Inhibition of Ethylene Biosynthesis and Associated Gene Expression by Aminoethoxyvinylglycine and 1-Methylcyclopropene and Their Consequences on Eating Quality and Internal Browning of ‘Starkrimson’ Pears

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ABSTRACT. ‘Starkrimson’ is a highly profitable red-skinned european pear (*Pyrus communis*) cultivar that has a short storage life due mainly to the development of a mealy texture upon ripening and an internal browning (IB) disorder during or after storage. In 2013, ‘Starkrimson’ pears were sprayed with aminoethoxyvinylglycine (AVG) at 0, 30, 60, and 120 mg·L⁻¹ 1 week before harvest or treated with 1-methylcyclopropene (1-MCP) at 0.3 µL·L⁻¹ for 24 hours shortly after harvest, then stored at -1.1 °C and evaluated over a 16-week period. The experiment was repeated in 2014. After 2 weeks of storage, control fruit (nontreated) had a higher respiration rate and ethylene biosynthesis than AVG or 1-MCP-treated fruit. Following 12 weeks of storage, control fruit exhibited a greater incidence of mealy texture and greater extractable juice (EJ) after ripening, and by 16 weeks significantly higher IB relative to AVG and 1-MCP-treated fruit. AVG at 30 mg·L⁻¹ had little effect on any of the storage responses measured compared with control. AVG at 60 mg·L⁻¹ reduced ethylene synthesis, respiration rate, and titratable acidity (TA) loss and maintained high eating quality with low EJ. Fruit treated with 60 mg·L⁻¹ AVG also developed markedly less IB following 16 weeks of storage than control or 30 mg·L⁻¹ AVG treatments. AVG at 120 mg·L⁻¹ did not improve storage quality achieved with 60 mg·L⁻¹ but delayed ripening capacity by 1 month. 1-MCP markedly inhibited ethylene synthesis and respiration rate and eliminated IB during 16 weeks of storage; however, 1-MCP-treated fruit required 14 days at 20 °C to ripen to high eating quality following 12 to 16 weeks of storage compared with 5 days for 60 mg·L⁻¹ AVG. Both AVG and 1-MCP suppressed the expressions of ethylene synthesis (*PcACS1*, *PcACS4*, *PcACS5*, and *PcACO1*) and perception genes (*PcETR1*, *PcETR2*, and *PcETR5*) although 1-MCP was more efficient than AVG. In conclusion, preharvest AVG applications at 60 mg·L⁻¹ or postharvest 1-MCP treatment at 0.3 µL·L⁻¹ extended storage life of ‘Starkrimson’; however, 1-MCP inhibited ripening capacity whereas 60 mg·L⁻¹ AVG did not.

‘Starkrimson’, a red-skinned sport of the cultivar Clapp’s Favorite, is a highly profitable european pear produced in the Pacific Northwest region of the United States due to its early maturation and attractive, bright red skin (Sugar and Lombard, 1989). Compared with other european pear cultivars, Starkrimson pear has a short storage life primarily due to the development of a mealy texture and an IB disorder after ripening and storage, respectively. ‘Starkrimson’ pears have a maximum storage life of ≈1.5 to 2 months in regular air storage at -1 °C (Sugar and Lombard, 1989) or 3 to 4 months in controlled atmospheric storage (CA) (Richardson and Kupferman, 1997). Consequently, ‘Starkrimson’ has a narrow marketing season that is limited predominantly to domestic markets. With

increasing export market demand and the increased of returns paid for high-quality pears, development or identification of technologies to extend the storage and marketing life of ‘Starkrimson’ would be valuable.

European pears are classified as climacteric fruit and ethylene, a gaseous plant hormone, contributes to their accelerated ripening/senescence processes (Villalobos-Acuña and Mitcham, 2008). To extend storage life of european pears, ethylene synthesis or action must be inhibited or reduced. Low storage temperature (i.e., -1.1 °C) and CA storage are the two commercial standard practices to extend storage life through reducing ethylene production and respiration rate of european pears. Two ethylene inhibitors AVG and 1-MCP are registered for commercial pre- or postharvest applications to reduce or prevent ethylene-dependent responses such as preharvest drop, ripening, and senescence of apples (*Malus ×domestica*), pears, and other crops.

AVG decreases ethylene biosynthesis by inhibiting aminocyclopropane-1-carboxylic acid synthase (ACC) synthase (ACS), one of the two key enzymes in the ethylene biosynthesis pathway (Kende, 1993; Yu and Yang, 1979). Preharvest AVG treatment suppressed ethylene production, reduced fruit drop, and delayed fruit ripening in apples (Autio and Bramlage, 1982; Bangerth, 1978; Bramlage et al., 1980; Child et al., 1984; Greene and Schupp, 2004; Halder-Doll and Bangerth, 1987;

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Salas et al., 2011; Schupp and Greene, 2004; Whale et al., 2008) and peaches (*Prunus persica*) (Bregoli et al., 2002; Ziosi et al., 2008). The response of pears to AVG has received less research attention. Previous studies showed that preharvest AVG treatment reduced ethylene production and increased storability of 'Bartlett' pears held at 20 °C (Romani et al., 1982, 1983) or stored at -1 °C (Clayton et al., 2000), and in two early-season european pears, 'Camusina di Genova' and 'Camusina di Bonarcado', stored at 18 °C (D'Aquino et al., 2010).

