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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.

Keywords: *Pyrus communis*, 1-MCP, ripening capacity, ethylene, gene expression

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., 2006; 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.

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., 2006; 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., 2006; 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 to 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 to 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., 2006; 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., 2006; 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 µL L^{-1}) was used for calibration. The limit of ethylene detection was approximately 0.08 µL 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 µg 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 juice extractor (Acme Model 6001) equipped with a uniform strip of milk filter (Chen et al., 1983). TA was determined by titrating 10 mL of the juice to pH 8.1 using 0.1 N NaOH with a commercial titration system (Model T80/20, Schott-Gerate, Hofheim, Germany) and expressed as meq L^{-1} of juice.

2.5. Analysis of α-farnesene, conjugated trienols (CTols), and superficial scald disorder

Hexane-extractable α-farnesene content of pear peel was analyzed as described by Anet (1972), with some modification. Two segments (1 cm diameter) of peel tissue were removed from opposite sides of each of five pear fruit peel, immersed in 12 mL of hexane in a transparent glass-vial (15 mL) and kept at room temperature for 10 min. After incubation, the solvent was centrifuged at 11,550 × g for 5 min. Absorbance at 232 nm (α-farnesene) and 281-290 nm (CTols) was recorded using a Ultrospec 3100 pro UV/Visible Spectrophotometer (Biochrom Ltd, Cambridge, England). Concentrations of α-farnesene and CTols were calculated using the molar extinction coefficients ε232nm = 27,740 for α-farnesene and ε281-290nm = 25,000 for CTols (Anet, 1972), and expressed on a fresh weight basis in mg kg^{-1}.

Scald was assessed visually in 60-70 fruit from each replicate 7 d after transferring from cold storage to 20 °C. Fruit having approximately 0.6 cm^2 or higher peel area affected were classified as commercially unacceptable scald. The incidence of scald was expressed as the percentage of fruit affected with commercially unacceptable scald.

2.6. Ripening capacity evaluation

On day 7 at 20 °C, 10 fruit were randomly selected from each of three replicate boxes and used to determine FF and extractable juice (EJ). FF was determined as described above. After FF determination, flesh tissue of 0.1 kg was ground for 3 min in a juice extractor (Acme Model 6001) equipped with a uniform strip of milk filter. EJ was measured in a 100 mL graduated cylinder and expressed on a fresh weight basis in mL kg^{-1}.

2.7. RNA extraction and isolation of cDNA

Peel tissue, including the epidermis and 2-3 mm of hypodermal cortex, was excised with a fruit peeler from the equatorial region of 10 fruit from each replication and immediately frozen in liquid nitrogen. The peel tissue samples were stored at -80 °C until used for extraction of RNA. For RNA isolation, 1-2 g of frozen pear peel was ground to a powder in liquid nitrogen, and total RNA was isolated with Plant Total RNA Kit (Sigma-Aldrich, USA) according to the manufacturer. First-strand cDNA was performed from 1 µg total RNA using Invitrogen's Superscript™ III First Strand Synthesis Systems for quantitative real-time PCR (qRT-PCR) using oligo (dT) as primers. Reactions for qRT-PCR on the cDNA were performed with iTaq™ Universal SYBR Green Supermix (Bio-Rad). The amplification protocol consisted of an initial step at 95 °C for 2 min, 40 cycles at 95 °C for 10 s, and 60 °C for 30 s. The specificity of the PCR amplification was checked routinely by the melting curve analysis. Data were analyzed using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) and are presented as the relative level of gene expression. The qRT-PCR efficiency (E) for each gene was obtained by calculating the kinetic curve (Liu & Saint 2002).

Transcript levels of ethylene biosynthesis, perception, and signaling genes were analyzed using 18S rRNA (Chen et al., 2004) as internal control. Sequences for primers are listed in Table 1. The primers for *PcACS1* (X87112), *PcACS4* (AF386518), *PcACS5* (AF386523) and *PcCTR1* (HM156629) were designed according to previous work from Villalobos-Acuña et al. (2011). *PcACS2* (AY388989), *PcACO1* (AJ504857), *PcETR1* (AF386509), *PcETR2* (HM61909) primers were designed based on previously published data of Fonseca et al. (2005) and Chiriboga et al. (2013).

Table 1 primers used for qRT-PCR analysis.

Gene	GenBank accession numbers	Oligonucleotide sequences	Size of PCR Product (bp)	References

PcACS1	X87112	F 5`-TGGCAGAGCAATCTAAGGC-3`	122	El-Sharkawy et al., 2004
		R 5`-AAGGAGAGGTGAGTGAGGCA		
PcACS2	AY388989	F 5`- CATGGAAAAGAGAGAGCGGG-3`	50	Chiriboga et al., 2013
		R 5`- GATAAAAGAGAGGAAACTTCATTCTAGCA-3`		
PcACS4	AF386518	F 5`-CTTGGTTGAAGAGTGGATTAG-3`	432	Villalobos-Acuña et al., 2011
		R 5`- ATGATCAAGCCCTTGACATTG-3`		
PcACS5	AF386523	F 5`-TTTCGACACAAACTCAGCATCT-3`	352	Villalobos-Acuña et al., 2011
		R 5`-AAAGCAACTTCCATGGTCTTGT-3`		
PcACO1	AJ504857	F 5`-AATGCACCACTCCATTGTCATA-3`	236	Fonseca et al. 2005
		R 5`-GCTTCATGTAGTCATCAAACACA-3`		
PcETR1	AF386509	F 5`-AGAACGAGGCGTTGTTGCAC-3`	50	Chiriboga et al., 2013
		R 5`-CCATCATCCCCCATTGCTC-3`		
PcETR2	HM61909	F 5`-TGGGTGCAATGTTAAAGGCC	50	Chiriboga et al., 2013
		R 5`-GGAGCAATGAAACCGATAGCC		
PcCTR1	HM156629	F 5`-GAAGTCAGATGTTTACAGTTTTGGTG-3`	405	El-Sharkawy et al., 2003
		R 5`-AAGAATACATATTGAAGGTAATGG-3`		
18s rRNA	AF386514	F 5`-CATGGCCGTTCTTAGTTGGTGGAG-3`	110	Chen et al., 2004
		R 5`-AAGAAGCTGGCCGCGAAGGGATAC-3`		

2.7. Statistical Analyses

The experimental units were boxes and there were three replications (boxes) per treatment at each evaluation period. The data were subjected to analysis of variance (ANOVA) using StatSoft® Statistica version 6 (StatSoft, Tulsa, OK). When appropriate, means were separated by Fisher's Protected LSD test at $P < 0.05$.

3. Results

When stored at 2.2 °C, 1-MCP treated 'd'Anjou' pears softened and yellowed in storage, and developed mealy texture after ripening at 20 °C after 3 months. Compared to the commercial harvest maturity, fruit harvested at the late maturity (FF = 55.0 N) did not differ in ripening capacity at 68 °F following 1-8 months of cold storage. Therefore, data for 1-MCP treated fruit at 2.2 °C and at the late maturity (except for ripening capacity data) are not presented further.

