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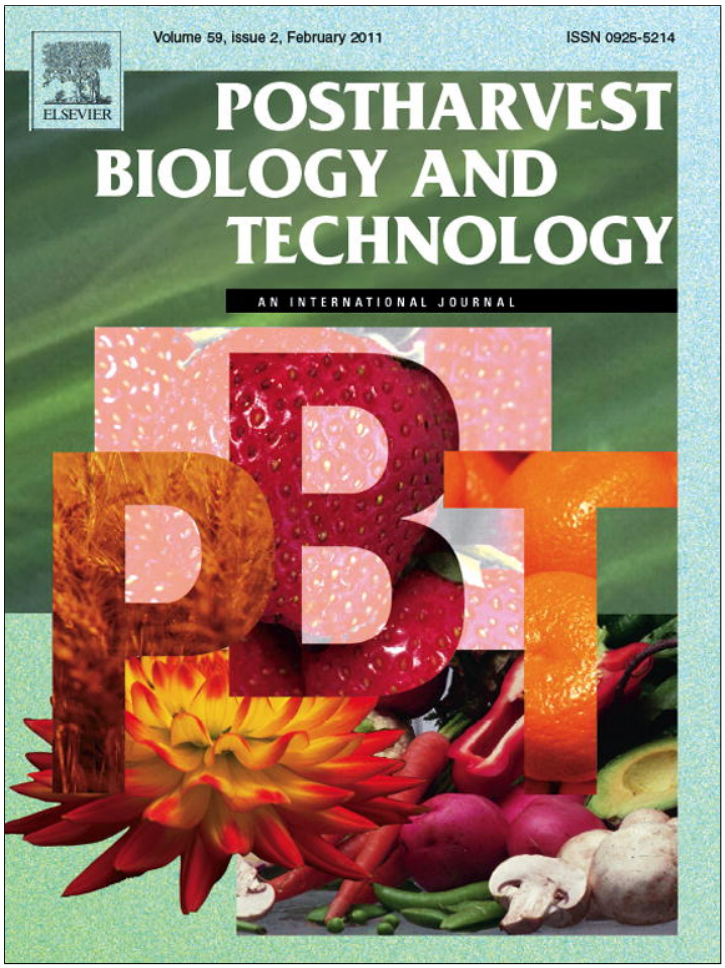

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Evaluation of the use of sulfur dioxide to reduce postharvest losses on dark and green figs

Celia M. Cantín[a,*], Lluís Palou[b], Vanessa Bremer[a], Themis J. Michailides[c], Carlos H. Crisosto[a]

[a] Department of Plant Sciences, University of California, Davis, One Shields Avenue, 95616 Davis, CA, United States
[b] Centre de Tecnología Postcollita, Institut Valencià d'Investigacions Agràries (IVIA), Apartat Oficial, 46113 Montcada, València, Spain
[c] Department of Plant Pathology, University of California, Davis, One Shields Avenue, 95616 Davis, CA, United States

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ABSTRACT

Postharvest diseases limit the storage period and market life of fresh figs (*Ficus carica* L.). The objective of this work was to determine the effect of sulfur dioxide (SO_2) applied by fumigation and/or by dual release SO_2 generating pads on postharvest decay and quality retention of 'Black Mission' and 'Brown Turkey' (dark skin), and 'Kadota' and 'Sierra' (green skin) figs. A protocol for the computer-controlled application of gaseous SO_2 has been developed which allows the application of very low specific concentration × time products of SO_2 and simultaneous monitoring of the application progress. In vitro tests with important fungal, yeast and bacterial postharvest pathogens plated on Petri dishes and exposed to a SO_2 concentration × time product (C × t) of 100 (μL/L) h at different temperatures showed fewer survived at 20 °C than at 0 °C. Therefore, fumigations were carried out at 20 °C in the rest of the experiments. The evaluation of different SO_2 concentration × time products showed that a product of 25 (μL/L) h provided the best compromise between decay control and fruit injury. The performance of SO_2 fumigations on warm or cold fruit, its combination with SO_2 generating pads, and the use of repeated fumigations during cold storage were also evaluated. All the SO_2 treatments tested reduced the percentage of decay, extending the market life of fresh figs. However, in some cases, the use of SO_2 generating pads increased the incidence of skin bleaching. Fumigation of warm fruit at 25 (μL/L) h of SO_2 reduced populations of *Alternaria* and *Rhizopus* spp. growing on the fig surface. The treatment was more effective against *Rhizopus* spp. than against *Alternaria* spp. Contamination of fruit by *Botrytis* spp. and *Penicillium* spp. was also reduced by SO_2. In conclusion, results showed that SO_2 can be a potential tool to control postharvest rots and therefore increase the market life of fresh figs.

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1. Introduction

The fig (*Ficus carica* L.), a member of the Moraceae family, is a pear-shaped infructescence called syconium that is widely marketed and consumed as fresh or dried. Although fig acreage in California declined in the past, the discovery of figs as a nutritious and nutrient-dense food has reverse this trend and sales are estimated to have grown 30% each year for the past five years (Ozkaya et al., 2008; California Fig Advisory Board, 2009). The bulk of fig production is marketed as a dried fruit because fresh figs have a short postharvest life (7–10 d). Figs are very perishable at room temperature, showing early senescence, fermentation, and decay that limits their storage period and marketing life (Karabulut et al., 2009). Fungal decay of figs can result in extensive losses for the grower (Michailides et al., 2008). The five major fruit diseases affecting figs are smut (caused in dried fruit by *Aspergillus niger* and closely related species), Alternaria rot (caused by *Alternaria alternata* or other *Alternaria* spp. and often associated with other fungi such as *Cladosporium herbarum* or *Ulocladium atrum*), gray mold or Botrytis rot (caused by *Botrytis cinerea*), fig endosepsis (caused by *Fusarium moniliforme* and other *Fusarium* spp.) and sour rot or souring (caused by various yeasts and bacteria including species in the genus *Hanseniaspora, Saccharomyces, Pichia* or *Bacillus*) (Doster et al., 1996; Doster and Michailides, 2007; Coviello et al., 2009). Many other fungi, especially in the genus *Aspergillus*, also decay figs although much less frequently (Doster et al., 1996). Of special importance are the species *Aspergillus flavus* and *A. parasiticus*, which can produce the mycotoxins known as aflatoxins (Doster and Michailides, 2007; Coviello et al., 2009). The most sensitive part of figs to fungal decay is the ostiole. The natural opening of fig fruit, the ostiole, serves as the avenue through which these fungal pathogens reach the internal cavity of fruit. In other instances, insects such as the fig pollinator, nitidulid beetles and thrips can carry the spores of these fungi to the inner tissues of the cavity. In addition, propag-

* Corresponding author. Tel.: +1 530 752 6474.
E-mail address: ccantin@ucdavis.edu (C.M. Cantín).

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ules of various fungi can reach the surface of the fruit and cause latent infections or reside as spore load on the surface. Any water-based treatment by dipping or spraying after harvest may leave free water in the ostiole. The water in the ostiole then can induce spore germination of pathogens which can then cause significant losses during the shelf life of fruit. Therefore, the most efficient way for disinfection of figs may be fumigation or fogging with appropriate chemicals to reduce fungal inoculum levels (Karabulut et al., 2004).