In contrast to AVG, 1-MCP is an ethylene action inhibitor that interferes with ethylene receptors, thereby preventing or reducing ethylene-dependent responses such as fruit ripening/senescence processes and ethylene-associated storage disorders (Blankenship and Dole, 2003; Watkins, 2006). 1-MCP controlled postharvest disorders and extended storage life of the european pear cultivars Anjou (Argenta et al., 2003; Xie et al., 2014), Bartlett (Villalobos-Acuña et al., 2011; Wang and Sugar, 2015), and Conference (Chiriboga et al., 2011). Calvo and Sozzi (2004) reported that 1-MCP at 0.2 $\mu\text{L}\cdot\text{L}^{-1}$ delayed ripening, reduced watery and/or core breakdown, and maintained quality during 60 d storage at -0.5 °C. Although 1-MCP generally provides valuable benefits in controlling postharvest disorders and extending storage life, 1-MCP did enhance the incidence and severity of some storage disorders in apples, including some CO₂ related injuries and IB disorders (Watkins, 2006). Specifically for european pears, its interference with the fruit's ability to ripen normally after storage (Argenta et al., 2003; Bai et al., 2006; Chen and Spotts 2005; Chiriboga et al., 2011; Gapper et al., 2006) has limited commercial utilization. Consumer preference of european pears was highest when fruit were ripened to a buttery, juicy texture at room temperatures following cold storage (Villalobos-Acuña and Mitcham, 2008).

This study was designed to assess the effects of a preharvest AVG application and postharvest 1-MCP treatment on ethylene synthesis, storage quality, and eating quality of 'Starkrimson' pear during 4 months of cold storage.

Materials and Methods

EXPERIMENTAL DESIGN. AVG (ReTain; Valent BioScience, Libertyville, IL) at 0, 30, 60, and 120 $\text{mg}\cdot\text{L}^{-1}$ and an organo-silicon surfactant (L-77; Helena Chemical Co., Collierville, TN) at 0.1% v/v, were applied to the entire canopy of 10-year-old 'Starkrimson' trees at the Oregon State University, 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), using a CO₂-pressurized sprayer to achieve uniform, complete coverage (i.e., sprayed to drip). All concentrations of AVG are expressed as active ingredient. Applications were performed 1 week before commercial harvest. Experimental units (trees) were arranged in a complete randomized design with three single-tree replications per treatment. Defect-free fruit were harvested at commercial harvest maturity [i.e., when the average flesh firmness (FF) decreased to ≈ 60 N] and packed in wooden boxes (80 fruit per box) with standard perforated polyethylene liners. Packed fruit were immediately stored in air at -1.1 °C (± 0.5) and >95% relative humidity.

For the 1-MCP treatment, defect-free fruit were harvested from non AVG-treated trees in the same orchard on the same day as AVG-treated fruit, packed as described above and cooled for 24 h at 0 °C. After 24 h, fruit were exposed to 0.3 $\mu\text{L}\cdot\text{L}^{-1}$ 1-MCP (SmartFresh®; AgroFresh, Spring House, PA) in an

airtight room (39.75 m³) at 0 °C for 24 h according to the application procedures recommended by the manufacturer. Following 1-MCP treatment, fruit were then stored in the same storage room with the AVG-treated fruit. Differences in ethylene production rate, respiration rate, and fruit quality were not found between fruit treated with water + surfactant or nontreated; therefore, nontreated fruit were used as the control.

INTERNAL ETHYLENE CONCENTRATION (IEC), ETHYLENE PRODUCTION RATE (EPR), AND RESPIRATION RATE (RR). IEC was measured on fruit immediately upon removal from cold storage. Gas was sampled from five individual fruit per treatment replication using a vacuum-immersion technique (Chen and Mellenthin, 1981), and injected into a gas chromatograph (GC-8A; Shimadzu, Kyoto, Japan). Nitrogen was used as the carrier gas at a flow rate of 50 $\text{mL}\cdot\text{min}^{-1}$. The injector and detector port temperatures were 90 and 140 °C, respectively. An external standard of ethylene (1.0 $\mu\text{L}\cdot\text{L}^{-1}$) was used for calibration. 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. Ethylene was measured by GC, and EPR was expressed as microliters per kilogram per hour. The headspace CO₂ concentration was measured using a CO₂ analyzer (model 900161; Bridge Analyzers, Alameda, CA). Fruit RR was expressed as milliliters of CO₂ per kilogram per hour.

STORAGE QUALITY. Ten fruit in each box were marked and weighed before and after 16 weeks of storage. Initial and final weight was determined with an electronic balance (PC4400; Mettler-Toledo, Zurich, Switzerland). Percent weigh loss was calculated according to the formula, $\text{WL} = (\text{W}_0 - \text{W}_f) / \text{W}_0 \times 100$, where W₀ is the initial weight (grams) and W_f is the final weight (grams). FF was determined after exposing fruit to 20 °C for 5 h immediately after removal from cold storage. 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. On day 5 at 20 °C, ≈ 50 fruit (one box) of each replication were evaluated for decay and then cut longitudinally and transversely to assess IB. Any pathological lesion was considered as decay and expressed as percentage of incidence. IB symptoms are described in the discussion section. Although two types of IB were identified, IB was expressed as percentage of incidence regardless of the degree of severity or type of IB.

