Adv. Hort. Sci., 2015 29(2-3): 97-102

Decay control of cold stored *Citrus clementina* Hort. ex Tan. fruit by pre- and postharvest application of potassium phosphite

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Key words: aging, chilling injury, citrus fruit, defence stimulators, pre- and postharvest decay.

Abstract: The effectiveness of pre- and postharvest application of potassium phosphite against naturally occuring postharvest decay and storage disorders on clementine Monreal fruit (*Citrus clementina* Hort. ex Tan.) was investigated. Phosphite solutions were applied according to the following experimental protocol: by spraying fruit on the trees (2.5 g/L), at fruit colour breaking and 15 days before harvest; by the combination of preharvest (2.5 g/L) and postharvest dipping application (4 g of a.i./L). Preharvest applications were compared to Phosethyl-Al solution (2.5 g/L) and water control. Decay and disorders were assessed after 30 days of cold storage at 6±1°C and 90-95% RH, followed by 7 days of shelf life at 20±2°C. The combination of pre- and postharvest application of phosphite was more effective in reducing green (*Penicillium digitatum* Sacc.) and blue mould (*P. italicum* Weh.) incidence, as compared to water control, but it was not so effective in reducing the incidence of minor decay. Potassium phosphite treatments, before harvest and in pre-postharvest combination, significantly reduced chilling injury and aging with respect to water control. Based on these results, pre- and postharvest application of potassium phosphite can be considered a useful strategy to be included in an integrated approach for controlling green and blue mould of citrus fruit in storage.

1. Introduction

Clementines (*Citrus clementina* Hort. ex Tan.), due to their high quality, are one of the most important cultivated citrus mandarins in southern Italy. Production in the last decade has increased considerably thanks to remarkable consumer preference. These fruits are very perishable and the occurrence of various fruit diseases and physiological disorders affect their marketing value.

The major postharvest diseases of citrus fruit, including clementines, can be separated into two categories based on their initial infections: preharvest infections including Brown rot (*Phytophthora* spp.), *Alternaria* rot (*Alternaria citri* Ellis et Pierce, *A. alternata* (Fr.) Keissl), Stem-end rot (*Diplodia natalensis* Pole-Evan, *Phomopsis citri* Fawcett), Grey mould (*Botrytis cinerea* Pers.), Anthracnose (*Colletotrichum gloeosporioides* Penz.); and postharvest infections including Green mould (*Penicillium digitatum* Sacc.), Blue mould (*P. italicum* Weh.) and Sour rot (*Geotrichum candidum* Link) (Ohr and Eckert, 1985; Brown and Miller, 1999; Schena *et al.*, 2011).

The most common and serious diseases, which occur in Italy, during storage and marketing of clementine fruit are green and blue moulds. Infection takes place only through wounds, where nutrients are available to stimulate spore germination and fruit decay begins at these infected injury sites (Eckert and Eaks, 1989; Smilanick *et al.*, 1997; 2006; Ismail and Zhang, 2004). The incidence of other pathogens is generally low, but can be a serious problem in warm, wet years. These diseases, however, can cause significant economic losses during storage, transport and marketing.

Chilling injury (CI) represents the major disorder of citrus fruit occurring during low non-freezing temperature storage (0-10°C), and it depends on species and cultivars; mandarin hybrids are sensitive to CI. The severity of CI is related to the temperature and the duration of exposure (Chalutz *et al.*, 1985; Eckert and Eaks, 1989; Lafuente and Zacarias, 2006). Aging is indicated by the shrivelling and collapse of the stem-end button tissue (Porat *et al.*, 2004).

The use of synthetic fungicides in packinghouses, before fruit storage, remains the major means of control for managing citrus postharvest diseases (Eckert and Ogawa, 1988; Ismail and Zhang, 2004; Smilanick *et al.*, 2006). However, the development of resistance in fungal pathogens to fungicides (Schwinn *et al.*, 1982; Viñas *et al.*, 1993; Holmes and Eckert, 1999) and the growing public concern regarding the potential impact on human health and environmental haz-

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Received for publication 26 September 2014
Accepted for publication 9 March 2015

ards, have resulted in a significant interest in the development of alternative methods of disease control.

The development of treatments to enhance plant defences is an attractive area to seek further improvements in postharvest disease control. Among preharvest treatments, phosphite products, which elicit biochemical defences against invading fungi, can offer an alternative means of decay control.

