Storage Temperature, Controlled Atmosphere, and 1-Methylcyclopropene Effects on α -Farnesene, Conjugated Trienols, and Peroxidation in Relation with Superficial Scald, Pithy Brown Core, and Fruit Quality of ‘d’Anjou’ Pears during Long-term Storage

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ABSTRACT. Alternatives to ethoxyquin (Etq) are needed for controlling superficial scald of ‘Anjou’ european pears (*Pyrus communis*) during long-term storage. The current commercial standard storage conditions [Etq + -1°C + controlled atmosphere (CA) with 1.5 kPa O_2] reduced scald occurrence compared with control fruit (-1°C + CA) during 6–8 months storage. At 1°C in air, 1-methylcyclopropene (1-MCP) fumigation at $0.15\ \mu\text{L}\cdot\text{L}^{-1}$ at harvest was more efficient on reducing scald than Etq but did not prevent scald during 6–8 months storage. The 1-MCP-treated fruit at 1°C in air developed their ripening capacity at 20°C following 6–8 months storage but had decreased shipping ability (softening and yellowing of fruit). Although Etq inhibition of scald was associated with the inhibition of α -farnesene oxidation to conjugated trienols (CTols); 1-MCP reduced α -farnesene synthesis and thereby the availability of substrate to oxidize to CTols. CA storage at 1.5 kPa O_2 totally prevented scald and retarded the loss of shipping ability without affecting the ripening capacity of 1-MCP-treated fruit at 1°C through further decreases in the syntheses of ethylene, α -farnesene and CTols during 6–8 months storage. In addition, 1-MCP prevented a CA-induced disorder, pithy brown core (PBC), in ‘Anjou’ pears possibly through enhancing an oxidative/reductive metabolic balance during extended storage. In conclusion, the combinations of 1°C + 1-MCP + CA is a potential commercial alternative to Etq for scald control while allowing the 1-MCP-treated ‘Anjou’ pears to recover ripening capacity during the shelf life period after 6–8 months storage.

‘Anjou’ is the most widely produced european pear cultivar in the Pacific northwestern United States with annual sales of ≈ 222 million kilograms (Northwest Horticultural Council, 2013). Superficial scald, resulting from necrosis of the hypodermal cortical tissue and manifested as brown or black patches on the fruit skin, is the most devastating physiological disorder of ‘Anjou’ fruit during long-term storage (Chen et al., 1990b; Whitaker, 2007). The cellular damage to the peel is thought to be induced and exacerbated by CTols, the oxidation products of the sesquiterpene (*E,E*)- α -farnesene (Chen et al., 1990b; Whitaker, 2007). CA storage at low O_2 (i.e., ≤ 1 kPa O_2) may control scald (Chen et al., 1993; Mellenthin et al., 1980); however, other disorders are increased by the same low- O_2 conditions [e.g., PBC and black speck (BS)] (Chen and Varga, 1989, 1997) as well as insufficient softening during the shelf life period following storage (Mattheis and Rudell, 2011). The current

commercial control of scald on ‘Anjou’ pear is a postharvest treatment with the antioxidant Etq (Hansen and Mellenthin, 1979). However, this treatment often causes considerable phytotoxicity, leaves Etq residue in fruit, and is often not adequate for scald control with long-term storage (Bai et al., 2009; Chen et al., 1990a, 1990b). The European Union withdrew authorization for plant protection products containing Etq in 2009. A continuing registration of Etq in the United States is not assured since european pear is the only horticultural crop registered for postharvest use. Therefore, alternatives to Etq for controlling scald of ‘Anjou’ pears are needed.

Ethylene biosynthesis is associated with superficial scald of apple (*Malus $\times$ domestica*) and ‘Anjou’ pear fruit through its regulation of the synthesis of α -farnesene in the fruit peel (Bai et al., 2006; Gapper et al., 2006; Jung and Watkins, 2008; Lurie and Watkins 2012). 1-Methylcyclopropene is an inhibitor of ethylene perception that retards ethylene-dependent responses such as ripening, senescence, and certain fruit disorders (Sisler and Serek, 1997; Sisler et al., 2003). Previous research demonstrated that 1-MCP inhibits ethylene synthesis and scald development in apples and european pears during storage (Argenta et al., 2003; Chen and Spotts, 2005; Fan and Mattheis, 1999; Isidoro and Almeida, 2006; Watkins et al., 2000). However, it interferes with ‘Anjou’ pear’s ability to ripen normally after storage (Bai et al., 2006; Chen and Spotts, 2005). ‘Anjou’ pear is enjoyed by consumers when the fruit have ripened to a buttery

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and juicy texture at warm temperatures following cold storage (Sugar and Basile, 2013; Villalobos-Acuña and Mitcham, 2008). Several strategies have been investigated to initiate ripening capacity in 1-MCP-treated European pears without consistent success, such as harvesting fruit at a more mature stage, post-storage ethylene conditioning, and warm temperature conditioning (Argenta et al., 2003; Bai et al., 2006; Xie et al., 2014). In European pears, storage temperatures ranging from -1 to 10 °C play a crucial role in the stimulation of ethylene biosynthesis during subsequent ripening at room temperatures (Sfakiotakis and Dilley, 1974; Sugar and Basile, 2013). Our previous research indicated that 1-MCP-treated 'Anjou' pears could recover their ripening capacity after long-term storage in air at storage temperature higher than -1 °C (i.e., 1 °C) but developed unacceptable scald, softening, and yellowing during long-term storage (Xie et al., 2014).