RIPENING CAPACITY, EXTRACTABLE JUICE, SOLUBLE SOLID CONTENT (SSC), AND TITRATABLE ACIDITY. Fruit were removed from cold storage and ripened for 5 d at 20 °C. Ten fruit were randomly selected from each of three replicate boxes for determination of ripening capacity (based on FF). 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 Co., Sierra Madre, CA) equipped with a uniform strip of milk filter (Schwartz Manufacturing Co., Two Rivers, WI). EJ was measured in a 100-mL graduated cylinder and expressed as milliliters per 100 g fresh weight. SSC of the juice was determined by a digital refractometer (Atago, Tokyo, Japan). 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.

RNA EXTRACTION, ISOLATION OF COMPLEMENTARY DNA (cDNA), AND REAL-TIME QUANTITATIVE POLYMERASE CHAIN REACTION (RT-qPCR). Peel tissue, including the epidermis and 2 to 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 to 2 g of frozen pear peel was ground to a powder in liquid nitrogen, and total RNA was isolated using a Plant Total RNA Kit (Sigma-Aldrich, St. Louis, MO) according to the manufacturer. First-strand cDNA was performed from 1 μg total RNA using SuperscriptTM III First Strand Synthesis Systems for RT-qPCR Systems (Invitrogen; Thermo Fisher Scientific, Waltham, MA) using oligo (dT) as primers. Reactions for RT-qPCR on the cDNA were performed with iTaqTM Universal SYBR Green Supermix (Bio-Rad, Hercules, CA). The amplification protocol consisted of an initial step at 95°C for 2 min, and 40 cycles at 95°C for 10 s and then at 60°C for 30 s. The specificity of the RT-qPCR amplification was checked routinely by melting curve analysis. Data were analyzed using the $2^{-\Delta\Delta\text{Ct}}$ method (Livak and Schmittgen, 2001) and are presented as the relative level of gene expression. Transcript levels of ethylene biosynthesis, perception, and signaling genes were analyzed using *PcActin* (El-Sharkawy et al., 2003) as an internal control. Sequences for primers were designed according to previous research and are provided in Table 1.

STATISTICAL ANALYSES. 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 test at $P < 0.05$.

Results

EFFECT OF AVG AND 1-MCP ON IEC, EPR, AND RR. In 2013, the control fruit required 4 weeks of cold storage before the IEC ($0.6 \mu\text{L}\cdot\text{L}^{-1}$) began to accumulate; thereafter, IEC

increased gradually to a maximum of $4.8 \mu\text{L}\cdot\text{L}^{-1}$ at 16 weeks of storage (Fig. 1A). The effect of AVG on IEC of fruit was rate dependent (Fig. 1A). Fruit treated with 30, 60, or $120 \text{ mg}\cdot\text{L}^{-1}$ AVG had an IEC of 1.7, 0.9, and $0.6 \mu\text{L}\cdot\text{L}^{-1}$ after 8 weeks and 4.7, 3.3, and $3.2 \mu\text{L}\cdot\text{L}^{-1}$ after 16 weeks of storage, respectively. There was not a significant difference ($P < 0.05$) between AVG at 60 and $120 \text{ mg}\cdot\text{L}^{-1}$ on IEC accumulation. Compared with preharvest AVG, 1-MCP applied shortly after harvest was more effective at inhibiting IEC during cold storage. The 1-MCP-treated fruit had only accumulated to $0.1 \mu\text{L}\cdot\text{L}^{-1}$ IEC after 8 weeks and levels did not exceed $1.0 \mu\text{L}\cdot\text{L}^{-1}$ throughout the entire 16 week storage period. In 2014, AVG and 1-MCP treatments had similar effects on IEC compared with 2013, except that AVG at $120 \text{ mg}\cdot\text{L}^{-1}$ reduced IEC ($P < 0.05$) after 12 weeks of storage compared with AVG at $60 \text{ mg}\cdot\text{L}^{-1}$ (Fig. 1B).

In 2013, the EPR of control fruit was detectable by 4 weeks of storage, slowly increased until week 12, then increased significantly to $18.8 \mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ by week 16. EPR was unaffected by AVG at $30 \text{ mg}\cdot\text{L}^{-1}$ but was significantly reduced ($P < 0.05$) by AVG at 60 and $120 \text{ mg}\cdot\text{L}^{-1}$ between 4 and 16 weeks of storage (Fig. 1C). In 2014, EPRs of all AVG concentrations were significantly reduced from control levels (Fig. 1D). 1-MCP completely inhibited EPR of 'Starkrimson' pears during 16 weeks of cold storage, irrespective of year. RR of control fruit generally increased during the 16 weeks of cold storage (Fig. 1E and F). In 2013, only 60 and $120 \text{ mg}\cdot\text{L}^{-1}$ AVG reduced RR relative to the control (Fig. 1E). However, in 2014 RR was significantly lower for all AVG treatments between 4 and 12 weeks (Fig. 1F). 1-MCP-treated fruit maintained the lowest RR, which slightly increased after 8 weeks of storage in both seasons (Fig. 1E and F).