3.1. IEC, ethylene production rate, and respiration rate

Control fruit started accumulating IEC at about 1.0 µL L^{-1} after 2 months. Thereafter, IEC reached the highest amount of 24.7 µL L^{-1} at 3 months, and maintained > 2 µL L^{-1} for 8 months at -1.1 °C. 1-MCP treated fruit stored at -1.1 °C (1-MCP@-1.1°C) had extremely low IEC (< 0.2 µL L^{-1}) throughout 8 months of storage. In contrast, the 1-MCP treated fruit that were stored at 1.1 °C (1-MCP@1.1°C) started accumulating IEC at about 1.5 µL L^{-1} after 5 months, and thereafter increased continuously between 5 to 8 months of storage (Fig. 1A).

Ethylene production on day 1 at 20 °C in control fruit after removal from cold storage increased significantly after 2 months, reached a maximum value after 5 months, and thereafter decreased. 1-MCP@-1.1°C fruit showed no ethylene production following 1-8 months of storage. 1-MCP@1.1°C fruit showed no ethylene production for 3 months, then started to produce ethylene at a low rate following 4 and 5 months, and produced a significant amount of ethylene after 6 months of storage (Fig. 1B).

The respiration rate of control fruit on day 1 at 20 °C after removal from cold storage increased during 8 months of storage and was higher than that of 1-MCP treated fruit stored either at -1.1 °C or 1.1 °C during 1-8 months of storage. 1-MCP@-1.1°C fruit maintained the lowest respiration rate which remained stable during 8 months of storage. 1-MCP@1.1°C fruit had a low but significantly ($p < 0.05$) higher respiration rate than 1-MCP@-1.1°C fruit in the first 5 months, and increased significantly after 6 months of storage (Fig. 1C).

3.2. Storage quality

Current commercial procedures prefer ‘d’Anjou’ pears to have an FF > 44.5 N upon entry into the shipment and distribution chain, designated as ‘shipping firmness’, to withstand mechanical damage (Sugar and Basile, 2013). Control fruit decreased FF from 64 to 53 N after 8 months of storage at -1.1 °C (Fig. 2A). 1-MCP treatment did not affect FF at -1.1 °C. 1-MCP@1.1°C fruit maintained FF similar to the control for 7 months, but firmness declined significantly to < 44.5 N after 8 months of storage (Fig. 2A). 1-MCP treatment did not affect I_{AD} during 8 months of storage at -1.1 °C. Fruit peel chlorophyll content (I_{AD}) in control fruit decreased from 1.9 to 1.7 for 8 months of storage at -1.1 °C (Fig. 2B). 1-MCP treatment did not affect I_{AD} at -1.1 °C. 1-MCP@1.1°C fruit maintained I_{AD} similar to the control for 5 months, but after 6 months I_{AD} was lower ($p < 0.05$) than in the control (Fig. 2B). An informal sensory evaluation indicated that the peel green color reduction observed in 1-MCP@1.1°C fruit at late storage did not affect consumer acceptance (data not shown). Control fruit lost TA by 50% during 8 months of storage. 1-MCP@-1.1°C and 1-MCP@1.1°C fruit maintained 65% and 42% higher TA than the control following 8 months of storage.

3.3. Superficial scald

In control fruit, α-farnesene accumulated from 14.3 mg kg^{-1} at harvest to a peak of 161.7 mg kg^{-1} after 3 months at -1.1 °C, and declined thereafter. 1-MCP effectively inhibited α-farnesene accumulation in the 1-MCP@-1.1°C fruit peel during 8 months of storage. 1-MCP@1.1°C fruit had 15.0 mg kg^{-1} of α-farnesene after 2 months, which increased to a maximum of 92.7 mg kg^{-1} after 5 months of storage, but did not have a significant peak like the control fruit (Fig. 2D). CTols in control fruit increased throughout the storage period, and reached a maximum of 28.5 mg kg^{-1} after 8 months of storage. 1-MCP effectively inhibited CTols accumulation in the 1-MCP@-1.1°C fruit peel throughout the storage period. The content of CTols in 1-MCP@1.1°C fruit increased only after 3 months, and reached 14.2 mg kg^{-1} at 8 months of storage (Fig. 2E). Compared to the control, 1-MCP@-1.1°C and 1-MCP@1.1°C fruit accumulated only 6% and 50% of CTols after 8 months of storage.

Control fruit developed 10.0 % scald after 3 months at -1.1 °C plus 7 d at 20 °C, which increased to 29.3% after 4 months and 100% after 5 months of storage. 1-MCP@-1.1°C fruit showed no scald during 8 months of storage. 1-MCP@1.1°C fruit developed scald incidence of 7.6%, 8.9% and 16.3% after 6, 7 and 8 months of storage, respectively. The severity of scald symptoms in 1-MCP@1.1°C fruit was very mild compared with those on the control fruit (Fig. 3).

3.4. Ripening capacity

The optimal eating quality of 'd'Anjou' pear is reached when fruit have ripened at warm temperatures to a buttery and juicy texture. For 'd'Anjou' pears, ripening capacity is defined as the ability of the fruit to soften to between 14 and 23 N with EJ content on a fresh weight basis of < 650 mL kg^{-1} within 7 d at 20 °C after removal from cold storage (Chen and Borgic, 1985). Control fruit developed ripening capacity following 3-5 months of storage at -1.1 °C, but developed mealy texture with increased FF and EJ within 7 d at 20 °C after 6 months of cold storage (Fig. 4A&B). 1-MCP@-1.1°C fruit did not develop ripening capacity within 7 d at 20 °C following 8 months of storage. 1-MCP@1.1°C fruit had no ripening capacity during the first 5 months, but were capable of ripening following 6-8 months of storage (Fig. 4A&B). 1-MCP@1.1°C fruit ripened with FF softened to 29.4, 16.2, and 8.3 N and EJ reduced to 653, 632, and 635 mL kg^{-1} following 6, 7, and 8 months of storage, respectively.

Late harvest maturity fruit responded to 1-MCP and storage temperature similarly to the commercial harvest maturity fruit with respect to ripening capacity (Fig. 4C). While 1-MCP treated fruit did not develop ripening capacity within 7 d at 20 °C following 8 months of storage at -1.1 °C, they were capable of ripening following 6-8 months of storage at 1.1 °C.

3.5. Ripening capacity related gene expressions

The expression of ethylene synthase genes *PcACS1*, *PCACS4*, *PCACS5* and *PCACO1* in control fruit increased about 24, 3252, 68 and 2-fold, respectively, during the first 3 months of storage (Fig. 5). *PcACS1, PCACS4* and *PCACS5* reached a maximum at 6 months and *PCACO1* reached a maximum at 4 months of storage. In 1-MCP@-1.1°C fruit, *PcACS1*, *PCACS4*, *PCACS5* and *PCACO1* gene expressions remained at basal levels during 8 months of storage. The transcript levels of *PcACS1, PCACS4*, *PCACS5* and *PCACO1* in 1-MCP@1.1°C fruit were considerably lower than in control fruit during the whole course of storage; however, *PcACS1* and *PcACO1* expressions in 1-MCP@1.1°C fruit were higher than 1-MCP@-1.1 °C fruit after 4 months of storage. There were no differences of expression in *PcACS4* and *PcACS5* between 1-MCP@-1.1°C and 1-MCP@1.1°C fruit. The expression of *PcACS2* decreased during the first few months of storage and then showed no difference in all treatment groups.

