Efficacy of liquid 1-methylcyclopropene to delay ripening of ‘Bartlett’ pears

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19 *Abstract*

20 1-Methylcyclopropene (1-MCP) has been a useful tool to extend the postharvest life of ‘Bartlett’
21 pears, but fruit response can be highly variable due to competition with ethylene. Application of
22 liquid 1-MCP after harvest was tested to determine its efficacy as compared with gaseous 1-
23 MCP. Fruit harvested from Sacramento and Lakeport, California at early-, mid- and late-
24 commercial harvest maturity were treated with 0.6 $\mu\text{L L}^{-1}$ gaseous 1-MCP at 0 °C for 24h or
25 dipped for 0, 15, 30, 45 or 60 s in 250, 500, 750 or 1000 $\mu\text{g L}^{-1}$ 1-MCP in four experiments
26 across three years of study. After treatment, pears were exposed to ethylene or kept in cold
27 storage at 1 °C for 5 weeks before ripening at 20 °C. Treatment with liquid 1-MCP delayed pear
28 ripening as evidenced by delayed softening for a minimum of 6 d compared to the control fruit,
29 delayed the increase in respiration and ethylene production rates, and reduced respiration and
30 ethylene production rates. Treatment was effective in a concentration- and dip time-dependent
31 manner. Overall, dipping in 1000 $\mu\text{g L}^{-1}$ liquid 1-MCP for 60 s was the most consistent treatment
32 among years and locations; however, the resulting time to ripen at 20 °C could be too long for
33 some commercial applications. Treatment at 500 $\mu\text{g L}^{-1}$ liquid 1-MCP is recommended for
34 ‘Bartlett’ pears as this dose controls the ripening process, and provides consistent response for
35 mid- and late-maturity fruit. A postharvest evaluation of a liquid formulation of 1-MCP provided
36 a more consistently effective treatment for ‘Bartlett’ pears (*Pyrus communis*) than the current
37 gaseous treatments.

38
39 *Key words:* color, ethylene, firmness, 1-MCP, *Pyrus communis*, respiration

1. Introduction

1-Methylcyclopropene (1-MCP) is a well-known inhibitor of ethylene action which has been widely used and evaluated for its capacity to prolong the storage life of many fruits and vegetables (Watkins, 2008). 1-MCP blocks ethylene perception, preventing the signaling mechanism that activates ripening-associated genes (Klee, 2004). Postharvest treatment of European pears with 1-MCP has been shown to decrease fruit respiration rate, ethylene production, softening, ACC synthase and ACC oxidase activities, and development of internal browning and storage scald (Argenta et al., 2003; Baritelle et al., 2001; Calvo and Sozzi, 2004; Hiwasa et al., 2003; Kubo et al., 2003; Ekman et al., 2004; Mwaniki et al., 2005; Trinchero et al., 2004).

Commercially, 1-MCP is applied to extend the storage life of ‘Bartlett’ pears, allowing shipment to distant markets. It is usually applied to fruit after harvest in its gaseous form (SmartFresh™) inside sealed rooms, containers or tents. However, the fruit response to SmartFresh™ can vary with season and harvest date (AgroFresh, Inc., personal communication). A number of factors have been shown to influence the success of SmartFresh™ treatment on pears, including concentration applied, treatment duration, temperature of the fruit during treatment and presence of exogenous ethylene (Argenta et al., 2003; Chen and Spotts, 2005; DeEll et al., 2002; Ekman et al., 2004; DeEll and Ehsani-Moghaddam, 2011; Villalobos Acuña et al., 2011b). Materials in the storage room can reduce treatment efficacy. According to Calvo and Sozzi (2009), treatment of pear fruit with SmartFresh™ in wooden bins that were wet following hydrocooling was much less effective than treatment of pears in plastic bins. Wet wooden bin material represents a major though unpredictable source of 1-MCP sorption that could bind a significant percentage of the 1-

64 MCP applied. Recently, Wang and Sugar (2015) reported that pears grown at high elevations,
65 with expected cooler preharvest temperatures, and/or held at 5 °C for 12 d between harvest and
66 1-MCP application frequently showed a reduced response to the treatment.

67 European pears naturally ripen in association with a climacteric rise in ethylene production
68 (Hansen, 1943). ‘Bartlett’ pears harvested early in the season produce low levels of ethylene at
69 harvest and ripen very slowly or even fail to ripen (Puig et al., 1996; Villalobos-Acuña and
70 Mitcham, 2008). Fruit harvested later in the season produce relatively higher rates of ethylene
71 and ripen more rapidly. In some growing areas, the commercial harvest can take place over a 3 to
72 4 week period; hence fruit are often picked at different degrees of maturity (Chen and
73 Mellenthin, 1981). To stimulate ripening of early-harvested ‘Bartlett’ pear fruit that are marketed
74 immediately, treatment with ethylene (100 $\mu\text{L L}^{-1}$ for 1 to 2 d at 20 °C) and/or exposure to low
75 temperature (0 to 10 °C for 5 to 14 d) conditioning is used (Agar et al., 2000; Mitcham et al.,
76 2000; Villalobos-Acuña and Mitcham, 2008).

77 Because of the competitive relationship between 1-MCP and ethylene for binding to ethylene
78 receptors (Macnish et al., 2012; Villalobos-Acuña et al., 2011a; Zhang et al., 2009), the efficacy
79 of 1-MCP to delay ethylene-mediated softening of climacteric fruits depends in part on the
80 physiological state of the fruit, with reduced efficacy on more mature fruit (Chiriboga et al.,
81 2013; Gamrasni et al., 2010; Jung and Watkins, 2014; Mir et al., 2001; Wang and Sugar, 2015;
82 Watkins, 2008). The increase in ethylene concentrations within ripening fruit tissues reduces the
83 competitive ability of 1-MCP to bind to available receptors, reducing 1-MCP’s effect, while an
84 excess of 1-MCP can lead to failure to ripen (Chen and Spotts, 2005; Ekman et al., 2004; Guillén
85 et al., 2007; Manenoi et al., 2007; Villalobos-Acuña et al., 2011a; Villalobos-Acuña and
86 Mitcham, 2008; Wang and Sugar, 2015; Zhang et al., 2009, 2010, 2011).