EFFECT OF AVG AND 1-MCP ON STORAGE QUALITY. Weight loss did not exceed 1% (data not shown) and no visible symptoms of shriveling were apparent regardless of treatments and storage duration.

In 2014, large fruit size at initial maturity resulted in an earlier commercial harvest date as evidenced by higher FF

Table 1. Gene-specific primers used for expression analysis of genes related to ethylene biosynthesis and perception in 'Starkrimson' pears.

Gene	GenBank accession no.	Gene	Oligonucleotide sequences	References
<i>PcACS1</i>	X87112	PCP011500.1	F 5'-TGGCAGAGCAATCTAAGGC-3' R 5'-AAGGAGAGGTGAGTGAGGCA-3'	El-Sharkawy et al. (2004)
<i>PcACS2</i>	AY388989	PCP013122.1	F 5'-CATGGAAAAGAGAGAGCGGG-3' R 5'-GATAAAAGAGAGGAACTTCATTCTAGCA-3'	Chiriboga et al. (2013)
<i>PcACS4</i>	AF386518	PCP023053.1	F 5'-CTTGGTTGAAGAGTGGATTAG-3' R 5'-ATGATCAAGCCCTTGACATTG-3'	Villalobos-Acuña et al. (2011)
<i>PcACS5</i>	AF386523	PCP009270.1	F 5'-TTTCGACACAACTCAGCATCT-3' R 5'-AAAGCAACTTCCATGGTCTTGT-3'	Villalobos-Acuña et al. (2011)
<i>PcACO1</i>	AJ504857	PCP011683.1	F 5'-AATGCACCACTCCATTGTCATA-3' R 5'-GCTTCATGTAGTCATCAAACACA-3'	Fonseca et al. (2005)
<i>PcETR1</i>	AF386509	PCP024250.1	F 5'-AGAACGAGGCGTTGTTGCAC-3' R 5'-CCATCATCCCCATTGCTC-3'	Chiriboga et al. (2013)
<i>PcETR2</i>	HM561909	PCP012383.1	F 5'-TGGGTGCAATGTAAAGGCC R 5'-GGAGCAATGAAACCGATAGCC	Chiriboga et al. (2013)
<i>PcETR5</i>	AF386511	PCP016805.1	F 5'-ATGATTCTCAAAGTAAAGTGAGAG-3' R 5'-CCAACACATCTGGGTCATTAATTG	El-Sharkawy et al. (2003)
<i>PcCTR1</i>	HM156629	PCP016676.1	F 5'-GAAGTCAGATGTTTACAGTTTGGTG-3' R 5'-AAGAATACATATTGAAGTAATGG-3'	El-Sharkawy et al. (2003)
<i>PcActin</i>	AF386514	PCP000044.1	F 5'-CTATGAACAAGAGCTAGAGACATC-3' R 5'-AGAGAAATCGACATGAATGAAATTC-3'	El-Sharkawy et al. (2003)

Fig. 1. Effects of aminoethoxyvinylglycine (AVG) rate (30, 60, or 120 mg·L⁻¹) and 1-methylcyclopropene (1-MCP) (0.3 μL·L⁻¹) on (A and B) internal ethylene concentration (IEC) determined immediately after removal from cold storage, (C and D) ethylene production rate (EPR) determined on day 1 at 20 °C after removal from cold storage, and (E and F) respiration rate (RR) determined on day 1 at 20 °C after removal from cold storage of 'Starkrimson' pears during a 16-week storage period at -1.1 °C in 2 production years (2013 and 2014). Values are means ± SD (n = 3); AVG30 = AVG at 30 mg·L⁻¹, AVG60 = AVG at 60 mg·L⁻¹, AVG120 = AVG at 120 mg·L⁻¹, 1-MCP = 1-MCP at 0.3 μL·L⁻¹.

relative to 2013 (Fig. 2A and B). There were no significant differences in FF at harvest among treatments in 2013 and 2014. FF of control fruit decreased from 58.7 to 53.1 N in 2013 and from 64.1 to 57.9 N in 2014 during the 16-week storage period; neither AVG nor 1-MCP affected FF (Fig. 2A and B).

In 2013, 12.3% of control fruit developed IB; 60 and 120 mg·L⁻¹ AVG and 1-MCP at 0.3 μL·L⁻¹ significantly reduced IB to 2.5%, 3.3%, and 0%, respectively, after 16 weeks storage (Fig. 2C). A slightly higher incidence of IB occurred in 2014, but treatment effects were nearly identical to those observed in 2013 (Fig. 2D). In 2013, 7.1% of control fruit had decay; AVG at 60 and 120 mg·L⁻¹ and 1-MCP reduced decay to 1.2%, 3.3%, and 1.1%, respectively, after 16 weeks storage (Fig. 2E). The natural decay incidence was low in 2014; control fruit had only 1.1% after 16 weeks storage (Fig. 2F). The differences of decay and IB for the control fruit between the two seasons might be due to differences in harvest maturation state.