In Italy, phosphite products (potassium, calcium and copper phosphite salts) are registered as fertilizers but not yet authorized as disease control agents; they require oxidation to phosphate prior to use by plants and this process is mediated by microbes (Adams and Conrad, 1953; Landschoot and Cook, 2005). Although foliar phosphite applications increased flower numbers and yields on 'Valencia' orange, their benefits may result from the control of fungal pathogens, as well as mitigating abiotic stresses, among other mechanisms, such as defence stimulators (Albrigo, 1999). Product activity is carried out primarily through two mechanisms: direct inhibition of the pathogen, with modification of the phosphate metabolism, and induction of host defence responses (induced systemic resistance mechanisms), such as the phytoalexins scoparone, scopoletin and umbelliferone (Smillie *et al.*, 1989; Guest and Bompeix, 1990; Guest and Grant, 1991).

Many growers of citrus fruit and other crops often apply phosphites before harvest to protect fruit from postharvest decay from fungal pathogens (Cerioni *et al.*, 2013 a). In fact, they are effective for the control of diseases caused by Oomycetes (*Phytophthora* and related fungi), particularly susceptible to inhibition by phosphite (Gaulliard and Pelossier, 1983; Cohen *et al.*, 1987; Guest and Grant, 1991; Martin *et al.*, 1998; McDonald *et al.*, 2001; Adaskaveg, 2009). On the other hand, few investigations, instead, describe control of *Penicillium* spp. by phosphites and also report the major efficacy of phosphites when applied in heated solution (Amiri and Bompeix, 2011; Bassay Blum *et al.*, 2007; Cerioni *et al.*, 2013 a). In the United States phosphites are exempt from residue tolerances (US EPA, 2006), and two commercial potassium phosphite formulations are registered for postharvest use.

The objective of the present research was to investigate the effectiveness of pre- and postharvest application of potassium phosphite against postharvest decay (in particular green and blue moulds), and physiological disorders (chilling injury and aging) on cold stored clementine fruits. The efficacy of the product was compared to Phosethyl-Al, a phosphate-generating fungicide. In order to simulate actual commercial conditions, experiments were conducted on naturally-infected fruit instead of on artificially inoculated specimens.

2. Materials and Methods

Plant material

Field trials were conducted in the fall 2013, on 20-year-old clementine trees (*Citrus clementina* Hort. ex Tan.) cv. Monreal, located in the "Palazzelli" experimental orchard (Sicily region, southern Italy) belonging to 'Consiglio per la Ricerca in Agricoltura e l'analisi dell'economia agraria - Centro di Ricerca per l'Agrumicoltura e le Colture Mediterranee (CRA-ACM)'.

Solution preparation

Commercial formulations of potassium phosphite (DeccoPhosk, Decco Italia s.r.l., Belpasso, Catania, Italy) and Phosethyl-Al (Aliette, Bayer CropScience) were dissolved manually in water to achieve a final concentration of 2.5 g L^{-1}.

Treatments and storage

Scheduled treatments are reported in Table 1. For preharvest treatments, trials were arranged in a completely randomized block design with three replicates of four plants each. Plants were selected for uniformity of fruit development, absence of evident symptoms of diseases and disorders, and sprayed with potassium phosphite, Phosethyl-Al and tap water. Treatments were carried out at fruit colour breaking and 15 days before harvest using a commercial motor-driven back sprayer (approximately 5 L $plant^{-1}$ of solution).

At commercial maturity, fruits were harvested from treated plants and placed into plastic boxes (one box per plant), each containing 50 fruits, with the exception of potassium phosphite treatments (two boxes per plant), in order to use the extra fruit for the postharvest treatment.

For the combination of pre- and postharvest treatments, a group of 600 fruits from plants A, already treated in the field, were immersed in a solution of potassium phosphite (4 g of a.i./L) at 40°C (±0.5°C) for 120 s. The fruits were not rinsed after treatment and were allowed to dry for 2 h at room temperature. All fruits, placed in three plastic boxes per treatment (each containing 200 fruits), were stored for 30 days at 6±1°C and 90-95% RH, followed by 7 days of shelf life at 20±2°C. These storage conditions were used to simulate actual commercial conditions.