The objectives of this study were to characterize the biochemical basis behind storage temperature, CA, and 1-MCP effects on controlling scald and PBC and to evaluate the combination of elevated storage temperature (1 °C) and CA (1.5 kPa O_2) on the ability of 1-MCP to control scald and extend the packing season while allowing the development of ripening capacity in 'Anjou' pears.

Materials and Methods

FRUIT AND TREATMENTS. 'Anjou' pears were harvested at commercial maturity [fruit flesh firmness (FF) = 65.7 N] from mature trees in the orchard of the Mid-Columbia Agriculture Research and Extension Center in Hood River, OR (lat. 45.7° N, long. 121.5° W, elevation 150 m, average annual rainfall ≈ 800 mm). Defect-free fruit were randomly packed in 40 wooden boxes (80 fruit per box) with standard perforated polyethylene liners and were immediately moved to a cold room at -1 °C and $> 90\%$ relative humidity. On the second day after harvest, 20 boxes of the cold fruit were exposed to $0.15 \mu\text{L}\cdot\text{L}^{-1}$ 1-MCP (SmartFresh®; AgroFresh, Spring House, PA) in an airtight room (39.8 m^3) with a circulation fan at 0 °C for 24 h according to the application procedures recommended by the manufacturer. Fruit of 10 boxes were drenched with Etq at $2700 \text{ mg}\cdot\text{L}^{-1}$ (Decco Cerexagri, Monrovia, CA) at 20 °C for 30 s. After treatments, 10 boxes of nontreated (as control) and 10 boxes of Etq-treated fruit were moved to a CA room at -1 °C; 10 boxes of 1-MCP-treated fruit to a cold room in air at 1 °C; 10 boxes of 1-MCP-treated fruit moved to another CA room at 1 °C. In each of the two CA rooms (35.6 m^3), a semistatic O_2 concentration of ≈ 1.5 kPa was established within 3 d of harvest via purified N_2 generated from a membrane gas generator (model CPA-5; Permea, St. Louis, MO) and CO_2 at 0.5 – 1.0 kPa was maintained by adding a sufficient amount of hydrated lime (0.5 kg/box). The O_2 and CO_2 concentrations were monitored using an O_2 and CO_2 analyzer (Storex, Gravendeel, The Netherlands). Physiological, biochemical, and fruit quality evaluations were carried out on fruit at harvest, and after 2, 4, 6, and 8 months of cold storage in air or CA.

ETHYLENE PRODUCTION RATE (EPR) AND RESPIRATION RATE (RR). EPR and RR were determined from a sample of five fruit per replicate on day 1 at 20 °C after removal from cold storage. The fruit were placed in a 3.8-L airtight jar for 1 h. Gas samples were withdrawn through a septum using a 1-mL gastight syringe. The headspace ethylene concentration was measured by injecting the gas sample into a gas chromatograph (GC-8A;

Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and a Porapack Q column (80/100 mesh, 3.0 mm i.d., 2.0 m long). The carrier gas was nitrogen at a flow rate of $50 \text{ mL}\cdot\text{min}^{-1}$, the oven temperature was 90 °C, and the injector and detector temperatures were 140 °C. An external standard of ethylene ($1.0 \mu\text{L}\cdot\text{L}^{-1}$) was used for calibration. EPR was expressed as microliters per kilogram per hour. The headspace CO_2 concentration was measured using a CO_2 analyzer (model 900161; Bridge Analyzers, Alameda, CA). RR was expressed as milliliters of CO_2 per kilogram per hour.

α -FARNESENE AND CTOLS. α -Farnesene and CTols were measured immediately upon removal from cold storage using the methods described by Anet (1972) and Bai et al. (2009). Peel tissue 2–3 mm in thickness (including the epidermis and hypodermal cortex) was removed from five fruit of each replicate. The peel tissue samples (40 cm^2) were immersed in 25 mL of hexane (HPLC-grade; Fisher Scientific, Fair Lawn, NJ) in a covered beaker. The beakers with peel tissue and hexane were shaken with an orbital shaker (VCOS-4P; PRO Scientific, Oxford, CT) at 120 rpm and 20 °C for 5 min. After extraction, the solvent was centrifuged at $11,550 g_n$ for 5 min. Absorbance at 232 (α -farnesene) and 281 – 290 nm (CTols) was recorded using a spectrophotometer (model Ultrospec 3100 pro; Biochrom, Cambridge, England). Concentrations of α -farnesene and CTols were calculated using the molar extinction coefficients $\epsilon_{232 \text{ nm}} = 27,740$ for α -farnesene and $\epsilon_{281-290 \text{ nm}} = 25,000$ for CTols and expressed as nanomole per square centimeter.

