

# Combination of postharvest antifungal chemical treatments and controlled atmosphere storage to control gray mold and improve storability of ‘Wonderful’ pomegranates

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## Abstract

Common food additives (sodium bicarbonate (SB), sodium carbonate (SC), and potassium sorbate (PS)) were compared to the fungicide fludioxonil for the control of gray mold on California-grown ‘Wonderful’ pomegranates artificially inoculated with *Botrytis cinerea* and stored at 7.2 °C in either air or controlled atmosphere (CA, 5 kPa O<sub>2</sub> + 15 kPa CO<sub>2</sub>) conditions. Fludioxonil was superior to other treatments. PS was the most effective additive. Synergistic effects between antifungal treatments and CA storage were observed. After 15 weeks of storage at 7.2 °C, the combination of PS treatment (3 min dip in 3% solution at 21 °C) and CA storage was as effective as the combination of heated fludioxonil (30 s dip in 0.6 g L<sup>-1</sup> of active ingredient at 49 °C) and air storage. Mixtures of PS with SB or SC did not improve the efficacy of either treatment alone. In tests conducted in commercial facilities, decay development and external and internal fruit quality were assessed on naturally infected pomegranates stored in either air or CA after application of a selected postharvest antifungal combined treatment (CTrt) integrating PS, SB + chlorine, and fludioxonil. CTrt was effective in controlling natural gray mold after 6 weeks of storage at 8.9 °C, but lacked persistence and it was not effective after 14 weeks. CA storage greatly enhanced decay control ability of CTrt. Skin red color was better maintained in CA-stored than in air-stored fruit. Juice color and properties (SSC, TA, and pH) were not practically affected by either postharvest treatment or storage condition. The integration of PS treatments with CA storage could provide an alternative to synthetic fungicides for the management of pomegranate postharvest decay.

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## 1. Introduction

Plantings of pomegranate in California, the leading state in the production of this crop in the United States, have been continuously increasing during recent years. From about 1000 ha at the beginning of the 1980s, current plantings approach 8000 ha, most of them located in central and southern San Joaquin Valley, especially in Fresno, Tulare, King, and Kern counties. This is basically a consequence of worldwide increasing consumer demand for nutritious and therapeutic high quality foods. Pomegranate arils, the edible parts of the fruit, comprise juice and seeds and are a rich

source of sugars, pectin, ascorbic acid, amino acids, minerals, fibers, phytoestrogens, and above all, polyphenolic flavonoids (Aviram et al., 2000). The important antioxidant activity of pomegranate is well-known (Gil et al., 2000; Noda et al., 2002; Wang et al., 2004) and many clinical studies demonstrate that its consumption contributes to prevent diseases such as coronary heart disease and some types of cancer (Lansky et al., 2000; Aviram et al., 2000; Sumner et al., 2005; Malik et al., 2005). Therefore, besides an increase in the volume of fruit assigned to traditional markets, new markets based on the manufacture of pomegranate-derived functional food products (nutraceuticals and dietary or health supplements) are arising.

‘Wonderful’ is by far the most widely planted pomegranate cultivar in California since it offers the best combination of

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yield and quality for the location. Mature trees of this cultivar, discovered about 1896 in Porterville in a quantity of cuttings received from Florida, can yield more than 6000 kg ha<sup>-1</sup>. The fruit is large and deep purple-red with a glossy appearance. The arils are tender, deep crimson with good flavor, and the skin is of medium thickness making the fruit well-adapted for both fresh consumption and processing for whole arils or juice (LaRue, 1980). Pomegranate is a nonclimacteric fruit that does not ripen off the tree even with ethylene treatment and should be picked when fully ripe to ensure its best flavor (Kader et al., 1984). Harvest period for 'Wonderful' in California typically extends from the beginning of September to the middle of November and there is commercial interest to prolong its postharvest life at least after the Christmas holiday season, when prices and demand are higher.

Chilling injury, decay, and weight loss are the most important problems limiting storability of pomegranate. According to Elyatem and Kader (1984), weight loss of 'Wonderful' pomegranates during cold storage is largely due to water lost through natural porosity of the skin and recommended storage in 95% or higher relative humidity (RH). Shriveling symptoms on fruit are noticeable only when weight loss exceeds 5% or more of the initial weight. These researchers observed that 'Wonderful' pomegranates develop chilling injury symptoms, namely brown discoloration or scald of the skin and surface pitting, if stored in air (conventional cold storage) at 5 °C or lower temperatures. Several studies showed that, compared to air, storage of 'Wonderful' pomegranates in different controlled atmosphere conditions significantly extended their postharvest life, not only by delaying fruit senescence but also by inhibiting the growth of microorganisms causing decay (Ben-Arie and Or, 1986; Holcroft et al., 1998). More recent work by Hess-Pierce and Kader (2003) suggested storage at 7.5 °C in 5 kPa O<sub>2</sub> + 15 kPa CO<sub>2</sub> as the optimum combination to maintain the original quality of 'Wonderful' pomegranates. Under these conditions, carefully sorted fruit were satisfactorily kept for up to 20 weeks. However, when the level of latent fungal infections at the time of harvest was high, a reduction of storage life of up to 8 weeks was reported. This result shows the importance of decay development as a main factor limiting storability of California-grown 'Wonderful' pomegranates, especially when fruit are held at temperatures above those that cause chilling injury (nonchilling temperatures).