87 Endogenous and exogenous ethylene has been shown to reduce the efficacy of 1-MCP to delay
88 ripening of ‘Bartlett’ pears (Macnish et al., 2012; Wang and Sugar, 2015). The higher rates of
89 ethylene production at harvest for late season ‘Bartlett’ pear fruit may explain the failure of 1-
90 MCP treatments to consistently delay fruit ripening (Macnish et al., 2012; Wang and Sugar,
91 2015). This phenomenon has also been described in other fruit, including tomatoes and apples
92 (Macnish et al., 2012; Watkins, 2008; Zhang et al., 2009, 2010, 2011). Zhang et al. (2009, 2010,
93 2011) found that the higher the internal ethylene at the time of tomato treatment with 1-MCP, the
94 smaller the effect on ripening. With apples, Watkins (2008) had earlier suggested the influence
95 of internal ethylene on the inconsistency of 1-MCP treatments. Macnish et al. (2012) illustrated
96 the importance of maintaining the appropriate ratio of 1-MCP to ethylene within the treatment
97 atmosphere. Using ‘Bartlett’ pears, the authors showed that a relatively high initial 1-MCP:
98 ethylene concentration ratio (e.g., 20-50:1) was necessary for maximum ripening inhibition of
99 early-harvested ‘Bartlett’ pear fruit. However, this initial ratio did not greatly extend the shelf
100 life of mid- and late-season pears because ethylene produced by these fruit accumulated more
101 substantially during the treatment.

102 These challenges of inconsistent fruit response to SmartFresh™ applications may potentially be
103 overcome by application of a liquid 1-MCP formulation on the harvested fruit. Such an
104 application would eliminate the need to occupy a sealed room during the 12 or more hours-
105 treatment, and would eliminate the reduction in 1-MCP efficacy due to accumulation of ethylene
106 in the sealed chamber atmosphere during gaseous applications. As discussed by Sisler (2006),
107 Sisler and Serek (2003) and Pongprasert and Srilaong (2014), liquid solutions could facilitate
108 broader agricultural applications. Harvista™ is a liquid formulation of 1-MCP that was designed
109 as a preharvest treatment to control preharvest drop in apples (Elfving et al., 2007; Yuan and

Carbaugh, 2007). It has also been widely tested as a preharvest treatment to control ethylene-related physiological processes and for its beneficial effects on maturity and postharvest quality of apples (Byers et al., 2005; Defilippi et al., 2010; Elfving et al., 2007; McArtney et al., 2008; Yuan and Carbaugh, 2007) and ‘Abate Fetel’ (Nock et al., 2009) and ‘Bartlett’ pears (Villalobos et al., 2010).

The use of liquid 1-MCP has recently been evaluated as a postharvest treatment, including as a topical dip in ‘Florida’ tomato and ‘Hass’ avocado (Choi and Huber, 2008), ‘Sanibel’ tomato (Choi et al., 2008) and ‘Joanna Red’ plums (Manganaris et al., 2008). All of these authors illustrated the potential of this formulation when applied after harvest. In all cases, the formulation delayed ethylene production, the increase in respiration rate, surface color development, and fruit softening. It was also very effective in delaying ripening of fruit harvested at an advanced maturity stage (Manganaris et al., 2008).

The principal objective of our study was to assess the postharvest application of a liquid formulation of 1-MCP to provide a more consistently effective postharvest treatment for ‘Bartlett’ pears than the current gaseous treatments. We determined the effects on fruit harvested from two growing locations at early-, mid- and late- commercial harvest maturity within each location. The effects of 1-MCP concentration and dip time on 1-MCP efficacy for different harvest maturities and seasons were tested.

2. *Materials and methods*

2.1. *Plant material*

For three subsequent years (2011 to 2013), mature green, size 110 ‘Bartlett’ pear (*Pyrus communis*) fruit were obtained on the day of harvest in packed boxes from packinghouses near Sacramento and Lakeport, California. The fruit were sampled near the time of the first commercial harvest and then every 5 to 8 d during the season to capture three stages of maturity from each growing region. All fruit were transported to the University of California, Davis in an air-conditioned vehicle. Upon arrival to the laboratory, the fruit were examined to eliminate damaged fruit, and selected for uniform quality and absence of sunburn, bruises or cuts. Fruit for each experiment were randomly divided into mesh bags for the following liquid or gaseous 1-MCP treatments.

2.2. *1-MCP treatments*

2.2.1. *Experiment 1: gaseous versus liquid 1-MCP*

In 2011, an initial comparison was made between gaseous and liquid 1-MCP in fruit harvested in Sacramento and Lakeport. The fruit were divided into three subsets of 150 fruit. One subset of fruit from each harvest was cooled to 0 °C overnight and treated with 0.6 µL L⁻¹ gaseous 1-MCP at 0 °C for 24h. A second subset was dipped for 1 min in 1000 µg L⁻¹ 1-MCP solution at an ambient temperature of 29-38 °C in a 37.8 L container. The concentration of liquid 1-MCP was selected to provide a similar dose of 1-MCP as the gaseous treatment, based on advice from the manufacturer, AgroFresh, Inc. Solutions were prepared from formulation AFxRD-038 (3.8% active ingredient, AgroFresh, Inc., Yakima, WA). The desired level of active ingredient (0.263 g L⁻¹) was suspended in 10 L of distilled water and a surfactant (Nu-Film P, 0.1%, Miller Chemical

& Fertilizer Corp., Hanover, PA, USA) for maximum absorption. The mixture was stirred gently to avoid off-gassing until complete dissolution of the powder. The solutions were used no later than 15 min after preparation, and all immersion treatments were completed within 10 min. Fruit were immersed into the solutions ensuring complete coverage of the fruit during the immersion period. The third subset of fruit was dipped in distilled water with the surfactant (Nu-Film P, 0.1%) as an untreated control. After removal, fruit were air dried at 20 ± 2 °C, packed back into vented boxes, and placed at 20 °C, >95% RH overnight.

Two boxes of 75 fruit each were used for each treatment; one box from each treatment was then exposed to $100 \mu\text{L L}^{-1}$ ethylene for 24 hours at 20 °C, a standard treatment for fruit ripened immediately after harvest. After the ethylene treatment, fruit were maintained at 20 °C for ripening evaluation. The second box from each treatment was stored at 1 °C for 5 weeks to simulate shipment and/or storage before being transferred to 20 °C for ripening.

