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Effects of Different Sulfur Dioxide Pads on *Botrytis* Mold in 'Italia' Table Grapes under Cold Storage

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Abstract: 'Italia' grape is one of the most important table grape cultivars grown worldwide. Gray mold, caused by *Botrytis cinerea* Pers. Fr., is one of the most important causes of postharvest decay of table grapes, and the control of this disease is very difficult because postharvest treatments with synthetic fungicides are not allowed in many countries. The objective of this study was to compare different types of pads releasing different doses of SO_2 during cold storage to control gray mold in 'Italia' table grapes grown under subtropical conditions. Grape bunches were harvested from a commercial field trained on an overhead trellis located at Cambira, state of Parana (PR), South Brazil. The grapes were packed into carton boxes (capacity, 4.5 kg) and subjected to the following SO_2 pad treatments (Uvasys®, Cape Town, South Africa) under cold storage (1.0 ± 1 °C) for 50 days: (i) Control; (ii) SO_2 slow release pad; (iii) SO_2 dual release pad; (iv) SO_2 dual release–fast reduced pad; (v) SO_2 slow release pad with grapes inoculated with *B. cinerea* suspension; (vi) SO_2 dual release pad with grapes inoculated with *B. cinerea* suspension; and (vii) SO_2 dual release-fast reduced pad with grapes inoculated with *B. cinerea* suspension. After cold storage, the grape boxes were maintained for 7 days at room temperature (25 °C). The incidence of gray mold on the grapes, firmness, shattered berries, stem browning, as well as other physicochemical variables, such as bunch mass, bunch mass loss, skin color, soluble solids (SS), titratable acidity (TA) and SS/TA were evaluated. Both SO_2 dual release pads were highly efficient in preventing the incidence of gray mold in 'Italia' grapes packed in clamshells during the 50-day period of cold storage and at room temperature, even with *Botrytis*-inoculated berries. The SO_2 slow release pad showed lower efficiency, but was higher than the control. The SO_2 dual release pad treatments provided the best results with respect to stem browning scores (fresh and green stems) during cold storage, and no differences were observed among the treatments with respect to the other physicochemical evaluations.

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

1. Introduction

'Italia' grape is one of the most important seeded table grapes worldwide. The major producing areas are located in Chile, Italy, Brazil and Peru, and it is commonly exported overseas because of its sweet and muscat flavor [1]. Table grapes are non-climacteric fruits with relatively low physiological activity, and they are subjected to major water-loss and 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.

Fungal decay is the main source of postharvest deterioration in table grapes; losses due to fungal decay in different countries (specifically in trade and industry) for different commodities range from 30 to 50%, and under some circumstances, the entire production has been lost due to the infection [2,3]. *Botrytis cinerea*, which causes gray mold, is the most important fungus of grapes, and it has the ability to grow and spread even at low temperatures (−0.5 °C) [4,5]. This fungus is second in the "world top 10 fungal pathogens in molecular plant pathology" based on scientific/economic importance [6], and it is quite destructive, as it can develop under different conditions such as in the field, at and after harvest, during storage and marketing, and even after customer purchase. Therefore, if the grapes are not managed well (especially during harvest and storage), then the chances of infection may lead to extensive damage of the grapes [7], especially in subtropical humid areas where fungal diseases are a common concern.

The severity of infection is dependent on the existence of the pathogen during harvest, handling, and cooling of the grapes in cold chambers [8,9]. At the beginning of the infection, a dark circular area is visible on tissues that are softer than the uninfected fruit parts, subsequently leading to abundant sporification (with the color ranging from white to gray), which can develop from the site of the infection; for example, natural openings or mechanical wounds that occur on fruit. Often, *B. cinerea* can spread from decayed fruit to neighboring fruit (in good condition), promoting the development of the infection and establishment of a nest of rotted berries and resulting in extensive loss of produce. The fungus has the ability to infect fresh fruit by entering damaged tissue at the stem end, which is rich in nutrient exudates; the infection then spreads and damages the entire fruit [10–12].

The control of gray mold is quite challenging, as most countries have banned postharvest treatments with synthetic fungicides because of human health considerations. Attempts to reduce the incidence of this fungus on grapes depend on having knowledge of its cause and mode of action to develop preharvest and postharvest control approaches. Among these approaches, the use of sulfur dioxide (SO_2) generator pads has been relatively successful worldwide because of their efficiency, ease of use, affordable cost and low health risk when compared with fungicides [13]. Different types of pads, in which the rate of SO_2 is controlled, have been developed according to industry needs, with one or two different release phases, quick and/or slow, and they are available in many different sizes, depending on the storage carton size used. Because some grape cultivars are sensitive to SO_2, new pads have been developed with different doses of the active ingredient (a.i.) to avoid unwanted alterations in the grapes.

The SO_2 pads contain sodium metabisulfite ($Na_2S_2O_5$) enclosed in a sheet of plastic and paper. This product is made for packing facilities that use SO_2 gas to treat and sterilize table grapes by releasing an appropriate dose of the a.i. to kill and eliminate any actively growing *B. cinerea* spores. Moisture within the carton of grapes is absorbed by the pads, and it reacts with the sulfite to release SO_2 [14]. However, a high-intensity dose of SO_2 can damage table grapes by causing bleaching or affecting sunken regions of the grape berry, and causing early browning of the rachis. In addition, berry cracking, fruit injury, unpleasant aftertaste, and allergies are also consequences of a high concentration of SO_2. Therefore, for export purposes, levels of SO_2 tolerance (10 ppm) have been proposed by EU countries, with the objective of protecting the consumer and environment.