EFFECT OF AVG AND 1-MCP ON RIPENING CAPACITY AND EATING QUALITY. Ripening capacity of European pears is defined as the ability of the fruit to soften below ≈23 N when continuously exposed to warm temperatures for 5 to 7 d. Control fruit developed ripening capacity after 2 weeks of storage at -1.1 °C when exposed to 20 °C for 5 d (Fig. 3A and B). Ripening capacity was unaffected by AVG at 30 and 60 mg·L⁻¹, but ripening of fruit treated with AVG at 120 mg·L⁻¹ was significantly delayed, requiring 2 and 4 additional weeks of low temperature in 2013 and 2014, respectively (Fig. 3A and B). 1-MCP-treated fruit did not develop ripening capacity throughout the entire 16 week storage period when placed in 20 °C for 5 d (Fig. 3A and B). Interestingly, fruit treated with 1-MCP did attain ripening capacity after 12 and 8 weeks of cold storage in 2013 and 2014, respectively, but required 14 d at 20 °C.

In 2013, EJ of control fruit was 60.3 and 66.0 mL/100 g following 5 d at 20 °C after 12 and 16 weeks of storage, respectively. EJ of fruit treated with AVG at 60 mg·L⁻¹ was reduced to 47.3 and 59.0 mL/100 g after ripening following 12 and 16 weeks of storage, respectively (Fig. 3C). Similar results were observed in 2014. AVG at 30 mg·L⁻¹ had little effect on EJ compared with control, and AVG at 120 mg·L⁻¹ increased EJ compared with AVG at 60 mg·L⁻¹ during 16 weeks of storage in both seasons. 1-MCP-treated fruit did not develop buttery, juicy texture (EJ < 60 mL/100 g) throughout the entire storage period when provided 5 d of 20 °C (Fig. 3C and D), but were

capable of ripening after 14 d at 20 °C following 12 to 16 weeks of cold storage (Table 2).

SSC levels remained similar throughout the 16-week cold storage duration and did not significantly differ among treatments in either year (Fig. 3E and F). The SSC levels appeared to be slightly higher in 2013 than 2014, which is probably attributed to the more mature nature of those fruit from that season. TA decreased gradually during storage, irrespective of year. For control fruit, TA losses of ≈40% and 34% occurred after 16 weeks of storage in 2013 and 2014, respectively (Fig. 3G and H). AVG at 60 or 120 mg·L⁻¹ and 1-MCP reduced TA loss to 20% and 23% in 2013 and 25% and 28% in 2014, respectively, after 16 weeks of storage.

EFFECT OF AVG AND 1-MCP ON EXPRESSION OF GENES ASSOCIATED WITH ETHYLENE BIOSYNTHESIS AND PERCEPTION. The expression of ethylene synthesis genes *PcACS1*, *PcACS2*, *PcACS4*, *PcACS5*, and *PcACO1* were characterized during the

Fig. 2. Effects of aminoethoxyvinylglycine (AVG) rate (30, 60, or 120 mg·L⁻¹) and 1-methylcyclopropene (1-MCP) (0.3 µL·L⁻¹) on (A and B) flesh firmness (FF) determined after exposing fruit to 20 °C for 5 h immediately after removal from cold storage, (C and D) internal browning (IB), and (E and F) decay of 'Starkrimson' pears during a 16-week storage period at -1.1 °C in 2 production years (2013 and 2014). Different letters indicate significant differences among treatments according to Fisher's protected least significant difference test at $P < 0.05$. Values are means $\pm$ SD (n = 3); AVG30 = AVG at 30 mg·L⁻¹, AVG60 = AVG at 60 mg·L⁻¹, AVG120 = AVG at 120 mg·L⁻¹, 1-MCP = 1-MCP at 0.3 µL·L⁻¹.

16-week storage period. In control fruit, transcription of *PcACS1*, *PcACS2*, *PcACS4*, *PcACS5*, and *PcACO1* increased respectively $\approx 22,530$ -, 10-, 1,500-, 1,413-, and 1,546-fold, during 4 to 8 weeks of storage and decreased thereafter (Fig. 4). In general, AVG reduced the expression of *PcACS1*, *PcACS4*, *PcACS5*, and *PcACO1* compared with control fruit. 1-MCP substantially suppressed *PcACS1*, *PcACS4*, *PcACS5*, and *PcACO1* during 16 weeks of storage. Interestingly, the transcript level of *PcACS2* was unaffected by AVG or 1-MCP treatments throughout the storage period.

The transcription of ethylene perception genes *PcETR5*, *PcETR1*, and *PcETR2* increased after 2, 4, and 8 weeks of storage and thereafter declined to very low levels in control fruit (Fig. 4). The expression of *PcETR1* was decreased by AVG treatments, and the inhibition was positively correlated with AVG concentration. *PcETR2* and *PcETR5* transcription levels were less influenced by AVG, although the expression peaks of both genes were delayed or reduced by AVG

treatments. 1-MCP inhibited *PcETR1*, *PcETR2*, and *PcETR5* gene expression throughout the storage period (Fig. 4).

