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Control of lemon postharvest diseases by low-toxicity salts combined with hydrogen peroxide and heat

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ABSTRACT

The effectiveness of potassium sorbate, sodium bicarbonate and potassium phosphite combined with heat and hydrogen peroxide in the presence of $CuSO_4$ to control major lemon postharvest diseases was investigated on artificially infected fruit. Green and blue molds, which both require wounds for infections to occur, were controlled by combination of hydrogen peroxide followed by inorganic salts, even when the temperature solutions were 25 °C. Control of sour rot was poor with salt solutions alone but significantly improved in treatments including hydrogen peroxide followed by potassium sorbate or sodium bicarbonate at 50 °C. Phomopsis stem-end rot was effectively controlled by potassium sorbate and potassium phosphite at 20 °C, and diplodia stem-end rot was partially controlled only by potassium sorbate. Applications of either potassium sorbate or a sequence of hydrogen peroxide followed by potassium phosphite were the most promising treatments, primarily because they controlled most of the diseases without the need to heat the solutions. These treatments controlled postharvest citrus diseases to useful levels and could be suitable alternative to conventional fungicides, or could be applied with them to improve their performance or to manage fungicide resistant isolates.

1. Introduction

Postharvest losses caused by green mold (*Penicillium digitatum*), blue mold (*P. italicum*), diplodia stem-end rot (*Lasiodiplodia theobromae*), phomopsis stem-end rot (*Diaporthe citri*), sour rot (*Geotrichum citri-aurantii*) and brown rot (*Phytophthora palmivora* or *P. nicotianae*) affect fresh fruit quality and marketing value of citrus fruit (Eckert and Eaks, 1989). Among them, green mold is the most important and sour rot, although less common, can cause significant losses in high rainfall years (Eckert and Eaks, 1989). Stem-end rot is a postharvest disease in warm and humid citrus growing regions such as Florida (US) and Tucumán (Argentina) and its incidence and severity can be considerably increased by ethylene degreening treatment (Brown and Eckert, 2000; Zhang, 2007).

The commercial control of green mold, stem-end rot, and other postharvest decay is conducted by integrated procedures with fungicides such as imazalil, sodium ortho-phenyl phenate, pyrimethanil, or thiabendazole as the core components (Ismail and Zhang, 2004). Stem-end rot is controlled by thiabendazole and more recently by a mixture of fludioxonil and pyraclostrobin (Zhang, 2007). Sour rot is not controlled with the currently registered fungicides i.e. imazalil and thiabendazole, and is only partially controlled by sodium o-phenylphenate (Eckert and Eaks, 1989). The widespread use of these chemicals has led to proliferation of resistant pathogen isolates and often an increase of fungicide residues on the fruit in attempts to control them, which risks exceeding residue maximum limits allowed by importing countries. Furthermore, some markets limit or ban the use of fungicides and their residues on fruit. Hence, there is an urgent need to control citrus postharvest diseases by alternative technologies, either used alone or in combination with conventional fungicides. Ideally, these treatments should have minimal registration issues because their safety to human health and environment is already known (Palou et al., 2002a,b; Venditti et al., 2005).

Among these alternatives, the number of several organic and inorganic salts (i.e. sodium bicarbonate and carbonate, potassium sorbate, calcium polysulfide, sodium silicate) have been comprehensively tested on a wide range of commodities, including citrus (Smilanick et al., 1999, 2008; Palou et al., 2009; Janisiewicz and

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Conway, 2010). Their effectiveness can reach commercially useful levels, although unlike most fungicides, they typically provide mostly curative action and little protection of fruit inoculated after they have been applied (Usall et al., 2008). These salts belong to the category of food additives or substances classified as GRAS (Generally Regarded as Safe) by the US Food and Drug Administration (FDA). Performance of these salts can be improved by combining them with other means, such as antagonistic microorganisms, hot water, sanitizers, low dose chemical fungicides, and waxes (Lima et al., 2005; Smilanick et al., 2008; Youssef et al., 2012).

Due to their low cost and availability, oxidizing biocides such as peroxides, are commonly used for general sanitation. Hydrogen peroxide was successfully applied in disinfection treatments of minimally processed fruit and vegetables and to control postharvest decay in fresh fruit (Sapers et al., 2001; Sapers and Simmons, 1998). Prior reports (Cerioni et al., 2012; Cerioni and Smilanick, 2012) indicated sequential treatments using salts and hydrogen peroxide to be particularly promising for the control of citrus green and blue molds; some of them matched the effectiveness of imazalil, the popular postharvest citrus fungicide used worldwide. The aim of this study was to determine the efficacy of several generic compounds and hydrogen peroxide to control the major postharvest diseases on lemons.