At the end of cold storage and after shelf life, decay incidence, chilling injury and aging were assessed. Decay incidence was expressed as the percentage of fruit infected by fungal pathogens. Diseases were visually identified and classified as green mould (*P. digitatum*), blue mould (*P. italicum*), mix of green and blue mould (*P. digitatum* and *italicum* present on the same fruit), and minor decay (*Phytophthora, Alternaria, Rhizopus*, *Botrytis*, *Phomopsis*, *Diplodia*, etc.). Severity of chilling injury (CI) was evaluated

Table 1 - Scheduled treatments on clementine fruits

Treatment	Dose	Period of treatment
Potassium phosphite (A)	2.5 g/L	Two preharvest treatments
Phosethyl-Al (B)	2.5 g/L	Two preharvest treatments
Water Control (W)		Two preharvest treatments
Potassium phosphite (Ap+p)	2.5 g/L 4 g of a.i./L	Two preharvest treatments and a postharvest treatment

using a four-grade scoring system. A subjective rating of 0 (none), 1 (light), 2 (moderate), and 3 (severe) was used to estimate damage of the rind. A light rating indicated damage <10% of peel area, not perceived to be objectionable to the discerning consumer, moderate (10-30%) was injury estimated to be objectionable, and severe (>30%) indicated damage that would cause consumers to reject the product. Aging was expressed as percentage of fruit damaged.

In order to evaluate the effect of treatments on fruit weight loss, 30 fruits per treatment were regularly weighed at the beginning, at the end of cold storage and after one week of shelf life. The percentage of weight reduction was recorded.

Statistical analysis

Data were analysed using one-way analysis of variance (ANOVA) procedures, using Statistica 6.0 software. Percentage data were arcsine transformed to normalize variance. Mean values of treatments were compared by using Tukey's test at P=0.05 level. Data in the figures are actual percentages of decayed fruit.

3. Results

Postharvest rots on clementines at the end of storage were mainly due to *P. italicum* (blue mould) and *P. digitatum* (green mould) alone and present in the same fruit (mix of green and blue mould). Minor decay was caused by *Geotrichum* spp., *Alternaria* spp., *Botrytis* spp., *Phytophthora* spp., etc. In all cases preharvest application of potassium phosphite and the combination of pre- and postharvest applications, showed variable effects in reducing decay incidence, depending on the pathogens involved. Since the trials were conducted on naturally occurring infections, disease incidence in the control treatments was not very high.

After 30 days of storage at 6±1°C followed by a week of shelf life at 20±2°C, preharvest application of potassium phosphite on clementines significantly reduced the percent infection of blue mould, the mix of green-blue mould, and minor decay as compared to the water control (Fig. 1B-1C-1D); no significant reduction was observed on the green mould incidence as compared to the water control (Fig. 1A).

The combination of pre- and postharvest applications of potassium phosphite was, instead, more effective in reducing the incidence of green and blue mould, as compared to water control (Fig. 1A-1B). The improved control of blue mould, known for its greater ability to grow at low temperature, was of particular interest. Conversely, its efficacy in reducing the incidence of minor decay, on preharvest treatments was not improved by postharvest application (Fig. 1D).