ASCORBIC ACID (ASA), MALONDIALDEHYDE (MDA), AND FLESH LEAKAGE. ASA, MDA, and flesh leakage were determined in the fruit immediately on removal from cold storage. Five fruit of each replicate were peeled and cut lengthwise. Four plugs (0.8 cm diameter) were taken using a cork borer from the cortex tissue just above the core from each fruit. The tissue plugs from each replicate (five fruit) were sliced to 2 mm thickness with a razor blade and mixed. Five-gram samples of the cortex tissue were immediately frozen in liquid N_2 and stored at -80 °C until ASA and MDA determinations. ASA was measured based on the methods of Logan et al. (1998). Briefly, 5 g of the frozen cortex tissue was ground in 10 mL ice-cold 6% (v/v) $HClO_4$. The extract was centrifuged at $10,000 g_n$ for 10 min at 2 °C and the supernatant used immediately for the measurement. About one-third volume of 1.5 M Na_2CO_3 was added in a portion of the extract to raise its pH to 1 – 2 . Thirty to $100 \mu\text{L}$ of the neutralized samples were used to assay the ASA spectrophotometrically at 265 nm in 100 mM potassium phosphate buffer (pH 5.6), before and after 15 min incubation with 5 units ASA oxidase. The ASA content was determined from the absorbance difference and compared with a standard curve with the results expressed as milligram per kilogram fresh weight. MDA concentration was measured according to the corrected thiobarbituric acid (TBA) method (Hodges et al., 1999). Two grams of the frozen fruit tissue was ground and extracted in 5 mL of 10% (w/v) trichloroacetic acid (TCA). After centrifugation at $10,000 g_n$ for 15 min, a 2 -mL aliquot of the supernatant was mixed with 2 mL 10% TCA containing 0.6% (w/v) TBA. The mixture was heated to 100 °C for 20 min, quickly cooled, and centrifuged at $10,000 g_n$ for 10 min. The supernatant was collected and absorbance was then measured at 450 , 532 , and 600 nm. The MDA concentration was calculated according to the formula: $6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}$ and the results expressed as micromole per kilogram fresh weight. Flesh leakage

was determined using the method described by Redman et al. (1986). Sliced plugs (5 g each) were rinsed twice in distilled water, then placed in 30 mL of distilled water and exposed to a -25 -kPa vacuum for 2 min. The sample was then shaken for 30 min. One milliliter of the solution was centrifuged at $10,000 g_n$ for 5 min and the absorbance of the supernatant measured at 280 nm using a spectrophotometer as described above. The samples were then frozen at -20 °C, thawed, and a second absorbance measurement of the medium taken. The ratio of the first and second absorbance measurements was defined as the relative leakage ratio (RLR).

DECAY AND PHYSIOLOGICAL DISORDERS. On day 7 at 20 °C, 50 fruit of each replication were evaluated for decay, scald, and then cut longitudinally and transversely to assess PBC. Any pathological lesion was considered as decay and expressed as percentage of incidence. The incidence of scald was expressed as the percentage of fruit affected with commercially unacceptable scald (>0.6 mm²). PBC was expressed as percentage of incidence regardless of the degree of severity (Chen and Varga, 1997).

SHIPPING ABILITY. Shipping ability of European pears is defined using FF (i.e., $FF > 44.5$ N) and skin color (i.e., degree of greenness) after cold storage and before shipping (Sugar and Basile, 2013). Fruit color and FF were measured on 10 fruit of each replicate on day 1 at 20 °C after removal from cold storage. The fruit peel color was determined on opposite sides of the equator of each fruit with a colorimeter (CR-400; Minolta Camera, Osaka, Japan) with an 8-mm-diameter aperture using CIE illuminant. Color values a^* and b^* were converted to hue angle $\{h^\circ = \arctangent [(b^*)/(a^*)^{-1}]\}$. FF was measured on 10 fruit per replication using a fruit texture analyzer (model GS-14; Guss Manufacturing, 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 equatorial region after removal of 20-mm-diameter peel discs.

PHYSICAL AND CHEMICAL ATTRIBUTES WHICH REPRESENT EATING QUALITY. Ripening capacity was determined as competency of fruit softening and decrease in the amount of extractable juice (EJ) at room temperature (Sugar and Basile, 2013; Xie et al., 2014). Ten fruit of each replicate were removed from cold storage and ripened for 7 d at 20 °C. FF was determined as described above. After FF determination, 100 g of flesh tissue was ground for 3 min in a juice extractor (model 6001; Acme Juicer Manufacturing, Sierra Madre, CA) equipped with a uniform strip of milk filter (Schwartz Manufacturing, Two Rivers, WI). EJ was measured in a 100-mL graduated cylinder and expressed as milliliter per kilogram fresh weight. Soluble solid content (SSC) of the juice was determined using a digital refractometer (Atago, Tokyo, Japan). Titratable acidity (TA) was determined by titrating 10 mL of the juice to an endpoint pH 8.1 using 0.1 N NaOH with a commercial titration system (model T80/20; Schott-Gerate, Hofheim, Germany) and expressed as percentage (grams per 100 mL juice) of malic acid equivalents.