2. Materials and methods

2.1. Chemicals

Two sources of phosphite (KP) were used: (i) commercial formulation A (54.5% potassium phosphite, KPhos^{TM}, Pace International, Seattle, WA) and (ii) commercial formulation B (45.5% potassium phosphite, AFITAL Fosfito de Potasio, AgroEMCODI, Buenos Aires, Argentina). Hydrogen peroxide (H_2O_2, 30% a.i.) was purchased from either Brenntag Pacific Inc. (Fresno, CA) or Reagents S.A. (Santa Fe, Argentina). Other chemicals used included sodium bicarbonate (SBC, 99% a.i.), potassium sorbate (KS, 99% a.i.) and cupric sulfate were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO).

2.2. Fruit

Eureka lemons [*Citrus limon* (L.) Burm] were collected from commercial orchards in Tucumán (Argentina) or California and stored at 5 °C and 90% RH. Lemons used in the study were free from previous treatment or coatings.

2.3. Fungi

P. digitatum isolate PD-90, *P. italicum* isolate PI-105 and *G. citri-aurantii* were cultured for 7–14 days on potato dextrose agar (PDA, Difco Laboratories, Detroit) at 25 °C. The pathogens were isolated from infected lemons of citrus packinghouses in California. *L. theobromae* isolate (D-1) and *Diaporthe citri* isolate (P-1) isolated from decayed lemons in Tucumán were grown on PDA, at 28 °C for 7–14 days. To prepare conidial suspensions of *P. digitatum* and *P. italicum*, a protocol adapted by Cerioni et al. (2012) was followed. Arthrospore suspensions of *G. citri-aurantii* were prepared similarly but were adjusted to contain 10^8 arthrospores mL^{-1}.

2.4. Fruit inoculation

For experiments to control green and blue molds, lemons were inoculated at one side with *P. digitatum* and at the opposite end with *P. italicum.* For fruit inoculation the tip of a stainless steel rod, 1 mm wide and 2 mm length, was immersed in a conidial suspension of the corresponding pathogen and inserted afterwards at the equatorial position in the fruit rind. In the test for sour rot control, lemons were wound-inoculated with arthrospore suspensions supplemented with 10% (v/v) lemon juice, 10 mg L^{-1} cycloheximide to retard wound healing and with 100 mg L^{-1} thiabendazole to prevent green mold from interfering with sour rot development. For evaluation of diplodia and phomopsis stem-end rots, a modification of an inoculation toothpick method employed by Crall (1952) was used. Five portions of 5 mm × 5 mm diameter of mycelium of each pathogen were placed in sterile plastic container with PDA. 50 quill-type wooden toothpicks were added and incubated at 28 °C about 5 days to ensure adequate contamination of the toothpicks. After that, toothpicks were inserted diagonally downward into the stylar-end of the lemon fruit to a depth of approximately 1.5 cm. In all cases inoculated fruit were maintained at 20 °C and 95% relative humidity (RH) for 24 h before treatments.

2.5. Treatments

P. digitatum, *P. italicum* and *G. citri-aurantii* inoculated 'Eureka' lemons were immersed for 1 min in 20 L of water (control) or 20 g L^{-1} KP (formulation A), SBC, or KS solutions alone or were immersed for 1 min in a 20 g L^{-1} solution of H_2O_2 containing 6 mmol L^{-1} copper sulfate at 25 °C, either alone or followed by 1 min immersion in 20 g L^{-1} solutions of KP (formulation A), KS or SBC. The treatments were done in 22 L capacity stainless steel tanks with a computer-controlled thermostat. The temperatures of salt solutions were 25 or 50 °C (±0.5 °C). For comparison purposes, fruit were also immersed for 15 s in a 50 °C solution of 200 mg L^{-1} imazalil (Deccozil, 22.5% a.i., DECCO US Post-Harvest, Inc., Monrovia, CA).

Similar tests were done to evaluate stem-end rot control, but the KP used was formulation B and the concentration of compounds was 10 g L^{-1} KP, 20 g L^{-1} SBC and KS, and 15 g L^{-1} H_2O_2. The temperatures of salt solutions were 20 or 50 °C (±0.5 °C).

