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Control of storage diseases of citrus by pre- and postharvest application of salts

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ABSTRACT

The effectiveness of sodium bicarbonate (SB), sodium carbonate (SC), sodium silicate (SS), potassium bicarbonate (PB), potassium carbonate (PC), potassium sorbate (PS), calcium chloride (CC), and calcium chelate (CCh) against naturally occurring postharvest decay on 'Comune' clementine and 'Valencia late' orange fruit was investigated. Aqueous salt solutions (2%, w/v, 20 hl ha^{-1}) were applied according to three strategies: (i) by spraying before harvest, (ii) by dipping after harvest, and (iii) by the combination of pre- and postharvest applications. Decay was assessed after two months at 4 ± 1 °C (oranges) or 6 ± 1 °C (clementines) and 95–98% RH, followed by 7 days of shelf life at 20 ± 2 °C. For both species, preharvest sprays and the combination of pre- and postharvest applications were more effective in suppressing decay than postharvest dipping. With regard to preharvest application, several salts completely inhibited the incidence of decay as compared to the water control, namely, SC and PC on both species, and SS on 'Valencia late' oranges. In combined applications, all salts were effective in reducing the decay as compared to the water control with an efficacy varying between 66–100 and 78–100% for oranges and clementines, respectively. When salts were applied after harvest, the activity was in general less pronounced, SC and PC being the most effective on both species. In *in vitro* tests, the minimum inhibitory concentration (MIC) for both *Penicillium digitatum* and *P. italicum*, was achieved at 0.25% SB, SC, PB, PC, PS, and SS. The filamentous fungal population on fruit treated once in the field and with the double treatment was reduced as compared to the water control, whereas no statistical differences were observed for postharvest application. Based on these results, field application of salts can be considered a useful strategy to be included in an integrated approach for controlling postharvest diseases of citrus fruit.

1. Introduction

Postharvest green mould, caused by *Penicillium digitatum* (Pers.:Fr.) Sacc., and blue mould, caused by *P. italicum* Wehmer, are the most important postharvest diseases affecting citrus production in arid climatic areas (Eckert and Eaks, 1989). Other pathogens can cause decay to stored citrus fruit but their incidence is generally low (Snowdon, 1990; Youssef et al., 2010a). Citrus postharvest diseases are commonly controlled worldwide by applying synthetic fungicides in packinghouses, before fruit storage. However, the development of pathogen resistance to fungicides and the growing public concern over health and environmental hazards associated with high levels of pesticide use have resulted in a considerable interest in developing alternative non-polluting control methods. Among these alternatives, the activity of several organic and inorganic salts has been comprehensively tested at 2–6% concentrations on a wide range of commodities, including citrus (Smilanick et al., 1997; Palou et al., 2008; Cunningham, 2010; Janisiewicz and Conway, 2010; Romanazzi et al., 2012). Organic and inorganic salts are "chemical means" belonging to the category of food additives or substances classified as GRAS (Generally Regarded as Safe) by the US Food and Drug Administration (FDA), and for this reason exempt from the usual Federal Food, Drug, and Cosmetic Act tolerance requirements (Senti, 1981). In spite of the interesting results obtained in laboratory and small scale experiments, salt usage limitations, i.e. inconsistent activity and limited persistence, lack of preventive effect, risk of fruit injury, and issues of disposal of exhausted salt solutions, make their postharvest commercial application still unreliable (Larrigaudiere et al., 2002; Palou et al., 2002; Smilanick et al., 2008; Smilanick, 2011). Performance of salts can be improved by combining them with other means, such as antagonistic microorganisms, hot water, sanitizers, low-dose chemical fungicides, and wax (El-Ghaouth et al., 2001; Lima et al., 2005; Palou et al., 2008; Cerioni et al., 2012; Youssef et al., 2012). However, combination may not encounter easy acceptance

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by operators because they are laborious and may need extra costs for labor, equipment, and energy (Lima et al., 2011). To overcome these problems and to attain commercially acceptable control levels, different application strategies as compared to fungicides are required.

Salts have been widely investigated as postharvest treatments by dipping or spraying; very few investigations on preharvest application have been conducted to control postharvest decay (Nigro et al., 2006; Teixidó et al., 2010). This strategy could be advantageous because of lesser fruit manipulation with a consequent decrease in damage and injuries commonly occurring during any postharvest treatment (Teixidó et al., 2010). Moreover, the presence of the active ingredient as an alternative control means as the injury is inflicted, may interfere with the first phases of the infection (Ippolito and Nigro, 2000). The objective of the present research was to compare the efficacy of a selection of salts applied according to three strategies: (i) by spraying before harvest, (ii) by dipping after harvest, and (iii) by the combination of pre- and postharvest applications on citrus fruit against storage decay. In order to simulate actual commercial conditions, experiments were conducted on naturally infected fruit instead of artificially inoculated ones. Preliminary results on 'Hernandina' clementines and 'Valencia late' oranges with the use of some salts and natural substances have been reported (Youssef et al., 2008a,b).