On table grapes, decay during storage is mainly caused by the fungus *B. cinerea* (gray mold) and its prevention is accomplished by the use of sulfur dioxide (SO_2), by either initial fumigation of fruit loads from the field followed by weekly fumigation of storage rooms or the presence of in-package pads containing sodium metabisulfite (Palou et al., 2010). SO_2 fumigation has been widely used for preventing decay during storage of table grapes in California since the 1920s (Winkler and Jacob, 1925). SO_2 generating sheets were also first developed in California in the 1960s (Gentry and Nelson, 1968) and today are widely used worldwide, especially in fruit used for export markets (Lichter et al., 2008; Zutahy et al., 2008). This technology is based on the reaction of the sulfite salt contained in the pads placed inside the boxes with water vapor from environmental humidity, which leads to a continuous emission of low SO_2 concentrations within the packages (Nelson and Ahmedullah, 1976). For grape fumigation, repeated applications of SO_2 are necessary because the treatment kills the fungal mycelia and spores present only on the berry surface, but do not affect internal *Botrytis* infections that may lead to gray mold nesting (Harvey, 1956). However, it has been shown that excessive levels of SO_2 can damage table grapes by bleaching or causing sunken areas on the berry surface or contributing to premature browning of the stems (Nelson and Ahmedullah, 1976; Crisosto and Mitchell, 2002). Other studies (Harvey et al., 1988; Zoffoli et al., 2008) have shown that grape hairline splits, commonly associated with significant water loss, are also induced by excessive SO_2 doses. SO_2 technology has also been tested to control postharvest decay on other fruit species such as banana (Williams et al., 2003), lemon (Smilanick et al., 1995), litchi (Lichter et al., 2000), raspberry (Spayd et al., 1984). However, to our knowledge, SO_2 has not been used on fresh figs for the time being.

The purpose of these trials reported here was to evaluate the potential of SO_2 as a technique to reduce postharvest losses and extend shelf life of dark- and green-skin fresh figs. Different application methods were tested on 'Kadota', 'Sierra', 'Black Mission' and 'Brown Turkey' commercial fig cultivars. Moreover, the effect of this technique in controlling specific genera of pathogenic microorganisms that may reduce the market life of fresh figs was evaluated.

2. Materials and methods

2.1. Fruit

Freshly harvested figs (*Ficus carica* L.) of four commercial cultivars, 'Black Mission' and 'Brown Turkey' (dark-skin), and 'Kadota' and 'Sierra' (green-skin), grown in the San Joaquin Valley (California), were used in this work. The figs were harvested early in the morning and transported to Kearney Agricultural Center (Parlier) on the same day. Figs were then selected by eliminating defective fruits (bruised, other physical damage, incorrect maturity, and odd color) and placed in plastic trays (40 cm × 60 cm × 12.5 cm) before receiving any treatment. Prior to the beginning of each test, fruit firmness was determined as a measure of initial quality and maturity. Different SO_2 exposure conditions were then applied to those trays as described next.

2.2. Assessment of SO_2 concentration × time (C × t) products

In order to test the effect of different SO_2 concentration × time (C × t) products on decay control and fruit quality of fresh figs, individual trays of 'Brown Turkey' and 'Kadota' figs were sealed in plastic containers attached to a flow-through fumigation system inside an environmental room at 20 °C, as described by Palou et al. (2002). Containers were exposed to a continuous flow of 500 μL/L of SO_2 for different periods of time calculated to result in concentration × time products of 0 (control), 25, 50, and 100 (μL/L) h. Actual concentration × time product was verified with passive dosimeter tubes placed in the boxes before the fumigation. Five trays of 22 fruit per treatment (total of 440 fruit), and six boxes of 25 fruit per treatment (total of 600 fruit) were used for 'Brown Turkey' and 'Kadota' cultivars, respectively. After fumigation, the figs were kept at 0 °C until evaluations were carried out. Decay, visual fruit quality evaluations, and firmness measurements were done as described below after 7 and 13 d at 0 °C, and every day during shelf life simulation at 20 °C for up to 4 d.

2.3. Effect of SO_2 treatments on fruit quality and decay

According to the previous test results, SO_2 was applied at a concentration × time product of 25 (μL/L) h. Fumigations were conducted in 330 L steel chambers with two 10 cm-diameter fans mixing the inner air to ensure homogeneous distribution of SO_2. The gas was released into the container through a 1.5 cm diameter polyvinyl chloride tube connected directly to the sulfur dioxide cylinder (Praxair, Los Angeles, CA). Concentration of SO_2 inside the 330 L chamber was continuously monitored with a gas-sampling pump (model 8014-400A, SE certified model 42CFR84; Matheson Kitagawa, East Rutherford, NJ) using a SO_2 meter/data logger (model Z1300XP, Environmental Sensors Co., Boca Raton, FL). The SO_2 meter was connected to the computer, where simultaneous calculations of the SO_2 concentration × time product applied were assessed, which allowed the application of very low precise concentration × time products of SO_2. The SO_2 concentration × time product applied during the fumigation was verified at the end of the fumigation with passive dosimeter tubes (tubes 5D with detection limits of 0.2–100 μL/L SO_2, Gastec Corporation, Ayase-Shi, Kanagawa) placed into each tray. Fumigations were conducted at 20 °C due to the results obtained on the pathogen survival rate assays performed at different fumigation temperatures (see below). After the treatment, the fruit were kept at 0 °C for 7 d. Fruit quality evaluations and firmness measurements were carried out immediately after cold storage and after 1, 2, 3, and 4 d of simulated display at 20 °C.

2.4. Effect of initial SO_2 fumigation combined with SO_2 generating pads

To compare the effect of SO_2 fumigations and the use of SO_2 generating pads during cold storage of figs, individual trays of 'Black Mission', 'Brown Turkey', 'Kadota', and 'Sierra' figs were placed in perforated plastic boxes (40 × 60 × 12.5 cm) resulting in four different treatments: (1) non-treated control fruit; (2) SO_2 application before cold storage; (3) use of SO_2 generating pads during cold storage; and (4) SO_2 fumigation before cold storage in combination with the use of SO_2 generating pads during cold storage. Two trays of 50 fruit per tray were placed on each perforated plastic box. Four boxes were used for each treatment (400 fruit per treatment).

In the boxes for treatments (3) and (4), commercial dual release SO_2 generating pads (UVASYS, Grapetek (Pty) Ltd., Cape Town, South Africa) were placed directly on top of the tray and the boxes were wrapped with 30 μm linear low-density polyethylene

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(LLDPE) film around this 'mini-pallet'. The SO_2 pads and bags were removed the first day of evaluation.

2.5. Effect application timing and multiple SO_2 fumigations

In order to study the effect of the moment of application and the effect of the exposure to repeated SO_2 fumigations over the storage period, four different treatments were applied to 'Black Mission' and 'Kadota' figs: (1) non-treated control fruit; (2) SO_2 fumigation before cold storage (warm fruit); (3) SO_2 fumigation after cold storage (cold fruit); and (4) SO_2 fumigation before cold storage combined with SO_2 fumigation after cold storage. Computer-controlled SO_2 fumigations on 330 L steel chambers were performed as described above. The concentration × time product of SO_2 applied in each fumigation was always 25 (μL/L) h.