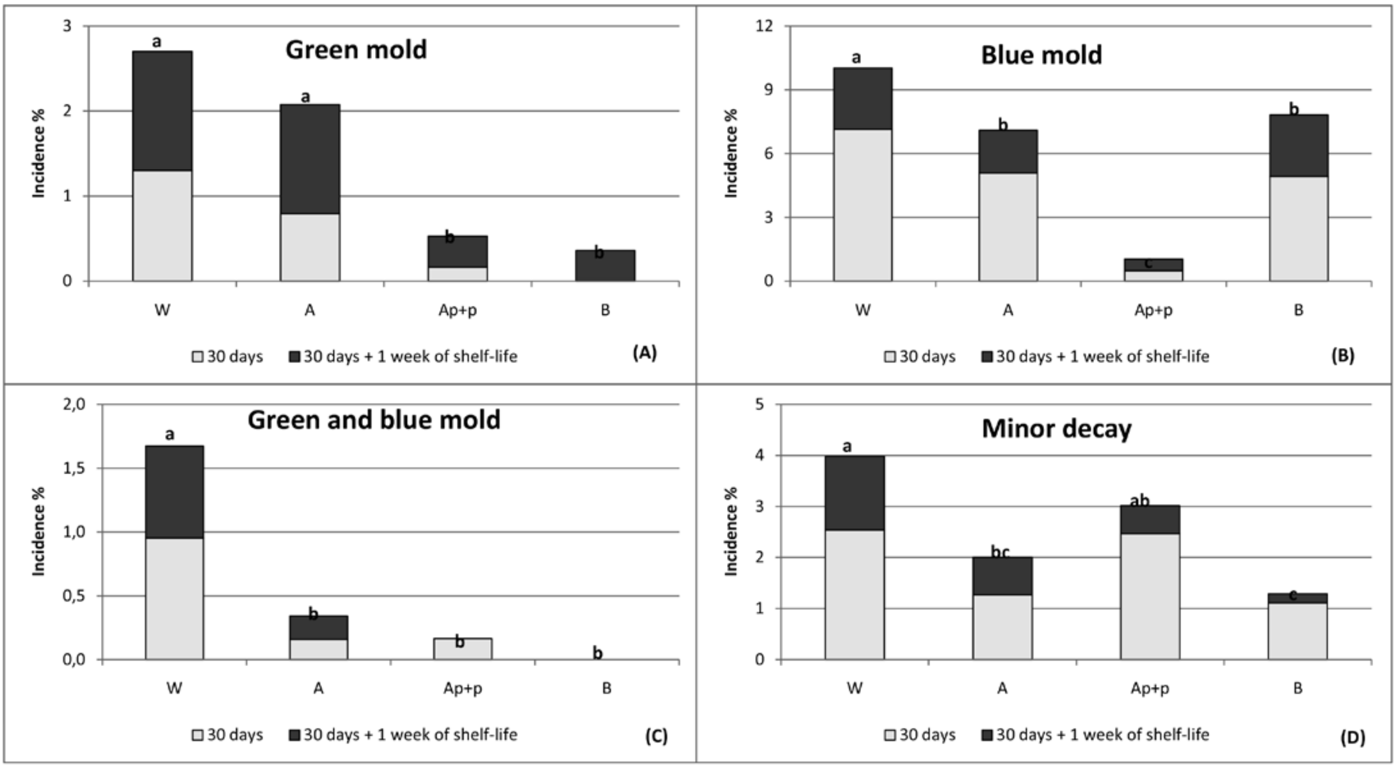

Fig. 1 - Incidence of green mold (*P. digitatum*) (A), blue mold (*P. italicum*) (B), mix of green-blue mold on the same fruit (C) and minor decay (D), on clementine, after 30 days of storage at 6±1°C followed by one week at 20±2°C. Each treatment was applied to three replicates of 200 fruit each. Water treatment was used as control. Columns marked with the same letters are not statistically different according to Tukey's test (P = 0.05). W= Water control, preharvest treatments; A= Potassium phosphite, preharvest treatments (2.5 g/L); Ap+p= Potassium phosphite, pre- (2.5 g/L) and postharvest treatments (4 g a.i./L); B= Phosetyl-Al, preharvest treatments (2.5 g/L).

Concerning CI, potassium phosphite treatments before harvest (A) and in pre-postharvest combination (Ap+p) significantly reduced light and moderate values, as compared to water control and severe values as compared to Phosetyl-Al (Fig. 2). All treatments (A, Ap+p and B) were significantly effective in reducing aging with respect to water control (Fig. 3).

Postharvest treatment with potassium phosphite had no phytotoxic effect on clementines. In addition, after 30 days of storage and one week of shelf life, the general external appearance of fruit was not affected by different treatments.

No statistically significant differences were found for weight loss, among all treatments, both at the end of cold storage and after a week of shelf life (*data not shown*).

4. Discussion and Conclusions

The main objective of the present study was to evaluate the efficacy of pre- and postharvest application of potassium phosphite, in controlling postharvest decay, particularly green and blue moulds of clementine, in order to extend its application for disease control of citrus fruits in Italy.

Reports describing the pre- and postharvest use of phosphite to control diseases caused by true fungi are few. Gutter (1983) reported that the posphite-generating compound Phosetyl-Al, *in vitro* and *in vivo*, had modest activity on the control of *P. digitatum*; Bassay Blum *et al.* (2007) reported that immersion of apple fruit in potassium phosphite solutions controlled blue mould caused by *P. expansum*. Cerioni *et al.* (2013 a) reported that improved control of green and blue mould, in postharvest treatments, was influenced by heating the solution (50°C), and by increasing the phosphite concentration (15 g/L). Regarding post-treatment storage temperature, 10°C were able to control green mould on citrus fruit, but had less effect on blue mould, even when the phosphite solution was heated to 50°C.

Our data showed that treatment with potassium phosphite was more effective against green and blue mould when applied before and after harvest, whereas, when applied only before harvest, it did not influence green mould incidence as compared to the water control. This different result is probably due to defence stimulation that treatment activates on the tree in field trials followed by the defence stimulation activated on fruit, in postharvest treatment (4 g of a.i./L), at the temperature of 40°C. The reduced efficacy of potassium posphite, on minor decay, in pre- and postharvest applications, was unexpected since its field application is effective against different pathogens.

Although not evaluated for the single control of *Phytophthora* brown rot, phosphites have long been known to control this fruit decay (Gaulliard and Pelossier, 1983; Cohen and Coffey, 1986; Graham and Timmer, 2011), which causes significant losses in wet years. Adaskaveg (2009) reported the excellent results obtained for the pre- and postinfection control of *Phythophthora citrophthora* on orange fruit dipped in 0.27 g/L of potassium phosphite. Thus, the phosphite treatments that controlled green and blue mould would be expected to control brown rot.