2.2.2. Experiment 2: liquid 1-MCP concentration

In 2012, we studied the effect of 1-MCP concentration in the liquid formulation on its efficacy. Fruit from early-, mid- and late-harvest maturity in Sacramento and early-harvest in Lakeport were immersed for 60 s in 0, 250, 500, 750, or $1000 \mu\text{g L}^{-1}$ liquid 1-MCP. Solutions were prepared as described for experiment 1, with the corresponding levels of active ingredient (0, 0.066, 0.132, 0.197 and 0.263 g L^{-1} , respectively). After treatment, fruit were maintained at 20 °C for ripening and evaluation. No fruit were stored.

2.2.3. Experiment 3: liquid 1-MCP dip time

173 In 2012, mid- and late-harvested pear fruit from Lakeport were immersed in 250 $\mu\text{g L}^{-1}$ for 0, 15,
174 30, 45, or 60 s. After treatment, fruit were kept at 20 °C for ripening assessment. No fruit were
175 stored.

176 *2.2.4. Experiment 4: interaction between liquid 1-MCP concentration and dip time*

177 In 2013, early-, mid-, and late-harvested fruit from Sacramento and Lakeport were treated with 0,
178 250, 500, or 1000 $\mu\text{g L}^{-1}$ 1-MCP for 0, 15, 30, or 60 s in a factorial design. All fruit were ripened
179 immediately after treatment. No fruit were stored.

180 *2.3. Fruit quality assessments*

181 Ethylene and CO₂ production, skin color, and flesh firmness were measured every 3 d for a
182 maximum of 21 d in 2011, and 18 d in 2012 and 2013 on three replications of six fruits until the
183 firmness level decreased to an average of ~13 N which was considered fully ripe.

184 *2.3.1 Measurement of firmness and color*

185 Flesh firmness was measured using a Fruit Texture Analyzer (GS-14, Güss Manufacturing Ltd.,
186 Strand, Western Cape, South Africa) fitted with an 8-mm diameter probe to penetrate the flesh to
187 a depth of 5mm on opposite sides of each fruit after removing a 1-mm diameter section of skin.

188 Skin color was determined on opposite sides of each fruit using a Minolta colorimeter (Model
189 CR-400, Ramsey, N.Y., USA) and described using the L*, a* and b* color space. The hue angle
190 (h°) represented the change in skin color from green (120 h°) to yellow (60 h°), calculated using
191 the formula $\arctg(b^*/a^*)$.

192 *2.3.2. Measurement of ethylene and CO₂ production*

Six fruit were placed into a 3.8 L glass jar as one replication, with three replications per evaluation. After the jars were sealed for 60 min at 20 °C, a headspace sample was withdrawn with a 10 mL syringe and subjected to analysis by gas chromatography (AGC Series 400; Chandler Engineering Company, Tulsa, OK, USA) with a flame ionization detector and alumina column for ethylene measurement. The ethylene concentration was calculated relative to a gas standard. A second headspace sample was analyzed for CO₂ with an infrared CO₂ analyzer (Horiba VIA-510; Horiba Instruments Inc., Irvine, CA) operated in a flow-through mode of 100 mL min⁻¹ with N₂ as the carrier gas. The CO₂ concentration was calculated relative to a certified standard (Matheson Tri-Gas, Inc.) containing 0.25% CO₂ balance N₂.

2.4. Data analysis

Statistical differences between treatment means were determined by the least significant difference test (LSD) using analysis of variance (ANOVA). The *P*-value was used to determine the level of significance (*P* = 0.05, *P* = 0.01 and *P* = 0.001) of the main effects of harvest, treatment, dip time and the interactions of harvest x treatment and harvest x dip time. Firmness and color data were also subjected to ANOVA and LSD means separation (Tukey test with *P* = 0.05 significance level) to identify the significant differences between treatments. A comparative analysis (t-test) of the regression equation slopes was applied to some data items to compare fruit changes over time. A Pearson correlation analysis (*P* = 0.05) was performed to study the skin color change in relation to pear softening. Statistical analyses were performed using the statistical software XL-Stat (Addinsoft SARL, 2014).

3. Results

In all three years, liquid 1-MCP treatment concentration had a highly significant ($P < 0.0001$) effect on the rate of fruit ripening. The dip time was also significant ($P < 0.001$) in all years and harvests, but with a lower level of significance in late harvests ($P < 0.05$). The fruit harvest date significantly affected the ripening response in all years and locations ($P < 0.001$), but there were no significant differences between mid- and late-harvests for most of the concentrations applied. The levels of internal ethylene at harvest in early-harvested Sacramento fruit differed by $0.261 \pm 0.07 \mu\text{L L}^{-1}$ among years, ranging from $0.018 \pm 0.007 \mu\text{L L}^{-1}$ in 2012 to $0.262 \pm 0.09 \mu\text{L L}^{-1}$ in 2011 (data not shown). There was a significant interaction between 1-MCP concentration and harvest ($P < 0.0001$) and 1-MCP concentration and dip time ($P < 0.0001$).

3.1. Experiment 1: gaseous versus liquid 1-MCP

Both gaseous and liquid 1-MCP treatments delayed flesh softening and thereby extended the shelf life of early-, mid- and late-harvested Sacramento (Fig. 1) and Lakeport (data not shown) fruit. A 60 s postharvest dip in $1000 \mu\text{g L}^{-1}$ liquid 1-MCP at 29-38 °C delayed Sacramento pear fruit softening by >15 d compared to the control fruit, while a 24 hour exposure to $0.6 \mu\text{L L}^{-1}$ gaseous 1-MCP at 0 °C delayed softening by up to 15 d (Fig. 1). Similar results were observed for Lakeport fruit (data not shown). A comparative analysis (t -test) of regression slopes showed that the softening process in pears treated with liquid and gaseous 1-MCP was not significantly different for mid-harvest Sacramento (softening rates 2.64 and 3.05 N d^{-1} , liquid and gaseous treatments, respectively) and all Lakeport harvests (average softening rates 2.46 and 2.60 N d^{-1} , liquid and gaseous treatments, respectively). However, early and late-harvested fruit from the Sacramento orchard treated with liquid 1-MCP remained firmer/were slower to soften ($P < 0.05$)

238 than gaseous 1-MCP-treated fruit. After 5-weeks of storage at 1 °C, the fruit ripening response
239 was similar to matching sets of fruit that were treated with ethylene after 1-MCP (Fig. 1 D-F). In
240 Lakeport, the only significant difference between gaseous and liquid 1-MCP-treated fruit was in
241 late-harvested fruit, which remained significantly firmer when treated with liquid 1-MCP (data
242 not shown).

