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Article

Active Packaging Systems to Extend the Shelf Life of 'Italia' Table Grapes

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Abstract: Sulfur dioxide (SO_2)-generating pads associated with perforated plastic liners are often used to control gray mold in table grapes during cold storage; however, SO_2 may cause bleaching, shattered berries, and an unwanted taste. To overcome this difficulty, a field ultrafast SO_2-generating pad was designed to be used for a few hours before packaging grape bunches as an alternative for eradicating spores of fungi from berry skin. This study aimed to assess the postharvest conservation and shelf life of 'Italia' table grapes packaged in plastic clamshells and perforated plastic liners using the field ultra-fast SO_2-generating pads before packaging, associated with or without slow- or dual-phase SO_2-generating pads during cold storage. The packaged grapes were cold stored (1.0 ± 1.0 °C; 95% relative humidity), and after 45 d, grapes were placed at room temperature (22.0 ± 1.0 °C) without plastic liners and SO_2-generating pads for 3 d. Before and after the grapes had been subjected to the field ultra-fast SO_2-generating pads, the quantification of filamentous fungi on the surface of the berries was assessed. The use of field ultra-fast SO_2-generating pads before packaging, associated with slow- or dual-phase SO_2-generating pads during cold storage, resulted in a lower incidence of gray mold after 45 d of storage, with low weight loss and shattered berries, good preservation of stem freshness, and no impairment in the color and firmness of the berries. Additionally, a significant decrease in disease incidence was observed when using only the dual-phase SO_2-generating pads in cold storage, with good maintenance of bunch quality.

Keywords: *Botrytis cinerea*; plastic liners; postharvest; sulfur dioxide; *Vitis vinifera* L.

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1. Introduction

In the production system of table grapes, bunches of cultivars that have excellent product performance, prolonged postharvest conservation, and desirable characteristics for consumption, such as sanitation, low shattered berry incidence, fresh and green stems, and characteristic berry color, are the most preferred [1]. Thus, the 'Italia' grape still maintains its position of preference in several regions worldwide.

Consumers increasingly expect grapes to be fresh and maintain their functional properties; however, it is a challenge to meet such market demands because bunches are exposed to injuries during handling, water loss, and pathogen attack. These factors can reduce the quality of the produce, impairing the commercialization of table grapes because the market requires a high-quality fruit.

Table grapes are manually harvested when fully ripe and properly handled to ensure high fruit quality. Injured berries are removed, and in most cases, the bunches are packed right after harvesting on the field or in a nearby facility to avoid further handling. Depending on the growing conditions, when this practice is not feasible, the bunches are transported to a packinghouse and cleaned and packed there. Usually, bunches are stored and shipped in cardboard boxes, and as soon as possible, they are pre-cooled at 0–1 °C to minimize water loss. Depending on the grape cultivar, bunches tolerate storage temperatures as low as −2 °C without significant damage from freezing. To ensure the fast cooling of the grapes, the packaging system, including the cardboard boxes and liners, should provide sufficient air movement over the berries via perforations in the packaging [2,3].

To maintain the postharvest quality of fresh grapes, packing bunches in vented plastic clamshells has become an innovative alternative that meets local and export market needs. This packaging prevents physical contact between the grapes and the external environment, resulting in less damage and extending the shelf life of the fruit [4]. Additionally, it allows the best integrity of the bunches to reach their destination and offers greater attractiveness and practicality to consumers [5,6].

However, *Botrytis cinerea* Pers., the causal agent of gray mold, can cause significant damage even when bunches are individually packed in clamshells and stored in refrigerated chambers. This fungus can remain latent in the field, can only be expressed during transport and storage of the fruit, and can develop at low temperatures (±0.5 °C) [7]. Further, it can be disseminated by consumers during marketing and even after purchase, resulting in bunches with rotten berries, which quickly affect other bunches and the final quality of the product [8,9].

The use of sulfur dioxide (SO_2)-generating pads inside cardboard boxes during cold storage has demonstrated good results in preventing the development of grey mold and prolonging the longevity of the bunches [10,11]. In addition to being efficient in disease control, this technology is easy to use, affordable, and has a lower health risk than fungicides [12]. Due to the delicate grape bunch architecture, unlike other fruits such as mango or oranges, washing the grapes after harvesting with hot water, chemicals, or bio-input solutions is not feasible because they should be dried afterwards to avoid the risk of decay development. Besides, some regulatory aspects should be considered regarding the use of SO_2 to preserve table grapes, like those established by Regulation (EC) No 1333/2008 of the European Parliament and of the council on food additives, which pose maximum permitted levels (MPLs) for sulfur-dioxide sulfites of 10 $mg \cdot L^{-1}$. Moreover, some countries have specific regulations. For instance, in Italy, table grapes cannot be treated with SO_2 for sale in local markets; it can only be done if the product is destined for foreign countries.