2.6. Decay and fruit quality assessment

Fig firmness was measured by compression on two cheeks of each fruit with a fruit texture analyzer (FTA) (Güss, Model: GS-14, Strand, South Africa) with a flat tip and expressed in Newtons (N). Visual fruit quality parameters determined in order to test the performance of different SO_2 treatments were percent of sound fruit (commercial fruit), percent of decayed fruit, percent of fruit with off color (color not typical for the cultivar), percent of fruit with growth cracks, percent of fruit with splits, and percent of fruit with other blemishes. The percent of bleaching per fruit (no purple or green coverage on black or green figs, respectively) was estimated using a visual scale after 7 d of cold storage at 0 °C and every day during simulated shelf life at 20 °C (day one through day four). Four replicates of 10 fruit each were used on every evaluation for each treatment-cultivar combination.

2.7. Effect of SO_2 fumigation on in vitro pathogen survival rates

The survival of potential causal agents of fig decay after 100 (μL/L) h SO_2 fumigation was tested in vitro. All the following microorganisms may cause significant postharvest diseases of fig fruit (Coviello et al., 2009). Pure cultures of isolates of an *Alternaria* sp. (RGAlt), *Aspergillus flavus* (AFP7), a *Penicillium* sp. (SWE5B) and *Rhizopus stolonifer* (WBD3 and 1123c) were grown on Petri plates containing potato dextrose agar (PDA; Difco Laboratories, Detroit, MI). Two strains of *Botrytis cinerea* (RGBc and OaBc) were grown on Kings B medium (Difco Laboratories, Detroit, MI). All these fungal cultures were incubated at 25 °C for about 7 d. Strains of the bacteria *Bacillus* sp. (RGP-3 and RGP-5) and *Bacillus subtilis* (B-18) and the yeast *Hanseniaspora guilliermondii* (Y-1) were incubated in nutrient yeast dextrose agar (NYDA; Difco Laboratories, Detroit, MI) dishes at 25 °C for 2 d. Petri dishes were incubated under a 12-h photoperiod of cold fluorescent light (350 μmol m^{-2} s^{-1}). Ten replications for each microorganism strain were used for this experiment.

Bacterial, yeast, and sporulated fungal cultures were uncovered and stacked in 7.8 L polypropylene containers (6 Petri dishes per container) with layers separated by galvanized mesh (1 cm^2). The vented containers were then sealed and the plates were fumigated with SO_2 at a concentration × time product of 100 (μL/L) h at 0 °C or 20 °C. The fumigation progress was monitored with SO_2 dosimeters placed in-line at the exhaust side of the containers. The 100 μL/L fumigation required an average of 44 min to be applied. When the dosimeter read the appropriate level of exposure, the SO_2 was turned off and the containers were purged with only air for 10–20 min. The plates were then placed within a laminar flow hood where fungal spores and yeast and bacterial cells were recovered with sterile water and thinly smeared in new Petri dishes containing fresh PDA, Kings B, or NYDA media. An aliquot of 20 μL from each aqueous spore/cell solution was plated into each of three replicate Petri dishes (replicates). After about 18 h of incubation at 25 °C, five sites on each dish were rated for germinating spores or dividing bacterial or yeast cells. Fungal spores were considered as germinating if the germ tube was visible, irrespective of its size. At least 100 spores or cells were counted for each of the five sites on each dish. The survival of the microorganisms was expressed as the percentage of germinating spores or living cells after 18 h of incubation.

2.8. Effect of SO_2 fumigation on fig epiphytic microorganisms

The effect of SO_2 on the survival of microorganisms present on the skin of fresh figs was evaluated in treated and untreated (control) 'Black Mission' and 'Kadota' figs. Treated fruit was fumigated twice: initial SO_2 fumigation before cold storage (warm fruit) at a concentration × time product of 25 (μL/L) h, and SO_2 fumigation after cold storage (cold fruit) also at 25 (μL/L) h. Computer-controlled SO_2 fumigations were performed on 330 L steel chambers as described above. Fumigations were conducted at 20 °C due to the results obtained on the pathogen survival rate assays performed at different fumigation temperatures (see below). After the treatment, the fruit were kept in cold storage at 0 °C for 7 d and then moved to 20 °C to simulate shelf life. Survival of epiphytic microorganisms was evaluated immediately after cold storage and after 1, 2, 3, and 4 d of simulated shelf life at 20 °C after cold storage.

Each evaluation day, a piece of the skin (1 cm^2) was collected of five replicates (figs) for each treatment. The pieces were immersed in 1 mL of sterile water into a 2-mL Eppendorf tube and vortexed for 30 s. One hundred microliter of the shaken water suspension was placed in Petri dishes containing PDA. Petri dishes were incubated at 25 °C under a 12-h photoperiod of cold fluorescent light (350 μmol m^{-2} s^{-1}) until microorganisms were examined. The number of fungal colony forming units (CFU) growing in a circumference of 13.5 mm diameter was counted from each Petri dish. Finally, to obtain pure cultures, hyphal tips from the colonies of each growing species were transferred to fresh PDA and incubated as described above to proceed to their identification. Sporulated cultures were identified at the genus level after macroscopic and microscopic observation of the morphological characteristics of the isolates.

2.9. Statistical analyses

Homogeneity of variances was determined using Levene's test. The quality traits were analyzed by ANOVA. For data expressed as percentage derived from counts (sound fruit, decay, browning, bleaching and incidence of survival), arcsine-transformation was performed before the analysis of variance. Means were separated by Fisher's protected least difference test ($P \leq 0.05$). Non-transformed means are presented in the tables. Analyses were performed using SPSS software (SPSS 15.0, SPSS Inc., Chicago, IL).

3. Results

3.1. Assessment of concentration × time product

In order to identify the best SO_2 concentration × time product to be applied on fresh figs to control decay without affecting the postharvest quality of the fruit, 25, 50, and 100 (μL/L) h SO_2 were tested and compared with untreated control figs [0 (μL/L) h]. No significant differences were found in the percentage of sound fruit among the four treatments at 0 and 1 d of shelf life at 20 °C after 6 d of cold storage (Table 1). However, differences were significant after 3 d of shelf life. The highest percentage of sound fruit

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Table 1
Effect of different SO_2 concentration × time products [(μL/L) h] on the percentage of sound fruit of 'Brown Turkey' and 'Kadota' figs, measured immediately after 7 d of cold storage at 0 °C, and after 1, 3, and 4 d of shelf life at 20 °C.