STATISTICAL ANALYSIS. The experimental design was completely randomized. There were three replications per treatment at each evaluation period. The data were subjected to analysis of variance using Statistica (version 6; StatSoft, Tulsa, OK). When appropriate, means were separated by Fisher's protected least significant difference (LSD) test at $P < 0.05$.

Results

ETHYLENE PRODUCTION AND RESPIRATION RATE. EPR was not detectable at harvest. For the control fruit (-1 °C + CA), EPR

was ≈ 0.3 $\mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ at 2 months and thereafter, increased gradually to a maximum of 7.6 $\mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ at 8 months of storage (Fig. 1A). Etq treatment (-1 °C + Etq + CA) did not affect EPR during the 8 months of storage. 1-MCP-treated fruit started to have EPR of 0.5 $\mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ at 4 months and increased gradually to 4.6 $\mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ at 8 months in air at 1 °C. CA reduced EPR in the 1-MCP-treated fruit to 0.2 and 2.2 $\mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ at 4 and 8 months of storage at 1 °C (Fig. 1A). Fruit RR at harvest was 5.1 $\text{mL}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ CO_2 . RR of the control and Etq-treated fruit remained relatively unchanged for 2 months, and thereafter increased gradually until 8 months. 1-MCP inhibited RR significantly for 8 months in air or CA at 1 °C. There was no significant difference in RR of the 1-MCP-treated fruit between air and CA storage at 1 °C over 8 months (Fig. 1B).

α -FARNESENE AND CTOLS. α -Farnesene concentration in 'Anjou' fruit peel was ≈ 6.0 $\text{nmol}\cdot\text{cm}^{-2}$ at harvest time. The control fruit accumulated α -farnesene to a peak of 37.0 $\text{nmol}\cdot\text{cm}^{-2}$ at 6 months and declined thereafter to 32.3 $\text{nmol}\cdot\text{cm}^{-2}$ at 8 months (Fig. 2A). Etq treatment did not affect α -farnesene accumulation in fruit peel over the 8 months in storage. 1-MCP reduced α -farnesene accumulation to 21.2 and 25.0 $\text{nmol}\cdot\text{cm}^{-2}$ after

Fig. 1. (A) Ethylene production rate (EPR) and (B) respiration rate (RR) influenced by ethoxyquin (Etq), 1-methylcyclopropene (1-MCP), storage temperature, and controlled atmosphere (CA) of 'Anjou' pear during long-term storage; Etq at 2700 $\text{mg}\cdot\text{L}^{-1}$, 1-MCP at 0.15 $\mu\text{L}\cdot\text{L}^{-1}$, CA conditions at 1.5 kPa $\text{O}_2 + <1\%$ CO_2 , storage temperature at -1 and 1 °C. Values are means $\pm$ SD ($n = 3$).

Fig. 2. (A) α -Farnesene and (B) conjugated trienols (CTols) influenced by ethoxyquin (Etq), 1-methylcyclopropene (1-MCP), storage temperature, and controlled atmosphere (CA) of 'Anjou' pear during long-term storage; Etq at 2700 mg·L⁻¹, 1-MCP at 0.15 μ L·L⁻¹, CA conditions at 1.5 kPa O₂ + <1% CO₂, storage temperature at -1 and 1 °C. Values are means $\pm$ SD (n = 3).

6 and 8 months storage in air at 1 °C, respectively. CA further reduced α -farnesene concentration in the 1-MCP-treated fruit to 10.5 and 17.9 nmol·cm⁻² at 6 and 8 months storage at 1 °C, respectively (Fig. 2A). CTols were not detectable in the fruit peel at harvest. CTols in control fruit increased gradually and significantly throughout the storage period and reaching a maximum of 6.2 nmol·cm⁻² after 8 months (Fig. 2B). Etq treatment decreased CTols production significantly; CTols accumulated after 4 months in storage and reached a maximum of 3.3 nmol·cm⁻² after 8 months. 1-MCP reduced CTols concentration to 1.1 and 2.5 nmol·cm⁻² after 6 and 8 months storage in air at 1 °C, respectively. CA further reduced CTols concentration to 0.5 and 1.7 nmol·cm⁻² in the 1-MCP-treated fruit after 6 and 8 months storage at 1 °C, respectively (Fig. 2B).