There are two main commercial types of SO_2-generating pads, slow (and continuous) release of gas and dual-phase (fast and slow release) [1]. The pad type must be chosen carefully because high concentrations of gas and/or residues can cause stem browning and affect the sensory characteristics of grapes, in addition to being harmful to humans and the environment [13,14].

To improve the efficacy of SO_2-generating pads during cold storage, grapes are packaged in perforated plastic liners composed of low-density polyethylene, which, when used correctly, facilitates the circulation and removal of gas in the package and prevents fruit weight loss [15,16]. However, even with this active packaging system, depending on the conditions under which the grapes are grown, symptoms of gray mold can still appear on berries, particularly when the grapes are stored for long periods in refrigerated chambers.

To overcome this difficulty, a field ultrafast SO_2-generating pad was designed to be used for a few hours before packing the bunches as an alternative for eradicating spores of fungi from the berry skin. This technology aims to further reduce the risk of gray mold because the field ultrafast SO_2-generating pad has an eradicating effect on *B. cinerea* spores and can enhance the effect of SO_2-generating pads used in packing grapes during cold storage, if necessary. Besides, this new type of SO_2-releasing pad does not lead to any safety risks to the consumption of grapes because of the low residual level of sulfur dioxide [17,18].

However, the effect of using field ultrafast SO_2-generating pads before packaging on the control of gray mold in table grapes, whether combined with different types of SO_2-generating pads used during cold storage or not, remains unknown. This study aimed to assess the impact of different packaging materials on the conservation and shelf life of 'Italia' table grapes packaged in plastic clamshells and perforated plastic liners using field ultra-fast SO_2-generating pads before packaging, associated with slow or dual-phase SO_2-generating pads during cold storage or not.

2. Materials and Methods

2.1. Location

Fully ripened bunches of 'Italia' grapes (*Vitis vinifera* L.) were harvested in a 10-year-old commercial vineyard where the vines were grafted onto the 'IAC-766 Campinas' rootstock, trained in an overhead trellis system covered with a black plastic mash (18% of shading), located in the municipality of Cambira, Paraná, Brazil (23°34′58″ S, 51°34′40″ W, elevation of 1.017 m a.s.l.). The climate of the region, according to the classification proposed by Köppen, was Cfa, subtropical, with a mean annual precipitation of 1633.5 mm, minimum mean temperature of 18 °C, and a maximum temperature of 22 °C [19]. The 'Italia' grapes were harvested in two consecutive seasons, 2019 and 2020. This area was selected because of the recurrent history of gray mold. In the field, grapes were subjected to treatments against gray mold during flowering stage by applying dicarboximide iprodione. In order to make the initial distribution of *Botrytis cinerae* homogeneous or avoid unpredicted differences between tests, grapes were harvested from several vines of the vineyard and then mixed up before applying the treatments.

2.2. Packaging Materials and Experimental Design

Treatments included the association of different packaging systems to improve the postharvest conservation and shelf life of 'Italia' grapes as follows: (a) control; (b) field ultrafast SO_2-generating pad (FieldSO_2) before packaging; (c) slow phase SO_2-generating pad (SlowSO_2) during cold storage; (d) dual-phase (fast and slow phases) SO_2-generating pad (DualSO_2) during cold storage; (e) FieldSO_2 before packaging associated with SlowSO_2 during cold storage; and (f) FieldSO_2 before packaging associated with DualSO_2 during cold storage. The cold storage was adjusted to 1.0 ± 1.0 °C and 95% of relative humidity.

The FieldSO_2 (1.4 g of active ingredient-AI, sodium metabisulphite), SlowSO_2 (4 g of AI), DualSO_2 (5 g of AI) (Uvas Quality Grape Guard®), and perforated plastic liners (0.3% of ventilation area) were supplied by Suragra S.A., San Bernardo, Chile. The size of all tested SO_2-generating pads was 46 × 26 cm.

Clamshells containing grapes were packed in cardboard boxes lined with perforated plastic liners for all treatments. No SO_2-generating pads were used in the control treatment; only perforated plastic liners with 0.3% of the ventilation area (VA) were used. A completely randomized design was used as the statistical model with four replicates. Each plot consisted of a corrugated cardboard box containing 10 bunches individually packaged in clamshells.

