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Preharvest Application of 1-Methylcyclopropene Influences Fruit Drop and Storage Potential of 'Bartlett' Pears

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Abstract. **Preharvest applications of 1-methylcyclopropene (1-MCP) were tested on California 'Bartlett' pears at 80 N maturity and at rates of 0, 28, and 56 mg·L^{-1} in 2006 and 0, 50, and 100 mg·L^{-1} in 2007. In 2007, a parallel experiment was conducted to compare 50 mg·L^{-1} 1-MCP with 96 g a.i./ha 1-naphthaleneacetic acid (NAA) used commercially to control or decrease premature fruit drop. Premature fruit drop, maturity, firmness at harvest, color, softening, and ethylene production during ripening and physiological disorders were studied in fruit harvested between 7 and 21 days after 1-MCP application and either ripened at 20 °C immediately after harvest or after 3.5 to 6 months storage at –1 °C. Overall, 50 mg·L^{-1} 1-MCP reduced the incidence of premature fruit drop when compared with the untreated fruit and fruit drop was similar to adjuvant-treated fruit and NAA-treated fruit, especially 28 days or longer after the treatment. 1-MCP was more effective in retarding color, softening, and ethylene production during ripening than delaying fruit maturation on the tree (loss of firmness), and both rates of 1-MCP tested each season yielded similar fruit responses on most evaluation times. 1-MCP's effect on ripening was lost if fruit remained on the tree 21 days or after the fruit were stored for 3.5 months in cold storage regardless of treatment concentration. A reduction of internal breakdown incidence was observed in 1-MCP-treated fruit.**

'Bartlett' pear is a climacteric fruit that is harvested at 80 to 89 N when the fruit is still green and firm but physiologically mature. After harvest, pears are conditioned with ethylene and marketed immediately or stored at low temperatures until ready to be marketed (Villalobos-Acuña and Mitcham, 2008). 1-Methylcyclopropene (1-MCP) is an ethylene action inhibitor that has been extensively studied in fruit and commercialized for some fruits as a postharvest gaseous application to control ripening (Sisler and Blankenship, 1996; Sisler and Serek, 2003). A liquid formulation (AFxRD-038-Agrofresh) is now being tested for preharvest use to control physiological processes influenced by ethylene in horticultural crops. Potential benefits of this application such as delaying maturation, expanding the harvest window, and slowing postharvest ripening rates have been shown in apple and tomato fruit (Byers et al., 2005; Choi and Huber, 2008; Choi et al., 2008; Elfving et al., 2007; McArtney et al., 2008; Yuan and Carbaugh, 2007).

In addition to ethylene, auxins play an important role in fruit abscission (Wertheim, 1973, 2000). The application of exogenous auxins delays the onset of fruit abscission or premature fruit drop in pome fruits, and ethylene promotes abscission. 1-Naphthaleneacetic acid (NAA), a synthetic auxin, is used commercially in California 'Bartlett' pears to reduce premature fruit drop, but it can induce ethylene production and fruit softening on the tree (Clayton et al., 2000). If 1-MCP is able to prevent or delay premature fruit drop and fruit softening, it could become a useful tool for growers to extend the harvest window, a benefit that becomes more valuable as labor availability for harvest is reduced.

Furthermore, preharvest treatments with 1-MCP might also produce a beneficial impact on postharvest fruit quality, delaying ripening for long-distance transport, and slowing the appearance of physiological disorders in storage. California 'Bartlett' pears can be stored for 2 to 3 months in air under ideal conditions at –1 to 0 °C. The postharvest life of 'Bartlett' pears generally is limited by the appearance of scald (storage or senescent scald) and internal breakdown, physiological disorders characterized by peel and flesh browning, respectively (Mitcham et al., 2009). Ethylene produced by the fruit during storage can exacerbate the incidence of these physiological disorders (Bower et al., 2003; Du and Bramlage, 1994; Ekman et al., 2004; Gapper et al., 2006; Ju and Curry, 2000; Watkins et al., 1995; Whitaker and Solomos, 1997).