	SO_2 concentration × time	After 7 d at 0 °C	7 d at 0 °C +1 d at 20 °C	7 d at 0 °C +3 d at 20 °C	7 d at 0 °C +4 d at 20 °C
Brown Turkey	0 (μL/L) h	91.4 a[z]	91.4 a	8.6 c	5.7 c
	25 (μL/L) h	100.0 a	97.1 a	42.9 a	22.9 a
	50 (μL/L) h	100.0 a	94.3 a	28.6 b	11.4 b
	100 (μL/L) h	97.1 a	85.7 a	11.4 c	5.7 c
Kadota	0 (μL/L) h	100.0 a	100.0 a	50.0 b	24.1 b
	25 (μL/L) h	100.0 a	100.0 a	74.1 a	50.0 a
	50 (μL/L) h	100.0 a	100.0 a	37.0 c	25.9 b
	100 (μL/L) h	100.0 a	100.0 a	53.7 b	44.4 a

[z] For each cultivar, means followed by the same letter in each column are not significantly different at $P \leq 0.05$, according to Fisher's Protected LSD. Statistical analysis was performed with arcsine-transformed data. Values presented are non-transformed means.

was obtained after the application of 25 (μL/L) h on both 'Brown Turkey' and 'Kadota' cultivars, whereas 50 and 100 (μL/L) h SO_2 resulted in lower percentage of sound fruit due to high incidence of SO_2 damage. Moreover, the percentage of bleaching on 'Brown Turkey' fruit after 6 d of cold storage increased with SO_2 concentration × time product (data not shown), and with the 25 (μL/L) h treatment affecting the color of the figs to the lowest extent. According to these results, a SO_2 concentration × time product of 25 (μL/L) h was used for the rest of the experiments conducted in this work.

3.2. Effect of initial SO_2 fumigation combined with SO_2 generating pads

The results of this study showed that firmness of the figs tested was not consistently affected by the different SO_2 treatments (Tables 2 and 3). On the other hand, since day two of shelf life, all SO_2 treatments significantly decreased the percentage of decay affecting fresh figs when compared with untreated control fruit (Tables 2 and 3). At day four of shelf life, initial SO_2 fumigation at 25 (μL/L) h reduced decay by about 40% and 30% for 'Black Mission' and 'Sierra' cultivars, respectively, compared to untreated fruit. Although the lowest incidence of decay was observed when both initial fumigation and SO_2 generating pads were applied, a high incidence of browning and bleaching was observed when SO_2 generating pads were used (Fig. 1).

3.3. Effect of application timing and multiple SO_2 fumigations

In order to optimize SO_2 applications and reach better control of decay, different timings of application and repeated SO_2 fumigations along the storage period were tested on dark 'Black Mission' (Table 4) and green 'Kadota' (Table 5) fig cultivars. Similarly to what was observed in the previous experiment, no consistent effect of SO_2 treatments on the firmness of the figs was observed on each cultivar.

Regarding to the control of decay by the SO_2 treatments, significant differences were observed at day two of shelf life with respect to the untreated fruit (Tables 4 and 5). For both 'Black Mission' and 'Kadota' figs, the percentage of decayed fruit was higher when SO_2 fumigation was applied after cold storage (cold fruit) than when applied before cold storage (warm fruit). Indeed, at the end of the shelf life at day four, no significant differences were found between untreated fruit and figs treated with SO_2 after cold storage for all of the figs tested. No significant differences between control and SO_2 treated fruit were found for either the incidence of browning or bleaching at any time of shelf life (Tables 4 and 5; Fig. 2). On the other hand, the percentage of decayed fruit of each fig after repeated SO_2 applications (initial SO_2 before cold storage plus SO_2 application after cold storage) was not significantly different from that determined for the fruit fumigated just before cold storage (Tables 4 and 5).

Table 2
Effect of initial SO_2 fumigation, SO_2 generating pads, or combination of initial SO_2 fumigation with SO_2 pads on the postharvest quality of 'Black Mission' figs, determined immediately after 7 d of cold storage at 0 °C, and after 1, 2, 3, and 4 d of shelf life at 20 °C.

Quality trait	Treatment	Day 0	Day 1	Day 2	Day 3	Day 4
Firmness (N)	Control	11.0 a[z]	9.6 ab	8.9 ab	7.8 a	8.5 a
	Initial SO_2[x]	10.6 a	11.6 a	9.4 a	7.6 a	8.0 ab
	SO_2 generating pad	11.0 a	9.6 ab	7.7 b	7.0 ab	6.8 ab
	Initial SO_2[x] + SO_2 generating pad	10.3 a	8.6 b	7.7 b	6.0 b	6.3 b
Decay (%)	Control	0.0 a[y]	0.0 a	27.5 a	45.0 a	85.0 a
	Initial SO_2[x]	0.0 a	0.0 a	12.5 b	32.5 ab	47.5 b
	SO_2 generating pad	0.0 a	0.0 a	2.5 b	17.5 be	17.5 c
	Initial SO_2[x] + SO_2 generating pad	0.0 a	0.0 a	0.0 b	5.0 c	5.0 c
Browning (%)	Control	0.0 a[y]	0.0 a	0.0 a	5.0 a	5.0 b
	Initial SO_2[x]	0.0 a	2.5 a	5.0 a	12.5 a	15.0 b
	SO_2 generating pad	0.0 a	0.0 a	5.0 a	30.0 a	90.0 a
	Initial SO_2[x] + SO_2 generating pad	0.0 a	0.0 a	2.5 a	32.5 a	95.0 a
Bleaching (%)	Control	0.0 b[y]	0.0 b	0.0 b	0.0 b	0.0 b
	Initial SO_2[x]	0.0 b	0.0 b	0.0 b	0.0 b	7.5 b
	SO_2 generating pad	70.0 a	90.0 a	87.5 a	92.5 a	92.5 a
	Initial SO_2[x] + SO_2 generating pad	70.0 a	82.5 a	87.5 a	87.5 a	95.0 a

[x] SO_2 concentration × time product = 25 (μL/L) h.
[y] Statistical analysis was performed with arcsine-transformed data. Values presented are non-transformed means.
[z] For each trait, means followed by the same letter in each column are not significantly different at $P \leq 0.05$, according to Fisher's Protected LSD.

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Table 3
Effect of initial SO_2 fumigation, SO_2 generating pads, or combination of initial SO_2 fumigation with SO_2 pads on the postharvest quality of 'Sierra' figs, determined immediately after 7 d of cold storage at 0 °C, and after 1, 2, 3, and 4 d of shelf life at 20 °C.