Usually, the SO_2 pads are placed over, but not touching, the grapes during shipping to avoid or limit damage. The use of dual release SO_2-generating pads (slow- and quick-release phases) in combination with a plastic liner and moisture-absorbing sheet is advised when the grapes are stored for longer than 10 days and during long periods of retail handling. The amount of SO_2 released is affected by the temperature; therefore, the efficiency of these pads depends on good cold-chain management [9,15].

Recently, packaging of grapes has experienced several changes and innovations, according to consumer preferences and demands of the international and domestic markets. New packaging materials have been created, and the use of plastic clamshells has become increasingly common, resulting in higher grape consumption and lower losses due to shattered berries. Although some

food market chains are equipped with cold chambers for storing fruit before exposing them to room temperature, the effects of different SO_2 pads on 'Italia' table grapes, grown in subtropical regions, and individually packed in clamshells, during cold storage have not yet been assessed. This packaging material may act as a barrier for gas circulation and distribution around the grapes, and an assessment of postharvest techniques to avoid berry losses due to gray mold is needed to maintain quality and profitability for both international and domestic markets.

Because SO_2 treatments can cause unwanted alterations in some grape cultivars, the objective of this study was to compare the effects of pads with different doses of SO_2 on controlling the incidence of gray mold in 'Italia' table grapes, grown under subtropical conditions, and individually packed in clamshells during cold storage.

2. Materials and Methods

2.1. Study Area

The experiment was performed at the Fruit Analysis Laboratory, Agricultural Research Center, State University of Londrina, PR, Brazil. Grape bunches were harvested from 7-year-old vines of 'Italia' grapes grafted onto 'IAC 766 Campinas' rootstock and trained on overhead trellis in a commercial field at Cambira, state of Parana (PR), South Brazil (23°35′ S, 51°34′ W; elevation, 1017 m). Samples were collected in the summer of 2017, and the selected bunches were free of defective or decayed berries. The area climate is classified as subtropical (Cfa), with an average temperature of below 18 °C in winter and above 22 °C in summer. The average annual rainfall is ~1700 mm. The area was selected because of the recurrence of the disease.

2.2. Treatments

The grapes were harvested early in the morning when they were fully ripe and the total soluble solids (TSS), titratable acidity (TA), and TSS/TA had reached around 14 °Brix, 1.0% and 14%, respectively.

The grapes were packed into carton boxes (4.5 kg capacity, see below) and subjected to the following SO_2 pad treatments under cold storage (1.0 ± 1 °C): (i) Control; (ii) SO_2 slow release pad; (iii) SO_2 dual release pad; (iv) SO_2 dual release–fast reduced pad; (v) SO_2 slow release pad with grapes inoculated with *B. cinerea* suspension; (vi) SO_2 dual release pad with grapes inoculated with *B. cinerea* suspension; and (vii) SO_2 dual release-fast reduced pad with grapes inoculated with *B. cinerea* suspension.

The different SO_2-generating pads (Uvasys®, Cape Town, South Africa) have 37.55% of the a.i., $Na_2S_2O_5$, with more than 98% purity and dimensions of 356 × 260 cm each, and were designed to provide 1 g of the a.i. per kg of grapes. The dual release-fast reduced pad was designed for SO_2-sensitive grape cultivars because it releases 40% of the a.i. over a 24–48 h fast phase (20% less than the dual release pad) and 60% over the slow phase.

The *B. cinerea* suspension used in this trial was isolated from infected grapes with typical symptoms of gray mold, purified, and identified morphologically and molecularly, according to Youssef and Roberto [16]. 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.

2.3. Packaging of the Grapes

After harvest and inoculation, the bunches were pre-cooled with forced air to a temperature of 2 °C within approximately four hours and cleaned, and damaged berries were removed. Then, they were standardized according to cluster shape, size, and mass (~0.5 kg), and each bunch was individually arranged in a plastic clamshell (20 × 10 cm) of 0.5-kg capacity. The grapes were packaged as follows: micro-perforated plastic liner films (1% of the ventilated area) were placed into carton boxes measuring 50 × 30 × 10 cm; a moisture-absorbing sheet measuring 37 × 28 cm was placed at the bottom of the liner; plastic clamshells with grapes were put into the cartons; an SO_2 pad was placed on top of the clamshells; and liners were sealed. A completely randomized experimental design was used, with seven treatments and four replications; each replicate plot consisted of one carton box containing eight bunches individually packed in plastic clamshells.

2.4. Cooling System

The grape boxes were cold stored for a period of 50 days. The cooling room was provided with four fans to maintain uniform air temperature of 1 °C. The cartons boxes, placed in layers of seven boxes each, were stacked on a pallet base and tightly positioned in front of a forced air cooler in a cold chamber at 1.0 ± 1 °C and 95% relative humidity. Cardboard (for sealing) was used to cover the corners and tops of the boxes and avoid any type of air escape when the cooler fans were turned on after pre-cooling the cold-storage room. After 50 days, the grape boxes were maintained for a period of 7 days at room temperature (25 °C).

