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Ethylene synthesis, ripening capacity, and superficial scald inhibition in 1-MCP treated 'd'Anjou' pears are affected by storage temperature

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ABSTRACT

A continuing challenge for commercializing 1-methylcyclopropene (1-MCP) to extend the storage life and control superficial scald of 'd'Anjou' pear (*Pyrus communis* L.) is how to initiate ripening in 1-MCP treated fruit. 'D'Anjou' pears harvested at commercial and late maturity were treated with 1-MCP at 0.15 μL L^{-1} and stored either at the commercial storage temperature −1.1 °C (1-MCP@−1.1 °C), or at 1.1 °C (1-MCP@1.1 °C) or 2.2 °C (1-MCP@2.2 °C) for 8 months. Control fruit stored at −1.1 °C ripened and developed significant scald within 7 d at 20 °C following 3–5 months of storage. While 1-MCP@−1.1 °C fruit did not develop ripening capacity due to extremely low internal ethylene concentration (IEC) and ethylene production rate for 8 months, 1-MCP@1.1 °C fruit produced significant amounts of IEC during storage and developed ripening capacity with relatively low levels of scald within 7 d at 20 °C following 6–8 months of storage. 1-MCP@2.2 °C fruit lost quality quickly during storage. Compared to the control, the expression of ethylene synthesis (*PcACS1*, *PcACO1*) and signal (*PcETR1*, *PcETR2*) genes was stable at extremely low levels in 1-MCP@−1.1 °C fruit. In contrast, they increased expression after 4 or 5 months of storage in 1-MCP@1.1 °C fruit. Other genes (*PcCTR1*, *PcACS2*, *PcACS4* and *PcACS5*) remained at very low expression regardless of fruit capacity to ripen. A storage temperature of 1.1 °C can facilitate initiation of ripening capacity in 1-MCP treated 'd'Anjou' pears with relatively low scald incidence following 6–8 months storage through recovering the expression of certain ethylene synthesis and signal genes.

1. Introduction

'D'Anjou' pear (*Pyrus communis L.*) is the most produced pear cultivar in the Pacific Northwest of the US. It is enjoyed by consumers when fruit have ripened to a buttery and juicy texture at warm temperatures following cold storage (Chen, 2004; Sugar and Einhorn, 2011). At the commercial standard storage temperature at −1.1 °C, 'd'Anjou' pears with optimum harvest maturity require 60–90 days of postharvest chilling in order to produce ethylene internally at a sufficient rate to activate and complete the ripening process with high eating quality including softening (Blankenship and Richardson, 1985; Chen et al., 1983). In general, the storage life of 'd'Anjou' pears is about 5 months in conventional air storage and 8 months in controlled atmosphere (CA) storage with 2% oxygen and <1% carbon dioxide (Hansen and Mellenthin, 1979). Under both storage conditions, superficial scald is a major physiological disorder which affects the external appearance of fruit during the marketing period. Symptoms of superficial scald result from necrosis of the hypodermal cortical tissue and the cell damage is thought to be induced by conjugated trienols (CTols), the oxidation products of α-farnesene (Chen et al., 1990; Gapper et al., 2006). The accumulation of α-farnesene in the peel of 'd'Anjou' pear fruit is regulated by ethylene production (Bai et al., 2009; Gapper et al., 2006). The primary commercial control of scald on 'd'Anjou' pears at the present time is a postharvest treatment with the antioxidant ethoxyquin (1,2-dihydro-6-ehoxy-2,2,4-trimethyle-quinoline) (Chen, 2004; Hansen and Mellenthin, 1979). This treatment, however, often causes considerable phytotoxicity when the ethoxyquin solution becomes more concentrated at contact points between fruit or between fruit and bins. In 2009, the European Union withdrew authorization for plant protection products containing ethoxyquin. Alternatives to ethoxyquin for controlling scald of 'd'Anjou' are needed.