Gray mold, caused by *Botrytis cinerea* Pers.: Fr., is the most economically important postharvest disease of pomegranate in California (Tedford et al., 2005). Other fungi causing fruit rot worldwide include *Aspergillus niger*, *Penicillium* spp., *Alternaria* spp., *Nematospora* spp., *Coniella granati*, or *Pestalotiopsis versicolor* (Wilson and Ogawa, 1979; Snowdon, 1990). As it occurs with other hosts (Droby and Lichter, 2004), *B. cinerea* can cause postharvest decay in pomegranate from surface-borne inoculum that infects the fruit through injuries or microwounds located on any part

of the skin, but decay originating from blossom latent infections is frequently more important. Typically, the pathogen infects the flowers or the crown (calyx containing the stamens and pistils) of young fruits in the field, remains latent, and after harvest develops from the crown to the rest of the mature fruit causing an apparent brown discoloration of the skin. Thus, we propose this particular form of gray mold be called botrytis crown decay of pomegranate. Additionally, *B. cinerea* is able to infect stored pomegranates by mycelial spread from infected fruit to adjacent healthy fruit, causing 'nests' of decay. In any case, gray mold development is favored by the usual pomegranate nonchilling storage conditions of 5–10 °C and >90% RH, and losses due to this disease of up to 30% of harvested pomegranates when no postharvest fungicides were applied have been reported in California (Tedford et al., 2005). In fact, such losses seriously jeopardized the viability of the California pomegranate industry during 1999–2002 growing seasons and suggested the need for the application of postharvest antifungal treatments. The 'reduced-risk' fungicide fludioxonil is registered for postharvest use on pomegranate in California since 2005 under Section 24(c) (Special Local Need, SLN) of the federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) as EPA SLN No. CA-050013 and the tolerance for residues in or on pomegranate is 5.0 mg kg<sup>-1</sup> (US EPA, 2005).

The application of fludioxonil has considerably reduced postharvest decay losses and is presently a key factor in the development of the pomegranate industry in California. However, general problems related to the use of fungicides such as the potential proliferation of resistant strains of the pathogens, concerns about public health, and environmental issues, make the search for alternative decay control methods advisable. Several treatments with food additives classified as Generally Recognized as Safe (GRAS) by the United States Food and Drug Administration (US FDA), especially carbonate salts, have been evaluated alone or in combination with other alternative treatments for the control of *B. cinerea* in vitro (Palmer et al., 1997; Fallik et al., 1997; Mlikota-Gabler and Smilanick, 2001; Karabulut et al., 2005) or on harvested fruits such as table grapes (Mlikota-Gabler and Smilanick, 2001), apples (Spadaro et al., 2004), sweet cherries (Karabulut et al., 2005), or bell peppers (Fallik et al., 1997). However, to our knowledge, none of them has been evaluated on pomegranates. Irrespective of the active ingredient used for chemical control of postharvest gray mold of pomegranate, dip applications are largely more appropriate than spray or drench applications because the antifungal solution must effectively contact the blossom tissues inside the crown in order to prevent the development of latent infections of *B. cinerea*. On the other hand, pomegranate postharvest handling to reduce the incidence of postharvest diseases should include a sanitizing chlorine wash prior to the application of any fungicidal treatment. A dip or drench chlorine wash followed by high volume washing on a brush bed cleans the fruit and improves its appearance, surface-sterilizes the fruit, and sanitizes the wash water. This consequently reduces the inoculum load and prevents

potential recontaminations of the fruit during packingline operation, but does not inactivate latent or wound infections (Adaskaveg, 1995). However, chlorine may be mixed with some compatible antifungal compounds to obtain some activity against certain wound pathogens. For instance, mixtures of sodium bicarbonate and sodium hypochlorite (used at a rate of 200 mg L<sup>-1</sup>) have provided enhanced control of table grape gray mold (Mlikota-Gabler and Smilanick, 2001) and citrus green mold caused by *Penicillium digitatum* (Smilanick et al., 1999, 2006).

We assume, in summary, that to extend the storage life of 'Wonderful' pomegranates, integration of postharvest sanitizing and fungicide treatments with controlled atmospheres and storage at optimal temperatures should be pursued. In the present work, we first evaluated the effectiveness of common food additives and mixtures to control gray mold on pomegranates artificially inoculated with *B. cinerea* and compared these treatments with that of the fungicide fludioxonil. Secondly, we applied a selected and potentially commercial combined treatment to naturally infected fruit and determined its impact on decay control and fruit quality. In both cases, storability of treated fruit was assessed at nonchilling temperatures in either conventional cold storage in air or the most recommended controlled atmosphere conditions.

## 2. Materials and methods

### 2.1. Fruit

Pomegranates (*Punica granatum* L.) cv. 'Wonderful' commercially-grown in the southern San Joaquin Valley (Kern County, California) were harvested at commercial maturity and transported in bins to a local packinghouse. Sound pomegranates of a uniform medium size were selected, randomized, and used in the experiments before any postharvest treatments were applied.

### 2.2. Experiments with artificially inoculated fruit

#### 2.2.1. Fruit inoculation with *B. cinerea*

*B. cinerea* isolate LCPC-12, obtained from a rotten pomegranate and previously selected for its high aggressiveness, was incubated on potato dextrose agar (PDA) medium in Petri dishes at 20 °C for 14–21 days. Spores were rubbed from the agar surface with a sterile glass rod after 5 mL of 0.05% (w/v) Triton X-100 in sterile water was added. The high-density spore suspension was passed through two layers of cheese cloth and, after counting the number of spores with a hemacytometer, diluted with sterile water to a spore inoculum concentration of 1 × 10<sup>8</sup> L<sup>-1</sup>. This conidial suspension was uniformly sprayed for about 2 s inside the crown of each pomegranate. When necessary, sepals closing the crown aperture were removed. Inoculated fruit were kept at room temperature and allowed to air dry for 1 day before chemical treatments were applied.