2.5. Incidence Analysis of Gray Mold

The incidence of gray mold on the berries (Figure 1) 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. The disease incidence was then calculated using the following formula: Disease incidence (% of diseased berries) = (number of decayed berries/total number of berries) × 100 [16].

Figure 1. Gray mold symptoms in 'Italia' table grape berries during cold storage.

2.6. Physical Analysis

Physical analysis of the grapes was performed 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 periodic weighing of bunches and was calculated by dividing the mass change during storage by the original mass: mass loss (%) = [(mi − ms)/mi] × 100, where mi = initial mass and ms = mass at the examined time [17]. The berry firmness, expressed in N, was measured with the texture analyzer TA.XT*plus* (Stable Micro Systems, Surrey, UK) at the equatorial position of 10 berry samples from each plot [18]. Each berry was placed on the stage of the analyzer and compressed using a cylindrical probe (35 mm diameter, P35). A constant force of 0.05 N at a speed of 1.0 mm s^{-1} was then used to deform the berry 20%.

Stem browning was evaluated by visual observation with the following score system: (1) fresh and green, (2) some light browning, (3) significant browning, and (4) severe browning [19]. The shattered berries (%) were evaluated by counting the separated berries from the bunch stem inside each clamshell.

The berry color attributes (CIELab scale system—International Commission on Illumination, Vienna, Austria), such as L^* (lightness), C^* (chroma), and h° (hue), were analyzed at the equatorial position of the berries with the color reader CR-10 (Konica Minolta®, Tokyo, Japan). L^* ranges from 0 (black) to 100 (white); C^* indicates the purity or intensity of color; and h° is measured in angles (green, yellow, and red correspond to 180°, 90°, and 0°, respectively) [20–22].

2.7. Chemical Analysis

The chemical analyses were performed at 50 days of cold storage and at 7 days at room temperature. For the chemical analyses, 10 berries were collected from each box (plot). The samples were crushed, and the juice was then used to determine soluble solids (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 an 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 [23]. The maturation index of the berries was then obtained from the TSS/TA value.

2.8. Statistical Analysis

The percentage data were arcsine square root-transformed to normalize the variance before analysis of variance (ANOVA). All data were subjected to ANOVA by using Sisvar® software (UFLA, Lavras, Brazil). The mean values of the treatments were compared using Tukey's test ($p \leq 0.05$).

3. Results

3.1. Gray Mold Incidence (%)

Regarding gray mold incidence in 'Italia' table grapes, significant differences were observed among the treatments when compared with the control at 50 days of cold storage and 7 days of storage at room temperature. After 50 days of cold storage, when the grapes were subjected to SO_2 slow release treatment, the incidence of gray mold was reduced by 99.4% and 93.2% under non-inoculated and inoculated conditions, respectively. SO_2 dual release treatments reduced the incidence of gray mold by 100% under both conditions (non-inoculated and inoculated) when compared with the control. SO_2 dual release–fast reduced pads reduced the incidence of gray mold by 99.8% and 100% under non-inoculated and inoculated conditions, respectively, when compared with the control (Figure 2).

After 7 days at room temperature, when grapes were subjected to SO_2 slow release treatment, the incidence of gray mold was reduced by 97.1% and 92.8% under non-inoculated and inoculated conditions, respectively. SO_2 dual release treatments reduced the incidence of gray mold by 99.7% and 97.4% under non-inoculated and inoculated conditions, respectively, when compared with the control. SO_2 dual release–fast reduced pads reduced the incidence of gray mold by 99.9% under both conditions (non-inoculated and inoculated) when compared with the control (Figure 3).

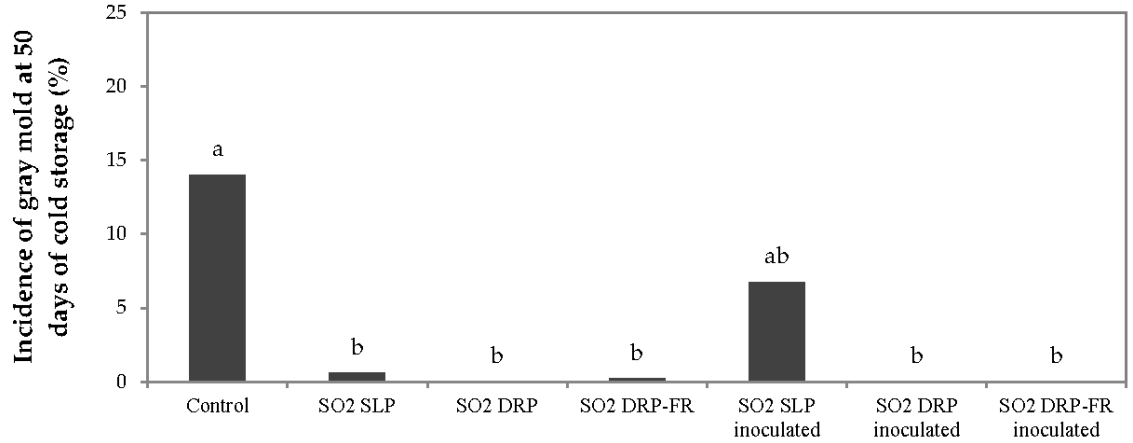

Figure 2. Incidence of gray mold (% of diseased berries) at 50 days of cold storage in 'Italia' table grapes. SO_2 SLP: SO_2 slow release pad; SO_2 DRP: SO_2 dual release pad; SO_2 DRP-FR: SO_2 dual release-fast reduced pad. Means within columns followed by the same letters are not statistically different by Tukey's test ($p \leq 0.05$).