The expression of ethylene signal genes *PcETR1* and *PcETR2* in control fruit decreased slightly in the first 3 months and then increased until 6 months of storage (Fig. 5). Compared to the control, 1-MCP@-1.1°C fruit exhibited a significant down-regulation of *PcETR1* and *PcETR2* during storage. In contrast, 1-MCP@1.1°C fruit had similar expression levels for *PcETR1* and *PcETR2* genes with 1-MCP@-1.1°C fruit in the first 3 months, but had a noticeable increase of expression after 4 months, reaching a peak after 6 months of storage. The *PcCTR1* expression in control fruit was down-regulated in the first 3 months and increased slightly thereafter. 1-MCP treatment decreased the expression of *PcCTR1*, and there was no significant difference between 1-MCP@-1.1°C and 1-MCP@1.1°C fruit during storage.

4. Discussion

4.1. 1-MCP and storage quality

In order for ‘d’Anjou’ pears to develop ripening capacity, they must reach an IEC of 1.5 to 2 $\mu L\ L^{-1}$ (Chen and Mellenthin, 1981). In this study, the control fruit accumulated IEC > 1.5 $\mu L\ L^{-1}$ after 3 months in storage at the commercial storage temperature of -1.1 °C. The accumulated IEC was accompanied by increases in ethylene production and respiration rates and a decrease in fruit TA content. Fruit FF and green color did not decrease significantly during 8 months of storage at -1.1 °C. 1-MCP pre-storage treatment markedly inhibited IEC accumulation, ethylene production rate, respiration rate, and TA loss in ‘d’Anjou’ pears during storage at -1.1 °C, as reported previously (Bai et al., 2006; Chen and Spotts, 2005). Storage quality of 1-MCP treated ‘d’Anjou’ pears was affected by storage temperature. While the fruit softened and yellowed quickly in storage at 2.2 °C, 1-MCP treated fruit stored at 1.1 °C maintained FF, green color, and TA with no significant difference ($p < 0.05$) from fruit stored at -1.1 °C during the first 7 months. However, they lost FF, green color, and TA significantly after 8 months of storage.

4.2. 1-MCP and superficial scald

Superficial scald is the most destructive postharvest disorder of ‘d’Anjou’ pears (Chen and Spott, 2005). It has been hypothesized that oxidation products of α-farnesene in the fruit peel, identified as several reactive oxygen species of CTols, are the main causes of scald development in ‘d’Anjou’ pears and in apples (Anet, 1972; Gapper et al., 2006; Whitaker, 2004). Synthesis and accumulation of α-farnesene in ‘d’Anjou’ peel is regulated by ethylene production (Bai et al., 2006; Gapper et al., 2006). While the concentration of α-farnesene increased to a maximum after

3 months and thereafter reduced in control fruit during 8 months of storage at -1.1 °C, CTols continued to accumulate in the peel of ‘d’Anjou’ throughout the storage period, as was observed by Chen et al. (1990) in ‘d’Anjou’ pear and by Isidoro and Almeida (2006) in ‘Rocha’ pear. Corresponding to the accumulation of CTols, scald developed on 10% and 100% of the fruit after 3 and 5 months of storage, respectively. 1-MCP was very effective in inhibiting α-farnesene and CTols accumulation and scald development in fruit stored at -1.1 °C during 8 months of storage. 1-MCP treated fruit stored at 1.1 °C synthesized more α-farnesene and CTols after 3 months than at -1.1 °C, but showed a significant delay in α-farnesene accumulation and a lower amount of CTols than control fruit during 8 months of storage. 1-MCP treated fruit stored at 1.1 °C developed little scald after 6 months and incidence increased thereafter, but with milder severity than in the control (Fig. 3). Argenta et al (2003) reported that 1-MCP at 0.1 $\mu L\ L^{-1}$ controlled scald of ‘d’Anjou’ pears for 8 months at 1 °C.

4.3. 1-MCP and ripening capacity

Storage temperatures affect the chilling requirement for developing ripening capacity of European winter pears (Villalobos-Acuña and Mitcham, 2008; Sugar and Basile, 2013). Storage temperatures higher than the commercial storage temperatures enhanced ethylene production and therefore reduced chilling requirement for developing ripening capacity in ‘d’Anjou’ (Sugar and Einhorn, 2011) and ‘Bosc’ pears (Sfakiotakis and Dilly, 1974). Villalobos-Acuña et al., (2011) reported that ‘Bartlett’ pears treated with 1-MCP and stored at 10 °C were better able to overcome ripening inhibition than fruit treated with 1-MCP and maintained at 0 °C. In this study, we found that 1-MCP treated ‘d’Anjou’ pears stored at 1.1 °C accumulated a significant IEC and continued a high ethylene production rate upon removal from storage. As a consequence, the fruit ripened with high eating quality following 6 to 8 months of storage.

Harvest maturity influences induction of ripening capacity of ‘d’Anjou’ pears (Chen and Mellenthin, 1981; Gerasopoulos and Richardson, 1997; Sugar and Einhorn, 2011). Chen and Mellenthin (1981) found that ‘d’Anjou’ pears harvested at 58-53 N were capable of ripening after 30 d, while those harvested at > 60.0 N needed 60 d at -1.1 °C. For 1-MCP treated fruit in this study, however, ‘d’Anjou’ pears did not develop ripening capacity at -1.1 °C and 6 months of cold storage were necessary to develop ripening capacity at 1.1 °C for both fruit harvested at > 60.0 N and at 55.0 N (Fig.3). Gapper et al. (2006) reported that ‘d’Anjou’ pears harvested at 58 ± 4 N did not develop ripening capacity during 216 d of storage in RA at -1 °C.

Argenta et al. (2003) reported that ‘d’Anjou’ pears grown in north central Washington and treated with 1-MCP at 0.1 or 1.0 μL L^{-1} could develop ripening capacity during 6-8 months of cold storage at 1 °C. Gapper et al (2006) speculated that the inconsistent results regarding the ripening capacity of 1-MCP treated ‘d’Anjou’ pears reported in the literature (Bai et al., 2006; Chen and Spott, 2005; Gapper et al., 2006) were due to different production locations of the fruit. This study indicated that storage temperatures even within the small range between -1.1 to 1.1 °C play an important role in developing ripening capacity of 1-MCP treated ‘d’Anjou’ pears. Porritt (1964) reported that the most effective storage temperature for maintaining quality in European pears is -1.1 °C and a slight increase significantly increased fruit respiration and ripening rates.