#### 2.2.2. Antifungal treatments and storage conditions

The following aqueous dip treatments were applied to inoculated fruit in the packinghouse using a stainless steel water tank fitted with electric heater and thermostat: (1) control (untreated fruit), (2) potassium sorbate (PS, 3 min dip in 3% (w/v) solution at 21 °C), (3) sodium bicarbonate (SB, 3 min dip in 3% (w/v) solution at 21 °C), (4) sodium carbonate (SC, 3 min dip in 3% (w/v) solution at 40.5 °C), (5) mixture of PS and SB (PS + SB, 3 min dip in 1.5 + 1.5% (w/v) solution at 21 °C), (6) mixture of PS and SC (PS + SC, 3 min dip in 1.5 + 1.5% (w/v) solution at 21 °C), (7) fludioxonil (F21, 30 s dip at 21 °C in Scholar<sup>®</sup> 50 WP (Syngenta Crop Protection Inc., Greensboro, NC, USA) at the recommended dose of 0.6 g L<sup>-1</sup> of active ingredient), and (8) heated fludioxonil (F49, 30 s dip at 49 °C in Scholar<sup>®</sup> 50 WP at 0.6 g L<sup>-1</sup> of active ingredient). Each treatment was applied to 16–20 replicates of 8 fruit each. Treated pomegranates were allowed to air dry at room temperature and packed in corrugated cartons that were transported to the Postharvest Laboratory at UC Kearney Agricultural Center in Parlier (California).

Half of the replicates (8–10 cartons of 8 fruit each) from each chemical treatment were kept for up to 15 weeks at 7.2 °C and 95% RH in a standard cold room in conventional cold storage conditions (air) and the other half in another room at the same temperature and RH in controlled atmosphere conditions (CA). For CA storage, cartons were placed in 338-L water-sealed aluminum tanks connected to a continuous flow-through system of 5 kPa O<sub>2</sub> + 15 kPa CO<sub>2</sub> (Hess-Pierce and Kader, 2003) that had been set up inside the cold storage room. Flow rates and gas mixtures were established using a mixing board with micrometering needle valves. Supply and exhaust gaseous composition was monitored using a paramagnetic analyzer for O<sub>2</sub> (model S-3A/II, Ametek Thermox, Pittsburgh, PA, USA) and an infrared gas analyzer for CO<sub>2</sub> (model VIA-510, Horiba, Irvine, CA, USA).

#### 2.2.3. Gray mold development assessment

The development of *B. cinerea* in the crown of artificially inoculated pomegranates was assessed in treated fruit from both storage conditions after 4, 8, and 15 weeks of cold storage. At each evaluation date, each fruit was scored for botrytis crown decay according to the following quantitative scale: 0 = no lesion (visible infected area) or fungal mycelium present, 1 = mycelium present in the crown, 2 = lesion ≤25% of skin surface, 3 = lesion on 26–50% of skin surface, 4 = lesion >50% of skin surface. At the same time, the number of fruit with spores of *B. cinerea* present was also determined. Following the last evaluation after 15 weeks of storage, pomegranates were cut in half and checked for presence of internal decay.

### 2.3. Experiments with naturally infected fruit

Another set of experiments was conducted with fruit that were not artificially inoculated with any pathogen. After arrival at the packinghouse from the field, sound

pomegranates were selected, randomized, treated, and stored in commercial conditions. A representative sample of fruit (5 replicates of 10 fruit each) was brought to the laboratory for determination of initial quality (quality at harvest) as described below.

### 2.3.1. Combined treatment and storage conditions

Considering the results of the previous set of experiments, a potentially commercial postharvest integrated treatment was selected and applied in a commercial packingline. This combined treatment intended to maximize prevention and control of postharvest decay by integrating washing and sanitizing operations with fungicidal treatments. The combined treatment (CTrt) consisted of the following sequence of three individual treatments: 3% (w/v) PS in pressure washer for 15 s at 21 °C, 3 min soak in 3% (w/v) SB + 200 mg L<sup>-1</sup> sodium hypochlorite at 26.7 °C, and 30 s dip in 0.6 g L<sup>-1</sup> of fludioxonil (a.i.) at 29.4 °C. Treated fruit were dried through the packingline dryer. The treatment was applied to 600 pomegranates that were packed in mesh bags (60 bags of 10 fruit each; each bag constituted a replicate). The same number of untreated fruit was used as control treatment.

The bags were weighed and half of them (30 bags for control and 30 replicates for CTrt) were stored for up to 14 weeks in a commercial cold storage room at 8.9 °C and 90% RH (air). The other half were stored with the same environmental conditions in a commercial controlled atmosphere room with 5 kPa O<sub>2</sub> + 15 kPa CO<sub>2</sub> (CA).

### 2.3.2. Fruit decay and quality evaluations

After 6 weeks of commercial storage in both air and CA conditions, 15 bags of fruit per treatment (control and CTrt) were removed from each storage room and transported to the laboratory to determine decay incidence and pomegranate external and internal quality. This procedure was repeated after 14 weeks of storage.

Upon receipt, each bag of pomegranates was weighed and the percentage of weight lost during storage calculated. The entire sample of fruit was visually inspected for decay and physiological disorders. The proportions of botrytis crown-infected fruit and of those with spores of *B. cinerea* were recorded. Pomegranates were scored for external physiological disorders, namely skin surface pitting and browning, according to the following scale: 0 = none visible; 1 = slight ( $\leq 25\%$  of the skin); 2 = moderate (26–50% of the skin); and 3 = severe ( $> 50\%$  of the skin).

Skin color was measured on opposite cheeks of healthy pomegranates using a spectrophotometer (model CM-2002, Minolta USA Co., Ramsey, NJ, USA) attached to a personal computer. Color was assessed according to the Commission Internationale de l'Eclairage (CIE) and described as the three independent attributes of lightness ( $L^*$ ), chroma ( $C^*$ , saturation), and hue angle ( $h^\circ$ ). Five replicates of 10 fruit each per treatment, storage condition, and evaluation date were used.