Discussion

EFFECT OF AVG AND 1-MCP ON STORAGE QUALITY OF 'STARKRIMSON' PEAR. The market has no tolerance for the presence of IB in 'Starkrimson' pear. IB displayed in different symptoms and causes significant economic loss for many European pear cultivars (Franck et al., 2007). In the present study, two types of IB were identified: senescent core breakdown (SCB) and brown heart (BH). SCB is a physiological disorder typically found in early maturing European pears (Calvo and Sozzi, 2004; D'Aquino et al., 2010; Wang and Sugar, 2013, 2015). SCB first appears as a brownish discoloration of the flesh, generally starting from the core tissue between the seed cavities and progressing outwards until the entire cortex tissue becomes brown (Fig. 5A). In the initial phase, flesh is firm and moist but becomes watery as the disorder progresses. Taste and aroma are strongly altered in SCB-affected fruit. SCB may occur during storage, but is typically observed after stored fruit are ripened at room temperature. In contrast, BH disorder begins when tissue of the flesh, centered between the core and epidermis, first appears dry and sponge-like, then progressively dries out and ultimately develops cavities (Fig. 5B). Browning is not a prerequisite of BH and taste and aroma are typically unaltered. BH is a common disorder reported

in several pear cultivars during cold storage and is thought to be due to CO₂ injury (Franck et al., 2007; Larrigaudière et al., 2004). IB is generally associated with a limited availability or depletion of energy and is aggravated by long periods of cold storage in which energy is used to sustain respiration and secondary metabolism (Pedreshi et al., 2009; Veltman et al., 2003; Villalobos-Acuña, et al., 2010). Both AVG and 1-MCP significantly reduced IB of 'Starkrimson' pears, possibly through reducing the respiration rate that results in metabolic savings and a longer storage life. AVG or 1-MCP decreased IB disorders of the summer pear cultivars Bartlett (Clayton et al., 2000; Villalobos-Acuña, et al., 2010; Wang and Sugar, 2015), Camusina di Genova, and Camusina di Bonarado (D'Aquino et al., 2010). Calvo and Sozzi (2004) reported that 1-MCP at 0.2 µL·L⁻¹ prevented SCB of 'Starkrimson' pear for 60 d in storage at -0.5 °C.

European pears are generally resistant to ripening at harvest and require exposure to a period of chilling to develop the

Fig. 3. Effects of aminoethoxyvinylglycine (AVG) rate (30, 60, or 120 mg·L⁻¹) and 1-methylcyclopropene (1-MCP) (0.3 μL·L⁻¹) on flesh firmness (FF), extractable juice (EJ), soluble solid content (SSC), and titratable acidity (TA) of 'Starkrimson' pears after 5 d at 20 °C throughout a 16-week storage period at -1.1 °C in 2 production years (2013 and 2014). Values are means $\pm$ SD (n = 3); AVG30 = AVG at 30 mg·L⁻¹, AVG60 = AVG at 60 mg·L⁻¹, AVG120 = AVG at 120 mg·L⁻¹, 1-MCP = 1-MCP at 0.3 μL·L⁻¹.

capacity to produce internal ethylene at a sufficient rate to activate and complete the ripening process when subsequently exposed to room temperatures (Blankenship and Richardson, 1985; Sugar and Basile, 2013; Villalobos-Acuña and Mitcham,

or EJ compared with control fruit. Based on FF and EJ levels, AVG at 120 mg·L⁻¹ required an additional 2 and 4 weeks to ripen compared with fruit of the control, AVG 30 mg·L⁻¹, and AVG 60 mg·L⁻¹ treatments in 2013 and 2014, respectively. No

2008). In this study, 2 weeks of -1.1 °C were required for 'Starkrimson' pear to develop its ripening capacity. AVG at 30 and 60 mg·L⁻¹ had no effect on ripening, but AVG at 120 mg·L⁻¹ delayed ripening capacity by 4 to 8 weeks, depending on the year. 1-MCP treated fruit never attained ripening capacity following 5 d at 20 °C throughout the entire 16-week storage duration (Table 2). However, these fruit softened when provided 14 d at 20 °C after 4 weeks of storage. Similarly, 'Starkrimson' pears treated with 1-MCP at 0.2 μL·L⁻¹ required 14 d to soften to 18–26 N following 60 d of cold storage (Calvo and Sozzi, 2004).

After gaining ripening capacity, European pears may attain a buttery, juicy texture or a mealy texture at warm temperatures following cold storage. Buttery, juicy, and mealy textures were not directly measured in this study; however, EJ served as an objective measure of texture given the strong correlation between EJ and buttery, juicy, and mealy textures (Chen and Borgic, 1985; Xie et al., 2014). For 'Starkrimson', a high eating quality was reached when EJ was reduced below $\approx$ 60 mL/100 g after ripening (Y. Wang, unpublished data). In the present study, control fruit ripened to a buttery, juicy texture following 2 to 12 weeks of cold storage; however, after 12 weeks fruit developed mealy texture. AVG at 60 mg·L⁻¹ extended 'Starkrimson' storage life producing high eating quality (buttery, juicy texture) fruit characterized by optimum FF and EJ to 16 weeks. AVG at 30 mg·L⁻¹ had no effect on IB, mealy texture,

Table 2. Flesh firmness (FF), extractable juice (EJ), soluble solid content (SSC), and titratable acidity (TA) of 1-methylcyclopropene-treated 'Starkrimson' pears following storage at -1.1 °C for 16 weeks and 14 d at 20 °C in 2 production years (2013 and 2014).

