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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_2 + 15 kPa CO_2) 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^{-1} 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.

Keywords: *Punica granatum*; *Botrytis cinerea*; Postharvest decay; Alternative chemical control; Food additives; Potassium sorbate; Fludioxonil

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^{-1}. 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_2 + 15 kPa CO_2 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^{-1} (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