243 *Fig. 1.* Experiment 1. Firmness (N) of ‘Bartlett’ pears during ripening at 20 °C following treatment with liquid or gaseous 1-methylcyclopropene
 244 (year 2011). Vertical lines represent the standard deviation of the means ($\pm$ SD, n = 18). A-B-C corresponds to early, mid and late harvests from
 245 Sacramento, respectively, without storage. D-E-F corresponds to early, mid and late harvest from Sacramento, respectively, after 5-weeks storage
 246 at 1 °C.

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Pear skin yellowing was delayed ($P < 0.05$) during ripening in non-stored fruit treated with liquid 1-MCP compared to gaseous 1-MCP in both locations and all harvests except early-harvested Lakeport fruit, and also compared to control fruit in both locations and all harvests except early-harvested Sacramento and late-harvested Lakeport fruit (data not shown). The average skin h° after 21 d of ripening was $100.4 \pm 2.1 h^{\circ}$ for liquid-1-MCP-treated fruit (not fully-ripe), $97.9 \pm 3.0 h^{\circ}$ for gaseous-1-MCP treated fruit (fully-ripe) and $96.7 \pm 4.2 h^{\circ}$ for control fruit (fully-ripe). There were no significant differences between the average skin color for gaseous 1-MCP-treated and control fruit when the fruit were fully-ripe ($P < 0.05$). After storage, pear skin yellowing was also delayed ($P < 0.05$) in the liquid-1-MCP-treated fruit compared to both gaseous 1-MCP-treated fruit and control fruit in early-harvested Sacramento fruit and mid- and late-harvested Lakeport fruit. (The average h° after 21 d of ripening was $95.2 \pm 1.4 h^{\circ}$ for liquid 1-MCP-treated fruit (not fully-ripe), $92.4 \pm 2.2 h^{\circ}$ for gaseous 1-MCP-treated fruit (fully-ripe) and $93.8 \pm 3.0 h^{\circ}$ for control fruit (fully ripe).

Both gaseous and liquid 1-MCP treated fruit similarly delayed and reduced rates of ethylene production at 20 °C compared to the control fruit in Sacramento (Fig. 2A-C). The results for Lakeport fruit were very similar (data not shown). However, ethylene production by gaseous 1-MCP-treated fruit started increasing before the liquid 1-MCP-treated fruit in most harvests. Liquid 1-MCP-treated fruit showed increases in ethylene production after 15 and 8 d for early- and late-harvested fruit, respectively, compared to after 12 and 8 d or 3 and 0 d for early- and late-harvested fruit treated with gaseous 1-MCP or untreated (control), respectively. After storage, ethylene production was higher in fruit from all harvests and locations relative to the rates when fruit were ripened at harvest (Fig. 2D-F). At early-harvest, gaseous 1-MCP-treated fruit showed increases in ethylene production after 13 d of ripening compared to after 16 or 0 d

272 for liquid 1-MCP-treated or untreated fruit, respectively. At late-harvest, fruit treated with liquid
273 and gaseous 1-MCP started to produce ethylene after 16 d. In both harvests, gaseous 1-MCP
274 produced more ethylene than liquid 1-MCP-treated fruit ($0.042 \text{ uL kg}^{-1} \text{ 2}^{-1}$ higher for early and
275 late harvests, on average).

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278 Fig. 2. Experiment 1. Ethylene production ($\mu\text{L kg}^{-1} \text{s}^{-1}$) of ‘Bartlett’ pears during ripening at 20 °C following treatment with liquid or gaseous 1-
 279 methylcyclopropene (year 2011). Vertical lines represent the standard deviation of the mean ($\pm$ S.D., $n = 18$). A-B-C corresponds to early, mid and
 280 late harvests from Sacramento, respectively, without storage. D-E-F corresponds to early, mid and late harvests from Sacramento, respectively,
 281 after 5-weeks storage at 1 °C.

A decrease in respiration rate was also observed in gaseous- and liquid-1-MCP-treated fruit compared to the control fruit in all harvests and locations (data not shown). Respiration rates of liquid-1-MCP treated fruit were significantly lower than gaseous-1-MCP treated fruit in late-harvested fruit from both Sacramento and Lakeport after 12 d of ripening ($P < 0.001$). After storage, differences in respiration rate between gaseous- and liquid-1-MCP treatments were significant ($P < 0.001$) in early-harvested fruit from both Sacramento and Lakeport.

3.2 Experiment 2: liquid 1-MCP concentration effects

The rate of fruit softening was dependent on the 1-MCP concentration applied during the 60 s dip treatments (Fig.3), delaying ripening for a minimum of 12 days in relation to the control. Fruit treated with $250 \mu\text{g L}^{-1}$ showed the least inhibition, becoming nearly fully ripe after 18 d at 20°C in all harvests. In general, those fruit treated with $\geq 500 \mu\text{g L}^{-1}$ 1-MCP softened more slowly, but fruit responded slightly differently depending on the harvest maturity. The comparative analysis (t-test) of regression slopes showed that $750 \mu\text{g L}^{-1}$ was as effective as $1000 \mu\text{g L}^{-1}$ in early-harvested Lakeport fruit, and mid- and late-harvested Sacramento fruit, while $500 \mu\text{g L}^{-1}$ was also similar to $1000 \mu\text{g L}^{-1}$ in early- and late-harvested Sacramento fruit ($P < 0.05$). Therefore, for early and late-harvested fruit from Sacramento, treatment with $500 \mu\text{g L}^{-1}$ 1-MCP saturated the response, while for mid-harvested fruit from Sacramento and early harvested fruit from Lakeport, $750 \mu\text{g L}^{-1}$ 1-MCP was needed to saturate the response. Mid- and late-harvested Lakeport fruit were not evaluated in this experiment.