2.3. Grape Packaging

The bunches subjected to treatments with FieldSO_2 before packaging were placed in plastic harvest boxes with a capacity of 20 kg, previously lined with perforated plastic liners containing 0.3% VA, according to Dantas et al. [17]. FieldSO_2 was placed over the grapes and the liners were sealed, leaving the bunches exposed to this condition for 5 h. During this period, the emission of SO_2 from FieldSO_2 was quantified using a dosimeter (Gastec Passive Dosi-Tube, Kanagawa, Japan). Readings were recorded at 160, 410, and 600 ppm after 1, 2, and 3 h, respectively. After treatments containing only slow or dual SO_2-generating pads (SlowSO_2 or DualSO_2), the bunches were placed directly on cardboard boxes. After 5 h, the plastic liners in the boxes containing field FieldSO_2 were opened. Following this, all harvested bunches were cleaned by removing the damaged berries, standardized to approximately 0.5 kg, and individually packaged in vented plastic clamshells with a capacity of 0.5 kg and dimension of 20 × 10 cm each.

The packaging steps of grapes before cold storage were performed as follows: corrugated cardboard boxes measuring 60 × 40 × 10 cm with a storage capacity for 10 vented plastic clamshells were internally lined with a perforated plastic liner of 0.3% VA. A moisture-absorbing single-layer pad paper measuring 37 × 28 cm was placed above the plastic liner. Plastic clamshells containing grape bunches subjected to FieldSO_2 were arranged in a cardboard box, and an SO_2-generating pad (SlowSO_2 or DualSO_2) was placed on top according to the treatment. Finally, the liner was sealed with an adhesive tape [3].

The cardboard boxes were kept under cold storage (1.0 ± 1.0 °C and 95% relative humidity) for 45 d. After this period, the cardboard boxes were removed from cold storage and kept at room temperature (22.0 ± 1.0 °C) for 3 d without perforated plastic liners and the SO_2-generating pads to simulate a supermarket shelf condition.

2.4. Grape Bunch Characteristics

Before and after treatment with FieldSO_2 before packaging, the quantification of filamentous fungi was performed on the skin surface of berries (log CFU/berry). After 45 d of cold storage and 3 d at room temperature, the incidence of gray mold (%) and quantification of filamentous fungi on the skin surface of berries were analyzed. After 45 d of cold storage, the following variables were analyzed: weight loss (%), berry firmness (N), stem browning, berry color index (CIRG), and percentage of shattered berries (%).

2.5. Gray Mold Incidence and Quantification of Filamentous Fungi on Berry Skin

The incidence of gray mold was calculated using the following formula: Incidence (%) = (number of diseased berries/total number of berries in the bunch) × 100 [20].

The epiphytic populations of filamentous fungi present on the skin surface of berries were quantified as described by Youssef and Roberto [21]. Ten berries from each plot were placed in 100 mL of sterile distilled water on a rotary shaker at 150 rpm for 30 min. Using a micropipette, 0.1 mL of the suspension was inoculated per plate into potato dextrose agar (PDA) containing ampicillin and streptomycin sulfate (250 $mg \cdot L^{-1}$ of each antibiotic). The inoculated plates were incubated at 24 °C, and after four days, the number of colonies was counted and expressed as log CFU/berry.

2.6. Physical Properties of Berries

Weight loss was determined by weighing the bunch at the beginning of cold storage and 45 d later using the following formula: Weight loss (%) = [(initial weight − weight at the time evaluated)/initial weight] × 100 [20].

The determination of firmness of the berries consisted of evaluating 10 berries per plot using the TA.XT Plus® Texture Analyzer (Stable Micro Systems, Surrey, UK), in which each berry was compressed on its equatorial axis by a cylindrical probe (35 mm diameter, P35), with a constant force of 0.05 N at a speed of 1.0 mm s^{-1}. Firmness was determined as the force required (N) to deform the berry by 20% of its equatorial diameter [22].

Stem browning was evaluated through visual evaluation, according to the methodology described by Ngcobo, Delele, Opara, & Meyer [14], assigning scores based on the level of browning: 1 (fresh and green); 2 (light browning); 3 (significant browning); and 4 (severe browning).

The shattered berries were evaluated by counting the loose berries inside each clamshell using the following formula: Shattered berries (%) = (number of loose berries/total number of berries in the bunch) × 100 [21].