Following external evaluations, each pomegranate was cut in half along the equator and hand-peeled. Internal symptoms

of physiological disorders (browning and/or pitting of internal surfaces: skin, pith, and integuments) were assessed in nondecayed fruit as a qualitative score in which 0 = none visible, 1 = slight symptoms, 2 = moderate symptoms, and 3 = severe symptoms.

Arils from 5 replicates of 10 fruit each were pooled and pressed through cheesecloth to extract the juice. Soluble solids concentration (SSC) was measured with a temperature compensating refractometer (model ATC-1, Atago Co., Tokyo, Japan). Titratable acidity (TA) and pH were determined with an automatic titrator (Radiometer, Copenhagen, Denmark). TA was expressed as percent citric acid. Juice color was measured with a colorimeter with a submersible probe (model CR-200, Minolta USA Co., Ramsey, NJ, USA) and reported as previously described. In addition, red color intensity of the juice was determined by measuring the optical density of a 20-time diluted aliquot of juice in a spectrophotometer (model UV160u, Shimadzu Co., Columbia, MD, USA) at 520 nm (OD<sub>520</sub>).

### 2.4. Statistical analysis

Scores in categorical scales were considered as quantitative variables and transformed to the square root of the value plus 0.5. Data from countings (e.g. decay incidence) were transformed to the arcsine of the square root of the proportion of decayed fruit. Data were subjected to two-way analysis of variance (ANOVA) with postharvest treatment and storage condition as factors using SAS software (SAS Institute Inc., Cary, NC, USA). Because of significant interactions, one-way analyses were performed for each factor at the different levels of the other factor. When appropriate, means were separated by Fisher's Protected L.S.D. test ( $P = 0.05$ ).

## 3. Results

### 3.1. Experiments with artificially inoculated fruit

The development of *B. cinerea* in the crown of artificially inoculated 'Wonderful' pomegranates after 4, 8, and 15 weeks of storage at 7.2 °C was significantly influenced by both postharvest treatment and storage condition, the interaction between these factors being significant at all three evaluation dates (Table 1). One-way ANOVAs indicated that regardless of the storage condition, the most effective antifungal treatments were F49, F21, and PS (Table 2). F49 was superior to F21, showing the benefit of heating the solution. PS lacked persistence and, although in general it was as effective as F49 after 4 and 8 weeks of storage, it was not after 15 weeks, especially in air. Botrytis crown decay was significantly more inhibited by all treatments in CA than in air, with the only exception of F49 after 8 weeks (Table 2).

Similar results were obtained when the sporulation of *B. cinerea* in crown-infected fruit was examined (data not shown). In general, the number of pomegranates with sporu-

Table 1

Analysis of variance of the development of gray mold in 'Wonderful' pomegranates artificially inoculated in the crown with *Botrytis cinerea*, treated with different antifungal chemicals, and stored in air or controlled atmosphere for 15 weeks at 7.2 °C and 95% RH

Source <sup>a</sup>	d.f.	Storage period					
		4 weeks		8 weeks		15 weeks	
		F	P>F	F	P>F	F	P>F
Treatment (T)	7	76.50	<0.0001	54.17	<0.0001	37.93	<0.0001
Storage condition (SC)	1	43.39	<0.0001	114.79	<0.0001	172.54	<0.0001
T × SC	7	6.50	<0.0001	5.43	<0.0001	5.68	<0.0001

<sup>a</sup> ANOVA applied to the transformed variable  $Y = (X + 0.5)^{1/2}$ , where X is a score in which 0 = no lesion (visible infected area) or fungal mycelium present, 1 = mycelium present in the crown, 2 = lesion ≤25% of skin surface, 3 = lesion on 26–50% of skin surface, and 4 = lesion >50% of skin surface.

Table 2

Influence of postharvest antifungal treatment and storage condition on the development of gray mold in 'Wonderful' pomegranates artificially inoculated in the crown with *Botrytis cinerea* and stored for 15 weeks at 7.2 °C and 95% RH

Treatment <sup>a</sup>	Botrytis crown decay (0–4 score) <sup>b</sup>					
	4 weeks <sup>c</sup>		8 weeks <sup>c</sup>		15 weeks <sup>c</sup>	
	Air <sup>d</sup>	CA <sup>d</sup>	Air <sup>d</sup>	CA <sup>d</sup>	Air <sup>d</sup>	CA <sup>d</sup>
Control	1.07a,r	0.87a,s	1.92a,r	0.97a,s	2.74a,r	1.47ab,s
PS	0.25e,r	0.00e,s	0.90c,r	0.13d,s	2.61a,r	0.74d,s
SB	0.75c,r	0.51c,s	1.54ab,r	0.95a,s	2.63a,r	1.11 c,s
SC	0.69c,r	0.97a,s	1.32b,r	1.00a,s	2.10b,r	1.24bc,s
PS + SB	0.79bc,r	0.56c,s	1.38b,r	0.93a,s	2.44ab,r	1.64a,s
PS + SC	0.93ab,r	0.72b,s	1.52b,r	0.93a,s	2.45ab,r	1.64a,s
F21	0.52d,r	0.25d,s	0.89c,r	0.47b,s	1.56c,r	0.70d,s
F49	0.37de,r	0.12de,s	0.51d,r	0.30c,r	0.80d,r	0.37e,s

<sup>a</sup> Control = untreated, PS = potassium sorbate, SB = sodium bicarbonate, SC = sodium carbonate, PS + SB = mixture of PS and SB, PS + SC = mixture of PS and SC, F21 = fludioxonil at 21 °C, and F49 = fludioxonil at 49 °C.