Quality trait	Treatment	Day 0	Day 1	Day 2	Day 3	Day 4
Firmness (N)	Control	9.81 b[z]	7.54 a	5.74 b	4.29 b	5.15 b
	Initial SO_2[x]	9.62 b	6.43 a	5.12 b	5.83 a	4.95 b
	SO_2 generating pad	12.84 a	6.94 a	7.40 a	6.09 a	6.49 ab
	Initial SO_2[x] + SO_2 generating pad	12.57 a	7.24 a	6.73 ab	5.99 a	7.48 a
Decay (%)	Control	0.0 a[y]	0.0 a	35.0 a	75.0 a	92.5 a
	Initial SO_2[x]	0.0 a	0.0 a	2.5 b	35.0 b	62.5 b
	SO_2 generating pad	0.0 a	0.0 a	0.0 b	0.0 c	22.5 c
	Initial SO_2[x] + SO_2 generating pad	0.0 a	0.0 a	0.0 b	0.0 c	15.0 c
Browning (%)	Control	67.5 a[y]	80.0 a	95.0 a	97.5 a	100.0 a
	Initial SO_2[x]	60.0 a	75.0 a	97.5 a	100.0 a	100.0 a
	SO_2 generating pad	0.0 b	30.0 b	30.0 b	85.0 a	100.0 a
	Initial SO_2[x] + SO_2 generating pad	0.0 b	30.0 b	32.5 b	87.5 a	97.5 a
Bleaching (%)	Control	0.0 a[y]	0.0 a	0.0 b	0.0 b	0.0 b
	Initial SO_2[x]	0.0 a	0.0 a	2.5 b	2.5 b	2.5 b
	SO_2 generating pad	0.0 a	10.0 a	100.0 a	100.0 a	100.0 a
	Initial SO_2[x] + SO_2 generating pad	0.0 a	7.5 a	80.0 a	100.0 a	100.0 a

[x] SO_2 concentration × time product = 25 (μL/L) h.
[y] Statistical analysis was performed with arcsine-transformed data. Values presented are non-transformed means.
[z] For each trait, means followed by the same letter in each column are not significantly different at $P \leq 0.05$, according to Fisher's Protected LSD.

Fig. 1. 'Black Mission' (a) and 'Sierra' figs (b) subjected to different SO_2 treatments after seven d of cold storage at 0 °C plus two d of shelf life at 20 °C. Treatments were (from left to right): (1) control (untreated); (2) 25 (μL/L) h SO_2 fumigation before cold storage; (3) SO_2 releasing pads during cold storage; and (4) 25 (μL/L) h SO_2 fumigation before cold storage + SO_2 releasing pads during cold storage.

Table 4
Effect of different timing of SO_2 fumigation and multiple applications on the postharvest quality of 'Black Mission' figs, determined immediately after 7 d of cold storage at 0 °C, and after 1, 2, 3, and 4 d of shelf life at 20 °C.

Quality trait	Treatment	Day 0	Day 1	Day 2	Day 3	Day 4
Firmness (N)	Control	14.7 a[z]	9.4 a	7.7 a	7.2 ab	6.6 a
	Initial SO_2[x]	12.7 ab	9.6 a	7.1 a	7.7 a	5.4 b
	SO_2 application after cold storage[x]	11.3 b	7.3 b	7.1 a	5.7 c	5.6 b
	Initial SO_2[x] + SO_2 application after cold storage[x]	11.9 ab	7.5 b	6.8 a	6.9 b	6.2 ab
Decay (%)	Control	0.0 a[y]	2.5 a	12.5 a	20.0 a	60.0 a
	Initial SO_2[x]	0.0 a	0.0 a	0.0 b	2.5 c	27.5 c
	SO_2 application after cold storage[x]	0.0 a	0.0 a	2.5 b	12.5 ab	47.5 ab
	Initial SO_2[x] + SO_2 application after cold storage[x]	0.0 a	0.0 a	0.0 b	5.0 be	30.0 be
Browning (%)	Control	0.0 a[y]	0.0 a	0.0 a	2.5 a	5.0 a
	Initial SO_2[x]	0.0 a	0.0 a	2.5 a	5.0 a	15.0 a
	SO_2 application after cold storage[x]	0.0 a	0.0 a	0.0 a	7.5 a	7.5 a
	Initial SO_2[x] + SO_2 application after cold storage[x]	0.0 a	0.0 a	2.5 a	2.5 a	15.0 a
Bleaching (%)	Control	0.0 a[y]	0.0 a	0.0 a	0.0 a	0.0 a
	Initial SO_2[x]	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
	SO_2 application after cold storage[x]	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
	Initial SO_2[x] + SO_2 application after cold storage[x]	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a

[x] SO_2 concentration × time product = 25 (μL/L) h.
[y] Statistical analysis was performed with arcsine-transformed data. Values presented are non-transformed means.
[z] For each trait, means followed by the same letter in each column are not significantly different at $P \leq 0.05$, according to Fisher's Protected LSD.

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Table 5
Effect of different timing of SO_2 fumigation and multiple applications on the postharvest quality of 'Kadota' figs, determined immediately after 7 d of cold storage at 0 °C, and after 1, 2, 3, and 4 d of shelf life at 20 °C.

Quality trait	Treatment	Day 0	Day 1	Day 2	Day 3	Day 4
Firmness (N)	Control	10.2 a[z]	6.5 a	5.2 a	3.9 b	3.6 a
	Initial SO_2[x]	7.7 b	5.9 a	5.7 a	4.7 a	3.6 a
	SO_2 application after cold storage[x]	8.4 b	6.1 a	5.8 a	3.9 b	4.1 a
	Initial SO_2[x] + SO_2 application after cold storage[x]	8.3 b	5.8 a	5.8 a	4.6 a	3.5 a
Decay (%)	Control	0.0 a[y]	0.0 a	25.0 a	40.0 a	87.5 a
	Initial SO_2[x]	0.0 a	0.0 a	2.5 b	15.0 b	60.0 be
	SO_2 application after cold storage[x]	0.0 a	0.0 a	10.0 ab	30.0 ab	75.0 ab
	Initial SO_2[x] + SO_2 application after cold storage[x]	0.0 a	0.0 a	7.5 ab	17.5 b	57.5 c
Browning (%)	Control	50.0 a[y]	85.0 a	95.0 ab	97.5 a	97.5 a
	Initial SO_2[x]	45.0 a	70.0 a	82.5 b	95.0 a	95.0 a
	SO_2 application after cold storage[x]	25.0 a	82.5 a	92.5 a	95.0 a	97.5 a
	Initial SO_2[x] + SO_2 application after cold storage[x]	47.5 a	85.0 a	92.5 ab	92.5 a	100.0 a
Bleaching (%)	Control	0.0 a[y]	0.0 a	0.0 a	0.0 a	0.0 a
	Initial SO_2[x]	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
	SO_2 application after cold storage[x]	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
	Initial SO_2[x] + SO_2 application after cold storage[x]	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a

[x] SO_2 concentration × time product = 25 (μL/L) h.
[y] Statistical analysis was performed with arcsine-transformed data. Values presented are non-transformed means.
[z] For each trait, means followed by the same letter in each column are not significantly different at $P \leq 0.05$, according to Fisher's Protected LSD.