The main objective of this study was to evaluate the effect of preharvest treatments with 1-MCP on premature fruit drop, maturity changes before harvest and fruit ripening, and on the incidence of physiological disorders after harvest and storage of 'Bartlett' pears. The relationships among 1-MCP concentration, application timing, and length of cold storage were also characterized.

Materials and Methods

2006 harvest season

Pear trees in a commercial orchard in Ukiah, CA, were sprayed with 0 (adjuvant only), 28, or 56 mg a.i./L of 1-MCP (AFxRD-038; AgroFresh Inc., Springhouse, PA) with 1% v/v adjuvant (Ultra-Fine Oil; Whitmire Micro-Gen Research Laboratories, Inc., St. Louis, MO) and 0.1% v/v Silwet L-77 (Helena Chemical Company, Collierville, TN). The application volume of 1800 L·ha^{-1} or 7.9 L per tree was applied until runoff with a low-pressure (≈0.31 MPa, Nozzle OC-12) sprayer. The sprayer was provided by AgroFresh Inc. and was pressurized using a high-pressure CO_2 cylinder attached to an 11.4-L tank containing the 1-MCP and/or adjuvant mix. Unsprayed trees were also selected as controls. Treatments were applied in the early morning between 0600 and 0930 HR (11 to 22 °C temperature, 60% to 94% relative humidity) using a large droplet size to encourage slow drying, and care was taken to reduce agitation of the solution before spraying. The tank was mixed using the following procedure: 1) the spray tank was filled with approximately two-thirds of the total volume of water required; 2) Ultra-Fine Oil and Silwet L-77 were added to the spray tank and swirled for 4 to 5 s; 3) AFxRD-038

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powder was added to the spray tank with swirling; 4) the remaining water was added; 5) spray solution was swirled for 2 min; and 6) spraying of the plots was performed no sooner than 5 min after completing Step 5 and no later than 15 min after tank mixing.

The experimental design was a randomized complete block with four blocks, each block containing three trees per treatment. Treatments were applied on 9 Aug. 2006 when the pears averaged ≈80 N firmness and the fruit were harvested 1, 2, or 3 weeks after the application (16, 23, or 30 Aug. 2006). Fruit were sorted to remove damaged or blemished fruit. A subset of fruit was treated immediately after harvest with ≈100 $\mu L \cdot L^{-1}$ ethylene for 24 h at 20 °C to stimulate ripening and then transferred to 20 °C for ripening. The remaining fruit were placed into pear fiberboard boxes with vented plastic liners and stored for 3.5 months or 6 months at –1 °C in air (85% to 95% relative humidity). Fruit condition was evaluated on removal from storage and again after ripening at 20 °C.

Phytotoxicity evaluation. Phytotoxicity, any necrotic damage visually detected on leaves or fruit, was evaluated at harvest. The leaves on each tree were evaluated and eight fruit per treatment per block were closely evaluated after harvest. Both leaf and fruit phytotoxicity were rated using the following scale: 0 = none; 1 = slight; 2 = moderate; 3 = severe.

Fruit evaluation. Ripening time varied depending on the storage time, with 8 d at harvest and 5 d after 3.5 and 6 months in regular air storage. All fruit were evaluated when the untreated fruit were fully ripe. Thirty-two fruit (eight per block) for each treatment were assessed for weight, color, and firmness at each evaluation time. In addition, 24 fruits (six per block) were used to determine ethylene and CO_2 production (respiration rate) for each treatment every 2 d during the ripening period at 20 °C.