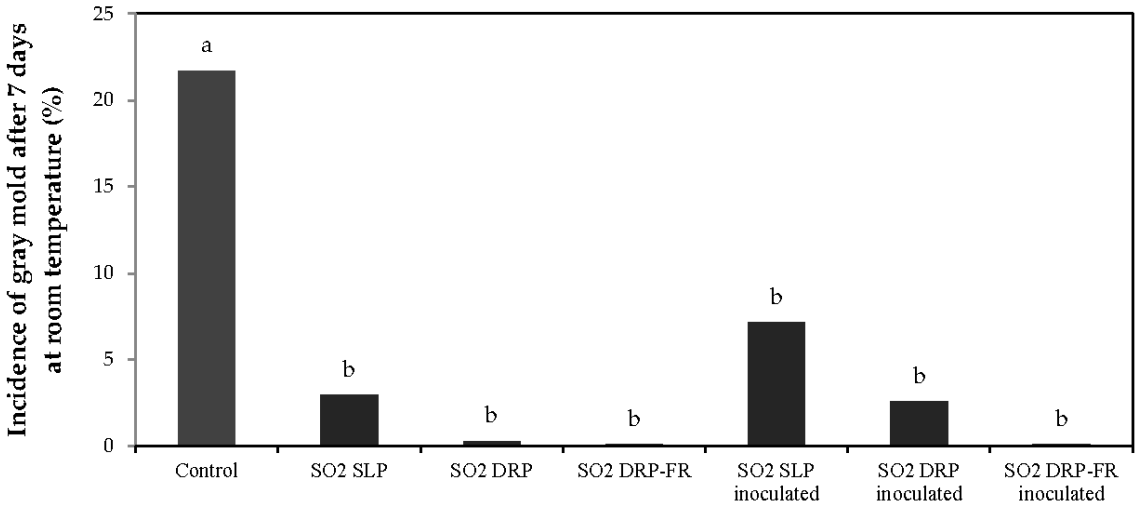

Figure 3. Incidence of gray mold (% of diseased berries) after 7 days at room temperature in 'Italia' table grapes. SO_2 SLP: SO_2 slow release pad; SO_2 DRP: SO_2 dual release pad; SO_2 DRP-FR: SO_2 dual release-fast reduced. Means within columns followed by the same letters are not statistically different by Tukey's test ($p \leq 0.05$).

3.2. Physical Analysis

No significant differences were observed among the treatments at 50 days of cold storage and after 7 days of storage at room temperature. The mass loss varied from 1.4 to 4.2% at 50 days of cold storage, and 0.5 to 2.7% at 7 days of storage at room temperature. No significant differences in berry firmness were observed among the treatments, and the means ranged from 7.0 to 8.6 N at 50 days of cold storage. However, a significant difference was observed between the SO_2 slow release pad and the SO_2 dual release-fast reduced pads inoculated with *Botrytis* after 7 days of storage at room temperature, with no significant differences among the remaining treatments (Table 1).

Table 1. Mass loss (%) and firmness (N) of 'Italia' table grapes at 50 days of cold storage and 7 days of storage at room temperature under different SO_2 pad treatments.

Treatments	Mass Loss (%)	Firmness (N)	Mass Loss (%)	Firmness (N)
	At 50 Days of Cold Storage		At 7 Days of Storage at Room Temperature	
Control	2.4	8.2	1.5	12.5 ab
SO_2 SLP	4.2	7.3	1.1	13.8 a
SO_2 DRP	1.4	7.9	0.8	11.0 ab
SO_2 DRP-FR	1.4	7.8	0.9	10.0 ab
SO_2 SLP inoculated	2.0	7.9	0.9	9.8 ab
SO_2 DRP inoculated	1.6	8.6	2.7	10.6 ab
SO_2 DRP-FR inoculated	2.1	7.0	0.5	9.0 b
F value	0.7 ^{ns}	1.2 ^{ns}	1.7 ^{ns}	2.8 *

SO_2 SLP: SO_2 slow release pad; SO_2 DRP: SO_2 dual release pad; SO_2 DRP-FR: SO_2 dual release-fast reduced pad. Means within columns followed by the same letters are not statistically different by Tukey's test ($p \leq 0.05$). ^{ns} = non-significant, * = significant at 5% level.

No significant differences in shattered berries were found among all treatments. The means ranged from 0.0 to 0.5% and 0.5 to 1.9% at 50 days of cold storage and 7 days of storage at room temperature, respectively. Significant differences in stem browning were observed between the control and SO_2 dual release pads inoculated with *Botrytis* at 50 days of storage at room temperature, with no significant differences among the remaining treatments. The means ranged from 1.0 to 1.6, whereas, after 7 days of storage at room temperature, no statistical differences were found among all treatments (Table 2).