303 Fig. 3. Experiment 2. Firmness (N) of ‘Bartlett’ pears during ripening at 20 °C following treatment with liquid 1-methylcyclopropene for 60 s
 304 (year 2012). Vertical lines represent the standard deviation of the means ($\pm$ S.D., n = 18). A-B-C corresponds to early, mid and late harvests from
 305 Sacramento, respectively, and D corresponds to early harvested fruit from Lakeport. Legend indicates aqueous 1-MCP concentrations ($\mu\text{L L}^{-1}$).

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1-MCP treatment concentrations were correlated to color development; after 18 d of ripening, yellow skin color development was correlated to fruit firmness in treated-fruit of Sacramento harvests ($P < 0.0001$ early and mid-harvests, $P < 0.01$ late harvest) and early Lakeport harvest ($P < 0.0001$) (data not shown). Early harvested Sacramento and Lakeport fruit treated with 250 (nearly fully-ripe), 500 and 750 $\mu\text{g L}^{-1}$ (not fully-ripe) liquid 1-MCP were more yellow (from 88.4 to 99.3 h° and 91.3 to 95.7 h° , Sacramento and Lakeport, respectively) after 18 d of ripening than the control fruit (fully-ripe) (107.8 h° Sacramento, and 96.5 h° Lakeport) ($P < 0.05$). Mid- and late-harvested fruit treated with 250 $\mu\text{g L}^{-1}$ 1-MCP were more yellow (92.1 and 90.7 h°) than the control fruit (92.6 and 91.8 h°) when they were all fully-ripe ($P < 0.05$).

All concentrations of liquid 1-MCP reduced ethylene production relative to the control fruit (data not shown). However, fruit treated with 250 $\mu\text{g L}^{-1}$ 1-MCP were differentiated from the other treatments for their early (on average 3 d earlier than fruit treated with $\geq 500 \mu\text{g L}^{-1}$) and higher (> 2-fold) production rate of ethylene. Ethylene production from early-harvested Sacramento fruit treated with $\geq 500 \mu\text{g L}^{-1}$ 1-MCP behaved similarly (started to increase after ~12 d, with the same production rate during ripening) to fruit treated with 250 $\mu\text{g L}^{-1}$ 1-MCP. In the remaining harvests, fruit treated with $\geq 500 \mu\text{g L}^{-1}$ 1-MCP started to increase in ethylene production after ~15 d, and the production rates increased accordingly with the concentration; 500 $\mu\text{g L}^{-1}$ 1-MCP treated-fruit always showed higher ethylene production than 1000 $\mu\text{g L}^{-1}$ 1-MCP treated-fruit, but for fruit treated with 750 $\mu\text{g L}^{-1}$ 1-MCP, it depended on the harvest.

All liquid 1-MCP concentrations reduced fruit respiration rate relative to the control fruit; however, respiration rate was less affected by treatment concentration than ethylene production (data not shown). In agreement with the ethylene production data, the highest respiration rate corresponded to the 250 $\mu\text{g L}^{-1}$ 1-MCP treatment in early- and mid-harvested Sacramento fruit,

but only early-harvested Lakeport fruit treated with $250 \mu\text{g L}^{-1}$ 1-MCP showed significant differences with respect to the other treatments ($P < 0.001$).

3.3 Experiment 3: liquid 1-MCP dip time effects

To investigate the minimum dip time required for liquid 1-MCP treatment, a low concentration of 1-MCP ($250 \mu\text{g L}^{-1}$) was selected, so as not to overwhelm the response, and tested on mid- and late-harvested Lakeport fruit. Dip time had a significant ($P < 0.001$) effect on the rate of fruit softening; the fruit dipped for 60 and 45 s took an additional 3 days to ripen compared to fruit dipped for 15 and 30 s (Fig. 4). The same effect was observed in both mid- and late-harvested fruit.

341 Fig. 4. Experiment 3. Firmness (N) of ‘Bartlett’ pears during ripening at 20 °C following treatment with 250 µg L⁻¹ liquid 1-methylcyclopropene
342 (year 2012). Vertical lines represent the standard deviation of the means (± S.D., n = 18). A and B correspond to mid- and late-harvests from
343 Lakeport, respectively. Legend indicates different dip times (s).

However, pear softening was not significantly correlated to pear fruit color after 18 d of ripening. For mid-harvest, all the dipping-times except 30 s showed the same fruit color (average 93.5 ± 1.4 h°) and were more yellow than the control fruit (97.3 h°) ($P < 0.05$); in late-harvested fruit there were no color differences between any treated or control fruit (average 92.3 ± 1.1 h°).

All dip times reduced ethylene production relative to the control fruit (data not shown). Fruit dipped for 15 s were differentiated from the other treatments for their earlier (3 d earlier than 30, 45 and 60 s) and higher production rate of ethylene (consistently ~ 0.16 uL kg⁻¹ s⁻¹ higher) during ripening. All liquid 1-MCP dipping times reduced fruit respiration rate relative to the control fruit (data not shown); however, comparative analysis (t-test) of regression slopes showed that dip time had no significant influence on respiration rate.

3.4 Experiment 4: liquid 1-MCP concentration x dip time interaction

Across all harvests and locations, higher 1-MCP concentrations led to greater reductions in the rate of softening; and when fruit were treated with the same concentration, longer dipping times further reduced the rate of softening (Fig. 5). Treatment efficacy was a function of concentration and time. For example, dipping pears from the Sacramento orchard in $250 \mu\text{g L}^{-1}$ 1-MCP for 60 s was statistically as effective as dipping pears in $500 \mu\text{g L}^{-1}$ for 15 and 30 s ($P < 0.05$; Table 1). The same effect was observed with higher concentrations of 1-MCP. Dipping pears in $500 \mu\text{g L}^{-1}$ 1-MCP for 60 s was as effective at slowing softening as dipping fruit in $1000 \mu\text{g L}^{-1}$ for 30 s in all harvests and locations and dipping for 60 s in early-and late-harvested Sacramento and Lakeport pears (Table 1). In late-harvested Lakeport fruit, there was no significant difference in firmness after 18 d of ripening between fruit dipped for 15, 30 or 60 s in $1000 \mu\text{g L}^{-1}$ and for 30 or 60 s in $500 \mu\text{g L}^{-1}$, as determined by comparing the regression slopes (data not shown) and

368 ANOVAs (Table 1). Overall, fruit firmness after 18 d of ripening was higher in Sacramento fruit
369 (ranging from 8.4 to 38.0 N) than in Lakeport fruit (from 8.0 to 30.7 N).