LIPID PEROXIDATION, FLESH LEAKAGE, AND AsA. MDA content decreased at 2 months and then increased gradually in all the treatments (Fig. 3A). In the control and Etq-treated fruit, PBC-damaged fruit had a higher MDA content than non-PBC fruit during 6–8 months storage. Etq treatment did not affect MDA content. After 4 months storage, 1-MCP retarded the

Fig. 3. (A) Malondialdehyde (MDA), (B) relative leakage ratio (RLR), and (C) ascorbic acid (AsA) influenced by ethoxyquin (Etq), 1-methylcyclopropene (1-MCP), storage temperature, and controlled atmosphere (CA) of 'Anjou' pear during long-term storage; Etq at 2700 mg·L⁻¹, 1-MCP at 0.15 μ L·L⁻¹, CA conditions at 1.5 kPa O₂ + <1% CO₂, storage temperature at -1 and 1 °C, PBC-damaged = fruit damaged by pithy brown core, no PBC = fruit with no pithy brown core. Values are means $\pm$ SD (n = 3).

increase of MDA in the fruit in air at 1 °C. The 1-MCP-treated fruit accumulated the least amount of MDA during 6–8 months storage in CA at 1 °C. The increased RLR observed with storage time (Fig. 3B) is likely due to the increasing senescence of the control and Etq-treated fruit. 1-MCP treatment slowed the RLR increase after 6 months. The 1-MCP-treated fruit had the lowest RLR during 6–8 months storage in CA at 1 °C. Fruit with PBC had significantly higher RLR values ($P \leq 0.05$) than undamaged fruit. AsA content decreased gradually in the

control fruit and the PBC-damaged fruit had a lower AsA than no-PBC fruit during 6–8 months (Fig. 3B). Etq treatment did not affect AsA content. 1-MCP slowed the AsA reduction in fruit stored in air at 1 °C during 6–8 months of storage. 1-MCP-treated fruit in CA at 1 °C had the highest AsA content during 6–8 months storage.

DISORDERS. Etq treatment reduced scald incidence to 8.3% and 12.2% from 53.2% and 87.7% of the control fruit after 6 and 8 months storage, respectively. Scald incidences were 3.6% and 6.5% in the 1-MCP-treated fruit after 6 and 8 months, respectively, in air at 1 °C. CA prevented scald incidence of the 1-MCP-treated fruit over 8 months of storage at 1 °C (Table 1). Scald incidence was highly correlated with CTols concentration in the fruit peel after 6 and 8 months storage (Fig. 4). The control fruit developed 3.2% and 5.9% PBC and 3.5% and 6.8% decay after 6 and 8 months storage, respectively. Although Etq treatment did not affect PBC and decay significantly, 1-MCP prevented PBC and reduced decay in air and in CA at 1 °C ($P < 0.05$) (Table 1).

SHIPPING ABILITY. The control fruit decreased in FF from 65.7 to 62.2 N gradually throughout the 8-month storage period (Fig. 5A). Etq treatment did not affect FF throughout the storage period. 1-MCP-treated fruit decreased in FF to 57.0 N in air at 1 °C after 8 months. 1-MCP-treated fruit in CA at 1 °C had 61.5 N firmness after 8 months storage. Control fruit peel h° decreased gradually from 116.0 to 109.1 during 8 months in storage. Etq treatment did not affect h° significantly during 8 months storage, whereas 1-MCP-treated fruit decreased h° to 101.2 in air at 1 °C after 8 months. However, at 1 °C CA retained h° at 110.0 in the 1-MCP-treated fruit after 8 months in storage (Fig. 5B).

EATING QUALITY. The control fruit softened to 45.0, 15.5, 15.8, and 20.6 N with EJ decreased to 685, 622, 625, and 636 mL·kg⁻¹ after 7 d at 20 °C following 2, 4, 6, and 8 months storage, respectively (Fig. 6A and B). Etq treatment did not affect fruit capacity to ripen. 1-MCP-treated fruit started softening to 28.4 and 23.8 N and EJ decreased to 646 and 635 mL·kg⁻¹ after 7 d at 20 °C following 6 and 8 months storage in air at 1 °C; CA did not affect FF and EJ significantly ($P < 0.05$) in the 1-MCP-treated fruit at 1 °C (Fig. 6A and B). SSC of the control fruit increased from 13.1% to 13.9% during 8 months storage; none of the treatments affected SSC (Fig. 6C). TA of the control fruit decreased 47% over 8 months of storage. Etq did not affect TA. TA of the 1-MCP-treated fruit decreased 33% in air and 20% in CA after 8 months storage at 1 °C (Fig. 6D).

Discussion

SUPERFICIAL SCALD. Scald is the most destructive storage disorder influencing the postharvest management of ‘Anjou’ pears (Chen and Spotts, 2005; Mattheis and Rudell, 2011). ‘Anjou’ pears may develop significant amount of scald and 100% in air after 3 and 5 months, respectively, at –1 °C (Xie et al., 2014). CA is a supplement to low temperature for extending storage life (mainly for eating quality) of European pears (Hansen and Mellenthin, 1979). A standard CA (1.5 kPa O₂) delayed or reduced scald in ‘Anjou’ pears in this and other studies (Chen et al., 1990a; Mattheis and Rudell, 2011; Mattheis et al., 2013). CA at low O₂ (≤ 1 kPa O₂) may control scald but can induce or increase other disorders such as BS, PBC, or losses of ripening capacity (Chen and Varga, 1989; Mattheis and Rudell, 2011; Mellenthin et al., 1980). A reliable

Table 1. Superficial scald, pithy brown core (PBC), and decay after 7 d at 20 °C following storage influenced by ethoxyquin (Etq), 1-methylcyclopropene (1-MCP), storage temperature, and controlled atmosphere (CA) in ‘Anjou’ pears after 6 and 8 mo. storage.

