 2009).

Stem browning was evaluated through visual assessment according to the methodology described by Ngcobo et al. (2011), assigning notes in accordance with the level of darkness: (1) fresh and green; (2) some light browning; (3) significant browning; and (4) severe browning.

The percentage of broken/shattered berries was evaluated by counting the loose berries from the bunch inside the clamshells and was expressed as a percentage.

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) using Statistica® Software Ver. 6.0 (Stat Soft, Inc., Tulsa, OK, USA). Fisher's protected least significant difference was used at $P \leq 0.05$ to distinguish the differences among various treatments.

Results and Discussion

Incidence of gray mold

In the summer crop, at 30 and 45 days of storage in a refrigerated chamber, the bunches of 'Rubi' table grapes subjected to treatments with SO_2-generating pads resulted in the lowest incidence, or even in the absence of gray mold, when compared to the control, composed only of microperforated liners (Table 1). In this season, the complete absence of symptoms of gray mold during cold storage was verified in the grapes packed with dual release SO_2-generating pads. At 3 days at room temperature, even though all treatments were stored in equal conditions (in plastic clamshells only), with no other packaging materials, the disease incidence in bunches of control was still high (Table 1). The treatments with SO_2-generating pads had an increase in the incidence of the disease when compared to its storage in a refrigerated chamber. Dual release-generating pads maintained a low incidence of the disease, while bunches packed with slow release pads resulted in between 6.3 and 7.2% berries with symptoms of gray mold.

In the off-season crop, at 30 and 45 days of cold storage, the control resulted in the highest percentage of gray mold incidence (Table 1). The grapes from the treatments with the dual release pads had no incidence of the disease. On the other hand, bunches packed with slow release pads resulted in between 4.8 and 8.5% and 5.9 and 9.5% berries with symptoms at 30 and 45 days of storage, respectively (Table 1). At 3 days at room temperature, the greatest efficiency in controlling the fungus continued to be observed for treatments composed of the dual release pad (Table 1). The treatment consisting of the slow release pad and microperforated liner resulted in 24.8% of berries with symptoms, not differing from the control, which had the highest incidence of the disease (30.5%).

Table 1
Incidence of gray mold of 'Rubi' table grapes grown under a two-cropping per year system after 30 and 45 days of storage in a cold chamber at 1.0 ± 1.0 ºC and 3 days at shelf life at 22.0 ± 1.0 ºC after the period of cold storage, individually packaged in clamshells with different SO_2-generating pads and perforated plastic liners

Treatments	Gray mold incidence (% of affected berries)					
	After 30 days in cold chamber		After 45 days in cold chamber		After 3 days in room temperature	
	Summer season	Off-season	Summer season	Off-season	Summer season	Off-season
Control [a]	5.9 a	12.1 a	15.3 a	19.8 a	19.1 a	30.5 a
SR/micro	0.1 c	8.5 b	1.0 b	9.5 b	7.2 b	24.8 a
SR/2.0 mm	0.8 bc	4.8 c	1.5 b	5.9 c	6.6 b	15.7 b
SR/4.0 mm	1.4 b	5.0 c	2.6 b	5.9 c	6.3 b	16.5 b
SR/5.0 mm	0.8 bc	6.4 bc	2.1 b	7.5 bc	6.5 b	15.1 b
DR/micro	0.0 c	0.0 d	0.0 c	0.0 d	0.1 c	1.7 c
DR/2.0 mm	0.0 c	0.0 d	0.0 c	0.0 d	0.3 c	0.7 c
DR/4.0 mm	0.0 c	0.0 d	0.0 c	0.0 d	0.3 c	1.0 c
DR/5.0 mm	0.0 c	0.0 d	0.0 c	0.0 d	1.2 c	1.3 c

[a] Without SO_2-generating pads, only microperforated liners. Means within columns followed by the same letters are not significantly different, as determined by Fisher's LSD test ($P \leq 0.05$). SR: slow release; DR: dual release; micro: micro perforated liner; 2.0 mm, 4.0 mm, 5.0 mm: diameters of plastic liners.

Postharvest quality of grape bunches

The mass loss observed at 30 and 45 days in a refrigerated chamber resulted in significant differences among treatments, with gradual loss over the evaluation period, in both season crops (Table 2). The highest means observed at the end of storage in the summer and off-season crops were 1.6 and 1.4%, respectively. In general, the mass loss remained low in all evaluated packaging.