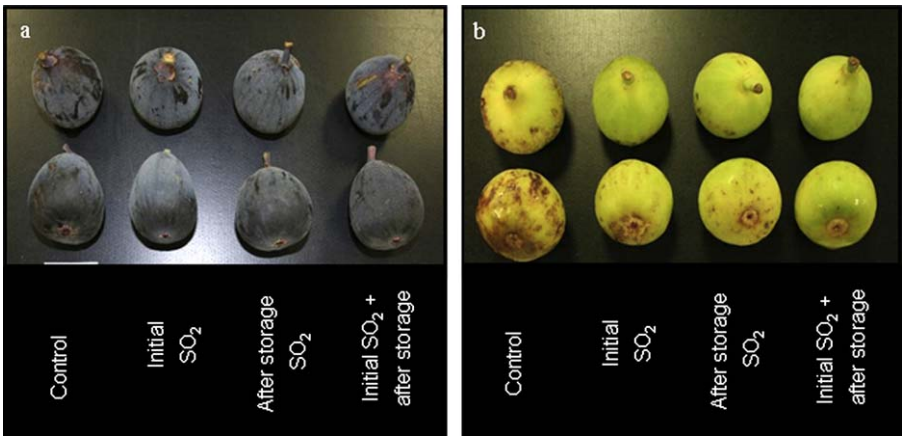

Fig. 2. 'Black Mission' (a) and 'Kadota' figs (b) subjected to different SO_2 treatments after 7 d of cold storage at 0 °C plus 2 d of shelf life at 20 °C. Treatments were (from left to right): (1) control (untreated); (2) initial 25 (μL/L) h SO_2 fumigation before cold storage; (3) 25 (μL/L) h SO_2 fumigation after cold storage; and (4) initial 25 (μL/L) h SO_2 fumigation before cold storage + 25 (μL/L) h SO_2 fumigation after cold storage.

3.4. Effect of SO_2 treatments on the control of contaminating microorganisms

The results of the in vitro work showed a significant reduction of the fungal and bacterial populations when a concentration × time product of 100 (μL/L) h SO_2 was applied at 20 °C, with the only exception of the strain B-18 of *B. subtilis*. However, the survival of all isolates was considerably higher when fumigation was performed at 0 °C (Table 6). The biocidal activity of SO_2 against fungal spores was significantly greater than against bacterial or yeast cells, and most of the fungal isolates did not survive the 100 (μL/L) h fumigation at 20 °C. The exceptions were *A. flavus* and *Penicillium* sp. which showed a survival percentage of 13% and 40%, respectively (Table 6) while the *Bacillus* sp. survival ranged from 53% to 100%. Since these results were obtained with colonies growing in agar media, it could be assumed that even higher mortality rates might be obtained with microorganisms completely exposed to the gas.

With respect to the in vivo assays, the repeated application of SO_2 [25 (μL/L) h before and after cold storage] showed a positive effect on the control of epiphytic microorganisms growing on the surface of both 'Kadota' and 'Black Mission' fig cultivars, especially

Table 6
Percentage of survival of common fig pathogenic microorganisms fumigated in vitro with 100 μL/L SO_2 at 0 and 20 °C. Fungal spores or bacterial and yeast cells were grown on potato dextrose agar or Kings B medium and the open plates were exposed to SO_2. SO_2 levels were monitored with SO_2 dosimeters.

Pathogen	Isolate	At 0 °C	At 20 °C
Alternaria sp.	RGAlt	84.8 a[z]	0.0 e
Aspergillus flavus	AfP7	44.5 b	13.0 d
Bacillus sp.	RGP3	100.0 a	67.0 b
Bacillus sp.	RGP5	56.0 b	53.0 c
Bacillus subtilis	B-18	100.0 a	100.0 a
Botrytis cinerea	RGBc	11.5 c	ND
Botrytis cinerea	OaBc	45.1 b	100.0 e
Hanseniaspora guilliermundii	Y-1	100.0 a	ND
Penicillium sp.	SWE5	53.4 b	40.0 c
Rhizopus stolonifer	WBD3	ND	0.0 e
Rhizopus stolonifer	1123c	ND	0.0 e

ND: Not determined.
[z] Means followed by the same letter in each column are not significantly different at $P \leq 0.05$, according to Fisher's Protected LSD. Statistical analysis was performed on arcsine-transformed data. Values presented are non-transformed means.

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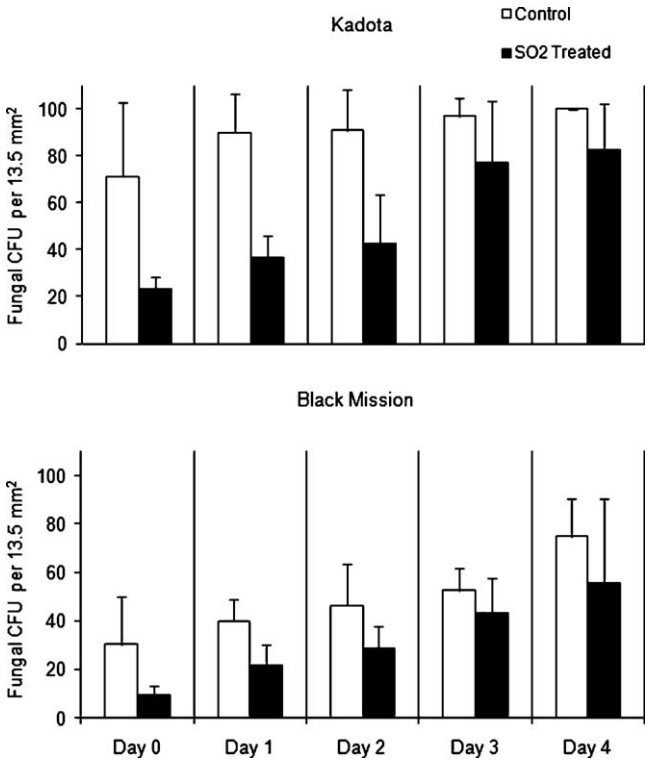

Fig. 3. Effect of SO_2 treatment on the total number of fungal colony forming units recovered from the skin of 'Kadota' and 'Black Mission' fig figs after 7 d of cold storage at 0 °C, and after 1, 2, 3, and 4 d of shelf life at 20 °C after cold storage. The number of fungal colony forming units (CFU) was counted in a circumference area of 13.5 mm diameter. Treated samples were fumigated with SO_2 at 25 (μL/L) h before cold storage (warm fruit) plus 25 (μL/L) h after cold storage (cold fruit). Vertical bars represent ± STD of means.

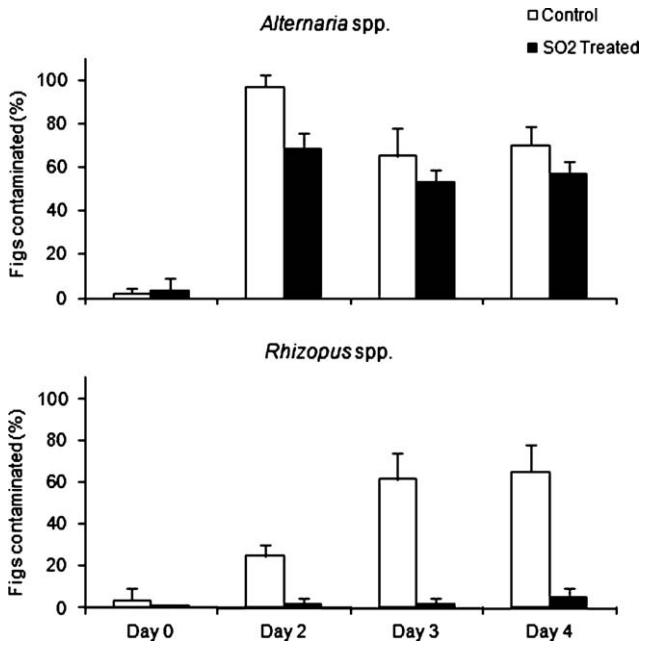

Fig. 4. Effect of SO_2 treatment on the surface contamination of 'Black Mission' figs by *Alternaria* spp. and *Rhizopus* spp. after 7 d of cold storage at 0 °C, and after 2, 3, and 4 d of shelf life at 20 °C after cold storage. Treated samples were fumigated with SO_2 at 25 (μL/L) h before cold storage (warm fruit) plus 25 (μL/L) h after cold storage (cold fruit). Vertical bars represent ± STD of means.