1-MCP inhibits ethylene function and synthesis of climacteric fruit by competing for the binding site of ethylene receptors, an irreversible process once a high enough dose of 1-MCP occupies the ethylene binding receptors (Blankenship and Dole, 2003). Plant tissues have been shown to vary widely in their ability to regenerate new ethylene receptors (Blankenship and Dole, 2003). 1-MCP treated ‘d’Anjou’ pears might totally shut down the formation of new binding receptors when stored at -1.1 °C, but could resume their formation after 6 months at 1.1 °C. In

Pelargonium peltatum, the half-life of 1-MCP treatment was about 2, 3 and 6 d at 25, 20.7, and 12 °C, respectively, which indicates that 1-MCP has shorter half-life at higher temperature (Cameron and Reid, 2001). Another possibility is that the recovery of ripening capacity of the 1-MCP treated 'd'Anjou' pears stored at 1.1 °C is produced by proteins other than ethylene receptor proteins involved in the ethylene perception pathway (Blankenship and Dole, 2003). On the other hand, when fruit are stored at a higher temperature (1.1 °C), 1-MCP might have less effect on binding the binding sites tightly. Further study is needed to elucidate these hypotheses.

4.4. 1-MCP and ripening related gene expression

'd'Anjou' pears are climacteric fruit and their chilling-induced ripening capacity is triggered and regulated by ethylene synthesis (Blankenship and Richardson, 1985; Chen et al., 1983). Ethylene biosynthesis in climacteric fruit is autocatalytic and mediated by the binding of ethylene to an ethylene receptor (Barry and Giovannoni, 2007). Ethylene in fruit is biosynthesized from methionine, the rate limiting steps being the conversion of s-adenosylmethionine (SAM) to 1-aminocyclopropene-1-carboxylic acid (ACC) via ACC synthase (ACS), and the subsequent conversion of ACC to ethylene via ACC oxidase (ACO) (Adams and Yang, 1979). Both the rate limiting steps in ethylene synthesis are highly transcriptionally regulated by multigene families in tomatoes (Barry and Giovannoni, 2007) and European pears (Chiriboga et al., 2013).

Cold-induced ethylene biosynthesis is correlated with ACS and ACO activities in 'd'Anjou' and 'Passe-Crassane' pears. Both cultivars require conditioning before they will ripen at warm temperatures (Blankenship and Richardson, 1985; Lelievre et al., 1997). El-Sharkawy et al. (2004) demonstrated at least four ACS genes and one ACO gene were expressed during pear development and ripening, and the transcription of *PcACS1* increased during cold storage at 0 °C in ‘Passe-Crassane’ pears suggesting that *PcACS1* is a control point in the onset of ethylene production and ripening in these cultivars. Based on the qRT-PCR analysis, we confirmed that transcription of both *PcACS1* and *PcACO1* in ‘d’Anjou’ pears was highly stimulated in control fruit during cold storage at -1.1 °C, and their expression levels were much higher in 1-MCP@1.1°C fruit than in 1-MCP@-1.1°C fruit in the later period of storage. The expression of *PcACS1* and *PcACO1* was down-regulated in 1-MCP@-1.1°C fruit in the whole course of storage, but increased in 1-MCP@1.1°C fruit after 6 months of storage, which corresponded with IEC accumulation and the development of ripening capacity. The high correlation of transcription levels of *PcACS1* and *PcACO1* with ethylene production and the development of ripening capacity in control, 1-MCP@1.1°C, and 1-MCP@-1.1°C fruit suggest that *PcACS1* and *PcACO1*are control points in the onset of ethylene production and development of ripening capacity in ‘d’Anjou’ pears for both cold-stored and 1-MCP treated fruit.

PcACS4 and *PcACS5* were also up-regulated in control fruit in cold storage at -1.1 °C and were down-regulated in 1-MCP@-1.1°C fruit. However, the transcription of *PcACS4* and *PcACS5* did not increase to correspond to the ethylene synthesis in 1-MCP@1.1°C fruit in the later stage of storage. Therefore, *PcACS4* and *PcACS5* may not play important role in ethylene biosynthesis and developing ripening capacity in ‘d’Anjou’ pears, at least in 1-MCP treated fruit.

Interestingly, the expression of *PcACS2* was up-regulated by 1-MCP and decreased during storage. The function of *PcACS2* in the ripening process is still unclear. It was reported that the expression of *PcACS2* was negatively regulated by ethylene in ‘Passe-Crassane’ pear (El-Sharkawy et al., 2004) and not induced by cold storage in ‘Bartlett’ pear (Villalobos-Acuña et al., 2011). In apple fruit, the expression of *MdACS3*, which belongs to one subfamily with *PcACS2* in pear fruit, was reduced during ripening or ethylene treatment (El-Sharkawy et al., 2004; Yang et al., 2013). It is possible that the similar sequence between *MdACS3* and *PcACS2* contributes to the ethylene or 1-MCP reaction in fruit. In tomato fruit, expression of *LeACS1A* and *LeACS6* was negatively regulated but *LeACS2* was positively regulated by ethylene (Barry et al., 2000). Nakatsuka et al. (1998) showed that the ACS gene family members displayed different responses to 1-MCP. The expression of *LeACS6* was induced but *LeACS2* largely prevented by 1-MCP.

Two systems of ethylene regulation have been proposed to operate in higher plants (Lelièvre et al., 1998). In tomato, system-1 ethylene synthesis is regulated via the expression of *LeACS1A* and *LeACS6*, and system-2 ethylene is subsequently initiated and maintained by ethylene-dependent induction of *LeACS2* (Barry et al., 2000). Our data show that increased IEC and ethylene production in 'd'Anjou' fruit after two months of cold storage are correlated with decreased *PcACS2* and increased *PcACS1*, *PcACS4* and *PcACS5* expression. *PcACS2* in pear fruit behaved in a similar fashion to the *LeACS1A* and *LeACS6* genes in tomato, and *PcACS1*, *PcACS4* and *PcACS5* similar to *LeACS2*. This suggests that *PcACS2* may belong to system-1 in pear, and its expression is inhibited by internal ethylene. On the other hand, *PcACS1*, *PcACS4* and *PcACS5* may belong to system-2 in pear, and its expression is induced by internal ethylene.

Ethylene signaling related genes, including two receptor genes (*PcETR1* and *PcETR2*) and a *PcCTR1*-like gene, have been reported in pears (El-Sharkawy et al., 2003). The transcription of *PcETR1* and *PcETR2* generally increases upon ripening in control fruit and inhibited by 1-MCP treatment during storage time, which is in agreement with the findings in pears and other fruit (El-Sharkawy et al., 2003; Yang et al., 2013). Yang et al. (2013) showed that in apple fruit, *MdETR1*, *MdETR2* transcription levels were decreased with 1-MCP treatment. Our results further showed that 1-MCP@1.1°C treatment increased the transcription level of

PcETR1 and *PcETR2* after 4 month of storage. Those data suggest that *PcETR1* and *PcETR2* may play an important role in the inhibition of ethylene production by 1-MCP in ‘d’Anjou’ pears, which is consistent with the findings in apple (Costa et al., 2010; Yang et al., 2013). It has also been shown that the change of *PcETR1* and *PcETR2* exactly corresponded with the expression of *PcACS1* and *PcACO1* and those results coincided well with ethylene production and developing of ripening capacity in control and 1-MCP@1.1°C fruit. It might be hypothesized that new ethylene receptors in 1-MCP@1.1°C fruit may actually synthesized after long time storage at 1.1 °C and therefore initiated the fruit ripening capacity.