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386 Fig. 5. Experiment 4. Firmness (N) of ‘Bartlett’ pears during ripening at 20 °C following treatment with various concentrations of liquid 1-
 387 methylcyclopropene for various dip times (year 2013). Vertical lines represent the standard deviation of the means ($\pm$ S.D., $n = 18$). A-B-C
 388 corresponds to fruit from Sacramento early, mid and late season, respectively, and D-E-F to early, mid and late harvests from Lakeport,
 389 respectively). Legend indicates aqueous 1-MCP concentration ($\mu\text{g L}^{-1}$) – immersion duration (s).

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Table 1. Experiment 4. Firmness of ‘Bartlett’ pears after treatment with liquid 1-methylcyclopropene followed by 18 d of ripening at 20 °C (fully ripe <13 N).

1-MCP ($\mu\text{g L}^{-1}$)	Immersion (s)	Firmness (N)					
		Sacramento			Lakeport		
		Early	Mid	Late	Early	Mid	Late
0	60	<13 e	<13 de	<13 d	<13 d	<13 e	<13 c
250	15	<13 e	<13 de	<13 d	<13 d	<13 e	<13 c
	30	<13 e	<13 de	<13 d	<13 d	<13 e	14.8 bc
	60	13.4 de	14.7 cd	13.5 cd	<13 d	16.7 bcd	14.4 bc
500	15	<13 e	13.6 cde	<13 d	<13 d	<13 e	16.9 b
	30	20.2 cd	17.8 c	16.7 c	14.5 bc	15.2 cde	29.7 a
	60	30.5 ab	23.7 b	27.9 ab	18.9 ab	19.3 bc	26.7 a
1000	15	24.3 bc	18.6 bc	27.7 ab	15.5 b	16.7 bcd	29.3 a
	30	33.1 a	24.5 b	25.3 b	17.4 b	21.8 ab	30.1 a
	60	38.0 a	34.1 a	33.8 a	23.7 a	25.7 a	30.7 a

Values within columns followed by the same letter are not significantly different at $P = 0.05$ (ANOVA-Tukey test).

Pear skin color changes from green to yellow were delayed in a dependent manner with 1-MCP treatment concentration, but dipping times had a much smaller effect on color in most treatments and harvests (Table 2). After 18 d of ripening, all Sacramento treated fruit showed high correlations between color and firmness (coefficients 0.48 $P < 0.0001$, 0.71 $P < 0.0001$ and 0.64 $P < 0.0001$ for early, mid- and late-harvest, respectively). When correlations were analyzed separately by dipping time and concentration, concentration had higher correlation coefficients (average 0.67 $P < 0.0001$) than dipping times (average 0.27 $P < 0.01$). Fully-ripe Sacramento fruit previously treated with 250 $\mu\text{g L}^{-1}$ for 15 and 30 s and 500 $\mu\text{g L}^{-1}$ for 15 s were more yellow than the fully-ripe control fruit in all harvests ($P < 0.05$). After 18 d of ripening, fruit treated with 250 $\mu\text{g L}^{-1}$ for 60 s and 500 $\mu\text{g L}^{-1}$ for 30 s (both not fully softened) were also more yellow than the fully-ripe control fruit ($P < 0.05$). Likewise for mid-harvested Lakeport fruit, all concentrations and dip times resulted in yellower fruit than the control fruit after 18 d of

ripening. However, almost all late-harvested Lakeport fruit presented the same color as the control ($P < 0.05$; Table 2).

Table 2. Experiment 4. Skin color (hue angle (h°)), of ‘Bartlett’ pears when fully-ripe or after 18 d ripening, whichever occurred first, following treatment with liquid 1-methylcyclopropene.

1-MCP ($\mu\text{g L}^{-1}$)	Immersion (s)	Hue angle (h°)					
		Sacramento			Lakeport		
		Early	Mid	Late	Early	Mid	Late
0	60	99.5 ab	98.1 b	96.6 ab	96.5 a	96.8 a	94.5 a
250	15	92.5 e	94.2 def	91.7 d	94.4 bcd	93.5 cd	91.9 ab
	30	91.8 e	92.7 ef	92.3 d	93.0 de	-	92.0 ab
	60	93.5 de	92.5 f	92.4 d	93.1 de	92.5 d	90.0 b
	15	91.3 e	92.9 ef	91.4 d	92.5 e	92.7 cd	92.2 ab
500	30	96.0 cd	94.3 def	93.2 cd	93.4 cde	92.7 cd	93.9 a
	60	97.8 bc	96.1 bcd	94.9 bc	93.9 bcde	93.2 cd	93.2 ab
	15	95.5 cd	94.9 cde	95.2 b	94.2 bcd	92.5 d	94.0 a
1000	30	99.2 ab	97.1 bc	96.0 ab	95.4 ab	93.8 c	93.3 ab
	60	100.7 a	100.5 a	97.4 a	94.8 abc	95.1 b	93.7 a

Values within columns followed by the same letter are not significantly different at $P = 0.05$ (ANOVA-Tukey test). Hue angle of 120° corresponds to green skin; the closer to 60° , the more yellow the fruit.