Table 2
Mass loss of 'Rubi' table grapes grown under a two-cropping per year system after 30 and 45 days of storage in a cold chamber at 1.0 ± 1.0 °C, individually packaged in clamshells with different SO_2-generating pads and perforated plastic liners

Treatments	Mass loss (%)			
	After 30 days in cold chamber		After 45 days in cold chamber	
	Summer season	Off-season	Summer season	Off-season
Control [a]	0.8 bc	0.9 b	1.2 bc	1.1 abc
SR/micro	0.9 bc	0.8 bc	1.2 bc	1.1 abc
SR/2.0 mm	0.7 c	0.5 d	0.8 c	0.7 d
SR/4.0 mm	0.8 bc	0.9 b	1.5 ab	1.2 ab
SR/5.0 mm	1.2 ab	1.4 a	1.6 a	1.4 a
DR/micro	1.0 abc	0.6 cd	1.3 ab	0.7 d
DR/2.0 mm	1.0 abc	0.7 bcd	1.3 ab	0.8 cd
DR/4.0 mm	1.3 a	1.0 b	1.6 a	1.2 ab
DR/5.0 mm	0.8 bc	0.7 bcd	1.2 bc	1.0 bcd

[a] Without SO_2-generating pads, only microperforated liners. Means within columns followed by the same letters are not significantly different, as determined by Fisher's LSD test ($P \leq 0.05$). SR: slow release; DR: dual release; micro: micro perforated liner; 2.0 mm, 4.0 mm, 5.0 mm: diameter of plastic liners.

Regarding stem browning (Table 3), in the summer crop, at 30 days of cold storage, the lowest scores were observed for the treatments consisting of SO_2-generating pads, while the treatment consisting only of the microperforated liner (control) resulted in the highest mean. Despite this difference, stems remained fresh and green in all treatments. At 45 days in the cold chamber and at 3 days at room temperature, in the summer crop, the control also resulted in the highest scores of stem browning (Table 3). However, even with significant differences among the means of all treatments, the grape stems were close to the light browning conditions. In the off-season crop, in general, grapes from all treatments maintained mean scores ranging between 1.0 and 1.1 during cold storage and between 1.1 and 1.2 during storage at room temperature, indicating that stems remained fresh and green during the whole period of storage (Table 3).

The percentage of shattered berries is shown in Table 4. In the summer crop, at 30 days in a refrigerated chamber, higher means were observed for the control treatment. At 45 days in cold storage and at 3 days at room temperature, this treatment remained higher in relation to the shattered berries, followed by bunches packed with slow release pads. Dual release pad treatments resulted in the lowest levels of shattered berries. In the off-season crop, throughout the evaluated period, the highest percentages of shattered berries were also observed for the treatment composed only of the microperforated liner (control treatment) (Table 4). However, despite significant differences, the other treatments had levels of shattered berries similar to the control, which were generally considered low.

Table 3

Stem browning scoring of ‘Rubi’ table grapes grown under a two-cropping per year system after 30 and 45 days of storage in a cold chamber at 1.0 ± 1.0 ºC and 3 days at shelf life at 22.0 ± 1.0 ºC after the period of cold storage, individually packaged in clamshells with different SO_2-generating pads and perforated plastic liners

Treatments	Stem browning[b]					
	After 30 days in cold chamber		After 45 days in cold chamber		After 3 days in room temperature	
	Summer season	Off-season	Summer season	Off-season	Summer season	Off-season
Control [a]	1.3 a	1.1 a	2.0 a	1.1 a	2.2 a	1.2 a
SR/micro	1.1 b	1.0 b	1.9 ab	1.0 b	2.1 ab	1.1 a
SR/2.0 mm	1.1 b	1.0 b	1.7 bc	1.0 b	1.8 bc	1.1 a
SR/4.0 mm	1.0 b	1.0 b	1.9 ab	1.1 a	2.0 ab	1.2 a
SR/5.0 mm	1.0 b	1.0 b	1.7 bc	1.0 b	2.0 ab	1.1 a
DR/micro	1.0 b	1.0 b	1.6 c	1.1 a	2.0 ab	1.2 a
DR/2.0 mm	1.0 b	1.0 b	1.6 c	1.0 b	1.7 c	1.1 a
DR/4.0 mm	1.1 b	1.0 b	1.8 abc	1.0 b	2.1 ab	1.1 a
DR/5.0 mm	1.1 b	1.0 b	1.7 bc	1.0 b	2.0 ab	1.1 a

[a] Without SO_2-generating pads, only microperforated liners. Means within columns followed by the same letters are not significantly different, as determined by Fisher’s LSD test ($P \leq 0.05$). SR: slow release; DR: dual release; micro: micro perforated liner; 2.0 mm, 4.0 mm, 5.0 mm: diameter of plastic liners. [b]Stem browning scores: (1) fresh and green; (2) some light browning; (3) significant browning; and (4) severe browning (Ngcobo et al., 2011).

The main objective of this study was to evaluate the effect of different SO_2-generating pads and perforated plastic liners to control the gray mold of ‘Rubi’ table grapes packaged in clamshells and to prevent other disturbances during storage. The use of SO_2-generating pads during cold storage is one of the main methods for controlling the gray mold of table grapes (Lichter et al., 2008; Palou, Serrano, Martínez- Romero, & Valero, 2010; Champa, 2015), and its use is necessary when the objective is to export the table grape ‘Rubi’ or even to store it for prolonged periods for commercialization in the domestic market.