	6 mo.			8 mo.		
	Scald (%)	PBC (%)	Decay (%)	Scald (%)	PBC (%)	Decay (%)
–1 °C + CA ^z	53.2 a ^w	3.6 a	4.0 a	87.7 a	6.5 a	7.3 a
–1 °C + Etq ^y + CA	8.3 b	3.2 a	3.5 a	12.2 b	5.9 a	6.8 a
1 °C + 1-MCP ^x	3.6 c	0 b	0 b	6.5 c	0 b	1.6 b
1 °C + 1-MCP + CA	0 d	0 b	0 b	0 d	0 b	0.3 b

^zCA conditions at 1.5 kPa O₂ + <1% CO₂.

^yEtq at 2700 mg·L⁻¹.

^x1-MCP at 0.15 μ L·L⁻¹.

^wDifferent letters indicate significant differences among treatments according to Fisher’s protected LSD test at $P < 0.05$.

Fig. 4. The correlation of superficial scald incidence with the concentration of conjugated trienols (CTols) in fruit peel of ‘Anjou’ pear after 6 and 8 months of storage. Fruit were treated with ethoxyquin (Etq) at 2700 mg·L⁻¹, 1-methylcyclopropene (1-MCP) at 0.15 μ L·L⁻¹, CA conditions at 1.5 kPa O₂ + <1% CO₂, and storage temperature at –1 or 1 °C.

CA protocol that prevents scald while avoiding the development of other disorders has not been identified or applied commercially. In the present study, the commercial standard Etq treatment (Etq + –1 °C + CA) reduced scald but did not prevent it for 6–8 months storage (Fig. 2); these results are in accordance with Bai (2010) and Chen et al. (1990b). Its efficacy is affected by many factors such as production lot, year, harvest maturity, and Etq application timing (Bai et al., 2009; Chen et al., 1990b).

The buildup of CTols, the oxidation products of α -farnesene, in fruit peel wax layers has been directly linked to tissue necrosis since application of certain CTols onto intact fruit provokes damage symptoms similar to superficial scald (Rowan et al., 2001). In this study, scald incidence had a positive correlation with the concentration of CTols in ‘Anjou’ fruit peel after 6 and 8 months storage (Fig. 5), as was observed by Bai et al. (2009) and Chen et al. (1990b) in ‘Anjou’ pears and by Isidoro and Almeida (2006) in ‘Rocha’ pears. Etq treatment did not affect the accumulation of α -farnesene in ‘Anjou’ fruit peel; however, based on the reduced accumulation of CTols (Fig. 1), it appeared to inhibit the oxidation of α -farnesene. However,

Fig. 5. (A) Fruit firmness (FF) and (B) skin color (hue) influenced by ethoxyquin (Etq), 1-methylcyclopropene (1-MCP), storage temperature, and controlled atmosphere (CA) of 'Anjou' pear during long-term storage; Etq at 2700 mg·L⁻¹, 1-MCP at 0.15 µL·L⁻¹, CA conditions at 1.5 kPa O₂ + <1% CO₂, storage temperature at -1 and 1 °C. Values are means ± SD (n = 3).

other factors likely also influence scald development (e.g., antioxidants in fruit peel) since CTols levels do not always correlate well with scald occurrence (Rowan et al., 2001; Tsantili et al., 2007). The synthesis of α -farnesene in fruit peel is regulated by ethylene production (Bai et al., 2006; Gapper et al., 2006; Jung and Watkins, 2008; Lurie and Watkins, 2012). We have reported that 1-MCP at 0.15 µL·L⁻¹ applied prestorage blocks ethylene production by decreasing the expression of ethylene synthesis and perception genes (i.e., *PcACS1*, *PcACO1*, *PcETR1*, *PcETR2*) and the accumulation of α -farnesene, thereby reducing the availability of substrate for oxidation to CTols, as a consequence, prevents scald in 'Anjou' pears during 8 months in air storage at -1 °C (Xie et al., 2014). Gapper et al. (2006) demonstrated that 1-MCP inhibited the ethylene-induced α -farnesene synthase gene *PcAFS1* expression in 'Anjou' pears stored at -1 °C, and both synthesis and oxidation of α -farnesene were substantially reduced, resulting in inhibition of scald. In this study, EPR and the syntheses of α -farnesene and CTols increased in the 1-MCP-treated fruit after 4 months in air at 1 °C, but was still lower than that in the Etq-treated fruit (-1 °C + Etq + CA) during 4–8 months storage. As a consequence, the 1-MCP-treated fruit at 1 °C developed