during the first 3 d of shelf life at 20 °C (Figs. 3–5). On 'Kadota' cultivar, the total number of fungal colony forming units (CFU) recovered from the fig surface and growing in plates with nutrient medium was drastically reduced by SO_2 treatment when compared with the untreated control figs. The effect of SO_2 treatment was weaker on 'Black Mission' figs, but still evident (Fig. 3). The most abundant pathogenic species recovered from the fig surface were species of *Alternaria* and *Rhizopus*. SO_2 repeated application resulted in a decrease of *Rhizopus* sp. presence on the skin of both 'Kadota' and 'Black Mission' figs (Figs. 4 and 5). After 4 d of shelf life at 20 °C, *Rhizopus* sp. was practically not found after repeated application of SO_2 on 'Black Mission' figs, while it was detected in more than 60% of untreated fruit (Fig. 4). In the case of 'Kadota' cultivar, the frequency of *Rhizopus* sp. after day four of shelf life was of about 35% and 100% on SO_2-treated and control figs, respectively (Fig. 5). In contrast, the percentage of figs in which *Alternaria* sp. was present was only slightly affected by SO_2 treatments and, after day three of shelf life of 'Kadota' figs, it was even higher on treated fruit than on untreated control fruit (Figs. 4 and 5).

Apart from species of *Alternaria* and *Rhizopus*, other less abundant pathogen species such as *Botrytis* sp. and *Penicillium* sp. were also isolated from the figs skin. These two species appeared later on than the species of *Alternaria* and *Rhizopus* (days three and four of shelf life on 'Black Mission' and 'Kadota', respectively). At day three of shelf life, 50% of the untreated 'Black Mission' figs were contaminated by *Botrytis* sp., whereas this fungus species was not isolated from any SO_2 treated fig. In the case of 'Kadota', 20% of the untreated figs were contaminated by *Botrytis* sp. by day four of shelf life, whereas SO_2 treatment decreased this percentage to 10%. *Penicillium* sp. was only isolated from untreated 'Black Mission' figs at days three and four after shelf life at 20 °C, while no contamination by this species was found on SO_2-treated 'Black Mission' figs or in both untreated and treated 'Kadota' figs (data not shown).

4. Discussion

After trying different SO_2 concentration × time products [25, 50, and (100 μL/L) h] to control decay on fresh figs, the highest percent-

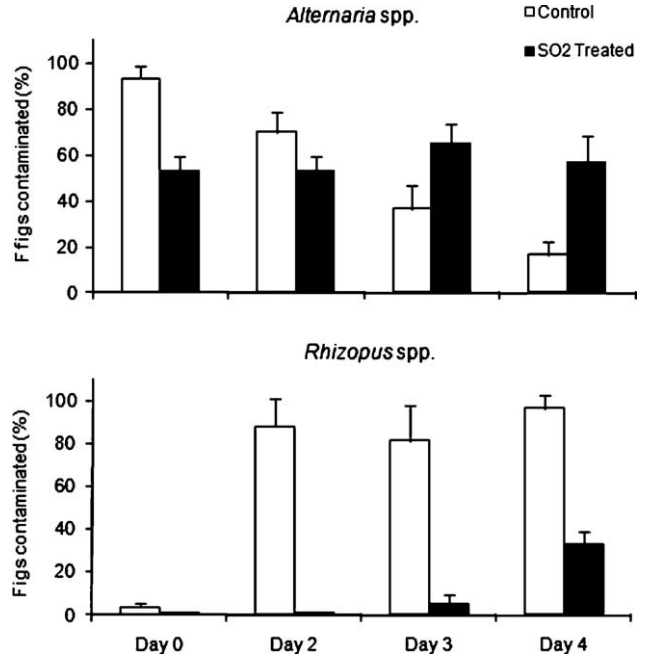

Fig. 5. Effect of SO_2 treatment on the surface contamination of 'Kadota' figs by species of *Alternaria* and *Rhizopus* after 7 d of cold storage at 0 °C (Day 0), and after 2, 3, and 4 d of shelf life at 20 °C after cold storage. Treated samples were fumigated with SO_2 at 25 (μL/L) h before cold storage (warm fruit) plus 25 (μL/L) h after cold storage (cold fruit). Vertical bars represent ± STD of means.

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age of sound fruit was obtained after the application of 25 (μL/L) h on both 'Brown Turkey' and 'Kadota' cultivars. According to these results, a SO_2 concentration × time product of 25 (μL/L) h was used for the rest of the experiments conducted in this work. Previous work has described the harmful effect excessive SO_2 may cause on postharvest quality of table grapes. Damage such as bleaching, sunken areas on the berries, premature browning of the stems, and hairline splits are caused by excessive SO_2 doses on table grapes (Nelson and Ahmedull, 1972; Harvey et al., 1988; Zoffoli et al., 2008). It has been reported also for grapes that concentrations higher than 20 (μL/L) h could induce bleaching on the berries (Laszlo et al., 1981). In their simulation of continuous exposure of table grapes to SO_2 during storage at 0 °C, Palou et al. (2002) observed that an SO_2 emission rate of 0.092 μmol kg^{-1} s^{-1} was enough to inhibit gray mold nesting without causing fruit injury.

The results of the current study showed that firmness was not consistently affected by the different SO_2 treatments for any of the figs tested (Tables 2 and 3). This is in agreement with previous results reported by Crisosto et al. (2007) for 'Brown Turkey' and 'Kadota' fig cultivars.

When testing the effect of SO_2 generating pads, alone or in combination with SO_2 fumigation, as a method to control decay on fresh figs, the lowest incidence of decay was obtained with a combination of initial fumigation and SO_2 generating pads. However, the high incidence of browning and bleaching observed when SO_2 generating pads were used makes this technology infeasible for use on fresh figs. These harmful effects could be explained by the fact that when SO_2 releasing pads are used, the fruit is continuously exposed to SO_2 released fumes, thus increasing the possibility of cumulative gas damage on the fruit. Similar results have been reported for table grapes, where the use of SO_2 generating pads controlled more effectively the development of decay than the use of SO_2 fumigation by its own (Mlikota-Gabler et al., 2010). Nevertheless, even though table grapes contain a significant layer of epicuticular wax which protects them from SO_2 damage, unacceptable bleaching injuries (Crisosto and Mitchell, 2002) and even induction of off-flavors (Chervin et al., 2005) have been reported to affect berries by the use of generating sheets that led to excessive exposure to SO_2.