The rate of SO_2 required to eliminate the spores or to inactivate the mycelium of the causal agent depends on the concentration of the active ingredient (a.i.) and the type/time of release (Fernández-Trujillo et al., 2008). Possibly, the dose of 60% of the a.i. release by the dual release pad in the first 48 hours, after contact of the pad with moisture, was sufficient to eliminate the fungus spores that were in active growth and, consequently, guaranteed the best performance in the control of gray mold. In addition, the combination of this fast phase with a dose of 40% of a.i. release in a slow and continuous way allowed the regular supply of SO_2, preventing the development of the fungus in the treatments in which the dual release pad was used. On the other hand, in the slow release pad, the release form associated with the low concentration of the a.i. (3.85 g) may have compromised the gas efficiency in

Table 4

Shattered berries of 'Rubi' table grapes grown under a two-cropping per year system after 30 and 45 days of storage in a cold chamber at 1.0 ± 1.0 ºC and 3 days at shelf life at 22.0 ± 1.0 ºC after the period of cold storage, individually packaged in clamshells with different SO_2-generating pads and perforated plastic liners

Treatments	Shattered berries (%)					
	After 30 days in cold chamber		After 45 days in cold chamber		After 3 days in room temperature	
	Summer season	Off-season	Summer season	Off-season	Summer season	Off-season
Control [a]	1.4 a	0.9 a	2.6 a	1.4 a	5.6 a	2.1 a
SR/micro	0.4 bc	0.3 b	1.5 abc	0.3 b	2.2 ab	0.8 ab
SR/2.0 mm	0.0 c	0.1 b	1.6 abc	0.8 ab	2.9 ab	1.2 ab
SR/4.0 mm	1.0 ab	0.3 b	2.0 ab	0.3 b	3.7 ab	0.3 b
SR/5.0 mm	0.5 bc	0.5 ab	1.8 abc	0.8 ab	2.5 ab	0.8 ab
DR/micro	0.4 bc	0.1 b	1.0 c	0.4 b	1.7 b	0.9 ab
DR/2.0 mm	0.5 bc	0.8 ab	1.0 c	1.0 ab	1.8 b	1.4 ab
DR/4.0 mm	0.4 bc	0.0 b	1.3 bc	0.3 b	1.6 b	0.4 b
DR/5.0 mm	0.0 c	0.1 b	1.0 c	0.7 ab	1.6 b	0.7 ab

[a] Without SO_2-generating pads, only microperforated liners. Means within columns followed by the same letters are not significantly different, as determined by Fisher's LSD test ($P \leq 0.05$). SR: slow release; DR: dual release; micro: micro perforated liner; 2.0 mm, 4.0 mm, 5.0 mm: diameter of plastic liners.

the initial control of the inoculum of the fungus. The results obtained herein are in agreement with Chaves et al. (2019), who found that a slow release pad with 4.0 g of a.i. led to the highest percentage of gray mold incidence after 30 and 45 days in cold storage.

'Rubi' table grape bunches remained individually inside the vented plastic clamshells throughout the evaluated period. This packaging, despite being considered a physical barrier to the absorption of SO_2, allowed the maintenance of a suitable environment for the action and retention of the gas released around the bunches. This fact can be confirmed by the low incidence of the disease, especially when the dual release pad was used, regardless of the type of perforation of the liner.

At 3 days of room temperature, the increase in the incidence of the disease in the treatments in which the SO_2-generating pads were used is possibly because the SO_2 gas does not penetrate the berry skins. Therefore, grapes must be continuously exposed to gas to control the disease by periodic elimination of growing mycelium (Smilanick et al., 1990; Chervin, Aked, & Crisosto, 2012). However, the absorption of SO_2 is cumulative (Palou et al., 2002), and the gas can be trapped within the plastic clamshells (Romanazzi, Lichter, Gabler, & Smilanick, 2012). Due to the fumigant effect of the fast phase of the dual release pads, the amount of SO_2 residue maintained the low incidence of the fungus in these treatments. In 'BRS Vitoria' table grapes packaged in plastic clamshells using slow and dual release pads, it was found that the dual release pads resulted in the lowest incidence of gray mold after 50 days of refrigerated storage. These pads also had the best results at 7 days at room temperature, being more promising in controlling the fungus than the slow release pads (Domingues et al., 2018).