<sup>b</sup> For each storage period and condition, columns with the same letter (series 'a–e'), and for each storage period and treatment, rows with the same letter (series 'r–s'), are not significantly different according to Fisher's Protected L.S.D. test applied after an ANOVA to the transformed variable  $Y = (X + 0.5)^{1/2}$ , where X is a 0–4 score (see Table 1). Nontransformed means are shown.

<sup>c</sup> Storage period.

<sup>d</sup> Storage condition. Air, conventional cold storage; CA, controlled atmosphere storage (5 kPa O<sub>2</sub> + 15 kPa CO<sub>2</sub>).

lating lesions was lower in CA than in air for the entire storage period. F49, F21 and PS were the most effective treatments in inhibiting sporulation. The percentage of sporulation on PS-treated fruit in air, after 4, 8, and 15 weeks of storage was about 5, 50, and 90%, respectively, while in CA it was about 0, 5, and 50%, respectively. In contrast to what was observed with food preservatives, the antispore effect of F49 was the same in air as in CA (about 5, 10, and 20% of fruit with spores after 4, 8, and 15 weeks of storage, respectively; data not shown).

Internal decay was only assessed at the last evaluation date, after 15 weeks of storage. Crown infections of *B. cinerea* spread inside the fruit causing brown discoloration of membranes and internal tissues and watery disintegration of the arils, which acquired a dark brown color. Structures of the pathogen were not usually visible in these internal symptomatic areas. In some cases, however, mycelia and spores of *B. cinerea* were noticed within the fruit, usually in fissures beneath rind cracks. The incidence of internal decay was affected by both postharvest treatment and storage condition (Fig. 1). Internal gray mold was consistently higher in air (about 70% in control fruit) than in CA (about 20% in control fruit). Although in air F21 reduced internal decay by

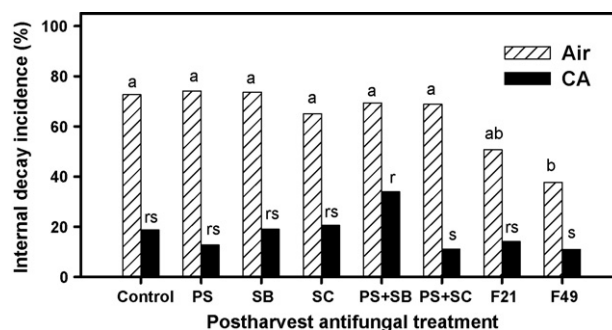


Fig. 1. Influence of postharvest antifungal treatment (untreated (control), potassium sorbate (PS), sodium bicarbonate (SB), sodium carbonate (SC), mixture of PS and SB (PS + SB), mixture of PS and SC (PS + SC), fludioxonil at 21 °C (F21), and fludioxonil at 49 °C (F49)) on the incidence of internal decay on 'Wonderful' pomegranates artificially inoculated in the crown with *Botrytis cinerea* and stored for 15 weeks at 7.2 °C and 95% RH in conventional cold storage (air) or in a controlled atmosphere (CA, 5 kPa O<sub>2</sub> + 15 kPa CO<sub>2</sub>). For each storage condition (series 'a–b' for air and series 'r–s' for CA), columns with the same letter are not significantly different according to Fisher's Protected L.S.D. test applied after an ANOVA to the arcsine-transformed data. Nontransformed means are shown.

Table 3

Influence of postharvest antifungal treatment and storage condition on the incidence of botrytis crown decay and sporulation of *Botrytis cinerea* in naturally infected ‘Wonderful’ pomegranates stored for 14 weeks at 8.9 °C and 90% RH in commercial facilities

Evaluation	Incidence (%) <sup>a</sup>			
	6 weeks <sup>b</sup>		14 weeks <sup>b</sup>	
	Air <sup>c</sup>	CA <sup>c</sup>	Air <sup>c</sup>	CA <sup>c</sup>
Botrytis crown decay				
Control (untreated)	88a,r	83a,r	100a,r	100a,r
CTrt <sup>d</sup>	29b,r	5b,s	91b,r	70b,s
<i>B. cinerea</i> sporulation				
Control	83a,r	78a,r	99a,r	94a,r
CTrt	23b,r	1b,s	83b,r	30b,s

<sup>a</sup> For each storage period and condition, values in columns with the same letter (series ‘a–b’), and for each storage period and treatment, values in rows with the same letter (series ‘r–s’), are not significantly different according to Fisher’s Protected L.S.D. test applied after an ANOVA to the arcsine-transformed data. Nontransformed means are shown.

<sup>b</sup> Storage period.

<sup>c</sup> Storage condition. Air, conventional cold storage; CA, controlled atmosphere storage (5 kPa O<sub>2</sub> + 15 kPa CO<sub>2</sub>).

<sup>d</sup> CTrt = combined treatment, which consisted of: 3% potassium sorbate in pressure washer for 15 s at 21 °C, 3 min soak in 3% sodium bicarbonate + 200 mg L<sup>-1</sup> sodium hypochlorite at 26.7 °C, and 30 s dip in 0.6 g L<sup>-1</sup> of fludioxonil at 29.4 °C.

28% compared to the control treatment, this reduction was not statistically significant. The only treatment that significantly reduced internal decay was F49 (47% reduction; Fig. 1).

No symptoms of browning or pitting were observed on internal surfaces of opened fruit.

### 3.2. Experiments with naturally infected fruit

#### 3.2.1. Decay incidence

The incidence of disease on naturally infected ‘Wonderful’ pomegranates after 6 and 14 weeks of storage at 8.9 °C was very high (80–100% in untreated fruit; Table 3) and most of the infections (more than 95%) were caused by *B. cinerea* in the fruit crown. The rest of the decayed fruit were infected mainly by *Penicillium* sp. through superficial skin wounds. After 14 weeks of storage, secondary infections by *Penicillium* sp. and to a lesser extent by *Cladosporium* sp. were observed on some fruit primarily infected by *B. cinerea* (data not shown).