2. Materials and methods

2.1. Salts, fungal isolates, and plant material

Eight organic and inorganic salts (Table 1) were evaluated for their activity *in vitro* against *P. digitatum* and *P. italicum* and *in vivo* on different citrus species. Potassium bicarbonate (99.5%, PB) was purchased from Sigma Aldrich S.r.l (Milan, Italy), sodium bicarbonate (99.9%, SB), sodium carbonate (99%, SC), potassium carbonate (99%, PC), potassium sorbate (99%, PS), sodium silicate (72%, SS) and calcium chloride (99%, CC) from Carlo Erba Reagenti S.p.A (Rodano, Mi, Italy), and calcium chelate (CCh) from Chimica D'Agostino S.p.A. (Agrikel-Ca, div. Agridast, Ba, Italy). A standard chemical compound, DECCOZIL® 50 [50% imazalil (IMZ)], was purchased from Decco Italia s.r.l. (Belpasso, Ct, Italy). Fungal strains were isolated from decayed citrus fruit and maintained on potato dextrose agar (PDA) at 4 ± 1 °C in the culture collection of the Department of Environmental and Agro-Forestry Biology and Chemistry, University of Bari "Aldo Moro", Italy. The *in vivo* experiments were conducted on 7-year-old clementine (*Citrus reticulata* Blanco) cv. Comune, and 30-year-old sweet orange [*Citrus sinensis* (L.) Osbeck] cv. Valencia late trees, located in Basilicata region, Southern Italy.

Table 1
Minimum inhibitory concentration (MIC) of salts and imazalil on *Penicillium digitatum* and *P. italicum* in a colony growth assay.

Salt	MIC (%, w/v)	
	P. digitatum	*P. italicum*
Na bicarbonate ($NaHCO_3$)	0.25	0.25
Na carbonate (Na_2CO_3)	0.25	0.25
K bicarbonate ($KHCO_3$)	0.25	0.25
K carbonate (K_2CO_3)	0.25	0.25
Ca chloride ($CaCl_2$)	> 2.0	> 2.0
Ca chelate ($C_{10}H_{12}N_2O_8CaNa_2{\cdot}2H_2O$)	1.0	> 2.0
K sorbate ($C_6H_7KO_2$)	0.25	0.25
Na silicate ($Na_2O_x2SiO_2{\cdot}2H_2O$)	0.25	0.25
Imazalil	0.025	0.025

The diameter of *P. digitatum* and *P. italicum* was determined after 6 days incubation at 24 ± 1 °C on PDA amended with different salt concentrations (0.0125, 0.025, 0.05, 0.1, 0.25, 0.5, 1.0, and 2%, w/v).

2.2. Effect of salts and IMZ on in vitro growth of Penicillium spp.

The effect of salts and IMZ on the mycelial growth of *P. digitatum* and *P. italicum* was evaluated. An aqueous solution of the salts and IMZ was sterilized by filtration (0.45 μm) and added to molten (45 °C) PDA before pouring into 90 mm Petri dishes, to achieve 0.0125, 0.025, 0.05, 0.1, 0.25, 0.5, 1.0, and 2.0% (w/v) final concentration; PDA without salts or IMZ served as a control. Dishes were seeded in the center with a 5 mm mycelial plug taken from the edge of actively growing colony of the pathogens and incubated for 6 days at 24 ± 1 °C. For each salt/concentration, five Petri dishes were utilized as replicates and the entire experiment was repeated twice. Colony diameter was calculated as the average of the longest and the shortest diameter; the results were expressed as minimum inhibitory concentration (MIC).

2.3. In vivo trials

Salt solutions (2%, w/v) were prepared at room temperature dissolving 300 g for each salt in 15 L of tap water. The pH of all the solutions was measured using a pH meter (Jenway 3510 bench, Staffordshire, UK). The application strategies used were: (i) preharvest treatment; (ii) pre- and postharvest treatments; and (iii) postharvest dipping. In all the trials, fruit treated with water served as a negative control and fruit treated after harvest with IMZ were included as a standard chemical control (Table 2).

For preharvest treatments, trials were arranged in a completely randomized block design with 3 replicates, containing 3 plants each. Plants were selected for uniformity of fruit development and absence of evident symptoms of diseases and disorders, and sprayed with salt solutions (approximately 5 L plant^{-1}, 20 hl ha^{-1}). Treatments were made 7 days before harvest using a commercial motor-driven back sprayer (Fox Motori mod. 320, Poviglio, Re, Italy). At commercial maturity fruit were harvested from treated plants and placed into covered plastic boxes (5 boxes per plant), each containing 40–50 fruit for clementines and 20–25 fruit for oranges. Fruits were stored for two months at 4 ± 1 °C (oranges) and 6 ± 1 °C (clementines) and 95–98% RH, followed by 7 days of shelf life at 20 ± 2 °C. These conditions of storage were used to simulate actual commercial conditions.

For the combination of pre- and postharvest treatments, fruit from plants treated 7 days before harvest in the field were placed in plastic boxes (5 boxes per plant). Then, in the laboratory fruit from each group of five boxes were dipped (45 boxes per treatment) for 10 min in a solution of the same salt used in the field. After dipping, fruit were left to dry at room temperature for 2 h, then placed again in the same boxes and stored as described for preharvest treatment.

Regarding postharvest dipping, clementines and oranges harvested from untreated plants of the same orchard used for preharvest treatment were thoroughly mixed and arranged in 45 boxes per treatment in a completely randomized design. The remaining procedures were the same described above for combined treatments.

At the end of shelf life, the incidence of decay was assessed and expressed as percentage of fruit infected by fungal pathogens. Pathogens were visually identified and in case of doubt, isolation and morphological identification were carried out.

2.4. Epiphytic populations occurring on citrus surface

For the three application strategies, at the end of storage, epiphytic populations of filamentous fungi, yeasts and yeast-like fungi occurring on the fruit surface were evaluated according to Ippolito et al. (2005) with minor modifications. In particular, for each treatment, three replicates of 5 fruit each were weighed and shaken in 200 mL of sterile distilled water on a rotary shaker at 150 rpm for