In another test, *P. digitatum*, *P. italicum*, *L. theobromae* and *D. citri* inoculated lemons were immersed for 1 min in 20 L of water (control) or 20 g L^{-1} solution of H_2O_2 containing 6 mmol L^{-1} copper sulfate either alone or followed by an incubation of 5, 10, or 20 g L^{-1} KP, (formulation A to green and blue molds, formulation B to diplodia and phomopsis stem-end rot). The temperature of the solutions was 25 °C (±0.5 °C).

2.6. Storage of fruit

The fruit were not rinsed after treatment and stored at 20 °C. The infected fruit were counted after 7 days to green mold, blue mold and sour rot and to stem-end rots the evaluation was after 4 days. The asymptomatic fruit were stored for 14 days before discharged.

2.7. Statistical analysis

Each treatment was applied to 5 replicates of 20 fruit each and the tests were done twice. Data were subjected to analysis of variance (ANOVA) followed by Tukey's test with Statitix 9.0 Analytical Software 2008 for Windows (USA). Differences at $P < 0.05$ were considered significant.

3. Results and discussion

3.1. Control of green mold, blue mold and sour rot on lemons

Applied alone, KS and KP were better than H_2O_2 or SBC to control green and blue molds (Fig. 1A and B). In an attempt to improve the effectiveness of these salts, we tried a sequence of treatments in which the fruit were first immersed in H_2O_2 plus copper sulfate at 25 °C, then passed through a second solution of KP, KS or SBC

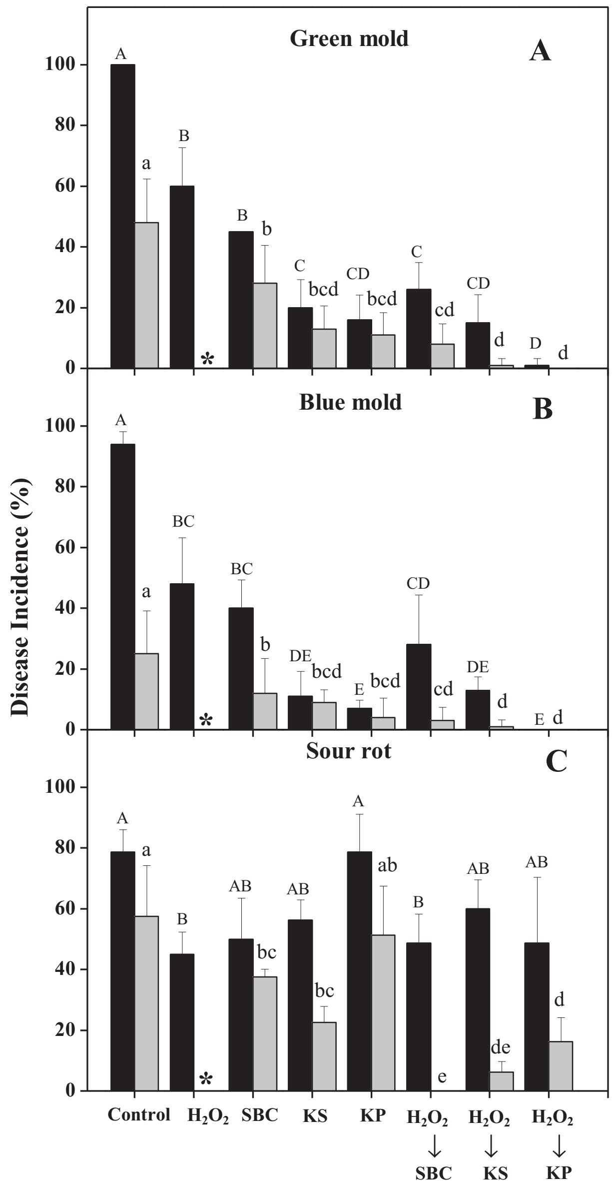

Fig. 1. Green mold (A), blue mold (B), and sour rot (C) incidence in 'Eureka' lemons immersed for 1 min in water (control) or 20 g L^{-1} of potassium sorbate (KS), sodium bicarbonate (SBC), potassium phosphite (KP) or hydrogen peroxide (H_2O_2) containing 6 mmol L^{-1} copper sulfate. Peroxide treatments at 25 °C were applied alone or followed by 1 min immersion in 20 g L^{-1} solutions of KP, KS or SBC (arrows). Salt solutions were applied at 25 °C (black) or 50 °C (grey). Columns with different letters (large cases for 25 °C and small cases for 50 °C) are significantly different according to Tukey's HSD (P = 0.05). *not determined.