When pear fruit were dipped for shorter times using the same 1-MCP concentration, ethylene production started to increase earlier during ripening in both Sacramento and Lakeport fruit (Fig.6, only Sacramento results shown). Fruit dipped in $250 \mu\text{g L}^{-1}$ 1-MCP for 15 s showed a sharp increase in ethylene production 3 d earlier than fruit dipped in the same concentration for 30 s, and 6 d earlier than fruit dipped for 60 s. Fruit from the early-harvest in Sacramento dipped in $500 \mu\text{g L}^{-1}$ 1-MCP for 15 s showed a sharp increase in ethylene production sooner and produced higher levels of ethylene ($1.07 \pm 0.23 \text{ uL kg}^{-1} \text{ s}^{-1}$) than fruit dipped in $250 \mu\text{g L}^{-1}$ 1-MCP for 60 s ($0.84 \pm 0.19 \text{ uL/kg h}$). Similarly, mid-harvested Sacramento and early-and late-harvested Lakeport (data not shown) fruit dipped in $1000 \mu\text{g L}^{-1}$ 1-MCP for 15 s showed higher ethylene production than fruit treated with $500 \mu\text{g L}^{-1}$ 1-MCP for 30 s. The differences in

ethylene production rates and number of days to the sharp increase in ethylene production as related to dip times were smaller when fruit were treated with 500 or 1000 $\mu\text{g L}^{-1}$ 1-MCP than for the 250 $\mu\text{g L}^{-1}$ 1-MCP treatment. Within a concentration, once ethylene production started increasing, the rate of ethylene production was related to dip times; shorter dip time, higher ethylene production rate.

443 Fig. 6. Experiment 4. Ethylene production ($\mu\text{L kg}^{-1} \text{s}^{-1}$) and respiration rate ($\text{mL kg}^{-1} \text{s}^{-1}$) of ‘Bartlett’ pears during ripening at 20 °C following
 444 treatment with various concentrations of liquid 1-methylcyclopropene for various dip times (year 2013). The vertical lines represent the standard
 445 deviation of the means ($\pm$ S.D., $n = 18$). A-B-C corresponds to early, mid and late harvests from Sacramento, respectively. Legend indicates
 446 aqueous 1-MCP concentration ($\mu\text{g L}^{-1}$) – dipping time (s).

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The increase in respiration rate of the 1-MCP treated fruit from all treatments was evidently delayed and in most cases the respiration rate was lower than the control fruit (Fig. 6 D-F, Sacramento results). In early- and mid-harvested Sacramento and Lakeport fruit, treatment with 250 $\mu\text{g L}^{-1}$ 1-MCP for 15 and 30 s resulted in significantly different respiration rates as compared with the other treatments, with earlier increases in respiration rate. Overall, the respiration rates of fruit treated at different concentrations and dip times were more divergent in early- and mid-season fruit. In late-harvested fruit, respiration rates were lower (showing very low rates in Lakeport; data not shown) and very similar between treatments.

3.5. Interactions: concentration $\times$ dip time $\times$ harvest $\times$ year (data not shown)

Across the three years of study, pear fruit from Sacramento and Lakeport were treated with 1000 $\mu\text{g L}^{-1}$ liquid 1-MCP for 60 s allowing a comparison across seasons. ANOVAs of 2011, 2012 and 2013 outcomes showed that for this concentration of 1-MCP, there were no differences in early-, mid- and late-harvested fruit firmness after 18 days of ripening among the three years ($P < 0.001$). Other 1-MCP concentrations and dip times showed significant differences between years. Early-harvest fruit treated with 250 $\mu\text{g L}^{-1}$ liquid 1-MCP for 60 s in 2012 showed differences in firmness after 18 d of ripening from fruit similarly treated in 2013 ($P < 0.001$), but mid- and late-harvested fruit firmness was not different between the two years where this concentration and dip time was applied ($P < 0.001$). Dipping pears for 15 s in 250 $\mu\text{g L}^{-1}$ liquid 1-MCP gave consistent firmness after 18 d of ripening in 2012 and 2013 ($P < 0.001$).

4. Discussion

Our results showed that applying liquid formulations of 1-MCP in ‘Bartlett’ pears delayed softening and reduced ethylene production in fruit from different harvest dates. In general, the liquid formulation at 1000 $\mu\text{g L}^{-1}$ for 60 s was slightly more efficacious than exposure to 0.6 $\mu\text{L L}^{-1}$ MCP gas for 24 h. According to Macnish et al. (2012), late-harvested ‘Bartlett’ pear fruit usually produce higher levels of ethylene at harvest, and this could be the reason for the difficulties found by researchers and producers to consistently delay ripening of pear fruit from late harvests with gaseous 1-MCP, since ethylene could interfere with 1-MCP binding to the ethylene receptors (Calvo and Sozzi, 2009; Ekman et al., 2004; Macnish et al., 2012; Trincherro et al., 2004; Villalobos-Acuña et al., 2011a, 2011b). Our results showed that liquid 1-MCP was very effective as an ethylene action inhibitor in all harvests, including late-harvested ‘Bartlett’ pears (which averaged 50x more ethylene production than mid-harvested pears) across the three years of our study. Our results showed an equal or superior efficacy of liquid 1-MCP treatments in more mature ‘Bartlett’ pear fruit compared to less mature fruit, in contrast to the reduced response in more mature fruit that has been reported previously for gaseous 1-MCP treatments (Chiriboga et al., 2013; Gamrasni et al., 2010; Jung and Watkins, 2014; Macnish et al., 2012; Mir et al., 2001; Villalobos-Acuña et al., 2011a, b; Wang and Sugar, 2015; Watkins, 2008).

The inhibitory effects of gas and liquid 1-MCP were only slightly reduced when fruit were stored for 5 weeks at 1°C, indicating a lasting effect of both treatments at low temperatures.

Comparable results between gaseous and liquid 1-MCP applications have also been observed in tomato and avocado (Choi et al., 2008; Choi and Huber, 2008), and liquid formulations have also proved to be effective in breaker and turning tomatoes (Choi and Huber, 2008) and plums

(Manganaris et al., 2008). To the best of our knowledge, this is the first report of postharvest application of liquid 1-MCP and comparison between gaseous and liquid 1-MCP formulations in ‘Bartlett’ pear.

The liquid 1-MCP formulation was effective in a concentration- and dip time-dependent manner. Ethylene and respiration followed the same trends as softening; higher concentrations of 1-MCP delayed the increase in and rates of ethylene production and respiration significantly more than lower concentrations, and these results match previous research with liquid 1-MCP performed on tomatoes and avocados (Choi et al., 2008) and plums (Manganaris et al., 2008). Choi and Huber (2008) also found that liquid 1-MCP was effective on tomato after immersion times as brief as 15 s, in agreement with our data.