Table 2. Shattered berries (%) and stem browning score for 'Italia' table grapes at 50 days of cold storage and 7 days of storage at room temperature under different SO_2 pad treatments.

Treatments	Shattered Berries (%)	Stem Browning Scores [a]	Shattered Berries (%)	Stem Browning Scores [a]
	At 50 Days of Cold Storage		At 7 Days of Storage at Room Temperature	
Control	0.2	1.6 a	0.5	2.5
SO_2 SLP	0.5	1.3 ab	0.7	2.1
SO_2 DRP	0.3	1.1 b	1.9	2.2
SO_2 DRP-FR	0.1	1.1 b	0.9	2.0
SO_2 SLP inoculated	0.4	1.4 ab	1.2	2.4
SO_2 DRP inoculated	0.0	1.0 b	0.5	2.1
SO_2 DRP-FR inoculated	0.1	1.1 b	0.5	2.0
F value	0.7 ^{ns}	2.7 *	2.1 ^{ns}	3.1 ^{ns}

SO_2 SLP: SO_2 slow release pad; SO_2 DRP: SO_2 dual release pad; SO_2 DRP-FR: SO_2 dual release-fast reduced pad. Means within columns followed by the same letters are not statistically different by Tukey's test ($p \leq 0.05$). ^{ns} = non-significant, * = significant at 5% level. [a]: (1) fresh and green, (2) some light browning, (3) significant browning, and (4) severe browning.

No significant differences in berry color were observed among all treatments at 50 days of cold storage and 7 days of storage at room temperature (Table 3). The L^* means ranged from 30.4 to 31.8 and 30.7 to 31.8 at 50 days of cold storage and 7 days of storage at room temperature, respectively. The C^* means ranged from 11.9 to 13.1 and 11.9 to 12.5 at 50 days of cold storage and 7 days of storage at room temperature, respectively. Similarly, h° means ranged from 107.2 to 119.9 and 113.3 to 116.6 at 50 days of cold storage and 7 days of storage at room temperature, respectively (Table 3) indicating that the SO_2 pads had no negative effects on 'Italia' berry color.

Table 3. Luminosity (L^*), chroma (C^*), and hue angle (h°) of 'Italia' table grapes at 50 days of cold storage and 7 days of storage at room temperature under different SO_2 pad treatments.

Treatments	L^*	C^*	h°	L^*	C^*	h°
	At 50 Days of Cold Storage			At 7 Days of Storage at Room Temperature		
Control	30.4	12.0	119.9	31.7	12.0	113.5
SO_2 SLP	31.8	13.1	107.2	31.7	12.5	114.0
SO_2 DRP	31.3	11.9	113.1	31.5	12.0	114.9
SO_2 DRP-FR	31.5	12.5	116.8	31.8	12.3	116.0
SO_2 SLP inoculated	31.4	12.7	115.1	30.8	12.3	115.3
SO_2 DRP inoculated	31.4	13.1	118.6	31.3	12.4	116.6
SO_2 DRP-FR inoculated	31.0	12.4	115.3	30.7	11.9	113.3
F value	2.4 ^{ns}	2.4 ^{ns}	1.8 ^{ns}	1.3 ^{ns}	0.3 ^{ns}	2.3 ^{ns}

SO_2 SLP: SO_2 slow release pad; SO_2 DRP: SO_2 dual release pad; SO_2 DRP-FR: SO_2 dual release-fast reduced pad. ^{ns} = non-significant.

3.3. Chemical Analysis

No significant differences in SS, TA, and SS/TA were detected in the 'Italia' table grapes at 50 days of cold storage and 7 days of storage at room temperature (Tables 3 and 4). The SS ranged from 13.1 to 14.4 and 13.2 to 14.0 °Brix at 50 days of cold storage and 7 days of storage at room temperature, respectively. The TA means ranged from 1.0 to 1.1% at 50 days of cold storage and 7 days of storage at room temperature. SS/TA ranged from 12.1 to 13.7 at 50 days of cold storage and 12.8 to 15.0 at 7 days of storage at room temperature (Table 4).

Table 4. Soluble solids (SS), titratable acidity (TA), SS/TA of 'Italia' table grapes at 50 days of cold storage and 7 days of storage at room temperature under different SO_2 pad treatments.

Treatments	Soluble Solids SS (°Brix)	Titratable Acidity TA (%)	SS/TA	Soluble Solids SS (°Brix)	Titratable Acidity TA (%)	SS/TA
	At 50 Days of Cold Storage			At 7 Days of Storage at Room Temperature		
Control	13.1	1.1	12.1	13.3	1.0	13.6
SO_2 SLP	14.0	1.1	13.1	13.6	1.0	15.0
SO_2 DRP	13.7	1.0	13.7	13.6	1.0	13.4
SO_2 DRP-FR	13.3	1.1	12.2	13.2	1.0	13.1
SO_2 SLP inoculated	13.5	1.0	13.0	13.5	1.1	12.8
SO_2 DRP inoculated	14.0	1.1	13.5	13.5	1.1	13.2
SO_2 DRP-FR inoculated	14.4	1.1	13.2	14.0	1.0	14.3
F value	2.1 ^{ns}	2.5 ^{ns}	1.1 ^{ns}	0.6 ^{ns}	0.7 ^{ns}	0.7 ^{ns}

SO_2 SLP: SO_2 slow release pad; SO_2 DRP: SO_2 dual release pad; SO_2 DRP-FR: SO_2 dual release-fast reduced pad. ^{ns} = non-significant.