In the ethylene signaling pathway, *CTR1* acts as a negative regulator of the ethylene response (Guo and Ecker 2004). *CTR1* preferentially interacts with *ETR1* as a negative regulator of ethylene responses in *Arabidopsis* (Clark et al., 1998). *PcCTR1* expression decreased in ‘Old Home’ and ‘A16’ pears, whereas in ‘Passe-Crassane’ fruit it increased during cold storage (El-Sharkawy et al., 2003). In ‘Conference’ pears, which are capable of producing ethylene immediately after harvest without the need of cold storage (Chiriboga et al., 2012), *PcCTR1* was not affected by chilling temperature (Chiriboga et al., 2013). We found that *PcCTR1* expression decreased in the first 3 months and increased slightly thereafter in control fruit of ‘d’Anjou’ pears stored at -1.1 °C. It is noteworthy that 1-MCP decreased the expression of *PcCTR1* in ‘d’Anjou’ pears, a negative regulator of the ethylene signal pathway, during cold storage. It is possible that a feedback-regulation mechanism in ‘d’Anjou’ fruit is stimulated by 1-MCP blocking the ethylene signaling and synthesis (Stepanova and Alonso, 2009).

In conclusion, ‘d’Anjou’ pears are highly sensitive to 1-MCP with respect to ethylene production and development of ripening capacity and superficial scald. The effect of 1-MCP on ethylene production and ripening capacity of ‘d’Anjou’ fruit is both storage temperature and time dependent. 1-MCP treatment of ‘d’Anjou’ pears followed by storage at an elevated temperature (1.1 °C) instead of the traditional storage temperature of -1.1 °C may be used as an alternative to ethoxyquin treatment for controlling scald while maintaining ripening capacity after long-term storage (i.e., 6-8 months). The recovery of ripening capacity in 1-MCP treated ‘d’Anjou’ fruit stored at 1.1 °C was regulated by ethylene synthesis (*PcACS1*, *PcACO1*) and signal (*PcETR1*, *PcETR2*) genes. Additional work is going on in our lab to further understand how 1-MCP treated d’Anjou’ pears regain their ability to synthesize or respond to ethylene and ripen prior to 6 months of storage at 1.1 °C.

References

Adams, D.O., Yang, S.F., 1979. Ethylene biosynthesis: identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. Proc. Natl. Acad. Sci. U.S.A. 76, 170-174.

Anet, E.F.L.J., 1972. Superficial scald, a functional disorder of stored apples. IX. Effect of maturity and ventilation. J. Sci. Food Agric. 23, 763-769.

Argenta, L.C., Fan, X.T., Mattheis, J.P., 2003. Influence of 1-methylcyclopropene on ripening, storage life, and volatile production by ‘d’Anjou cv. pear fruit. J. Agric. Food Chem. 51, 3858-3864.

Bai, J., Yin, X., Whitaker, B.D., Deschuytter, K., Chen, P.M., 2009. Combination of 1-methylcyclopene and ethoxyquin to control superficial scald of ‘Anjou’ pears. HortTechnol. 19, 521-525.

Barry, C.S., Llop-Tous, M.I., Grierson, D., 2000. The regulation of 1-aminocyclopropane-1-carboxylic acid synthase gene expression during the transition from system-1 to system-2 ethylene synthesis in tomato. Plant Physiol. 123, 979-986.

Barry, C.S., Giovannoni, J.J., 2007. Ethylene and fruit ripening. J. Plant Growth Reg. 26, 143-159.

Blankenship, S.M., Richardson, D.G., 1985. Development of ethylene biosynthesis and ethylene-induced ripening in ‘d’Anjou’ pears during the cold requirement for ripening. J. Amer. Soc. Hortic. Sci. 110, 520-1520.

Cameron, A.C., Reid, M.S., 2001. 1-MCP blocks ethylene-induced petal abscission of *Pelargonium peltatum* but the effect is transient. Postharvest Biol. Technol. 22, 169–177.

Chen, P.M., 2004. Pear. In: Gross, K.C., Wang, C.Y., Saltveit, M. (Eds.). The Commercial Storage of Fruits, Vegetables, and Florist and Nursery Crops. USDA, ARS, Agriculture Handbook 66, <http://www.ba.ars.usda.gov/hb66/107pear.pdf>.

Chen, P.M., Borgic, D.M., 1985. Changes in water-soluble polyuronides in the pulp tissue of ripening 'Bosc' pears following cold storage in air or in 1% oxygen. J. Amer. Soc. Hort. Sci. 110, 667-671.

Chen, P.M., Spotts R.A., 2005. Changes in ripening behaviors of 1-MCP-treated ‘d’Anjou' pears after storage. Inter. J. Fruit Sci. 5, 3-17.

Chen, P.M., Mellenthin, W.M., 1981. Effects of harvest date on ripening capacity and postharvest life of ‘d’Anjou’ pears. J. Amer. Soc. Hort. Sci. 106, 38-42.

Chen, P.M., Mellenthin, W.M., Borgic, D.M., 1983. Changes in ripening behavior of ‘d’Anjou’ pears (Pyrus communis L.) after cold storage. Sci. Hortic. 21, 137-146.

Chen, P.M., Varga, D.M., Mielke, E.A., Facteau,T.J., Drake, S.R., 1990. Control of superficial scald on ‘d’Anjou’ pears by ethoxyquin: Effect of ethoxyquin concentration, time and method of application, and a combined effect with controlled atmosphere storage. J. Food Sci. 55, 167-170.

Chiriboga, M., Saladie, M., Bordonaba, J., Recasens, I., Garcia-Mas, J., Larrigaudiere, C. 2013. Effect of cold storage and 1-MCP treatment on ethylene perception, signaling and synthesis: Influence on the development of the evergreen behavior in ‘Conference’ pears. Postharvest Biol. Technol. 86, 212-220.

Chiriboga, M., Recasens, I., Schotsmans W.C., Dupille E., Larrigaudière, C. 2012. Cold-induced changes in ACC metabolism determine softening recovery in 1-MCP treated ‘conference’ pears. Postharvest Biol. Technol. 68, 78-85.

Clark, K.L., Larsen, P.B., Wang, X.X., Chang, C., 1998. Association of the Arabidopsis CTR1 Raf-like kinase with the ETR1 and ERS ethylene receptors. Proc. Natl. Acad. Sci. U.S.A. 95, 5401-5406.

Costa, F., Alba, R., Schouten, H., Soglio, V., Gianfranceschi, L., Serra, S., Musacchi, S., Sansavini, S., Costa, G., Fei, Z., Giovannoni, J., 2010. Use of homologous and heterologous gene expression profiling tools to characterize transcription dynamics during apple fruit maturation and ripening. BMC Plant Biol. 10, 229.