In both storage conditions (air or CA), the application of the combined treatment (CTrt) significantly reduced both the incidence of botrytis crown decay and the sporulation of *B. cinerea* (Table 3). These reductions, however, were of larger magnitude on fruit stored in CA than on fruit stored in air. Furthermore, CA storage significantly reduced disease incidence and sporulation of CTrt-treated fruit, but not of untreated control fruit. Although all these tendencies were maintained after 14 weeks of storage, gray mold was not satisfactorily controlled at this evaluation date even by the combination of CTrt and CA (Table 3).

#### 3.2.2. Fruit quality

Initial quality and quality of control and CTrt-treated ‘Wonderful’ pomegranates after 6 weeks of storage at 8.9 °C in both air and CA are presented in Table 4. Fruit quality after 14 weeks of storage was not determined because the incidence of decay was too high.

Weight loss of pomegranates ranged 5–7.5%. It was not influenced by postharvest treatment, but it was about 2% higher in CA than in air. While skin color attributes ( $L^*$ ,  $C^*$ ,  $h^\circ$ ) of pomegranate stored in air were slightly higher (the color was lighter, more saturated, and less reddish) after storage than at harvest, those of fruit stored in CA practically did not change. The use of CTrt did not affect skin color on CA-stored fruit. When skin external physiological disorders were evaluated after storage, a slight to moderate skin pitting was detected (0.8–1.8 score). Its magnitude was significantly higher in air than in CA for both control and CTrt-treated fruit and, regardless the storage condition, it was slightly higher on treated than on control fruit. Therefore, the origin of this pitting was not chilling injury. No extensive uniform skin browning was consistently observed.

No internal symptoms of chilling injury (browning or pitting of internal skin and membranes) were observed in any fruit after 6 weeks of storage at 8.9 °C. Juice color, as determined by both CIE parameters and OD<sub>520</sub>, was slightly lighter and more red saturated after storage than at harvest (higher values of  $L^*$ ,  $C^*$ ,  $h^\circ$ , and OD<sub>520</sub>). In general, juice color attributes after storage were not affected by either postharvest treatment or storage condition. SSC of the juice increased during cold storage from 15.2 to 16.4–17.1% while initial values of TA (1.41%) and pH (3.04) were not substantially modified. Despite finding some significant differences between SSC and pH between air and CA storage, the magnitude of these differences had no practical impact (16.4% versus 17.1% for SSC and 3.01 versus 3.07 or 3.10 for pH) and thus neither postharvest treatment nor storage condition influenced these properties of the juice.

## 4. Discussion

Treatments with fludioxonil were superior to GRAS compounds in reducing botrytis crown decay, sporulation of *B. cinerea*, and internal gray mold after prolonged storage of artificially inoculated ‘Wonderful’ pomegranates. This was an expected result since fludioxonil is a phenylpyrrole fungicide whose mode of action is known and effective not only against *B. cinerea* but also against other important plant pathogens such as *Penicillium* spp., *Monilinia* spp., *Rhizopus* spp., or *Gilbertella* spp. (Olaya and Tally, 2004; Errampalli, 2004; Schirra et al., 2005). It inhibits a fungal protein kinase that catalyzes phosphorylation of the regulatory enzyme of glycerol biosynthesis (Rosslénbroich and Steubler, 2000). In our tests, the activity of fludioxonil was enhanced by heating the solution. The synergistic effect between numerous postharvest fungicides and heat has been reported (Schirra et

Table 4

Influence of postharvest antifungal treatment and storage condition on external and internal quality of 'Wonderful' pomegranates stored for 6 weeks at 8.9 °C and 90% RH in commercial facilities

Quality attribute <sup>a</sup>	At harvest (unit ± S.D.)	Treatment <sup>b</sup>	Storage condition <sup>c</sup>	
			Air	CA
Weight loss (%)	NA	Control CTrt	5.4a,r 4.9a,r	7.4a,s 7.5a,s
Skin color				
<i>L</i> <sup>*</sup>	51.16 ± 4.38	Control CTrt	53.66r –	51.94a,r 50.46a
<i>C</i> <sup>*</sup>	38.03 ± 3.96	Control CTrt	40.56r –	37.20a,s 35.48b
<i>h</i> <sup>°</sup>	26.92 ± 3.11	Control CTrt	30.73r –	28.01a,s 27.96a
Skin external disorders (0–3 score) <sup>d</sup>	0 ± 0	Control CTrt	1.6a,r 1.8a,r	0.8a,s 1.1b,s
Internal surface disorders (0–3 score) <sup>e</sup>	0 ± 0	Control CTrt	0 0	0 0
Juice color				
<i>L</i> <sup>*</sup>	18.61 ± 1.52	Control CTrt	21.52a,r 18.67a,r	20.20a,r 20.50a,r
<i>C</i> <sup>*</sup>	8.52 ± 0.34	Control CTrt	10.98a,r 7.99a,r	9.03a,r 8.43a,r
<i>h</i> <sup>°</sup>	322.33 ± 3.08	Control CTrt	338.17a,r 335.50a,r	341.02a,r 338.87a,r
OD <sub>520</sub>	0.70 ± 0.13	Control CTrt	0.89a,r 0.75b,r	0.68a,s 0.75a,r
Juice properties				
SSC (%)	15.2 ± 1.3	Control CTrt	16.4a,r 16.6a,r	17.1a,s 16.8a,r
TA (%)	1.41 ± 0.13	Control CTrt	1.47a,r 1.38a,r	1.52a,r 1.41a,r
pH	3.04 ± 0.04	Control CTrt	3.01a,r 3.01a,r	3.07a,s 3.10a,s

NA, not applicable; (–) no data available.