4. Discussion

The use of different SO_2-releasing pads on 'Italia' table grapes, especially the dual release types, was an efficient technique for controlling *B. cinerea* (gray mold) in bunches packed in clamshells under cold storage. Natural infection (non-inoculated) in this experiment was caused mostly by *B. cinerea*, as the incidence of other pathogens (e.g., *Aspergillus* spp. and *Alternaria* spp.) was negligible. *B. cinerea* is considered one of the most serious postharvest pathogens of table grapes worldwide, and it causes extensive losses [24].

The control of gray mold is not easy, as most countries have banned the use of synthetic fungicides; as a consequence, different techniques have been used to reduce its infection. Among these practices, appropriate postharvest management of the crop before harvest and the use of SO_2 generator pads in cold storage after harvest have been found to be very effective in controlling gray mold [6,8,25,26].

The results showed that the incidence of gray mold in 'Italia' table grapes was reduced by the different SO_2 pad treatments. When the grapes were subjected to the slow release pads, the incidence of gray mold was reduced by 99.4% and 97.1% at 50 days of cold storage and 7 days of storage at room temperature, respectively. The best results were observed when the SO_2 dual release pads were used, as gray mold incidence was reduced by 100% and 99.9% at 50 days of cold storage and 7 days of storage at room temperature, respectively, even when the grapes had been inoculated. The contribution of gray mold infection is dependent on the incidence of the infection during harvest, handling, and cooling of grapes in cold chambers. The symptoms of gray mold can be observed on berry skin as "skin-skip" (upon touching, the skin is separated from the flesh), resulting in the establishment of "nests" [8,9].

Our results confirm that both SO_2 dual release pads can be used for 'Italia' table grapes, as this cultivar was not sensitive to the higher amounts of SO_2 in the fast-release phase and no unwanted effects were observed. In addition, these treatments provided the best results on stem browning scores (fresh and green stems) during the cold storage.

The slow release pad has only a slow release layer containing the a.i. enclosed in a sheet of plastic and paper. This pad is designed to be used in packing facilities that use SO_2 or dosing chambers to treat and sterilize table grapes by releasing an appropriate and uniform dose of the a.i. to kill and eliminate any actively growing *B. cinerea* spores over time. The SO_2 dual release pads have a fast release and contain the a.i. between paper sheets of differing permeabilities composed of a slow and fast release phase. Moisture within the grape package is absorbed by the pads, and it reacts with the particles to release SO_2.

The fast release phase of the pad sterilizes the table grapes by releasing a large enough dose of SO_2 over a 24- to 48-h period to kill and eliminate any actively growing *B. cinerea* spores, and it then declines in the subsequent 7 days. The slow release phase remains active for a long period for export purposes [14].

Because of the better marketing index of healthy grapes, such as 'Italia' (which has been a good option because of its muscat flavor and sweetness, even though it is seeded, in South American countries such as Chile and Brazil, it takes around 30 days from harvest for the grapes to reach the consumer's hands in Europe. Therefore, this cultivar can be easily cold-stored in clamshells with a combination of SO_2 pads and liners. Even in the case of price fluctuations or especially for long-distance export marketing where the grapes are in an ocean transport for long periods, this grape cultivar can be maintained for around 50 days in cold storage with SO_2-generating pads and no quality-loss problems. The continual dose of SO_2 in these pads is concentrated enough to inhibit any latent or inherent *B. cinerea* spores from growing, and also to ensure low sulfite residues in the table grapes.

The quality of grape berries is the prime consideration in both domestic and export markets. Grape berries grow as bunches; each one is connected to the bunch stem. The bunch of 'Italia' grapes was adequate with respect to visual appearance, quality, flavor, color, firmness, and shape of the berries because of the limited water loss and absence of stem browning. These are important quality characteristics that comprise both bunch as well as berry properties, and these include phytosanitary

conditions, uniform bunch color, bunch mass, size and shape, stem quality, berry size and shape, firmness, and SS and TA content [27].

In addition, bleaching, hairline cracking (almost invisible, small, fine, longitudinal, cracking lines), berry softening, and berry cracking also did not occur in this cultivar when it was treated with the different SO_2 pads. These are the main limitations for long-term storage of the grapes and not only affect the quality of the produce but also consumer quality perception [28].

The bunch stem is an important part of the grape, as it does not just add to the general look but also provides a support for the customer while eating the grapes. When the grapes are maintained under cold storage, water loss is observed, and as a result the stem starts to change color from green to brown, affecting grape quality. However, in the case of 'Italia' table grapes, stem browning remained green (score of 1) at 50 days of cold storage, and the score changed to 2 (light browning) at 7 days of storage at room temperature. Stem browning is related to water loss, and bunches showing severe stem-browning symptoms lose more water than the bunches with significant stem-browning symptoms [29].