Storage time (wk)	2013				2014			
	FF	EJ	SSC	TA	FF	EJ	SSC	TA
	(N)	(mL/100 g)	(%)	(%)	(N)	(mL/100 g)	(%)	(%)
	Mean $\pm$ SD				Mean $\pm$ SD			
2	38.4 $\pm$ 4.6	72.5 $\pm$ 0.6	11.7 $\pm$ 0.2	0.31 $\pm$ 0.01	—	—	—	—
4	27.4 $\pm$ 1.9	71.3 $\pm$ 1.3	11.6 $\pm$ 0.2	0.26 $\pm$ 0.01	20.4 $\pm$ 1.4	72.3 $\pm$ 1.5	11.9 $\pm$ 0.2	0.26 $\pm$ 0.01
8	26.4 $\pm$ 1.7	68.6 $\pm$ 1.3	12.2 $\pm$ 0.1	0.25 $\pm$ 0.01	16.7 $\pm$ 1.5	68.6 $\pm$ 1.1	11.5 $\pm$ 0.2	0.25 $\pm$ 0.01
12	16.9 $\pm$ 0.6	60.3 $\pm$ 1.2	12.4 $\pm$ 0.2	0.25 $\pm$ 0.02	17.1 $\pm$ 1.3	61.3 $\pm$ 1.1	11.3 $\pm$ 0.2	0.23 $\pm$ 0.01
16	12.9 $\pm$ 1.3	59.6 $\pm$ 1.2	12.6 $\pm$ 0.1	0.22 $\pm$ 0.01	10.7 $\pm$ 0.8	60.6 $\pm$ 0.8	11.1 $\pm$ 0.1	0.22 $\pm$ 0.00

Fig. 4. Effects of aminoethoxyvinylglycine (AVG) rate (30, 60, or 120 mg·L⁻¹) and 1-methylcyclopropene (1-MCP) (0.3 µL·L⁻¹) on transcription levels of ethylene biosynthesis genes (*PcACS1*, *PcACS2*, *PcACS4*, *PcACS5*, and *PcACO1*) and ethylene action genes (*PcETR1*, *PcETR2*, and *PcETR5*) of 'Starkrimson' pears throughout a 16-week storage period at -1.1 °C in 2013. Values are means ± SD (n = 3); AVG30 = AVG at 30 mg·L⁻¹, AVG60 = AVG at 60 mg·L⁻¹, AVG120 = AVG at 120 mg·L⁻¹, 1-MCP = 1-MCP at 0.3 µL·L⁻¹.

deleterious effects on ripening were evident during mid to long-term storage (8 to 16 weeks). In contrast, 1-MCP-treated fruit were incapable of ripening to an acceptable texture unless the ripening period was prolonged 9 additional d following 12 to 16 weeks of storage. This presents a significant challenge to 1-MCP use for 'Starkrimson', given consumer preference of ready-to-eat products and general disinterest in allowing pears extended time to ripen after purchase (K. Moffitt, personal communication).

FF of European pears after cold storage and before shipping is referred to as shipping FF (Sugar and Basile, 2013). Shipping FF and TA of 'Starkrimson' decreased and SSC increased slightly during cold storage. After 16 weeks of storage, there were no treatment differences in shipping FF and SSC, but AVG and 1-MCP treatments slowed the loss of TA. Preharvest AVG delayed the loss of shipping FF, but did not affect SSC or TA in 'Camusina di Genova' and 'Camusina di Bonarcado' pears beyond 10 d of storage at 18 °C (D'Aquino et al., 2010). 1-MCP at 0.2 µL·L⁻¹ did not influence shipping FF, SSC, or TA of 'Starkrimson' pears during storage at -0.5 °C for 60 d (Calvo and Sozzi, 2004). The disparate results between those studies and ours may be attributed to genotypic effects and/or differences in storage conditions.

EFFECT OF AVG AND 1-MCP ON ETHYLENE SYNTHESIS AND ASSOCIATED GENE EXPRESSION IN 'STARKRIMSON' PEAR. The short storage life and increased propensity of early season European pear cultivars to develop storage disorders is associated with high metabolic activity and increased ethylene production induced by cold storage (Ekman et al., 2004; Villalobos-Acuña, et al., 2011). In this study, untreated 'Starkrimson' pear showed a marked increase in IEC, EPR, and RR during 16 weeks of storage; therefore, control measures applied before (i.e., AVG) or after harvest (i.e., 1-MCP), which lowered ethylene synthesis and RR prolonged 'Starkrimson' pear storage life. Ethylene in fruit is biosynthesized from methionine. The conversion of *S*-adenosylmethionine to 1-ACC via ACS and the subsequent conversion of ACC to ethylene via ACC oxidase (ACO) are the two key steps in ethylene synthesis (Adams and Yang, 1979). Both steps were highly transcriptionally regulated by multigene

families in tomatoes [*Solanum lycopersicum* (Barry and Giovannoni, 2007)] and European pears (Chiriboga et al., 2013). Ethylene biosynthesis in climacteric fruit is autocatalytic and mediated by the binding of ethylene to ethylene receptors (Barry and Giovannoni, 2007). In pears, at least four ACS genes and one ACO gene (El-Sharkawy et al., 2004), and four ethylene receptor genes (El-Sharkawy et al., 2003) have been described during pear development and ripening.