less scald incidence than Etq-treated fruit after 6 and 8 months. The standard CA storage further reduced EPR and the syntheses of α -farnesene and CTols in the 1-MCP-treated fruit at 1 °C, completely preventing scald occurrence during 8 months in storage; these results are in agreement with those of Rizzolo et al. (2005) that CA prolonged or enhanced the effect of 1-MCP on inhibiting scald and other fruit quality attributes in 'Conference' pears. Argenta et al. (2003) reported that 1-MCP at 0.1–1.0 µL·L⁻¹ prevented scald in 'Anjou' pears for 8 months in air at 1 °C. The differences in results between other studies and ours may be due to number of factors (e.g., production location, year, harvest maturity), which are known to influence the susceptibility of 'Anjou' pears to scald (Calvo et al., 2015; Mattheis and Rudell, 2011; Mattheis et al., 2013).

PITHY BROWN CORE. PBC and BS are the major physiological disorders affecting 'Anjou' pears in long-term CA storage (Chen and Varga, 1989, 1997; Mattheis and Rudell, 2011). While BS (brown spots scattered randomly on the peel tissue and affecting five to six layers of hypodermal cells) arises from low O₂, PBC (pithy and brown tissue in the core area between the carpels or encompassing the entire core) is a CO₂-induced disorder that occurs when fruit are stored in low O₂ (Chen and Varga, 1997; Hansen and Mellenthin, 1979). Although <1 kPa O₂ substantially increases the risk, PBS and BS are observed frequently under standard commercial CA storage conditions (1–2 kPa O₂ + <1 kPa CO₂) after extended storage and their occurrences are affected by factors such as harvest maturity, tree vigor, growing season temperature, delayed cooling, storage temperature, and extended CA (Chen and Varga, 1989). In the present study, PBC but not BS was observed in the standard CA (1.5 kPa O₂ + <1.0 kPa CO₂) after 6 and 8 months storage, as was reported by Mattheis and Rudell (2011) in certain lots of 'Anjou' pears in 1.5 kPa O₂ + 0.5 kPa CO₂. 1-MCP treatment totally prevented PBC in 'Anjou' pears for 8 months in CA storage (Table 1).

The browning disorders of European pears may result from a disruption in the typical oxidative/reductive metabolic balance caused by low-O₂ environments, particularly in the presence of high CO₂ (Larrigaudière et al., 2001a, 2001b). CA conditions result in an accumulation of ethanol in 'Anjou' pears (Mattheis et al., 2013) and active oxygen species (AOS) such as O₂⁻ and H₂O₂ in 'Conference' pears (Larrigaudière et al., 2001a, 2001b). These compounds are toxic to fruit tissues causing lipid peroxidation and protein denaturation that in turn result in membrane disintegration and cellular decompartmentalization (Kays, 1997; Veltman et al., 1999). Plants have several enzymatic and nonenzymatic antioxidant defense mechanisms that counteract membrane oxidative damage caused by AOS (Kays, 1997; Larrigaudière et al., 2001a). Among the antioxidant systems, AsA appears to play an important role in the occurrence of the browning disorder in 'Conference' pears (Eccher Zerbini et al., 2002; Franck et al., 2003). It has been suggested that AsA protects against browning and that browning in pears is initiated when AsA drops below a threshold value (Eccher Zerbini et al., 2002; Veltman et al., 1999). This study demonstrated that the CA-induced PBC in 'Anjou' fruit appears to be related to membrane lipid peroxidation as indicated by increased MDA and RLR. MDA is widely used as an indicator of membrane lipid peroxidation during plant senescence and environmental stress (Hodges et al., 1999) and RLR is an indicator of tissue and membrane integrity in fruit and other plant tissues (Lu and Toivonen,

Fig. 6. Ripening capacity expressed as (A) fruit firmness (FF) and (B) extractable juice (EJ), (C) soluble solid content (SSC), and (D) titratable acidity (TA) after 7 d at 20 °C following storage influenced by ethoxyquin (Etq), 1-methylcyclopropene (1-MCP), storage temperature, and controlled atmosphere (CA) of 'Anjou' pear during long-term storage; Etq at 2700 mg·L⁻¹, 1-MCP at 0.15 µL·L⁻¹, CA conditions at 1.5 kPa O₂ + <1% CO₂, storage temperature at -1 and 1 °C. Values are means ± SD (n = 3).

2000; Redman et al., 1986). 1-MCP is believed to extend storage life of European pears in part through reduced oxidative stress (Larrigaudière et al., 2004). In this study, 'Anjou' pears decreased AsA significantly during CA storage, as previously reported in 'Rocha' (Silva et al., 2010; Veltman et al., 2000) and 'Conference' (Franck et al., 2003; Veltman et al., 2000) pears. The 1-MCP-treated fruit retained a higher AsA content and lower MDA and RLR compared with the non-1-MCP-treated fruit, especially the PBC-damaged fruit, during 6–8 months CA storage. PBC-damaged fruit had a lower AsA and higher MDA and RLR than the non-PBC-damaged fruit. What predisposes some fruit in the same CA atmosphere to maintain a higher concentration of AsA and resistance to PBC than other fruit in the same production lot remains to be elucidated.