Individual fruit weights were measured using an electronic balance (Sartorius, AG Gottingen, Germany). Firmness was measured objectively using a Gűss FTA Penetrometer (Gűss, Strand, Western Cape, South Africa) fitted with an 8-mm probe. CO_2 and ethylene production rates at 20 °C were measured by placing six fruit from each treatment and block into a 3.8-L jar and sealing it for 10 to 60 min. The headspace gas was analyzed for CO_2 and ethylene concentrations using rapid gas analysis (VIA510; Horiba, Fukuoka, Japan) and flame ionization gas chromatography (Model 211 Series S; Hach-Carle Co., Fullerton, CA) using two columns (1.22 m and 0.305 m, 8% NaCl on Alumina F-1 80/100 DV; Chandler Engineering–Carle Chromatography, Tulsa, OK). Nitrogen was used as the carrier gas at a flow rate 30 $mL \cdot min^{-1}$, and the injector port, detector port, and oven temperature was 80 °C. A 10-mL headspace sample was injected into a 2-mL fixed sample volume valve. Color was measured subjectively using the California Department of Food and Agriculture Color Chart (1 = green; 2 = light green; 3 = light yellow; 4 = yellow; CDFA, Sacramento, CA). Internal browning and scald (including storage scald and senescent scald) severity were evaluated subjectively using the following scale: 0 = none; 1 = slight; 2 = moderate; 3 = severe.

The experiment was analyzed using SAS statistical software (Version 9.1, SAS Institute Inc., Cary, NC). Means were compared using contrast or least significant difference (LSD) to calculate the LSDs for each data set ($\alpha = 0.05$). Power, log, or arcsine transformations were used to fulfill analysis of variance assumptions of ethylene production, internal breakdown, and scald data.

2007 harvest season

Fruit drop experiment. An independent experiment was performed in 2007 to evaluate the effect of 1-MCP on fruit drop. This experiment was conducted in the same orchard as that used for the fruit quality experiment (see subsequently) but with separate trees. Four treatments were established: 1) control, no application of NAA or 1-MCP; 2) adjuvants only as for the quality experiment in 2007; 3) 1-MCP 50 mg/L + adjuvants; and 4) NAA (96 g a.i./ha in a volume of 2300 $L \cdot ha^{-1}$; Liqui-Stik Concentrate; Loveland Products, Greeley, CO).

NAA was applied commercially on 24 July 2007, whereas all the remaining treatments were applied on 27 July 2007 when fruit was at 91 N maturity. Fruit drop was evaluated weekly during 5 weeks by counting the number of fruit on the ground around each tree. The experimental design was a randomized complete block with four blocks total (one tree per block per treatment). Trees in this experiment were not sampled or harvested at any time after the application of the treatments to decrease fruit drop caused by human intervention.

Fruit quality experiment. The experiment in 2007 had similar treatments as in 2006 with the following modifications. Pear trees were located in a commercial orchard in Finley, CA, and treated with 0, 50, or 100 mg a.i./L of 1-MCP (AFxRD-038; AgroFresh) Adjuvant concentrations were like in 2006 except that HI Supreme Spray Oil (Independent Agribusiness Professionals, Fresno, CA) and foam buster (Doc Farwell's, silicone antifoam ≈3 drops; Farwell Products Inc., Wenatchee, WA) were used instead of Ultra Fine Oil. Application volume was 9.8 to 10.6 L per tree and application pressure was ≈0.33 to 0.35 MPa. Spray solution preparation was similar except that Step 5 was changed to provide 10 to 15 s of swirling and Step 6 changed to allow spraying of the plots up to 30 min after mixture preparation.

The experimental design for 2007 was a randomized complete block design with four blocks and one tree per block per treatment. Treatments were applied on 13 Aug. 2007 between 0600 to 1000 HR (6 to 19 °C, 53% to 96% relative humidity) when the pears averaged ≈80 N. Fruit were harvested 7 and 17 d after the application on 20 and 30 Aug. 2007, respectively. After each harvest, fruit were transported to the postharvest laboratory in Davis, CA, in a cargo van with air conditioning, stored overnight at room temperature, and sorted the next day. Fruit condition was evaluated before and after ripening at harvest and on removal from storage at –1 °C after 4.5 months (H1 = Harvest 1) or 4 months (H2 = Harvest 2). Ripening time at 20 °C varied depending on the storage time with 8 d for H1 and 6 d for H2 at the time of harvest and 4 d after 4.5 (H1) or 4 months (H2) of cold storage. All the fruit were evaluated when the untreated fruit were fully ripe. Means were compared using LSD to calculate the LSDs for each data set ($\alpha = 0.05$). In 2007, contrast was not used because this experiment had other treatments not presented in this report that did not follow orthogonality assumptions.