<sup>a</sup> For each attribute and storage condition, values in columns with the same letter (series 'a–b'), and for each attribute and treatment, values in rows with the same letter (series 'r–s'), are not significantly different according to Fisher's Protected L.S.D. test.

<sup>b</sup> Control, untreated; CTrt, combined treatment (see Table 3).

<sup>c</sup> Air, conventional cold storage; CA, controlled atmosphere storage (5 kPa O<sub>2</sub> + 15 kPa CO<sub>2</sub>).

<sup>d</sup> 0 = none visible, 1 = slight (≤25% of the skin), 2 = moderate (26–50% of the skin), and 3 = severe (>50% of the skin).

<sup>e</sup> 0 = none visible, 1 = slight symptoms, 2 = moderate symptoms, and 3 = severe symptoms.

al., 2000). In contrast to fludioxonil, PS, SB and SC are food additives, which have inhibitory action against fruit postharvest pathogens and are more fungistatic than fungicidal and not very persistent (Palou et al., 2001, 2002). Their mode of action is complex and in most cases still not clear, but in general combines a weak direct toxic effect to the pathogen with indirect effects related to nonpermanent modifications of the conditions within the site of infection (pH, physical changes, or induction of antifungal compounds; Palou et al., 2001; Venditti et al., 2005). Furthermore, all of these effects are specific for each pathosystem and the toxicity of these salts against a particular pathogen may vary considerably when tested in vitro or in different hosts. This could reasonably explain why the effectiveness of SB was poor against

*B. cinerea* in our tests with pomegranates while it was high against the same pathogen in vitro or in vivo on other fruit hosts (Palmer et al., 1997; Mlikota-Gabler and Smilanick, 2001; Karabulut et al., 2005). Another unanticipated result was the effectiveness of PS compared to that of SB and SC. Contrarily to carbonates, which are mineral salts, PS is an organic acid salt and its antimicrobial activity is primarily due to the undissociated form of the acid, which is naturally more abundant at low pH (Davidson, 1997). We presume that, once the aqueous PS solutions (pH 7.8) had been applied, they became more active within the relatively low pH of the sites of infection in the pomegranate fruit. PS has shown good inhibitory activity against *P. digitatum* and *P. italicum* on citrus fruit (Hall, 1988; Palou et al., 2002) and although

PS treatments have not been widely adopted for use by the citrus industry because more effective and persistent postharvest treatments with synthetic fungicides are still available, they are currently valuable tools to control fungicide-resistant strains of *Penicillium*. In this research with pomegranates, as it was observed in previous work with citrus fruit (Palou et al., 2002), mixtures of PS with other GRAS compounds did not provide a significant benefit compared to PS alone. In the case of PS + SB or PS + SC, the high pH of the sodium carbonates (about 8.5 for SB and 11.5 for SC in aqueous solutions) might raise the pH in the host infection sites and therefore reduce PS effectiveness.

A clear synergism between antifungal treatments and CA storage was observed for prevention of growth and sporulation of *B. cinerea* on artificially inoculated fruit. The fungistatic action of long-term storage in different CA conditions has been previously observed on 'Wonderful' (Holcroft et al., 1998; Hess-Pierce and Kader, 2003) and other pomegranate cultivars such as 'Mollar de Elche' (Artés et al., 1996) or 'Hicaz' (Küpper et al., 1995). Significant fungal decay reductions have also been obtained after storage of pomegranates in modified atmospheres (MA) created by wrapping the fruit with micro-perforated plastic films. For example, the use of polypropylene (Artés et al., 2000), polyolefin (Nanda et al., 2001), or polyethylene (Talaie et al., 2004) bags led to atmospheres rich in CO<sub>2</sub> and poor in O<sub>2</sub> that to some extent delayed fruit senescence and prevented decay development. In our work, CA storage also reduced the incidence of internal decay in artificially inoculated fruits. As expected, the synergistic effect of CA was more evident for GRAS compounds than for fludioxonil because of their weaker fungicidal activity. In the case of PS, which showed acceptable effectiveness but lacked persistence, storage in CA prolonged the fruit storage life to levels similar to those obtained with heated fludioxonil and storage in air. Therefore, the integration of PS treatments with CA storage could provide an alternative to fludioxonil for the management of pomegranate postharvest decay.

In our tests with naturally infected 'Wonderful' pomegranates stored in commercial facilities, the incidence of gray mold was very high. Although this incidence obviously depends on grove location and growing season, these result and those of other authors (Hess-Pierce and Kader, 2003; Tedford et al., 2005) illustrated the importance of field latent infections of *B. cinerea* in the environmental conditions of the San Joaquin Valley. CTrt was highly effective in reducing the incidence and sporulation of *B. cinerea* after 6 weeks of storage, especially on CA-stored pomegranates, but it was not effective after 14 weeks. Since the commercial application of fludioxonil was a component of CTrt, we expected better effectiveness and persistence of this combined treatment. Reasons that may account for this disappointing finding include the extremely high proportion of natural infections on the fruit used for the experiments and the storage temperature (8.9 °C) that undoubtedly favored gray mold development in comparison to lower temperatures. This high tempera-

ture was selected to definitely avoid chilling injuries during storage under commercial conditions. On the other hand, CTrt, comprised of three consecutive treatments including PS, SBC + chlorine, and fludioxonil, was an overly aggressive chemically, thermally, and mechanically integrated treatment that damaged the skin and accelerated the senescence process and that made the pomegranates more susceptible to gray mold. The fact that CTrt-treated fruit had a slightly higher degree of skin pitting (not due to chilling injury) than control fruit and, moreover, that treated fruit stored in CA showed lower incidence of botrytis crown decay than treated fruit stored in air seem to support this interpretation. Another possibility is the existence of some kind of negative interaction between two or more of the components of CTrt that led to a loss of antifungal activity. Precedents of such incompatibility were nevertheless not found in the literature. On the contrary, Smilanick et al. (1999, 2005, 2006) reported enhanced control ability of citrus green mold on oranges and lemons by combining applications of SBC and chlorine with applications of postharvest synthetic fungicides such as imazalil or thiabendazole.