at 25 °C or 50 °C. H_2O_2 treatment followed by KS or SBC was moderately effective at 25 °C and improved markedly at 50 °C (Fig. 1A and B). This sequence of treatments matched imazalil effectiveness at 50 °C, which controlled 85 and 95% of the green and blue mold infections, respectively. In order to control sour rot, KS and SBC were partially efficient at both temperatures assayed (Fig. 1C). When the sequence of H_2O_2 followed by salt compounds at 50 °C was applied on inoculated lemons, the control of this disease was significantly improved. Several alternatives to control postharvest diseases show promise, although few used alone approached the effectiveness of conventional fungicides. In prior reports, SBC, KS and KP have been shown to successfully control citrus postharvest diseases to partial but useful levels (Smilanick et al., 1999, 2008; Cerioni et al., 2013). Cerioni et al. (2012) showed that H_2O_2 in the presence of copper can partially control green mold, but should be followed by a second treatment, such as sodium bicarbonate, to maximize its effectiveness.

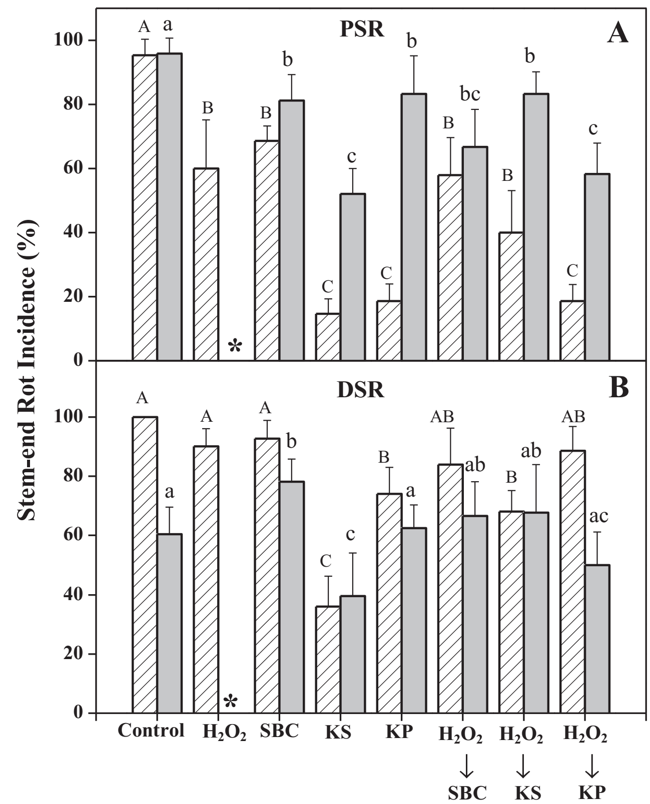

Fig. 2. Phomopsis (A) and diplodia (B) stem-end rots incidence in 'Eureka' lemons immersed for 1 min in water (control) or 20 g L^{-1} of potassium sorbate (KS), sodium bicarbonate (SBC), 10 g L^{-1} of potassium phosphite (KP) or 15 g L^{-1} hydrogen peroxide (H_2O_2) containing 6 mmol L^{-1} copper sulfate. Peroxide treatments at 20 °C were applied alone or followed by 1 min immersion in KP, KS or SBC (arrows). Salt solutions were applied at 20 °C (dashed) or 50 °C (grey). Columns with different letters (large cases for 20 °C and small cases for 50 °C) are significantly different according to Tukey's HSD (P = 0.05). *not determined.

3.2. *Control of diplodia and phomopsis stem-end rots on lemons*

Phomopsis stem-end rot was most effectively controlled by KP and KS at 20 °C (Fig. 2A). In contrast to results with green and blue molds, when the temperature increased the effectiveness of salts to control stem-end rots decreased. The combination of hydrogen peroxide and KP at 20 °C was as effective as KP alone. Only KS at 20 °C was moderately effective to control diplodia stem-end rot (Fig. 2B). Partial control of these diseases is relevant due to the limitations in substances approved for the use in packinghouses available to control these pathogens. Moreover, this is the first report where control of diplodia or phomopsis stem-end rots by artificial inoculation has been reported. Further studies will be necessary to analyze the effectiveness of these compounds under conditions of natural inoculation, with other cultivars, and when used in simultaneous application with commercial fungicides.