DECAY. 1-MCP at 0.15 µL·L⁻¹ reduced naturally inoculated decay of 'Anjou' pears after 6 and 8 months in storage (Table 1). Argenta et al. (2003) and Spotts et al. (2007) also reported that 1-MCP ≥ 0.1 µL·L⁻¹ reduced decay of 'Anjou' pears during storage. Although the mechanism of action is not understood, it is possible that delayed senescence and an enhanced antioxidant system in fruit treated with 1-MCP may increase the fruit's resistance to fungal pathogens. As a consequence, 1-MCP may be considered as a complementary component in an integrated decay control strategy for 'Anjou' pears.

SHIPPING ABILITY. The current commercial procedures prefer 'Anjou' pears maintained a higher FF and green color on entry into the shipment and distribution chain to withstand mechanical

damage and as indicators of high fruit quality (Sugar and Basile, 2013). Low storage temperature at -1 °C is critical for retaining FF and green color of 'Anjou' pears during long-term storage; both FF and green color decreased significantly at elevated storage temperatures (Porritt, 1964; Xie et al., 2014). 1-MCP at 0.15 µL·L⁻¹ did not retard the decrease in FF and green color of 'Anjou' pears in air at 1 °C (Fig. 6A and B). Standard CA storage reduced ethylene production and RRs and retained FF and green color of the 1-MCP-treated fruit during 8 months of storage at 1 °C. Ethylene synthesis may not be the only trigger for the losses of FF and green color since 1-MCP-treated fruit in air at 1 °C had a lower EPR but lower FF and green color than the control fruit in CA at -1 °C during 8 months storage. It seems that the low storage temperature (-1 °C) plays a more important role than 1-MCP in retaining FF and green color in 'Anjou' pears. Porritt (1964) reported that the most effective storage temperature for maintaining quality in European pears is -1 °C and a slight increase in temperature significantly increased fruit respiration and ripening rates.

EATING QUALITY. Consumer acceptance for European pears is mainly dependent on the development of ripening capacity, sugar content, and acidity (Kappel et al., 1995). European pears are enjoyed for their buttery-juicy texture with fully characteristic flavor after ripening. For 'Anjou' pears, ripening capacity is defined as the ability of the fruit to soften to between 14 and 23 N with an EJ of <650 mL·kg⁻¹ within 7 d at 20 °C after removal from cold storage (Xie et al., 2014). However, European pears are generally resistant to ripening at harvest

(Sugar and Basile, 2013; Villalobos-Acuña and Mitcham, 2008; Xie et al., 2014). In order for 'Anjou' pears to develop an appropriate ripening capacity, they must develop competency to produce ethylene internally at a sufficient rate to activate and complete the ripening process. In air at -1°C , 'Anjou' pears with optimum harvest maturity require $\approx 60\text{--}90$ d of post-harvest chilling to develop their ripening capacity and a high eating quality (Sugar and Basile, 2013; Xie et al., 2014). In this study, 'Anjou' pears did not develop ripening capacity until 4 months in the standard CA storage at -1°C .

1-MCP inhibits ethylene function and synthesis in 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 'Anjou' pears might totally shut down the formation of new binding receptors when stored at -1°C since 1-MCP-treated fruit do not develop their ripening capacity until 8 months in storage (Xie et al., 2014). A continuing challenge for commercializing 1-MCP on European pears is how to reinitiate the ripening capacity of 1-MCP-treated fruit following cold storage. Increasing storage temperature to 1°C recovered ripening capacity but increased scald incidence, softening, and yellowing of the 1-MCP-treated 'Anjou' pears after 6–8 months storage in air. The recovery of ripening capacity from 1-MCP-induced inhibition may result from production of new receptors at 1°C after long-term storage (Xie et al., 2014). In this study, we found that the standard CA storage conditions ($1.5\text{ kPa O}_2 + <1.0\text{ kPa CO}_2$) further inhibited ethylene production and thereby prevented scald, retained FF and green color, and did not affect the ripening capacity of 1-MCP-treated 'Anjou' pears after 6–8 months in storage at 1°C . The combinations of $1^{\circ}\text{C} + 1\text{-MCP} + \text{CA}$ with 1.5 kPa O_2 , therefore, may be an alternative to Etq for controlling scald while allowing the 1-MCP-treated fruit to ripen during the shelf life period after long-term storage. In addition, increasing storage temperature from -1 to 1°C will provide other potential benefits such as energy and cost savings since maintaining low temperature during fruit storage is an important aspect regarding energy consumption (Kitemann et al., 2015). Future investigations are warranted to, for examples, optimize storage temperature and 1-MCP application rate at commercial CA storage scales/conditions.

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