Results

Premature fruit drop. Fruit drop increased in all treatments, especially after 21 d of the application (Fig. 1). Twenty-one d after application, NAA was most effective in decreasing fruit drop. After 28 and 35 d following application, NAA was statistically as effective as 1-MCP in reducing fruit drop. Treatment with 1-MCP and adjuvant alone did not present statistically significant differences and both treatments with the exception of the adjuvant after 35 d of treatment reduced fruit drop when compared with the untreated control.

Effects on fruit firmness on the tree and during cold storage. In 2006, there were significant differences in fruit firmness among the treatments at H1 and 2, but 1-MCP treatments were as effective in delaying maturation (loss of firmness on the tree) as the adjuvant only treatment (Fig. 2). Immediately after 3.5 months of storage, fruit treated with 56 $mg \cdot L^{-1}$ 1-MCP were statistically firmer in comparison with the adjuvant

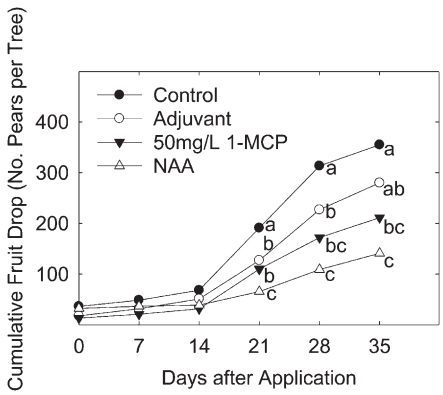

Fig. 1. Cumulative fruit drop in untreated, 1-methylcyclopropene (1-MCP) + adjuvant or 1-naphthaleneacetic acid (NAA)-treated (96 g a.i./ha) 'Bartlett' pears measured weekly after treatment application. Different letters within each evaluation time represent statistical differences using the least significant difference test ($P < 0.05$). Commercial harvest occurred 20 d after application.

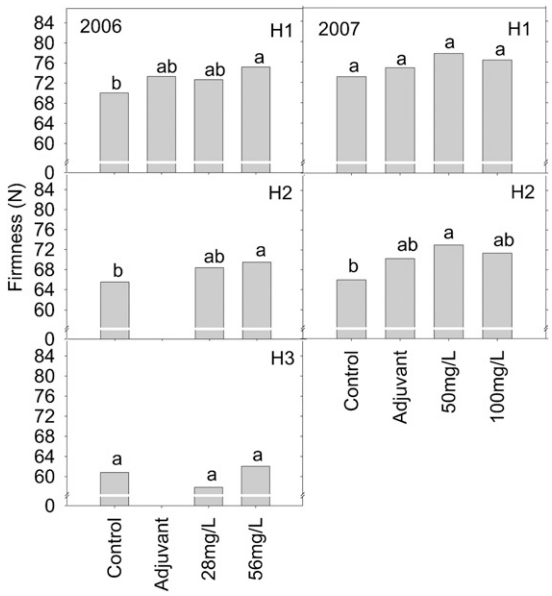

Fig. 2. Pear fruit firmness (N) at harvest. Different letters within each evaluation time represent statistical differences using the least significant difference ($P < 0.05$).

Table 1. Firmness (N) means for unripened pear fruit after 3.5 (2006), 4.5 (H1, 2007), and 4 months (H2, 2007) storage at 1 °C.