Ekman, J.H., Clayton, M., Biasi, W.V., Mitcham, E.J., 2004. Interactions between 1-MCP concentration, treatment interval and storage time for ‘Bartlett’ pears. Postharvest Biol. Technol. 31, 127-136.

El-Sharkawy, I., Jones, B., Gentzbittel, L., Lelievre, J.M., Pech, J.C., Latché, A., 2004. Differential regulation of ACC synthase genes in cold-dependent and independent ripening in pear fruit. Plant Cell Environ. 27, 1197-1210.

El-Sharkawy, I., Jones, B., Li Z.G., Lelièvre, J. M., Pech J.C., Latché A., 2003. Isolation and characterization of four ethylene perception elements and their expression during ripening in pears (Pyrus communis L.) with/without cold requirement. J. Exp. Bot. 54, 1615-1625.

Fan, X.T., Mattheis, J.P., 1999. Development of apple superficial scald, soft scald, core flush, and greasiness is reduced by MCP. J. Agric. Food Chem. 47, 3063-3068.

Fonseca, S., Monteiro, L., Barreiro, M.G., Pais, M.S., 2005. Expression of genes encoding cell wall modifying enzymes is induced by cold storage and reflects changes in pear fruit texture. J. Exp. Bot. 56, 2029-2039.

Gapper, N.E, Bai, J., Whitaker, B.D., 2006. Inhibition of ethylene-induced α-farnesene synthase gene PcAFS1 expression in 'd'Anjou' pears with 1-MCP reduces synthesis and oxidation of α-farnesene and delays development of superficial scald. Postharvest Biol. Technol. 41, 225-233.

Guo, H., Ecker, J.R., 2004. The ethylene signaling pathway: new insights. Curr. Opin. Plant Biol. 7, 40-49.

Hansen, E., Mellenthin, W.M., 1979. Commercial handling and storage practices for winter pears. Oregon Agr. Expt. Sta. Spec. Rpt. 550.

Isidoro, N., Almeida, D.P.F., 2006. α-Farnesene, conjugated trienols, and superficial scald in 'Rocha' pear as affected by 1-methylcyclopropene and diphenylamine. Postharvest Biol. Technol. 42, 49-56.

Ju, Z.G., Curry, E.A., 2000. Evidence that α-farnesene biosynthesis during fruit ripening is mediated by ethylene regulated gene expression in apples. Postharvest Biol. Technol. 19, 9-19.

Kondo, S., Isuzugawa, K., Kobayashi, S., Mattheis, J., 2006. Aroma volatile emission and expression of 1-aminocyclopropane-1-carboxylate (ACC) synthase and ACC oxidase genes in pears treated with 2,4-DP. Postharvest Biol. Technol. 41, 22-31.

Lelievre, J.M., Latche, A., Jones, B., Bouzayen, M., Pech, J.C., 1997. Ethylene and fruit ripening. Physiol. Plant. 101, 727-739.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. Methods 25, 402–408.

Liu, W., Saint, D.A., 2002. A new quantitative method of real time reverse transcription polymerase chain reaction assay based on simulation of polymerase chain reaction kinetics. Analytical Biochem. 302, 52–59

Nakatsuka, A., Murachi, S., Okunishi, H., Shiomi, S., Nakano, R., Kubo, Y., Inaba, A., 1998. Differential expression and internal feedback regulation of 1-aminocyclopropane-1-carboxylate synthase, 1- minocyclopropane- 1-carboxylate oxidase, and ethylene receptor genes in tomato fruit during development and ripening. Plant Physiol. 118, 1295–1305.

Porritt, S.W., 1964. The effect of temperature on postharvest physiology and storage life of pears. Can. J. Plant Sci. 44, 568-579.

Sfakiotakis, E.M., Dilley, D.R., 1974. Induction of ethylene production in ‘Bosc’ pears by postharvest cold stress. HortScience 9, 336-338.

Sisler, E.C., Alwan, T., Goren, R., Serek, M., Apelbaum, A., 2003. 1-Substituted cyclopropenes: effective blocking agents for ethylene action in plants. Plant Growth Reg. 40, 223-228.

Sisler, E.C., Serek, M., 1997. Inhibitors of ethylene responses in plants at the receptor level: recent developments. Physiol. Plant. 100, 577-582.

Stepanova, A.N., Alonso, J.M., 2009. Ethylene signaling and response: where different regulatory modules meet. Current Opinion in Plant Biol. 12, 548–555.

Sugar, D., Einhorn, T.C., 2011. Conditioning temperature and harvest maturity influence induction of ripening capacity in ‘d’Anjou’ pear fruit. Postharvest Biol. Technol. 60, 121-124.

Sugar, D., Basile, S.R., 2013. Integrated ethylene and temperature conditioning for induction of ripening Capacity in ‘Anjou’ and ‘Comice’ pears. Postharvest Biol. Technol. 83, 9-16.

Trinchero, G.D., Sozzi, G.O., Covatta, F., Fraschina, A.A., 2004. Inhibition of ethylene action by 1-methylcyclopropene extends postharvest life of ‘Bartlett’ pears. Postharvest Biol. Technol. 32, 193-204

Villalobos-Acuña, M., Biasi, W.V., Mitcham, E.J., Holcroft, D., 2011. Fruit temperature and ethylene modulate 1-MCP response in ‘Bartlett’ pears. Postharvest Biol. Technol. 60, 17-23.

Villalobos-Acuña, M., Mitcham, E.J., 2008. Ripening of European pears: the chilling dilemma. Postharvest Biol. Technol. 49, 187-200.

Watkins, C.B., 2006. The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. Biotechnol. Adv. 24, 389-409.

Watkins, C.B., Nock, J.F., Whitaker, B.D., 2000. Responses of early, mid and late season apple cultivars to postharvest application of 1-methylcyclopropene (1- MCP) under air and controlled atmosphere storage conditions. Postharvest Biol. Technol. 19, 17-32.

Whitaker, B.D., 2004. Oxidative stress and superficial scald of apple fruit. HortScience 39, 933-937.

Yang, X.T., Song, J., Campbell-Palmer, L., Fillmore, S., Zhang, Z., 2013. Effect of ethylene and 1-MCP on expression of genes involved in ethylene biosynthesis and perception during ripening of apple fruit. Postharvest Biol. Technol. 78, 55-66.

Ziosi, V., Noferini, M., Fiori, G., Tadiello, A., Trainotti, L., Casadoro, G., Costa, G., 2008. A new index based on vis spectroscopy to characterize the progression of ripening in peach fruit. Postharvest Biol. Technol. 49, 319-329.

Fig. 1. Internal ethylene concentration (µL L^{-1}) during cold storage (A), ethylene production (B) and respiration rates (C) on d 1 at 20 °C following storage of 1-MCP treated ‘d’Anjou’ pears at -1.1 or 1.1 °C for 8 months. Values are means ± SD, n=3.