Fig. 5. Transverse cross-sections of 'Starkrimson' pears depicting a range of severity of (A) senescent core breakdown and (B) brown heart.

Regarding the ethylene synthesis genes, *PcACS1*, *PcACS4*, *PcACS5*, and *PcACO1* of untreated 'Starkrimson' pears increased their expression dramatically during cold storage, which is in agreement with previous reports for 'Bartlett' (Villalobos-Acuña et al., 2011) and 'Anjou' (Xie et al., 2014). In contrast, *PcACS2* increased slightly in 'Starkrimson' a result which differs from that reported for 'Bartlett' (Villalobos-Acuña et al., 2011) and 'Anjou' pears (Xie et al., 2014) whereby *PcACS2* either decreased or was not induced by chilling. El-Sharkawy et al. (2004) reported the transcription accumulation of *PcACS2* to be negatively regulated by ethylene in the late-season pear cultivar Passe-Crassane, and positively regulated by ethylene in the early-season cultivar Old-Home. Thus, the disparate responses indicate a genotypic effect. AVG reduced the transcription levels of *PcACS1*, *PcACS4*, *PcACS5*, and *PcACO1*, resulting in lower EPR and RR in 'Starkrimson' pear during storage. Yuan and Li (2008) reported that preharvest AVG suppressed expression of *MdACS1* and *MdACO1* and delayed preharvest fruit drop in 'Delicious' apple. Similarly, though to a much greater degree, 1-MCP treatment led to a significant decrease in the expressions of *PcACS1*, *PcACS4*, *PcACS5*, and *PcACO1*, thereby significantly inhibiting EPR and RR of 'Starkrimson' pear. These findings are generally consistent with previous reports that ethylene synthesis genes are inhibited by 1-MCP in pears (El-Sharkawy et al., 2004; Villalobos-Acuña et al., 2011; Xie et al., 2014). Interestingly, the transcript level of *PcACS2* was unaffected by AVG or 1-MCP throughout the storage period. Our previous results showed that *PcACS2* was upregulated by 1-MCP treatment in 'Anjou' pear during cold storage (Xie et al., 2014). In tomato fruit, the ACS gene family members displayed different responses to 1-MCP (Nakatsuka et al., 1998). These results indicate that *PcACS1*, *PcACS4*,

PcACS5, and *PcACO1* play important roles, but *PcACS2* may not be related to ethylene biosynthesis in 'Starkrimson' pear during ripening/senescence.

In the ethylene signal pathway, ethylene receptors negatively regulate ethylene response. An inverse relationship has been shown between ethylene receptor level and ethylene sensitivity in *Arabidopsis thaliana* (Guo and Ecker, 2004). In general, the expressions of *PcETR1*, *PcETR2*, and *PcETR5* in 'Starkrimson' pear increased during early storage and decreased thereafter. Similarly, a noted increase in these ethylene receptor genes during fruit ripening or storage has been reported in apple (Yang et al., 2013), and other European pear cultivars (El-Sharkawy et al., 2003; Xie et al., 2014). AVG and 1-MCP treatments reduced their expression in 'Starkrimson' pear, which corroborates with previous findings for other pear (El-Sharkawy et al., 2003) and apple (Dal Cin et al., 2008) cultivars. Ripening recovery from AVG inhibition was associated with increased accumu-

lation of *PcETR1*, *PcETR2*, and *PcETR5* transcription in 'Starkrimson' pear. Tassoni et al. (2006) reported increased expression of ETR4/5/6 was associated with ripening recovery from inhibition by 1-MCP. As the degree of the inhibition in ETRs expression by AVG weakened, the ripening process advanced accordingly. Collectively, these data indicate an association between the recovery of ethylene receptor gene expression of AVG-treated 'Starkrimson' and reinitiation of ripening competency.

In conclusion, 1-MCP was more effective than AVG in reducing ethylene biosynthesis, eliminating IB, and extending storage life of 'Starkrimson' pear. However, the 1-MCP-treated fruit required additional time (i.e., 14 d) at 20 °C to ripen to high eating quality following cold storage. 1-MCP was more effective than AVG at inhibiting expression of ethylene synthesis genes and especially the signal genes in 'Starkrimson' pear during storage. 1-MCP bound the ethylene receptors as previously shown and thus blocked the ethylene signal and suppressed autocatalytic synthesis. AVG, on the other hand, counteracted ethylene biosynthesis by inhibiting ACS activity but the ethylene receptors remained open to bind free ethylene in the fruit and surrounding atmosphere. Thus, preharvest AVG application at 60 mg·L⁻¹ and postharvest 1-MCP treatment at 0.3 μL·L⁻¹ extended storage life of 'Starkrimson' pears, though from a consumer perspective, the effect of 1-MCP on lengthening the ripening period to 14 d may be undesirable. More research is warranted to investigate technologies such as ethylene conditioning to adequately ripen 1-MCP-treated European pears after cold storage.

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