For long-term storage, new techniques should be developed for packing grapes; water loss as well as deterioration problems when the concentration of SO_2 is too low or too high should be reduced [30]. For this purpose, plastic clamshell punnets (perforated) were used in this study, and each has the capacity for one grape bunch inside. The grape bunches were placed in these clamshells, which were then placed in carton boxes. Even though the grapes were packed in the clamshells, air circulation was sufficient for continuous SO_2 diffusion. The objective of using these packaging materials was to retain grape quality as well as furnish a shelter that can reduce mechanical damage, water loss, and gray mold incidence [31].

The use of dual release pads was effective in controlling the incidence of gray mold in other grapes, such as 'BRS Vitoria' [32]. This cultivar is sold in both domestic and international markets, and this method prevented decay caused by *B. cinerea* for more than 45 days. Dual release SO_2-generating pads in polyethylene with macro-perforations can be used for a period of four months or even longer for 'Red Globe' grapes, without any quality loss or infection [15].

5. Conclusions

The dual SO_2 pads were highly efficient in preventing the incidence of gray mold in 'Italia' grapes packed in clamshells during a 50-day period of cold storage and then 7 days at room temperature, even when inoculated with *Botrytis*. The slow release SO_2 pad showed a lower efficiency than the dual pads, but was better than the control. The SO_2 dual release pad treatments provided the best results with respect to the stem browning scores (fresh and green stems) during cold storage, and no differences in physicochemical quality parameters were observed among the treatments.

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References

1. Kishino, A.Y.; Carvalho, S.L.C.; Roberto, S.R. *Viticultura Tropical: O Sistema de Produção do Paraná*; IAPAR: Londrina, Brazil, 2007; pp. 117–140. ISBN 978-85-881-8428-2.
2. Smilanick, J.L.; Brown, G.E.; Eckert, J.W. The biology and control of post-harvest diseases. In *Fresh Citrus Fruits*, 2nd ed.; Wardowski, W.F., Miller, W.M., Hall, D.J., Grierson, W., Eds.; Florida Science Source Inc.: Longboat Key, FL, USA, 2006; pp. 339–396.

3. Ladaniya, M.S. *Citrus Fruit: Biology, Technology and Evaluation*; Academic Press: San Diego, CA, USA; Elsevier Inc.: Atlanta, GA, USA, 2008; pp. 1–10. [CrossRef]
4. Crisosto, C.H.; Mitchell, F.G. Postharvest handling systems: Small fruits (Table grapes). In *Postharvest Technology of Horticulture Crops*; Kader, A., Ed.; University of California, Agriculture and Natural Resources: Oakland, CA, USA, 2002; pp. 357–363.
5. Elad, Y.; Vivier, M.; Fillinger, S. Botrytis: The good, the bad and the ugly. In *Botrytis—The Fungus, the Pathogen and Its Management in Agricultural Systems*; Fillinger, S., Elad, Y., Vivier, M., Eds.; Springer: Heidelberg, Germany, 2015; pp. 1–15. ISBN 978-3-319-23371-0.
6. Dean, R.; Van Kan, J.A.L.; Pretorius, Z.A.; Hammond-Kosack, K.E.; Di Pietro, A.; Spanu, P.D.; Rudd, J.J.; Dickman, M.; Kahmann, R.; Ellis, J.; et al. The Top 10 fungal pathogens in molecular plant pathology. *Mol. Plant Pathol.* **2012**, *13*, 414–430. [CrossRef] [PubMed]
7. Droby, S.; Lichter, A. Post-harvest botrytis infection: Etiology development and management. In *Botrytis: Biology, Pathology and Control*; Elad, Y., Williamson, B., Tudzynski, P., Delen, N., Eds.; Kluwer Academic Publishers: London, UK, 2004; pp. 349–367.
8. Luvisi, D.; Shorey, H.; Smilanick, J.; Thompson, J.; Gump, B.; Knutson, J. *Sulfur Dioxide Fumigation of Table Grapes*; University of California: Oakland, CA, USA, 1932; p. 22.
9. Chervin, C.; Aked, J.; Crisosto, C.H. Grapes. In *Crop Post-Harvest: Science and Technology*; Rees, D., Farrell, G., Orchard, J., Eds.; Blackwell Publishing Ltd.: Oxford, UK, 2012; pp. 187–211.
10. Michailides, T.J.; Elmer, P.A.G. Botrytis gray mold of kiwifruit caused by *Botrytis cinerea* in the United States and New Zealand. *Plant Dis.* **2000**, *84*, 208–223. [CrossRef]
11. Romanazzi, G.; Lichter, A.; Gabler, F.M.; Smilanick, J.L. Recent advances on the use of natural and safe alternatives to conventional methods to control postharvest gray mold of table grapes. *Postharvest Biol. Technol.* **2012**, *63*, 141–147. [CrossRef]
12. Teles, C.S.; Benedetti, B.C.; Gubler, W.D.; Crisosto, C.H. Pre-storage application of high carbon dioxide combined with controlled atmosphere storage as a dual approach to control *Botrytis cinerea* in organic 'Flame Seedless' and 'Crimson Seedless' table grapes. *Postharvest Biol. Technol.* **2014**, *89*, 32–39. [CrossRef]
13. Melgarejo-Flores, B.G.; Ortega-Ramírez, L.A.; Silva-Espinoza, B.A.; González-Aguilar, G.A.; Miranda, M.R.A.; Ayala-Zavala, J.F. Antifungal protection and antioxidant enhancement of table grapes treated with emulsions, vapors, and coatings of cinnamon leaf oil. *Postharvest Biol. Technol.* **2013**, *86*, 321–328. [CrossRef]
14. Mustonen, H.M. The efficacy of a range of sulfur dioxide generating pads against *Botrytis cincerea* infection & on out-turns quality of 'Calmeria' table grapes. *Aust. J. Exp. Agric.* **1992**, *32*, 389–393. [CrossRef]
15. Zutahy, Y.; Lichter, A.; Kaplunov, T.; Lurie, S. Extended storage of 'Red Globe' grapes in modified SO_2 generating pads. *Postharvest Biol. Technol.* **2008**, *50*, 12–17. [CrossRef]
16. Youssef, K.; Roberto, S.R. Applications of salt solutions before and after harvest affect the quality and incidence of postharvest gray mold of 'Italia' table grapes. *Postharvest Biol. Technol.* **2014**, *87*, 95–102. [CrossRef]
17. Mattiuz, B.; Miguel, A.C.A.; Galati, V.C.; Nachtigal, J.C. Efeito da temperatura no armazenamento de uvas apirênicas minimamente processadas. *Revista Brasileira de Fruticultura* **2009**, *31*, 44–52. [CrossRef]
18. Lijavetzky, D.; Carbonell-Bejerano, P.; Grimplet, J.; Bravo, G.; Flores, P.; Fenoll, J.; Hellín, P.; Oliveros, J.C.; Martínez-Zapater, J.M. Berry flesh and skin ripening features in *Vitis vinifera* as assessed by transcriptional profiling. *PLoS ONE* **2012**, *7*, e39547. [CrossRef]
19. Ngcobo, M.E.K.; Delele, M.A.; Opara, U.L.; Meyer, C.J. Performance of multi-packing for table grapes based on airflow, cooling rates and fruit quality. *J. Food Eng.* **2013**, *116*, 613–621. [CrossRef]
20. Mcguire, R.G. Reporting of objective color measurements. *HortScience* **1992**, *27*, 1254–1255.
21. Lancaster, J.E.; Lister, C.; Reay, P.F.; Triggs, C.M. Influence of pigment composition on skin color in a wide range of fruits and vegetables. *Am. Soc. Hortic. Sci.* **1997**, *122*, 594–598.
22. Peppi, M.C.; Fidelibus, M.W.; Dokoozlian, N. Abscisic acid application timing and concentration affect firmness, pigmentation and color of 'Flame Seedless' grapes. *HortScience* **2006**, *41*, 1440–1445.
23. Youssef, K.; Roberto, S.R. Salt strategies to control Botrytis mold of 'Benitaka' table grapes and to maintain fruit quality during storage. *Postharvest Biol. Technol.* **2014**, *95*, 95–102. [CrossRef]
24. Cappellini, R.A.; Ceponis, M.J.; Lightner, G.W. Disorders in table grapes shipments to the New York market, 1972–1984. *Plant Dis.* **1986**, *70*, 1075–1079. [CrossRef]