To determine the skin color attributes of berries, 10 berries were collected per plot. Readings were performed with a CR-10 Plus® colorimeter (Konica Minolta, Tokyo, Japan) to obtain L^* (brightness), C^* (chroma), and h° (hue). From these variables, the berry color index (CIRG) was calculated using the following formula: CIRG = $(180 - h^\circ)/(L^* + C^*)$ [23].

2.7. Statistical Analysis

The percentage data were expressed as log or $\sqrt{x+1}$ and then subjected to analysis of variance using Statistica 6.0® software (Statsoft, Hamburg, Germany). Treatment means were compared using Fisher's least significant difference (LSD) test at a 5% probability level.

3. Results

In both seasons, bunches of 'Italia' table grapes that were subjected to treatments with field ultrafast SO_2-generating pads (FieldSO_2) before packaging as well as those with slow release or dual-phase (SlowSO_2 or DualSO_2) during cold storage, whether associated or not, had the lowest gray mold incidence compared to the control treatment, which contained only the perforated plastic liner, indicating that SO_2 was effective in controlling gray mold (Table 1).

Table 1. Gray mold incidence of 'Italia' table grapes at 45 d of cold storage (1.0 ± 1.0 °C) and at 3 d at room temperature (22.0 ± 1.0 °C), individually packaged in plastic clamshells. Grapes were subjected to field ultra-fast SO_2-generating pads before packaging, associated or not with slow and dual-phase SO_2-generating pads during cold storage. Seasons of 2019 and 2020.

Treatments	**Gray Mold Incidence (% of Diseased Berries)**			
	At 45 d of Cold Storage		**At 3 d of Room Temperature**	
	2019	**2020**	**2019**	**2020**
Control [a]	15.00 a	6.73 a	19.75 a	8.72 a
FieldSO_2	4.44 b	1.12 b	4,87 c	2.70 b
SlowSO_2	3.29 bc	1.48 b	7.43 b	3.27 b
DualSO_2	4.25 b	0.61 bc	5.26 c	1.38 c
FieldSO_2 + SlowSO_2	2.20 c	0.10 c	2.97 d	0.26 c
FieldSO_2 + DualSO_2	0.00 d	0.00 c	1.80 d	0.31 c

[a] Without SO_2-generating pads, just a perforated plastic liner. Means followed by the same letters within columns are not different according to the LSD Fisher's test ($p \leq 0.05$). FieldSO_2: field ultra-fast SO_2-generating pad before packaging; SlowSO_2 and DualSO_2: slow and dual-phase SO_2-generating pads, respectively, during cold storage.

At 45 d of cold storage, in the two seasons evaluated, the lowest incidence of gray mold on 'Italia' grape berries was observed when FieldSO_2 was used before packaging associated with SlowSO_2 or DualSO_2 during cold storage, and no symptoms of the disease were observed when DualSO_2 was used in combination (Table 1). After 3 d of storage at room temperature in 2019, the best gray mold control was obtained when the association of SO_2-generating pads before packaging was used with those during cold storage, which was similar to the 2020 season, where the isolated use of DualSO_2 had high efficacy.

In all cases, the use of only FieldSO_2 before packaging resulted in lower disease control efficacy when compared with its use in combination with SlowSO_2 or DualSO_2 during cold storage, and the highest incidence of the disease was found in the control; at the end of the experiment, abundant gray sporulation covered part of the surface of the berries (Figure 1). These results are similar to those of the evaluation of the population of filamentous fungi on the surface of 'Italia' table grape berries, where FieldSO_2 associated with SlowSO_2 or DualSO_2 had higher efficacy (Figures 2 and 3).

The treatments FieldSO_2 + SlowSO_2 and FieldSO_2 + DualSO_2 exhibited different levels of control between the 2019 and 2020 seasons. Depending on the climate conditions of each season including temperature, precipitation, and air humidity, as well as the field management used, including the type and the number of fungicide applications, the development of Botrytis on the berry surface can vary notably, which explains the lower efficacy of these treatments to eradicate the filamentous fungi spores during the 2019 season compared with the 2020 season (Figure 3). However, both of these treatments led to the lower incidence of gray mold during the cold storage of 'Italia' grapes (Table 1).