Regarding to the control of decay occurred by the SO_2 treatments, the significant differences found between all SO_2 treatments and the untreated fruit from the day two of shelf life, agree with previous results published on table grapes (Nelson, 1983; Crisosto et al., 2002; Mlikota-Gabler et al., 2010). The results obtained on this work showed that the effect of SO_2 on the development of decay was affected by the time of application and that better control is obtained when the gas is applied immediately after harvest before cold storage.

On the other hand, there was no significant improvement in decay control effectiveness when SO_2 fumigation before cold storage was followed by a second SO_2 fumigation after cold storage. In California, grapes are typically subjected to an initial fumigation with SO_2 and subsequent weekly fumigations during storage (Nelson, 1985; Harvey et al., 1988). Although repeated applications of SO_2 during storage have been recommended for table grapes to control latent infections by *Botrytis cinerea* that develop inside the berries (Harvey, 1956; Smilanick and Henson, 1992; Mlikota-Gabler et al., 2010), no evidence of significant improvement was observed in this work using repeated fumigation of green and black fresh figs. Therefore, a SO_2 fumigation before cold storage (on warm fruit) was the most appropriate treatment to control decay on fresh figs without affecting the postharvest quality of the fruit.

The results of the in vitro work indicate important differences in the effects of SO_2 exposure among pathogenic strains and also a clear influence of the application temperature. While at 20 °C SO_2 fumigation at a concentration × time product of 100 (μL/L) h significantly reduced the proportion of fungal and bacterial populations in the exposed Petri dishes, with the only exception of the strain B-18 of *B. subtilis*, at 0 °C the survival of all isolates was considerably higher. The biocidal activity of SO_2 against fungal spores was significantly greater than that against bacterial or yeast cells, and most of the fungal isolates did not survive the 100 (μL/L) h fumigation at 20 °C, with the exceptions of *A. flavus* and *Penicillium* sp. These results are in agreement with previous observations made on commercially stored table grapes in California. While the application of SO_2 technologies effectively controlled gray mold and other fungal diseases of cold-stored 'Redglobe' table grapes, it was ineffective against the devastating disease named as postharvest sour rot, grape breakdown or melting decay, caused by a complex of yeast and bacterial strains including *H. guilliermondii*, *Cryptococcus laurentii*, *Aureobasidium pullulans* or *B. subtilis* (Palou et al., 2002; Morgan and Michailides, 2004). SO_2 diffuses through membranes and accumulates in microorganisms by an ionization-entrapment mechanism (Smilanick et al., 1990). Morphological and/or physiological differences between fungal spores and yeast and bacterial cells probably influence such mechanism and account for the observed differences in SO_2 effectiveness. Also differences between those organisms on the SO oxidase and other enzymes involved in the SO metabolism, could contribute to the different SO_2 efficacy observed. On the other hand, recommendations for the control of sour rot on figs do not include the use of chemical fungicides, but control of the field populations of dried fruit beetles and vinegar fruit flies is recommended because these insects are primary disseminators of the disease (Coviello et al., 2009). On the other hand, the fact that the levels of survival were much higher for all isolates treated at 0 °C compared to 20 °C, corroborates with fumigation recommendations in the literature that describe that more SO_2 is required when fumigating at lower temperatures to effectively control grape pathogens (Nelson, 1967, 1983; Smilanick et al., 1990; Smilanick and Mansour, 2007). Furthermore, the increase in SO_2 toxicity with increasing temperature may also explain why the results were better when SO_2 was applied to warm fruit before cold storage than when it was applied to cold fruit after cold storage. The present studies on fig support early reports concerning the rates of SO_2 fumigation required for effective control of pathogens (McMallan and Weedon, 1940; Couey and Uota, 1961).

Regarding to the in vivo assays, the repeated application of SO_2 [25 (μL/L) h before and after cold storage] showed a positive effect on the control of epiphytic microorganisms growing on the surface of both 'Kadota' and 'Black Mission' fig cultivars, especially during the first 3 d of shelf life at 20 °C. SO_2 repeated applications resulted in a decrease of the most abundant pathogenic species found on the surface of the figs, such as species of *Alternaria* and *Rhizopus.* These results are in agreement with previous work in which Alternaria rot, caused by *Alternaria* sp., and decay caused by *Rhizopus* sp. were among the most common types of decay in main-crop figs (Montealegre et al., 2000; Doster and Michailides, 2007). Contamination by *Rhizopus* sp. during shelf life at 20 °C was dramatically decreased after repeated application of SO_2 on both 'Black Mission' and 'Kadota' figs. In contrast, the percentage of figs in which *Alternaria* sp. was present was only slightly affected by SO_2 treatments and, after day three of shelf life of 'Kadota' figs, it was even higher on treated fruit than on untreated control fruit. Besides the low biocidal effect of SO_2 to *Alternaria* sp., confirmed by a survival rate of 85% in the in vitro tests at 0 °C, this result could also be due to competition and displacement of *Alternaria* sp. populations on untreated fruit by other more abundant, aggressive and fast-growing microorganisms such as *Rhizopus* sp. Since Alternaria rot (caused mainly by *Alternaria alternata*) is primarily a problem on ripe fruit, especially when rains occur during harvest, the recommendation is to pick the fruit before it is overripe

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(Coviello et al., 2009). The positive effect of SO_2 treatment on reducing the contamination by other fungal species less abundant on the fig skin such as species of *Botrytis* and *Penicillium* was also observed both in in vitro and in vivo assays, especially when the fumigation was performed at 20 °C. The effectiveness of SO_2 to control decay caused by *B. cinerea* by killing both mycelia and spores has been largely demonstrated on table grapes (Smilanick and Henson, 1992; Palou et al., 2002), where the gray mold caused by this fungus is the most economically important postharvest disease affecting grapes (Palou et al., 2010). In figs, *B. cinerea* may infect the fruit through skin wounds, but it has also been reported as one of the most important pathogens causing decay on healthy non-wounded fruit. In addition, this fungus also causes the development of Botrytis limb blight, also referred to as Botrytis dieback, an important field disease that leads to shoot cankers (Coviello et al., 2009).

5. Conclusion

The results presented here show that SO_2 fumigation seems to be a promising technology to reduce decay and increase the shelf life of fresh figs. However, continuous application has significant impact and requires further research, at the same time that application conditions must be optimized to minimize secondary negative effects such as fruit bleaching and browning. SO_2 fumigation with 25 (μL/L) h was a less harmful method to reduce decay than SO_2 generating pads or the combination of an initial fumigation with the use of SO_2 pads. In addition, repeated fumigations during fig cold storage did not significantly improve control of decay compared to a single 25 (μL/L) h fumigation before cold storage. Moreover, our results indicated that SO_2 fumigation was, to some extent, able to kill the pathogens present on the surface of fresh figs that cause decay under favorable conditions. To our knowledge, little work has been done in the use of SO_2 to extend the marketing life of fresh figs, so future efforts should be develop in this direction. Currently, research on SO_2 usage is included in the Federal IR-4 program and studies on gas residues are ongoing to develop a protocol for future registration of this technology on fresh figs. Intense work is being developed by our group on the use of both SO_2 and SO_2 alternatives as tools to extend the shelf life of fresh figs.

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