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1-Methylcyclopropene (1-MCP) is an inhibitor of ethylene perception that prevents ethylene-dependent responses such as ripening and senescence of vegetative and fruit tissues (Sisler and Serek, 1997; Sisler et al., 2003; Watkins, 2006). 1-MCP inhibits ethylene production and scald development in 'd'Anjou' pears and apples by inhibiting α-farnesene production and as a result prevents the accumulation of CTols (Bai et al., 2009; Gapper et al., 2006; Fan and Mattheis, 1999; Isidoro et al., 2006; Ju and Curry, 2000; Watkins et al., 2000). Gapper et al. (2006) demonstrated that 1-MCP inhibited ethylene-induced α-farnesene synthase gene *PcAFS1* expression in 'd'Anjou' pears, and both synthesis and oxidation of α-farnesene were substantially reduced, resulting in inhibition of scald.

Although postharvest 1-MCP application to pears provides valuable benefits in controlling scald and extending storage life, it interferes with the fruit's ability to ripen normally after storage (Bai et al., 2009; Chen and Spotts, 2005; Gapper et al., 2006). In recent years, there have been several research articles elucidating the effects of 1-MCP treatment on ripening capacity of European pear fruit (Argenta et al., 2003; Chiriboga et al., 2013; Ekman et al., 2004; Isidoro et al., 2006; Trinchero et al., 2004; Villalobos-Acuña et al., 2011). Chen and Spotts (2005) reported that 'd'Anjou' pears treated with 1-MCP at the dosages which control scald (0.05–0.3 $\mu L\,L^{-1}$) did not ripen normally at 20 or 25 °C following cold storage; fruit treated at lower dosages (0.01–0.02 $\mu L\,L^{-1}$) maintained ripening capacity, but developed unacceptable scald incidence. While the ripening capacity of 'd'Anjou' pears is completely blocked by 1-MCP at rates higher than 0.1 $\mu L\,L^{-1}$ even following up to 7 months of cold storage (Bai et al., 2009; Chen and Spotts, 2005; Gapper et al., 2006), Argenta et al. (2003) reported that d'Anjou' pears treated with 1-MCP at 0.1 to 1 $\mu L\,L^{-1}$ could develop ripening capacity following 6–8 months of cold storage. To initiate ripening capacity in European pears following 1-MCP treatment, several strategies have been investigated without consistent success, such as postharvest ethylene conditioning and warm temperature conditioning (Argenta et al., 2003; Bai et al., 2009; Trinchero et al., 2004).

In European pears, storage temperatures ranging from −1.1 to 10 °C play a crucial role in the stimulation of ethylene biosynthesis during subsequent ripening at room temperatures (Villalobos-Acuña et al., 2011). Exposure of 'Bosc' pears to intermediate temperatures (5–10 °C) stimulated the capability of producing adequate levels of ethylene during ripening at room temperatures more quickly than exposure to low temperatures (−1.1 to 0 °C) (Sfakiotakis and Dilley, 1974). 'D'Anjou' pears stored at 5 or 10 °C for 30 days developed ripening capacity in a shorter time than fruit stored at −0.5 °C (Sugar and Einhorn, 2011). Sugar and Basile (2013) also found that 'd'Anjou' and 'Comice' pear ripening capacity developed considerably faster at 10 °C than at −0.5 °C. Based on those results, we hypothesized that a storage temperature higher than −1.1 °C may allow development of ripening capacity in 1-MCP treated d'Anjou' pears during long-term storage.

D'Anjou' pear develops ripening capacity during chilling due to the induced synthesis of the enzymes involved in ethylene biosynthesis: ACC synthase (ACS) and ACC oxidase (ACO) (Chen et al., 1983; Blankenship and Richardson, 1985; Chiribboga et al., 2012). There are at least four ACS and one ACO gene sequences that have been isolated from pears (El-Sharkawy et al., 2004; Kondo et al., 2006). Four ethylene receptors (*PcETR1*, *PcETR2*, *PcETR5* and *PcCTR1*) have also been reported in pears (El-Sharkawy et al., 2003). Ethylene receptors are less affected by chilling, although all of them increase during ripening and negatively regulate the ethylene signal transduction pathway (El-Sharkawy et al., 2003; Guo and Ecker, 2004). Their transcript levels in 1-MCP treated 'd'Anjou' pears during storage have not yet been described.