3.3. *Effect of hydrogen peroxide followed by potassium phosphite to control postharvest diseases*

KP at several concentrations in combination with H_2O_2 was evaluated to control major postharvest diseases in lemons. The results in Table 1 show that KP was very effective to control blue mold at all concentrations tested. This compound partially controlled phomopsis stem-end rot on lemons and was ineffective to

Table 1
Mold incidence (% ± SD)[a] in 'Eureka' lemons after treatments with different concentrations of potassium phosphite (KP) and hydrogen peroxide (H_2O_2).

Treatment	Green mold	Blue mold	DSR	PSR
Water control	100a	82 ± 8a	100a	97 ± 5a
KP 5 g L^{-1}	91 ± 2ab	21 ± 11b	77 ± 11bc	33 ± 10b
KP 10 g L^{-1}	81 ± 11bc	4 ± 4cd	82 ± 10ab	23 ± 6bc
KP 20 g L^{-1}	67 ± 12cd	3 ± 4cd	ND	ND
H_2O_2 20 g L^{-1}	56 ± 7d	13 ± 8bc	90 ± 4ab	18 ± 3c
H_2O_2 20 g L^{-1} → KP 5 g L^{-1}	36 ± 14e	5 ± 4cd	90 ± 4ab	25 ± 7c
H_2O_2 20 g L^{-1} → KP 10 g L^{-1}	21 ± 10ef	0d	68 ± 9c	17 ± 2c
H_2O_2 20 g L^{-1} → KP 20 g L^{-1}	9 ± 7f	1 ± 2cd	ND	ND

DSR: diplodia stem-end rot. PSR: phomopsis stem-end rot. ND: not determined.

[a] Lemons were inoculated with *P. digitatum*, *P. italicum*, *L. theobromae* or *D. citri* isolates 24 h before treatments. Values within columns followed by the same letter are not significantly different according to Tukey's HSD (P = 0.05).

control green mold and diplodia stem-end rot. In previous work, Cerioni et al. (2013) reported KP solutions effectively controlled green mold only when they were heated or combined with fungicides.

When the inoculated lemons were treated with the sequence of hydrogen peroxide followed by potassium phosphite, the effectiveness to control blue mold, green mold, and phomopsis significantly increased. The treatment with 15 g L^{-1} H_2O_2 followed to 10 g L^{-1} KP produced an excellent control of blue mold and phomopsis stem-end rot. Green mold was controlled when the concentration of KP was 20 g L^{-1}. Diplodia stem-end rot was not controlled with the concentrations of KP assayed.

This work is the first report about the effect of the low toxicity salts and H_2O_2 to control pathogens causing stem-end rots in citrus fruit. In previous works the mechanism of action of these compounds was informed in phytopathogenic fungi. Cerioni et al. (2010) reported a direct mechanism of action of H_2O_2 that involves oxidative damage on *P. digitatum* conidia to different cellular levels. Sodium bicarbonate has been widely evaluated for postharvest disease control and generally, it is used in association with other treatments (Smilanick et al., 1999; Palou et al., 2001). $NaHCO_3$ salt prevents infection by fungal pathogens, which need an injury on the fruit surface, by leaving a protective film against future infections (Venditti et al., 2005). The mechanism of action of phosphite salts in Oomycetes was studied (Dercks and Buchenauer, 1987; Smillie et al., 1989). The toxicity of phosphite to *Phytophthora spp.* comes from the activation of defense mechanisms in plants and/or by direct inhibitory action (Smillie et al., 1989; Guest and Bompeix, 1990; Olivieri et al., 2012). Forbes-Smith et al. (1998) showed that the potassium phosphite induced resistance in orange fruit to *P. digitatum* was associated with increase in the phenylpropanoid phytoalexin scoparone. It would be interesting to study how the proposed treatments control the stem-end rots. It is possible that direct and/or indirect mechanisms play an important role in the control of diseases caused by *L. theobromae* and *D. citri* pathogens.

4. Conclusions

Applications of KS or a sequence of hydrogen peroxide followed by KP were the most promising treatments identified in this study. Control of most diseases occurred without heating the solutions, which avoids considerable expense and an increased risk of fruit injury. Many concerns of dietary safety, disposal, worker safety, and other regulatory issues have already been addressed for these compounds, which should facilitate approval for their use in postharvest. These treatments are potential alternatives to conventional fungicides for control of postharvest diseases on citrus fruit. They could be used alone, or in integrated strategies with conventional fungicides to improve their efficacy or manage fungicide resistant isolates.

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