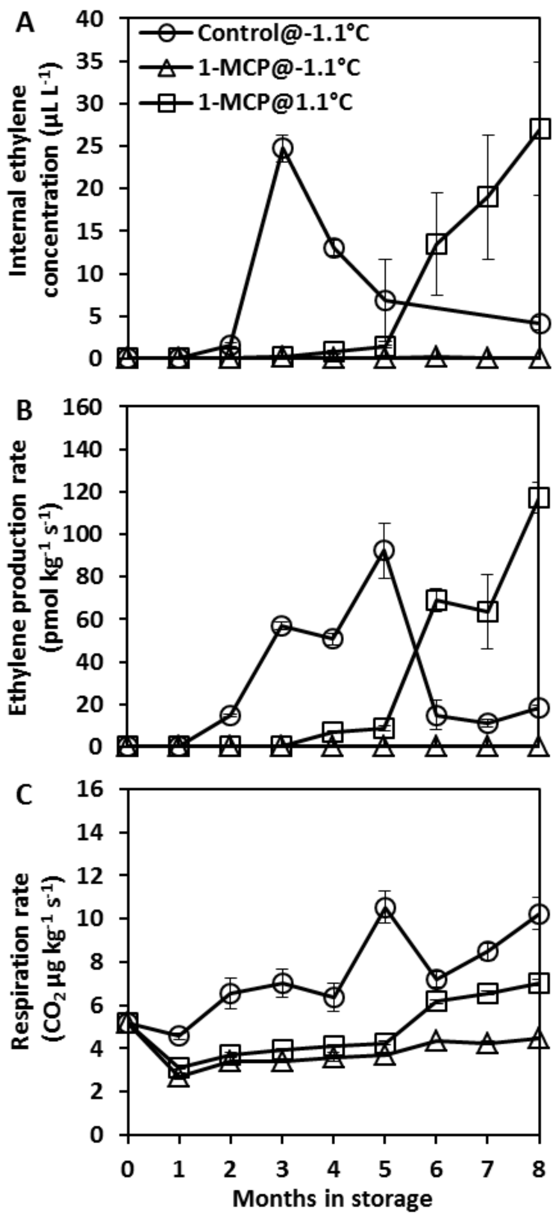


Fig. 2. Effect of 1-MCP and storage temperatures on flesh firmness (FF) (A), fruit peel chlorophyll content (I_{AD}) (B), titratable acidity (TA) (C), α-farnesene (D), conjugated trienols (E), and superficial scald incidence (F) in ‘d’Anjou’ pears during 8 months of storage at -1.1 or 1.1 °C. Values are means ± SD, n=3.

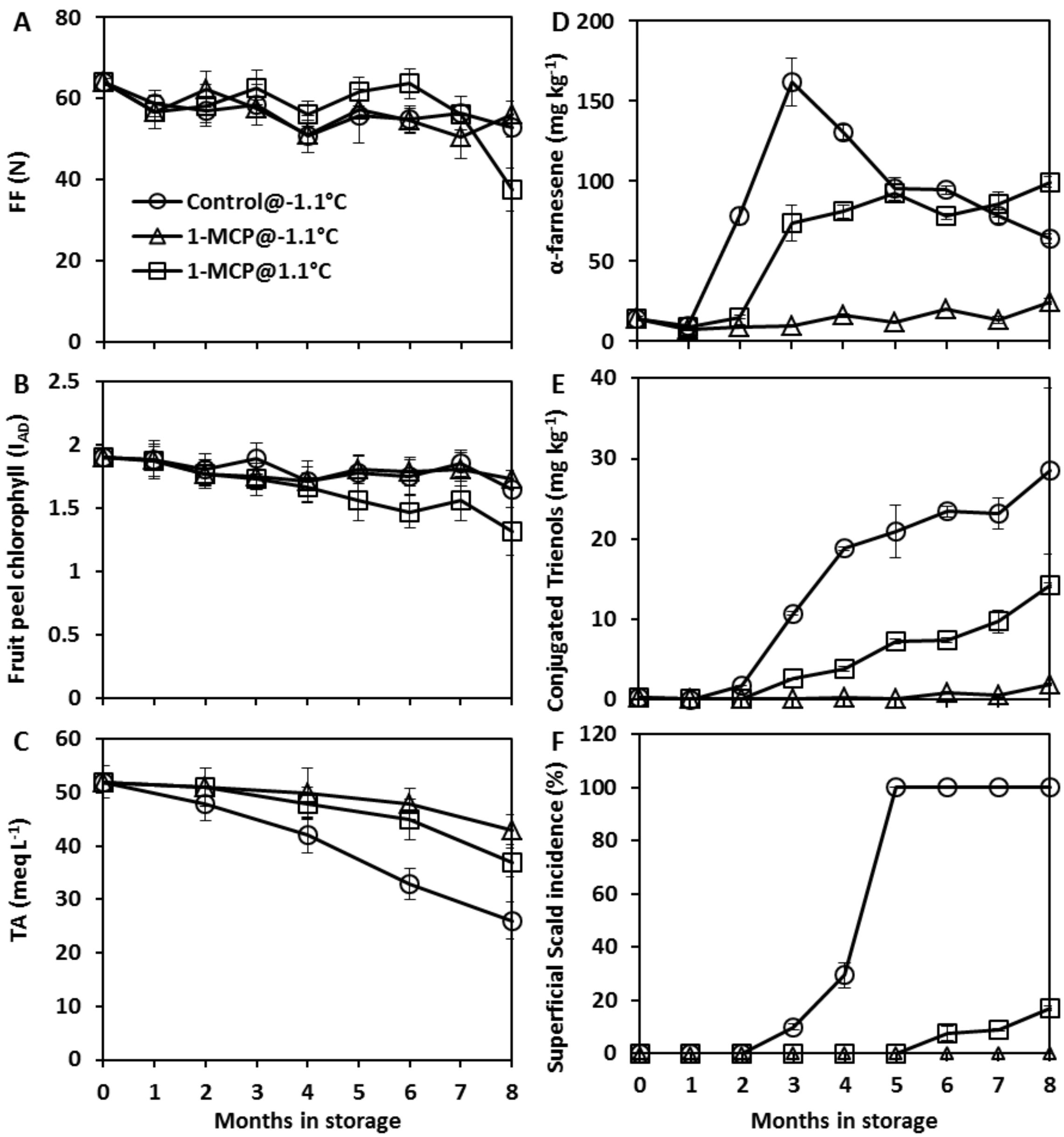


Fig. 3. Appearance of control and 1-MCP treated ‘d’Anjou’ pears after 7 months of storage at -1.1 °C or 1.1 °C plus 7 d ripening at 20 °C.

Fig. 4. Ripening capacity expressed as flesh firmness (FF) (A) and extractable juice (EJ) (B) of the normal harvested fruit and ripening capacity expressed as FF of the late harvested fruit (C) after 7 d at 20 °C following storage of 1-MCP treated ‘d’Anjou’ pears at -1.1 or 1.1 °C for 8 months. Values are means ± SD, n=3.