The objectives of this study were to characterize the physiological and biochemical responses of 1-MCP treated 'd'Anjou' pear fruit to different storage temperatures and to evaluate the effect of increased storage temperature on the ability of 1-MCP to control scald while allowing the development of ripening capacity.

2. Materials and methods

2.1. Fruit material

'D'Anjou' pears were harvested at commercial maturity in 2012 from mature trees in the orchard of the Mid-Columbia Agriculture Research and Extension Center in Hood River, OR, USA (45.7° N, 121.5° W, elevation 150 m, average annual rainfall ~800 mm). Commercial harvest maturity was defined as when the average flesh firmness (FF) of 'fruit decreased to 62.1 N (±2.8), the late maturity to 55.0 N (±2.2). Defect-free 'd'Anjou' pears from three orchard blocks were harvested and randomized at commercial and late maturity and packed in 180 wooden boxes (80 fruit per box) with standard perforated polyethylene liners. The experimental design was completely randomized. Packed fruit were immediately stored in air at −1.1 °C (±0.5) and >95% relative humidity.

2.2. 1-MCP treatment

On the second day after harvest, cold fruit were exposed to 0.15 $\mu L\,L^{-1}$ 1-MCP (SmartFresh®, AgroFresh, Spring House, PA, USA) in an airtight room (39.75 m^3) with a circulation fan at 0 °C for 24 h. Following 1-MCP treatment, fruit with or without 1-MCP treatment were then stored at −1.1, 1.1, and 2.2 °C in air for up to 8 months.

2.3. Determinations of internal ethylene concentration (IEC), ethylene production rate and respiration rate

IEC was measured on fruit immediately upon removal from cold storage. Gas was sampled from five fruit individually using a vacuum-immersion technique (Chen and Mellenthin, 1981), and injected into a gas chromatograph (Shimadzu GC-8A, Kyoto, Japan). Nitrogen was used as the carrier gas at a flow rate of 0.8 mL s^{-1}. The injector and detector port temperatures were 90 and 140 °C, respectively. An external standard of ethylene (1.0 $\mu L\,L^{-1}$) was used for calibration. The limit of ethylene detection was approximately 0.08 $\mu L\,L^{-1}$.

Ethylene production and respiration rate were measured in five fruit of each replicate after 24 h at 20 °C. The fruit were placed in a 3.8 L airtight jar for 1 h at 20 °C. Gas samples were withdrawn through a septum on the top using a 1 mL gas-tight syringe. Ethylene was measured with the same GC system used for IEC determination. Ethylene production rate was expressed as pmol $kg^{-1}\,s^{-1}$. The headspace CO_2 concentration was measured using an O_2 and CO_2 analyzer (Model 900161, Bridge Analyzers Inc., Alameda, CA, USA). Fruit respiration rate (CO_2 evolution rate) was expressed as $\mu g\,kg^{-1}\,s^{-1}$.

2.4. Fruit storage quality evaluations

Fruit peel chlorophyll content, FF, and flesh titratable acidity (TA) were measured on 10 fruit of each replicate on day 1 after removal from cold storage. Peel chlorophyll content was estimated using a DA meter (Sinteleia, Bologna, Italy) and expressed as I_{AD} value (Ziosi et al., 2008). Measurements were taken on opposite sides of the equator of each fruit. FF was measured using a fruit texture analyzer (model GS-14, Guss Manufacturing Ltd., Strand, South Africa) with an 8 mm probe that penetrates 9 mm in 0.9 s. Two measurements were obtained per fruit on opposite sides of the equator after removal of 20 mm diameter peel discs. After chlorophyll and FF determination, flesh tissue of 0.1 kg was ground for 3 min in a