Figure 1. 'Italia' grape bunches subjected to field ultra-fast SO_2-generating pads before packaging, associated or not with slow and dual-phase SO_2-generating pads during cold storage, at 3 d of room temperature (22.0 ± 1.0 °C) after being cold stored for 45 d (1.0 ± 1.0 °C), individually packaged in vented clamshells. (**A**): Control (without SO_2-generating pads, just a perforated plastic liner); (**B**): FieldSO_2; (**C**): SlowSO_2; (**D**): DualSO_2; (**E**): FieldSO_2 + SlowSO_2; (**F**): FieldSO_2 + DualSO_2. FieldSO_2: field ultra-fast SO_2-generating pad before packaging; SlowSO_2 and DualSO_2: slow and dual-phase SO_2-generating pads, respectively, during cold storage.

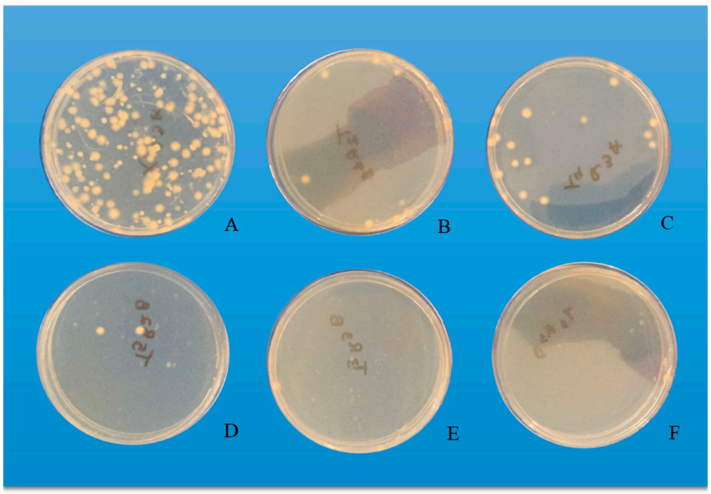

Figure 2. Growth of filamentous fungi colonies in PDA medium recovered from berry skin of 'Italia' grapes subjected to field ultra-fast SO_2-generating pads before packaging, associated or not with slow and dual-phase SO_2-generating pads during cold storage, at 3 d of room temperature (22.0 ± 1.0 °C) after being cold stored for 45 d (1.0 ± 1.0 °C). Season of 2020. (**A**): Control (without SO_2-generating pads, just a perforated plastic liner); (**B**): FieldSO_2; (**C**): SlowSO_2; (**D**): DualSO_2; (**E**): FieldSO_2 + SlowSO_2; (**F**): FieldSO_2 + DualSO_2. FieldSO_2: field ultra-fast SO_2-generating pad before packaging; SlowSO_2 and DualSO_2: slow and dual-phase SO_2-generating pads, respectively, during cold storage.