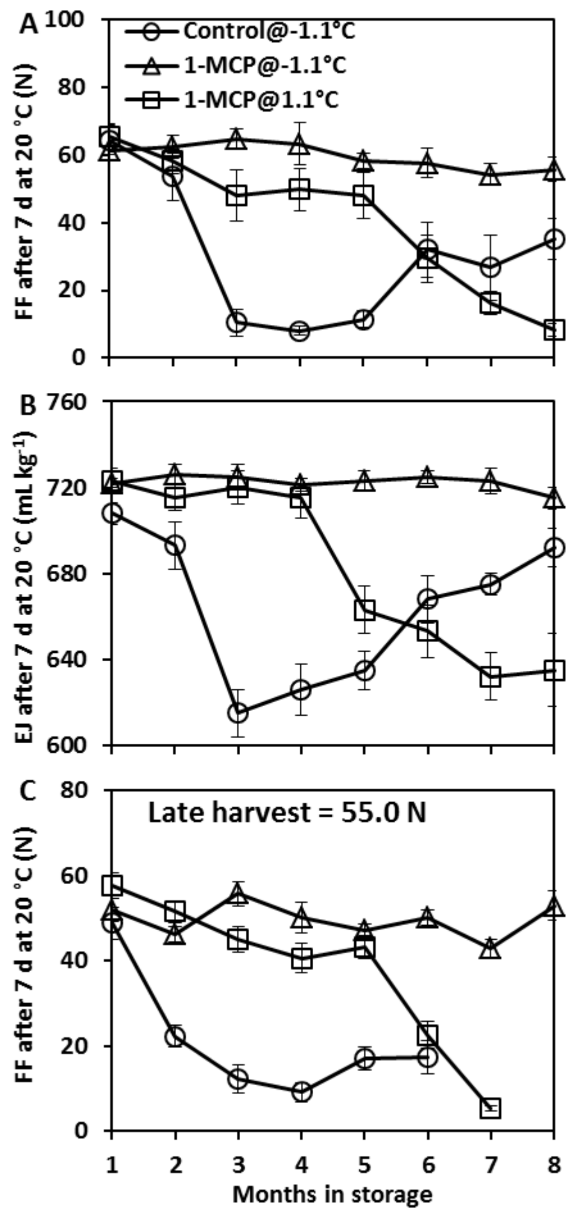
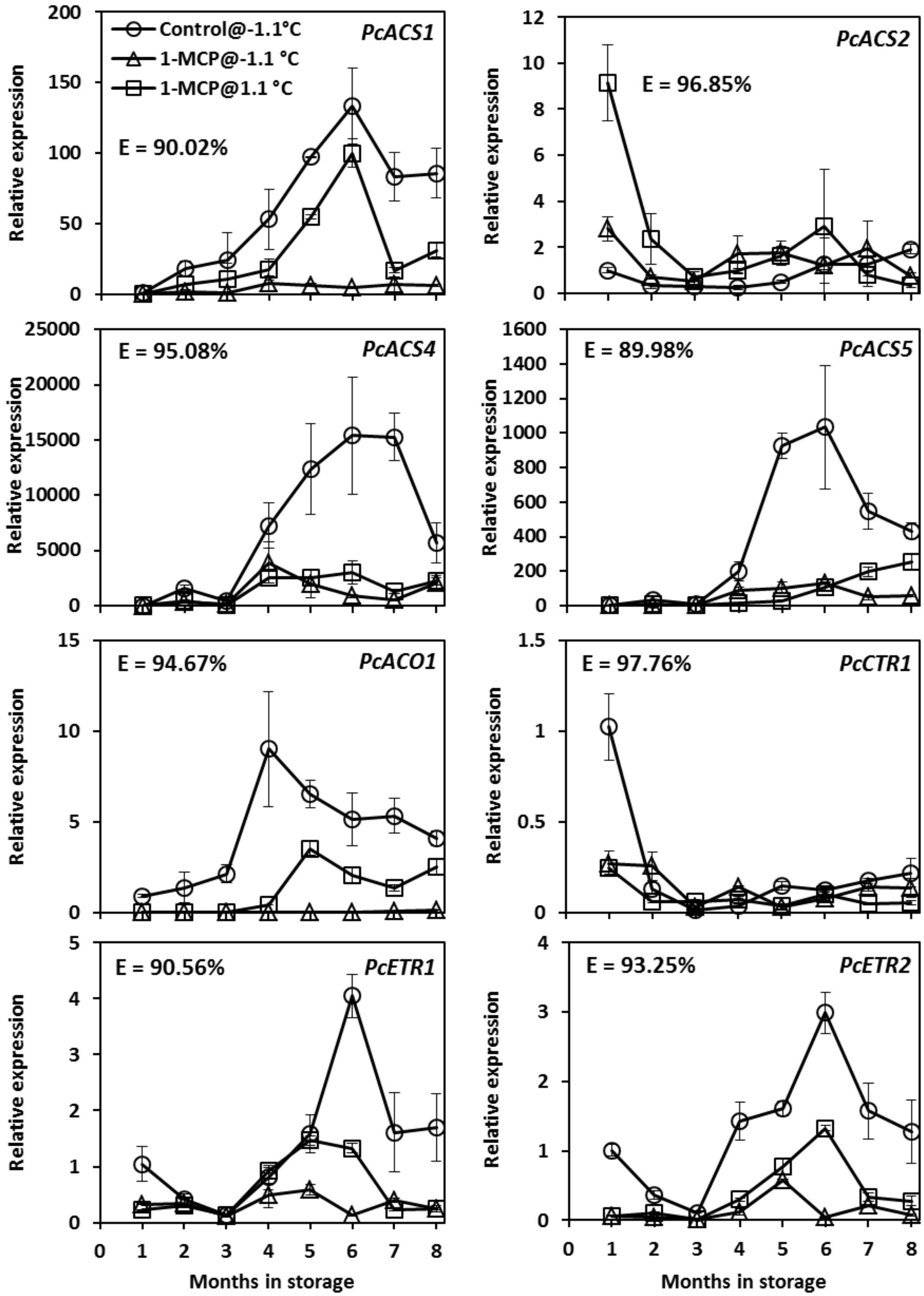


Fig. 5. Transcript levels of *PcACS1*, *PcACS2*, *PcACS4*, *PcACS5*, *PcACO1*, *PcETR1*, *PcETR2* and *PcCTR1* in ‘d’Anjou’ pear fruit stored at -1.1 °C (Control@-1.1°C), 1-MCP treated fruit stored at -1.1 °C (1-MCP@-1.1°C), and 1-MCP treated fruit stored at 1.1 °C (1-MCP@1.1°C). Values are means ± SD, n=3


Control@-1.1°C
1-MCP@-1.1 °C
1-MCP@1.1 °C
PcACS1
E = 90.02%
PcACS2
E = 96.85%
PcACS4
E = 95.08%
PcACS5
E = 89.98%
PcACO1
E = 94.67%
PcCTR1
E = 97.76%
PcETR1
E = 90.56%
PcETR2
E = 93.25%
Relative expression
Months in storage