2006				2007		
Treatments	H1	H2	H3	Treatments	H1	H2
Control	64.1	53.0	55.6	Control	44.5	49.3
Adjuvant (Adj.)	64.0			Adj.	44.4	51.5
28 mg·L^{-1} 1-MCP	66.4	57.7	59.5	50 mg·L^{-1} 1-MCP	57.1	55.4
56 mg·L^{-1} 1-MCP	67.6	62.2	58.3	100 mg·L^{-1} 1-MCP	57.2	53.0
Contrast				LSD mean comparison		
Control versus 28 mg·L^{-1}	NS	NS	NS	Control versus 50 mg·L^{-1}	**	*
Control versus 56 mg·L^{-1}	*	**	NS	Control versus 100 mg·L^{-1}	**	NS
Control versus Adj.	NS	NA	NA	Control versus Adj.	NS	NS
28 mg·L^{-1} versus 56 mg·L^{-1}	NS	NS	NS	50 mg·L^{-1} L versus 100 mg·L^{-1}	NS	NS
Adj. versus 28 mg·L^{-1}	NS	NA	NA	Adj. versus 50 mg·L^{-1}	*	NS
Adj. versus 56 mg·L^{-1}	*	NA	NA	Adj. versus 100 mg·L^{-1}	*	NS

**$P < 0.01$; *$P < 0.05$; NS = nonsignificant.
H1 = Harvest 1; H2 = Harvest 2; H3 = Harvest 3; 1-MCP = 1-methylcyclopropene; NA = not analyzed; LSD = least significant difference.

and/or untreated control fruit for H1 and 2 but not for H3 (Table 1). There was no significant difference in firmness between fruit treated with 56 mg·L^{-1} and 28 mg·L^{-1} 1-MCP at any evaluation time (Fig. 2; Table 1). No phytotoxicity was observed on the leaves or the fruit for any of the treatments.

In 2007, 1-MCP was applied at a similar maturity stage. There were no significant differences in fruit firmness among the treatments at H1 (Fig. 2). At H2, 50 mg·L^{-1} had statistically higher firmness than the untreated fruit but was not statistically different from the adjuvant or 100 mg·L^{-1}-treated fruit. When fruit from these same treatments were compared immediately after 4.5 months storage (H1, 7-d interval between application and harvest), the 1-MCP-treated fruit (both 50 mg·L^{-1} and 100 mg·L^{-1}) were significantly different and had 12 to 13 N higher firmness than untreated control or adjuvant-treated fruit (Table 1). For H2 (17 d between application and harvest), only fruit treated with 50 mg·L^{-1} 1-MCP had statistically higher firmness after 4 months of cold storage (Table 1). No phytotoxicity was observed on the leaves or the fruit in any of the treatments.

In 2006, none of the treatments had a significant effect on fruit weight (data not shown). In 2007, fruit weight was studied to determine if the fruit would be significantly larger after the 10-d delay between H1 and H2 (Table 2). Fruit from all the treatments with the exception of the untreated fruit did not have significantly higher mean weight at H2 compared with H1 as measured with fruit samples immediately after harvest (Table 2) and after storage at –1 °C (data not shown). There were some significant differences between treatments for both evaluations, but these differences were also present before harvest (data not shown).

Effect on fruit ripening. Ripening behavior was studied immediately after harvest and after storage at –1 °C for 3.5 months (2006) or 4 to 4.5 months (2007). Preharvest treatment with 1-MCP significantly increased fruit firmness during ripening after harvest in both years except for H3 in 2006 (Fig. 3). Ethylene and skin color changes from green to yellow were also reduced during ripening after harvest in both seasons (Fig. 4; Table 3) with the exception of skin color changes at H2 in 2007 in which only the 100 mg·L^{-1}-treated fruit but not 50 mg·L^{-1} had statistically significant differences. After cold storage, there was an effect on fruit softening during ripening in H2 fruit in 2006 (Fig. 3). In 2007, the control fruit remained slightly firmer than some of the 1-MCP-treated fruit, which likely resulted from a higher incidence of internal breakdown in the untreated fruit, especially in fruit from H1 (Figs. 3 and 5). After cold storage, ethylene production was generally similar or slightly reduced in 1-MCP-treated fruit relative to the control fruit (Fig. 4). Skin color change from green to yellow was very slightly delayed after cold storage in 1-MCP-treated fruit, especially for fruit from H1 and H2 in 2006 (Table 3).