We found that weight loss of 'Wonderful' pomegranates stored for 6 weeks at 8.9 °C in commercial facilities was relatively high (5–7.5%). However, CTrt did not include any wax and thus the fruit were not protected against dehydration during cold storage. Furthermore, RH in the commercial cold rooms was approximately 90%, which is lower than the recommended values of 95% or higher for 'Wonderful' pomegranates (Elyatem and Kader, 1984). Weight loss was not affected by the application of CTrt but was slightly higher in CA than in air. This unexpected result is not in agreement with previous research reporting on quality of CA-stored (Küpper et al., 1995; Artés et al., 1996) or MA-packed pomegranates (Artés et al., 2000; Nanda et al., 2001; Talaie et al., 2004). Variable weight loss reductions, some of them reaching even more than 10%, were observed by these workers after storage of pomegranates in CO<sub>2</sub>-enriched atmospheres as compared to conventional cold storage. The more plausible explanation for our contradictory result could be that instead of weighing healthy individual marked fruits, we weighed unopened mesh bags that contained 10 fruit and the presence of decay and watery fruit decomposition could influence the actual weight. Changes in pomegranate skin color during storage were not affected by the application of CTrt and, though the magnitude of the differences was small, initial red color was better maintained in CA than in air. A decrease in skin redness during conventional air storage has been previously reported by several authors working with different pomegranate cultivars (Elyatem and Kader, 1984; Gil et al., 1995; Küpper et al., 1995). Likewise, similar retarding effects of CA storage or high CO<sub>2</sub> treatments on skin color loss have been observed (Küpper et al., 1995; Artés et al., 1996; Holcroft et al., 1998). Chilling injury symptoms were not detected in fruit internal surfaces after either 15 weeks of storage at 7.2 °C or 6 weeks of storage at 8.9 °C, which could be anticipated by the use of such temperatures of stor-



age. In contrast to skin color, we and other workers (Ben-Arie et al., 1984; Artés et al., 1996; Holcroft et al., 1998; Hess-Pierce and Kader, 2003) found that red color intensity of pomegranate juice was slightly higher after cold storage than at harvest. In our tests, such increase was not significantly influenced by gas composition of the storage atmosphere. In some reports no juice color changes during storage in air were noticed (Kader et al., 1984; Gil et al., 1995), while in other research work an increase in red color intensity was significantly slowed or inhibited by exposure to CA in comparison to air (Holcroft et al., 1998; Hess-Pierce and Kader, 2003). These authors discussed that this was probably due to greater synthesis of anthocyanins in air-stored than in CA-stored fruit. On the other hand, pomegranate skin and juice colors seem not to be independent and, as shown by Gil et al. (1995) in their study with ‘Mollar de Elche’ pomegranates, fruit with more reddish skin contained less pigmented juice. Regarding juice properties, SSC slightly increased during the 6-week storage period in a similar way for both air-stored and CA-stored pomegranates while TA and pH remained basically unchanged regardless storage condition and antifungal treatment. Although contradictory results have been reported in relation to changes in pomegranate maturity index during storage at different conditions for different periods of time, in all studies these changes were not substantial and it is concluded that pomegranates are nonclimacteric fruits that do not further ripen after harvest (Ben-Arie et al., 1984; Kader et al., 1984; Elyatem and Kader, 1984; Gil et al., 1995). Moreover, most of the experiments comparing storage in air with different CA conditions conclude, in agreement with our results, that changes in SSC, TA, or pH during cold storage are not considerably influenced by gas composition (Artés et al., 1996; Holcroft et al., 1998; Hess-Pierce and Kader, 2003).

In summary, conclusions of the present work are that treatments with fludioxonil were highly effective, especially if heated, for the control of postharvest gray mold of pomegranates caused by *B. cinerea*. When food additive alternatives to fungicides were examined on artificially inoculated fruit, PS was, among the range of food additives tested, the most promising compound to control gray mold. Synergistic effects between antifungal treatments and CA storage (5 kPa O<sub>2</sub> + 15 kPa CO<sub>2</sub>) at nonchilling temperatures were observed and the combination of PS treatment and CA storage was as effective in reducing disease development as the combination of fludioxonil treatment and conventional storage in air. Under commercial conditions (treatment and storage of naturally infected fruits in commercial facilities), our selected postharvest combined treatment integrating washing and sanitizing procedures with a fungicide treatment was effective in controlling gray mold but probably affected negatively the external fruit condition and, as a consequence, lacked persistence. High natural decay incidence was the main factor limiting the storage period at nonchilling temperatures (8.9 °C) of California-grown ‘Wonderful’ pomegranates. CA storage greatly improved fruit storability due more to its

fungistatic effects than to its effects on fruit condition. Further research is needed to establish a more appropriate integrated antifungal treatment and also to define the optimum dip application conditions (concentration, temperature, duration) for a potential postharvest treatment based on the use of PS. It is clear from this work that the integration of PS treatments with CA storage could provide an alternative to fludioxonil or other synthetic fungicides for the management of pomegranate postharvest decay. Situations in which the availability of such alternative would be of interest for the California pomegranate industry include the production of organic fruit, the destination of fruit to markets with zero tolerance to agrochemicals, or the establishment of alternative decay control programs or rotations to minimize the risks of resistance development.

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