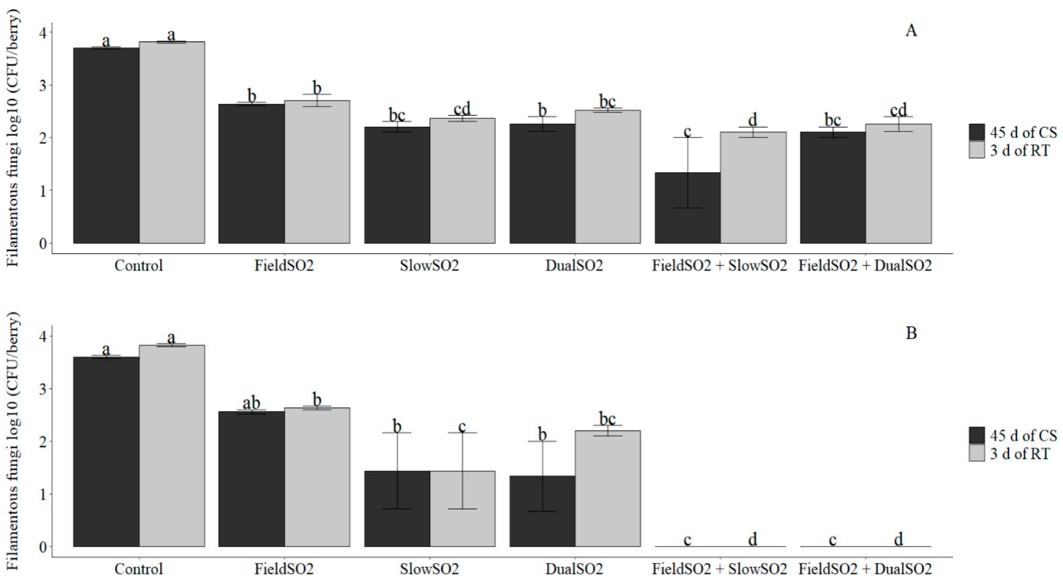

Figure 3. Filamentous fungi population recovered from berry skin of 'Italia' grapes subjected to field ultra-fast SO_2-generating pads before packaging, associated or not with slow and dual-phase SO_2-generating pads during cold storage, at 3 d of room temperature (22.0 ± 1.0 °C) after being cold stored for 45 d (1.0 ± 1.0 °C). Means followed by the same letters in the same storage periods do not differ by LSD Fisher's test ($p \leq 0.05$). (**A**): Season of 2019; (**B**): Season of 2020. Control (without SO_2-generating pads, just a perforated plastic liner); FieldSO_2: field ultra-fast SO_2-generating pad before packaging; SlowSO_2 and DualSO_2: slow and dual-phase SO_2-generating pads, respectively, during cold storage. CFU: colony forming unit; CS: cold storage; RT: room temperature.

The lowest weight loss in the 2019 season was observed when the grapes were subjected to treatment with SlowSO_2 during cold storage, whereas there was no difference among the treatments in the 2020 season. Regarding stem browning scores, no differences were found among the treatments during the 2019 season. However, in the 2020 season, the treatments containing SO_2-generating pads had the lowest scores compared with the control (Table 2).

Table 2. Weight loss and stem browning of 'Italia' table grapes at 45 d of cold storage (1.0 ± 1.0 °C) and at 3 d at room temperature (22.0 ± 1.0 °C), individually packaged in plastic clamshells. Grapes were subjected to field ultra-fast SO_2-generating pads before packaging, associated or not with slow and dual-phase SO_2-generating pads during cold storage. Seasons of 2019 and 2020.

Treatments	Weight Loss (%)		Stem Browing [b]	
	2019	2020	2019	2020
Control [a]	2.53 ab	3.70 a	1.00 a	1.33 a
FieldSO_2	2.88 a	4.03 a	1.00 a	1.13 b
SlowSO_2	2.21 b	4.23 a	1.00 a	1.15 b
DualSO_2	2.79 a	3.83 a	1.00 a	1.13 b
FieldSO_2 + SlowSO_2	2.64 a	4.17 a	1.00 a	1.05 b
FieldSO_2 + DualSO_2	2.77 a	4.11 a	1.00 a	1.08 b

[a] Without SO_2-generating pads, just a perforated plastic liner. Means followed by the same letters within columns are not different according to the LSD Fisher's test ($p \leq 0.05$). FieldSO_2: field ultra-fast SO_2-generating pad before packaging; SlowSO_2 and DualSO_2: slow and dual-phase SO_2-generating pads, respectively, during cold storage. [b] Stem browning scoring: (1) fresh and green; (2) light browning; (3) significant browning; and (4) severe browning [13].

In both seasons, the control treatment resulted in the highest number of shattered berries. The isolated use of FieldSO_2 before packaging did not result in loose berries in

2019. During the 2020 season, this treatment demonstrated efficacy in combination with SlowSO_2 during cold storage (Table 3).

Table 3. Shattered berries, firmness, and color index of 'Italia' table grapes at 45 d of cold storage (1.0 ± 1.0 °C) and at 3 d at room temperature (22.0 ± 1.0 °C), individually packaged in plastic clamshells. Grapes were subjected to field ultra-fast SO_2-generating pads before packaging, associated or not with slow and dual-phase SO_2-generating pads during cold storage. Seasons of 2019 and 2020.

Treatments	Shattered Berries (%)		Berry Firmness (N)		Berry Color Index (CIRG)	
	2019	2020	2019	2020	2019	2020
Control [a]	0.38 a	1.50 a	7.90 ab	8.82 ab	1.64 a	1.60 a
FieldSO_2	0.00 b	0.95 bc	7.57 ab	9.20 a	1.55 b	1.70 a
SlowSO_2	0.18 ab	1.10 ab	7.68 ab	8.61 b	1.55 b	1.70 a
DualSO_2	0.13 ab	0.90 bc	8.12 a	8.88 ab	1.62 ab	1.60 a
FieldSO_2 + SlowSO_2	0.13 ab	0.43 c	7.52 b	9.18 a	1.63 a	1.70 a
FieldSO_2 + DualSO_2	0.18 ab	0.68 bc	7.81 ab	8.92 ab	1.59 ab	1.60 a

[a] Without SO_2-generating pads, just a perforated plastic liner. Means followed by the same letters within columns are not different according to the LSD Fisher's test ($p \leq 0.05$). FieldSO_2: field ultra-fast SO_2-generating pad before packaging; SlowSO_2 and DualSO_2: slow and dual-phase SO_2-generating pads, respectively, during cold storage.