Table 2. Fruit weight (g) at harvest in 2007.[z]

	Harvest 1	Harvest 2
Control	180 bc	228 a
Adjuvant	157 cd	184 bc
50 mg·L^{-1} 1-MCP	139 d	159 cd
100 mg·L^{-1} 1-MCP	179 bc	180 bc

[z]Distinct letters represent means with statistical differences between harvest times using least significant difference ($P < 0.05$).

In 2006, there were interactions between treatment × harvest for the variables firmness and color ($P < 0.0001$ in both cases) and treatment × storage for the variables firmness ($P = 0.0103$) and color ($P = 0.0227$), indicating that the effect of the treatments changed at the different harvest and storage periods. In 2007, the same significant interactions were found using the variables firmness and color ($P < 0.0001$ in all cases). The interaction between treatment × harvest is illustrated in Figure 3, which presents firmness after ripening at harvest and after storage. For fruit ripened at harvest, the shorter the time the fruit remained on the tree after 1-MCP application, the more effect 1-MCP had in slowing softening during ripening. However, in contrast to the softening behavior during ripening after harvest, 1-MCP-treated fruit softened similarly to untreated and adjuvant-treated fruit when ripened after 3.5 months storage (2006) or 4.5 to 4 months storage (2007), which explains the statistically significant treatment by storage interaction.

In 2006, there were no differences between fruit treated with 28 and 56 mg·L^{-1} of 1-MCP in softening behavior (except for H3 in 2006) (Fig. 3) or ethylene production (Fig. 4) during ripening. However, in 2006, skin color changes from green to yellow were delayed to a greater extent in fruit treated with 56 mg·L^{-1} 1-MCP compared with those treated with 28 mg·L^{-1} in pears from H1 ripened at harvest or after 3.5 months cold storage or pears from H2 after 3.5 months storage (Table 3). No significant differences in firmness or ethylene production were observed at any evaluation time among fruit treated with 50 and 100 mg·L^{-1} of 1-MCP in 2007 (Figs. 3 and 4) and there were only slight differences in skin color (Table 3).

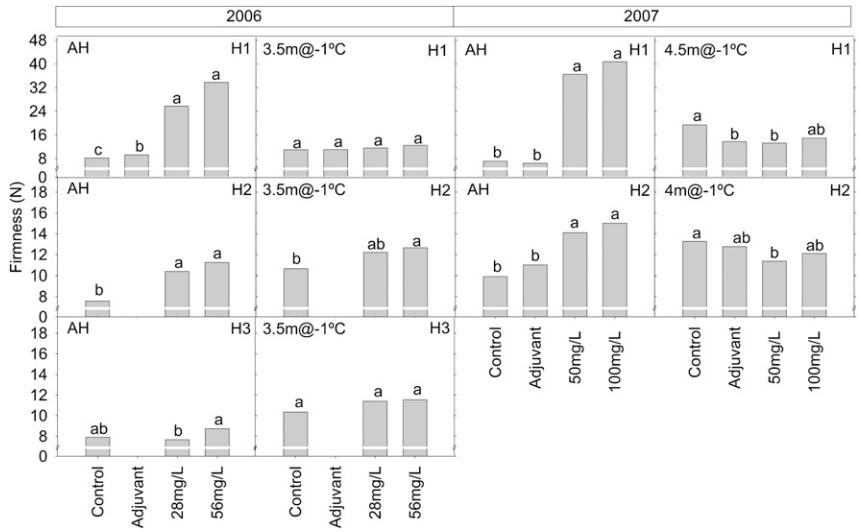

Fig. 3. Pear fruit firmness (N) after ripening at harvest and after cold storage. Fruit from all harvests (H1, H2, H3) in 2006 were ripened for 8 and 5 d at 20 °C at harvest and after 3.5 months cold storage, respectively. In 2007, fruit were ripened between 8 (H1) and 6 d (H2) at 20 °C at harvest and 4 d at 20 °C after 4.5 (H1) or 4 months (H2) cold storage. Different letters within each evaluation time represent statistical differences using least significant difference (P value < 0.05). Note differences in the scale between H1 and later harvests. AH = at harvest.