Phosphite is more costly than other alternatives used in packinghouses (SBC), but are compatible with SBC and with all of the fungicides currently registered for postharvest use such as Imazalil (IMZ) and Thiabendazole (TBZ), improving their performance (Cerioni *et al.*, 2013 a, 2013

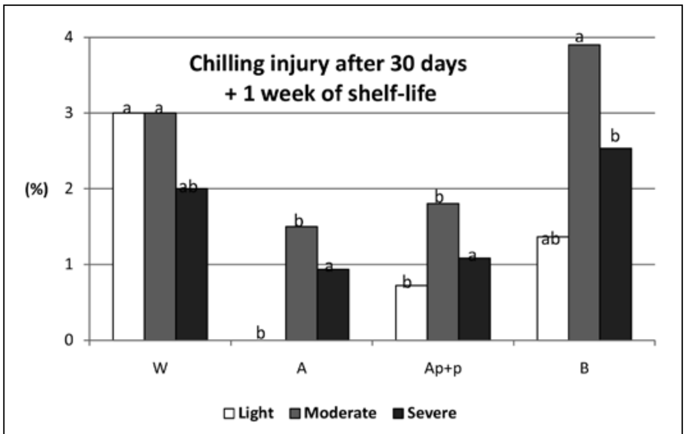

Fig. 2 - Effect of treatments on the severity of chilling injury on clementine, after 30 days of storage at 6±1°C followed by one week at 20±2°C. Each treatment was applied to three replicates of 200 fruits each. Water treatment was used as control. Columns marked with the same letters are not statistically different according to Tukey's test (P = 0.05). W= Water control, preharvest treatments; A= Potassium phosphite, preharvest treatments (2.5 g/L); Ap+p= Potassium phosphite, pre- (2.5 g/L) and postharvest treatments (4 g a.i./L); B= Phosetyl-Al, preharvest treatments (2.5 g/L).

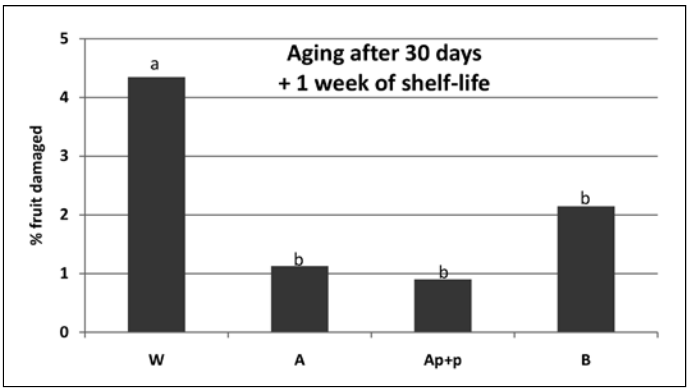

Fig. 3 - Effect of treatments on aging percentage on clementine, after 30 days of storage at 6±1°C followed by one week at 20±2°C. Each treatment was applied to three replicates of 200 fruits each. Water treatment was used as control. Columns marked with the same letters are not statistically different according to Tukey's test (P = 0.05).
W= Water control, preharvest treatments; A= Potassium phosphite, preharvest treatments (2.5 g/L); Ap+p= Potassium phosphite, pre- (2.5 g/L) and postharvest treatments (4 g a.i./L); B= Phosetyl-Al, preharvest treatments (2.5 g/L).

b; Palou *et al.*, 2001; 2002). Thus, the combination of potassium phosphite with SBC could be used to reduce costs, and in combination with IMZ could improve effectiveness for the control of IMZ-resistant isolates of *P. digitatum* (Kinay *et al.*, 2007).

In conclusion, our results have demonstrated that the incidence of green and blue mould on clementine fruit can be reduced by applying potassium phosphite twice before harvest and in postharvest treatments. Pre- and postharvest application of potassium phosphite can be considered a useful strategy to be included in an integrated approach for controlling postharvest diseases of citrus fruit. In any case, less infected fruit on packing lines should also reduce the demand for sanitizers during washing procedures (Lanza and Strano, 2009).

Practical application of potassium phosphite on citrus fruit needs to be further optimized as the obtainable level of protection is affected by various factors, first of all citrus variety, timing and number of applications. Additional research is in progress on different citrus varieties to improve the application strategy.

Acknowledgements

We thank Michele Scirè for technical assistance.

The work reported in this paper was presented at the “POSTHARVEST2014 Reducing Postharvest Losses to Feed the World Congress” held in Barletta, Italy, on 22-23 May 2014.

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