The highest mean berry firmness in the 2019 season was found in bunches subjected to treatment DualSO_2 during cold storage, whereas the lowest mean was observed in grapes subjected to FieldSO_2 before packaging and SlowSO_2 during cold storage. In the 2020 season, the highest means were found when FieldSO_2 before packaging was used alone and in combination with SlowSO_2. However, the lowest mean berry firmness was observed in the latter treatment (Table 3).

When used in isolation, FieldSO_2 before packaging and SlowSO_2 during cold storage resulted in a lower color index for berries in the 2019 season. When these treatments were used together, they resulted in the highest mean and control treatment. However, the color index of the berries found for all treatments was quite similar, ranging from 1.55 to 1.64, i.e., green-yellow berries according to the classification of Carreño et al. [23]. There were no differences in this characteristic among the treatments during the 2020 season (Table 3).

4. Discussion

The objective of this study was to assess the impact of different packaging systems on the conservation and shelf life of 'Italia' table grapes packaged in plastic clamshells and perforated plastic liners using ultra-fast SO_2-generating pads (FieldSO_2) before packaging, with or without slow- or dual-phase SO_2-generating pads (SlowSO_2 or DualSO_2) during cold storage.

Gray mold was more efficiently controlled in both seasons (2019 and 2020) when 'Italia' grapes were subjected to FieldSO_2 before packaging associated with SlowSO_2 or DualSO_2 during cold storage because of the different forms of gas released and amount of AI in the SO_2-generating pads. Fresh bunches exposed to a concentration of 1.4 g of AI, released by FieldSO_2 before packaging for approximately 5 h, were able to kill active spores of *B. cinerea*, allowing their eradication from the surface of the berries and sanitizing the bunches before they were packaged in the packinghouse (Figure 4) [17,18].

The eradicating effect of FieldSO_2 before packaging is associated with continuous gas release (4 g of AI) by SlowSO_2 during cold storage, maintaining a low incidence of gray mold. However, when FieldSO_2 was used in combination with DualSO_2 during cold storage, fungal development was inhibited for up to 45 d of cold storage in both seasons. The higher amount of SO_2 released in the first 48 h (fast phase) during cold storage (1 g of AI) eradicated spores that FieldSO_2 had not previously eliminated. Combining the fast phase with 4 g of AI released slowly and continuously resulted in the best control of gray mold, as observed previously [1,24,25].

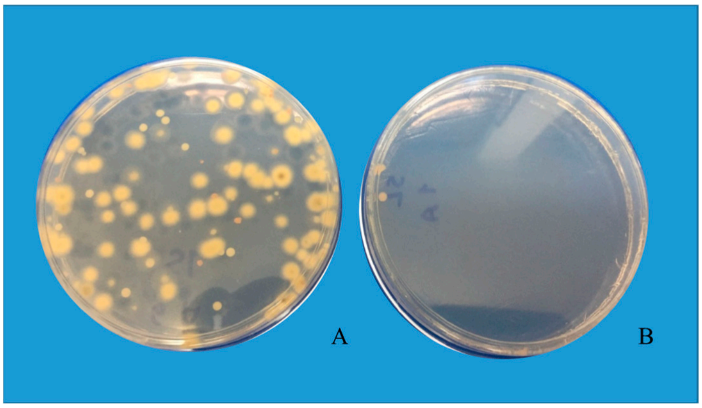

Figure 4. Growth of filamentous fungi colonies in PDA medium recovered from berry skin of 'Italia' table grape, before (**A**) and after (**B**) bunches being subjected to the treatment with the field ultra-fast SO_2-generating pad before packaging. Season of 2020.

The highest concentration of AI of FieldSO_2, associated with DualSO_2 (1.4 g and 5.0 g of AI, respectively), did not prevent the development of disease symptoms in berries after 3 d at room temperature, probably because the residual effect of SO_2 was insufficient to suppress fungal growth at room temperature. Youssef et al. [11] reported that table grapes subjected to a dual-phase SO_2-generating pad containing 8 g of AI showed a complete absence of gray mold after 3 and 6 d of storage at room temperature, respectively, and after cold storage for 45 d. In these cases, the form of gas release associated with a higher concentration of AI was decisive in keeping the bunches free of the disease. The AI concentration must ensure a regular supply of SO_2 until the end of the storage period as it does not penetrate the epicarp. Grapes must be continuously exposed to the gas so that the disease is controlled by periodically killing the mycelial growth of the fungus [26,27].