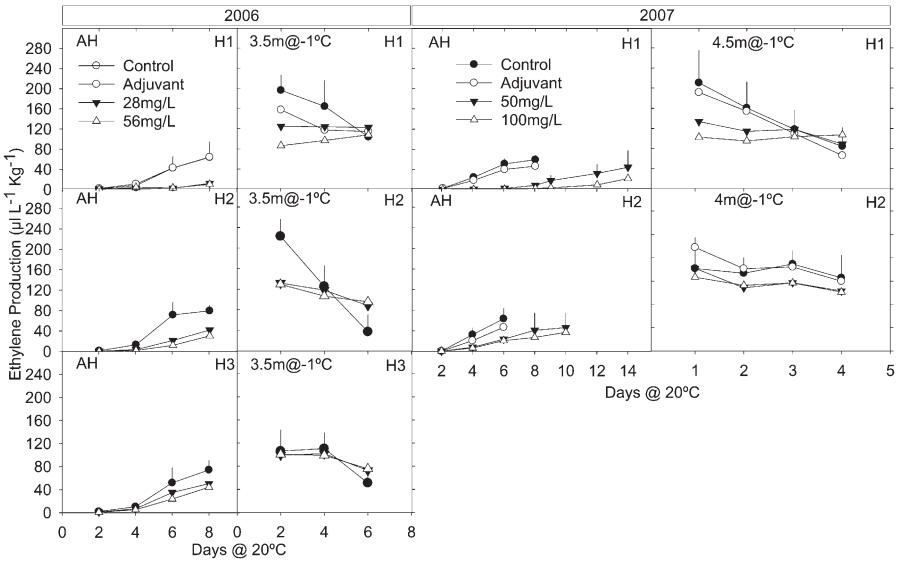

Fig. 4. Ethylene production during ripening at harvest and after cold storage. Vertical line represents minimum significant difference among treatment means using least significant difference (P < 0.05). AH = at harvest.

These data together suggest that the effect of 1-MCP on postharvest pear ripening was highly dependent on the interval between application and harvest with the greatest effect observed at the shortest interval tested in these experiments (7 d). Additionally, after cold storage of 3.5 m or longer, the effect of 1-MCP on fruit ripening was reduced compared with that obtained immediately after harvest.

Poststorage quality. Internal breakdown was observed after storage at most of the evaluation times in 2006 and 2007 (Fig. 5). In 2006, after 3.5 months for H2 and 6 months for H1 and H2, 1-MCP treatment significantly reduced internal breakdown (IB) incidence and in some cases prevented it. In 2007, untreated fruit from H2 had much less IB incidence than fruit from H1 (P = 0.0006). This difference could be the result of the somewhat shorter storage time for fruit from H2 (4 m) compared with fruit from H1 (4.5 m). Pears from H1 treated with 100 mg·L^{-1} 1-MCP in 2007 showed significant differences in IB incidence compared with the control but not to the adjuvant only fruit. For H2, both 1-MCP treatments presented statistical differences with the adjuvant only fruit but not with the untreated fruit.

In 2006, 1-MCP did not significantly reduce scald incidence at any evaluation time (Fig. 6), except for H2 in which only the 28 mg·L^{-1} concentration was statistically significant when compared with the untreated fruit, but 56 mg·L^{-1} was not. In 2007, 1-MCP treatment decreased scald incidence in comparison with the untreated fruit when fruit were harvested 7 d after treatment (H1), but not in fruit harvested 17 d after treatment. However, for H1, the adjuvant-treated fruit also exhibited a similar reduction in scald incidence as the 1-MCP-treated fruit (Fig. 6).