25. Gubler, W.D.; Marois, J.J.; Bledsoe, A.M.; Bettiga, L.J. Control of Botrytis bunch rot of grape with canopy management. *Plant Dis.* **1987**, *71*, 599–601. [CrossRef]
26. Palou, L.; Crisosto, C.H.; Garner, D.; Basinal, L.M.; Smilanick, J.L.; Zoffoli, J.P. Minimum constant sulfur dioxide emission rates to control gray mold of cold stored table grapes. *Am. J. Enol. Vitic.* **2002**, *52*, 110–115.
27. Harindra, C.W.A. Pre and postharvest practices for quality improvement of table grapes (*Vitis vinifera* L.). *J. Natl. Sci. Found. Sri Lanka* **2015**, *43*, 3–9. [CrossRef]
28. Crisosto, C.H.; Smilanick, J.L.; Dokoozlian, N.K.; Luvisi, D.A. Maintaining table grape post-harvest quality for long distant markets. In Proceedings of the International Symposium on Table Grape Production, Anaheim, CA, USA, 28–29 June 1994; American Society for Enology and Viticulture, University of California: Davis, CA, USA, 1994; pp. 195–199.
29. Crisosto, C.H.; Smilanick, J.L.; Dokoozlian, N.K. Table grapes suffer water loss, stem browning during cooling delays. *Calif. Agric.* **2001**, *55*, 39–42. [CrossRef]
30. Lichter, A.; Zutahy, Y.; Kaplunov, T.; Lurie, S. Evaluation of table grape storage in boxes with sulfur dioxide releasing pads with either an internal plastic liner or external wrap. *HortTechnology* **2008**, *18*, 206–214.
31. Opara, U.L. *From Hand Holes to Vent Holes: What's Next in Innovative Horticultural Packaging?* Stellenbosch University: Stellenbosch, South Africa, 2011; 24p.
32. Youssef, K.; Roberto, S.R.; Chiarotti, F.; Koyama, R.; Hussain, I.; De Souza, R.T. Control of Botrytis mold of the new seedless grape 'BRS Vitoria' during cold storage. *Sci. Hortic.* **2015**, *193*, 316–321. [CrossRef]