The number and size of the holes of the liners also influence the reaction of a.i. of the SO_2-generating pads and should be taken into account when selecting the most appropriate pad (Harvey, Harris, Hanke, & Hartsell, 1988; Boersig, Hartsell, & Smilanick, 2003; Leesch, Smilanick, & Tebbets, 2008). When the liner had a very small perforation, as in the case of microperforated film, the a.i. of the SO_2-generating pads may be fully finished before the end of the storage period (Zutahy et al., 2008). Most likely, in the treatment composed of the slow release pad and the microperforated liner, in the off-season crop, all the a.i. present in the pad was exhausted during refrigerated storage, leaving no residual gas that could control the fungus during storage at room temperature.

The results obtained in this research demonstrated that SO_2 pads were able to control gray mold in both seasons, giving satisfactory results in which the weather conditions were relatively different. It is well known that under intensive production systems, such as Brazil, harvest can take place during highly complimentary conditions for the development of gray mold.

From a commercial point of view, the differences between the treatments for the loss of mass of the bunches are considered irrelevant because in general, the loss of mass remained low in all packages. It is noteworthy that the wilting aspect of the berries, as a consequence of the loss of mass, occurs when the loss reaches approximately 4 to 5%, thus affecting the ideal appearance and firmness for consumption (Gorgatti et al., 1993). In addition, the apparent effects of mass loss on grape bunches may also be related to the morphological and anatomical characteristics of each cultivar (Mattiuz, Miguel, Nachtigal, Durigan, & Camargo, 2004), and for the ‘Rubi’ grape bunches under the conditions of the present study, the loss of mass was not significant. The packaging components also affect the transmission of water vapor, and the lower the transmission rate is, the greater the relative humidity inside the packaging, which reduces transpiration and, consequently, the loss of mass (Zagory & Kader, 1988; Cia et al., 2010). For this reason, the use of a perforated plastic liner combined with an SO_2-generating pad is recommended as a successful way to reduce water loss during postharvest handling of grapes (Crisosto, Smilanick, Dokoozlian, & Luvisi, 1994). In this research, the low values found for the mass loss of grape bunches are probably due to the associated effects of the adequate temperature and humidity conditions of storage, combined with the appropriate use of the packages and the modified atmosphere.

Regarding stem browning, although there were differences among treatments, the ‘Rubi’ stem conditions were considered light browning. The appearance of table grapes is seriously affected when the stem is dry and brown (Nelson, 1983), and this condition was not observed in this work. In another study, after 50 days of cold storage, it was possible to observe that the bunches of ‘Italia’ table grapes packaged with different dual release pads had the lowest stem browning scores when compared to the treatment composed only of the microperforated liner (control).

After 7 days at room temperature, there were no significant differences among treatments, and all stems were slighted brown (Ahmed et al., 2018). SO_2 gas, in addition to controlling fungi, also influences physiological processes, such as maintaining the green color of the stems and turgidity of the berries (Mansour, El-Tobshy, Nelson, & Fahmy, 1984; Nelson, 1983), and this is due to the inhibitory action of SO_2 on the catalytic mechanism of some enzymes that favor the respiration process (Gorgatti et al., 1993).

The highest percentage of shattered berries observed for the control treatment, followed by the bunches packaged with a slow release pad, assumes that the incidence of gray mold may have been one of the factors that influenced this characteristic, as the presence of the fungus in grapes causes the berries to soften and, consequently, favors the greater occurrence of loose berries (Bulit & Dubos, 1990; Celik et al., 2009). However, despite the significant differences, the levels of shattered berries were considered low and acceptable in these trials. The occurrence of shattered berries, depending on the intensity or position of occurrence, can often go unnoticed by the consumer. In addition, the plastic clamshells used to provide a combination of practicality, protection and marketing for table grapes (Saito & Xiao, 2017) also avoid shattered berries being a problem in the markets because even though the berries are loosened from the stem, they will remain in the packaging. It should also be taken into account that the ‘Rubi’ table grapes show a dense bunch (Kishino et al., 2019) that protects the berries and the stem, turning them resistant to shattered berries and stem browning (Gomes, Ferraz, & Cipolli, 2013). This may be one of the reasons why this grape cultivar did not show significant postharvest losses as a result of these disorders.

To summarize, the use of vented plastic clamshells that individually store the clusters of the ‘Rubi’ grape was demonstrated to be suitable for refrigerated conservation, mainly because it allowed SO_2 generated by the pads to have good circulation inside the package, which was aided by the use of perforated plastic liners. Finally, the gray mold was more efficiently controlled during the entire cold storage period when dual release pads were used.

Conclusions

The dual release SO_2-generating pads are efficient for controlling the gray mold of ‘Rubi’ table grapes regardless of the type of perforation of the plastic liners, with low mass loss and shattered berries, with good conservation of the freshness of the stems. The disease can be controlled efficiently both in the summer and in the off-seasons. The slow-release SO_2-generating pads, regardless of the type of perforation of the plastic liners, resulted in intermediate efficiency of gray mold control, with good physical quality of the grape bunches.

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