Concentrations of 250, 500 or 1000 $\mu\text{g L}^{-1}$ 1-MCP could be used to treat ‘Bartlett’ pears after harvest depending on the intended shelf life. Fruit treated for 15-30 s with 250 $\mu\text{g L}^{-1}$ liquid 1-MCP were able to ripen in less than 18 d in all years, harvests and locations, extending shelf life for a minimum of 5 d beyond the control fruit. Treatment with 500 $\mu\text{g L}^{-1}$ liquid 1-MCP extended shelf-life for a minimum of 10 d (15 s dip time) beyond the control fruit across years and locations. Treatment with 1000 $\mu\text{g L}^{-1}$ liquid 1-MCP gave the longest shelf life, independent of the dip time (treated fruit did not ripen before 18 d). Ripening of these fruit was very slow, but not irreversibly impaired, in contrast to the strong and irreversible effects sometimes reported after gaseous 1-MCP treatments in ‘Bartlett’ pear (Ekman et al., 2004; Villalobos-Acuña and Mitcham, 2008) and in ‘d’Anjou’ pear (Chen and Spotts, 2006) .

Our results indicate that low liquid 1-MCP concentrations with longer dip times were more effective to suppress softening, respiration rate and ethylene production than higher

concentrations with shorter dip times. These results confirm what Choi and Huber (2008) suggested about the mechanism by which the liquid formulation delivers biologically active levels of 1-MCP to the receptors. They hypothesized that saturating concentrations of 1-MCP would provide molecules in excess of those necessary to saturate all ethylene receptors while low concentrations applied for longer times would maximize binding to ethylene receptors, and hence the treatment efficacy.

Most of the fruit from both locations and all harvests dipped in 500 $\mu\text{g L}^{-1}$ liquid 1-MCP for 15 or 30 s ripened before or around 18 d. For practical use with ‘Bartlett’ pears, application of 500 $\mu\text{g L}^{-1}$ liquid 1-MCP with short controlled dip durations could be the best approach to delay fruit ripening in a controlled manner without overly inhibiting the ripening process. The dipping duration would be the key to determine the rate of fruit ripening; a difference of ± 15 s would delay/advance ripening by 3 d in early harvested fruit and 2 d in mid and late harvested fruit. The optimum protocol would depend on commercial facilities; shorter dip times could be advantageous in facilities with a high volume of production while longer dip times would reduce the effect of variance in dip times under commercial practice.

The response of ‘Bartlett’ pear fruit to gaseous 1-MCP-treatment has been reported to be highly variable from harvest to harvest and year to year (unpublished data; AgroFresh, Inc., personal communication). Our data compares for the first time postharvest application of liquid 1-MCP in consecutive years. Fruit treated with liquid 1-MCP (1000 $\mu\text{g L}^{-1}$) gave a consistent response year to year, but remained dependent on harvest maturity and growing location. For example, all early-harvested fruit from the same growing location that were treated with liquid 1-MCP softened similarly in 2011, 2012 and 2013. Similar results were observed for mid- and late-harvested fruit. However, this consistency depended on the concentration applied; lower

concentrations (250-500 $\mu\text{g L}^{-1}$) resulted in more variable effects on the rate of softening between years for early-harvested fruit, while fruit from mid- and late-harvest showed similar rates of softening among the years. This may be related to the fact that in some years, early harvested fruit produced measureable ethylene and in other years they did not, while mid- and late-harvested fruit always produce some ethylene at harvest. Differences in the fruit stage of maturity at harvest could have a more significant impact on 1-MCP treatment efficacy at lower concentrations. These results also suggest that the maturity of the fruit does have an effect independent of the accumulation of ethylene in the headspace competing with 1-MCP, which could indicate the importance of monitoring ethylene concentrations in treatment rooms before applying 1-MCP, as suggested by Macnish (2012).

In our studies, treated fruit skin yellowing was significantly linked to fruit softening; high treatment concentrations resulted in firmer and less yellow fruit than those treated with low concentrations after the same number of days of ripening. Similar results were observed by Choi and Huber (2008) with tomato and Manganaris et al., (2008) with avocado. Villalobos-Acuña et al. (2011a, 2011b) reported that application of 0.3 $\mu\text{L L}^{-1}$ gaseous 1-MCP also delayed yellow skin color development along with firmness during ripening, and when fruit were fully-ripe, treated and untreated fruit were the same color. However, in our study, fully-ripe fruit pears that had been treated with liquid 1-MCP were more yellow than fully-ripe control fruit in all locations, harvests and years. Moreover, most of the fruit treated with 500-1000 $\mu\text{g L}^{-1}$ liquid 1-MCP after 18 d of ripening (not fully-ripe yet), were also visibly more yellow than fully-ripe control fruit. Our results suggest that liquid 1-MCP treatment enhances color development in ‘Bartlett’ pear fruit. This effect could be a misleading indicator of ripeness for the pear industry and consumers. This differential ripening effect has been shown before in 1-MCP treated

560 bananas (Golding et al., 1998). It presumably reflects different sensitivities between ethylene-
561 dependent skin coloration and flesh softening. Further study is required to unravel the
562 biochemical and molecular factors that link the ethylene signal transduction pathway in liquid 1-
563 MCP-treated fruit and the chlorophyll degradation pathway.

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5. Conclusion

Application of 1-MCP as a liquid could be a useful alternative to gaseous application of this ethylene action inhibitor to 'Bartlett' pear fruit after harvest. Treatment with liquid 1-MCP across a range of concentrations and dip times delayed pear ripening in all harvests, including late-harvested pears, across the three years of our study. Dipping in 1000 $\mu\text{g L}^{-1}$ liquid 1-MCP for 60 s was the most consistent treatment among years and locations; however, the time required to ripen the fruit at 20 °C could be too long for some commercial applications. Treatment at 500 $\mu\text{g L}^{-1}$ liquid 1-MCP is recommended for 'Bartlett' pears as this dose slows the ripening process, and provides a consistent response among years and locations for mid- and late-harvested fruit. Application of liquid 1-MCP is fast and easy and does not require a sealed room. However, challenges could arise in preventing off-gassing and loss of active ingredient from the solution. Careful control of dip time will also be important for some applications. In addition, to achieve storage benefits, fruit would need to be dipped in bins or run over a packing line and re-binned before storage. For the commercial benefits to be realized, further research and treatment refinement will be necessary to overcome these challenges.

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