As 'Italia' table grape bunches were packaged in vented clamshells, SO_2 was in contact with the fruit longer, extending the protective effect. This packaging, despite being considered a physical barrier to the uniform circulation of SO_2, allowed the maintenance of a suitable environment for the action and retention of SO_2 around the bunches, which was confirmed by the low incidence of disease, mainly when FieldSO_2 before packaging was associated with the SO_2-generating pads during cold storage.

When grapes were subjected only to FieldSO_2 before packaging, the amount of gas released was insufficient to control the development of the fungus throughout the storage period, especially in the presence of a higher inoculum content, as in the 2019 season. Therefore, this study demonstrates that the association of both types of SO_2-generating pads provides better control of gray mold because, after the latency phase, the growth of microorganisms on the surface of the berries becomes exponential [28].

No differences were observed between the SlowSO_2 and DualSO_2 treatments until day 45 of cold storage. However, after 3 d at room temperature, the DualSO_2 treatment had a better efficacy in controlling gray mold, mainly in the presence of a lower inoculum (season of 2020), which can be attributed to its eradicating effect in the first 48 h of storage [25]. The SlowSO_2 treatment, owing to its release form associated with the low concentration of AI, compromised the initial control of fungal development.

In this study, it was demonstrated that the use of active packaging system SO_2-generating pads could be used in combination with 'Italia' table grapes to better control gray mold, as this cultivar responded well to higher concentrations of SO_2 released mainly by the field ultra-fast pad before packaging. In excess, this gas can cause damage to grapes, such as bleaching, early stem browning, shattered berries, and unwanted taste [29]. These factors, in addition to compromising the quality of the produce, also reduce the postharvest period of table grapes during storage and transport, and therefore, they must be considered while evaluating new packaging techniques or materials [29,30].

Grapes in treatments containing SO_2 had lower stem browning scores than those in the control treatment, but the bunched stems remained fresh and green in all treatments. 'Italia' table grapes have slightly dense bunches, making them resistant to shattered berries and stem browning [31]; however, it is difficult to spray fungicides uniformly, thus favoring the growth of fungi or germination of latent spores inside the bunches. The appearance of table grapes is seriously compromised when the stem becomes dry and brown [32]. SO_2, owing to its ability to bind enzymes responsible for browning, influences the physiological processes of the fruit, such as maintaining the green color of the bunch and its freshness [33], as previously reported [11,34].

The control treatment had the highest number of shattered berries in both seasons because of the higher incidence of gray mold, which softened the berries and consequently favored a greater occurrence of loose berries [35,36]. However, the incidence of shattered berries was considered low and acceptable because 'Italia' is a seeded grape with berries firmly attached to the pedicels, thus providing greater resistance to this condition.

'Italia' grapes have large berries, reaching 15 g, which confer greater resistance to postharvest losses. For this reason, although significant differences were observed in weight loss (2019 season) and berry firmness, they did not compromise fruit quality attributes, such as shriveling, which is associated with weight loss in fresh products. The perforated plastic liner maintains a high relative humidity inside the packaging, thus reducing the transpiration rate and weight loss [37,38]; however, higher relative humidity favors the development of pathogens that promote fruit deterioration. Therefore, the use of perforated plastic liners in association with SO_2-generating pads is recommended to reduce water loss during the postharvest handling of table grapes [39].

The amount of SO_2 released by the different pads (SlowSO_2 and DualSO_2) was adequate for good postharvest conservation of 'Italia' table grapes, as they could reduce the incidence of gray mold without side effects. Similar results have been reported for other table grapes such as 'BRS Isis', 'BRS Nubia', and 'BRS Vitoria' [10,11]. Thus, these pad treatments still give satisfactory and comparable results to control gray mold in table grapes during the cold storage period from a commercial point of view. However, in situations of a recurrent history of the disease in a given area, the combined use of these pads with a FieldSO_2 pad before packaging may provide a safer environment for a longer preservation of the grapes.

5. Conclusions

Controlling the gray mold in table grapes is challenging, and new technologies are required to meet consumer requirements. The proposed active packaging system associated with an easy and affordable SO_2 pretreatment extended the shelf life of 'Italia' table grapes. It is a promising technology for grapes because it controls gray mold, weight loss, and stem browning and maintains the color and firmness of the berries.

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