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, 1989; Smilanick et al., 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; 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 years. These diseases, however, can cause significant economic losses during storage, transport and marketing.

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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.

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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 phosphate (Gauliard 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; Bas-say 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 phosphate 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 phosphate, 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 phosphate 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 phosphate (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>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Period of treatment</th>
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<tbody>
<tr>
<td>Potassium phosphate (A)</td>
<td>2.5 g/L</td>
<td>Two preharvest treatments</td>
</tr>
<tr>
<td>Phosethyl-Al (B)</td>
<td>2.5 g/L</td>
<td>Two preharvest treatments</td>
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<tr>
<td>Water Control (W)</td>
<td></td>
<td>Two preharvest treatments</td>
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<tr>
<td>Potassium phosphate (Ap+p)</td>
<td>2.5 g/L</td>
<td>Two preharvest treatments</td>
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<tr>
<td></td>
<td>4 g of a.i./L</td>
<td>and a postharvest treatment</td>
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Table 1 - Scheduled treatments on clementine fruits
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 phosphate to control diseases caused by true fungi are few. Gutter (1983) reported that the phosphite-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 phosphate 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 phosphate 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 phosphite, 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 (Gauliardi 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 Phytophthora citrophthora on orange fruit dipped in 0.27 g/L of potassium phosphate. Thus, the phosphate treatments that controlled green and blue mould would be expected to control brown rot.

Phosphate 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...
b; Palou et al., 2001; 2002). Thus, the combination of potassium phosphate with SBC could be reduced to use 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 phosphate twice before harvest and in postharvest treatments. Pre- and postharvest application of potassium phosphate 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 phosphate 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.

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References