Discussion

1-MCP (50 mg·L^{-1}) reduced premature fruit drop when compared with the untreated but not with the adjuvant-treated fruit. Our data show that NAA alone was statistically as effective as 1-MCP in reducing fruit drop, beginning 28 d after treatment application and later, presumably because both auxins and ethylene change the transcription and activity of important enzymes responsible for cell wall degradation in the abscission zone (Del Campillo and Bennett, 1996; Kalaitzis et al., 1995; Li and Yuan; 2008). The significance of an interaction between ethylene and auxins on fruit abscission has been shown by other studies in which NAA plus 1-MCP or aminoethoxyvinylglycine have been shown to have a synergistic effect in inhibiting fruit abscission compared with each treatment alone (Byers et al., 2005; Li and Yuan; 2008; Yuan and Carbaugh, 2007). Furthermore, we found in this study that the adjuvant control treatment also reduced fruit drop similarly to 1-MCP, but to a lesser extent than did NAA. It is likely that the adjuvants modified the internal atmosphere of the pears by partially blocking gas exchange, thereby reducing ethylene production and action (Kader, 1995).

1-MCP + adjuvant significantly delayed fruit maturation on the tree, especially for H1 and H2 in 2006 and H2 in 2007 when compared with the untreated fruit, but there were no differences detected when 1-MCP-treated fruit were compared with the adjuvant-treated fruit. This suggests that the adjuvant alone likely played an important role in the firmness delay observed in this study. We hypothesize two potential scenarios to explain the mild effect of 1-MCP on fruit softening when pears are attached to the tree. First, endogenous ethylene production while pears are attached to the tree appears to be very low (Hiwasa et al., 2003a; Kondo et al., 2006; Murayama et al., 1998, 2006). This ethylene may not be essential for fruit softening on the tree. Furthermore, fruit attachment to the tree and fruit growth involves the continuous flux, deposition, and processing of water and solutes in fruit tissues, resulting in structural changes in the cell wall among other changes (Bargel and Neinhuis, 2005; Considine and Brown, 1981; Gibert et al., 2005; Hiwasa et al., 2003b; Murayama et al., 1998). Many of these changes in fruit growth and development are driven by

Table 3. Mean skin color of 'Bartlett' pear fruit after ripening at harvest and after storage at –1 °C.[z]

2006

	Harvest 1		Harvest 2		Harvest 3	
Treatments	AH	3.5 mo. storage	AH	3.5 mo. storage	AH	3.5 mo. storage
Control	4.0	4.0	4.0	4.0	4.0	4.0
Adjuvant (Adj.)	3.9	3.7				
28 mg·L^{-1} 1-MCP	2.0	3.6	3.6	3.9	3.9	3.6
56 mg·L^{-1} 1-MCP	1.7	3.3	3.6	3.5	3.8	3.7
Contrast						
Control versus 28 mg·L^{-1}	***	***	*	NS	*	NS
Control versus 56 mg·L^{-1}	***	***	*	***	**	NS
Control versus Adj.	NS	***				
28 mg·L^{-1} versus 56 mg·L^{-1}	*	***	NS	***	NS	NS
Adj. versus 28 mg·L^{-1}	***	NS				
Adj. versus 56 mg·L^{-1}	***	***				

2007

	Harvest 1		Harvest 2	
Treatments	AH	4.5 mo. storage	AH	4 mo. storage
Control	4.0	4.0	4.0	4.0
Adj.	4.0	4.0	3.9	4.0
50 mg·L^{-1} 1-MCP	2.4	3.9	3.8	4.0
100 mg·L^{-1} 1-MCP	2.9	3.8	3.6	3.9
LSD				
Control versus 50 mg·L^{-1}	***	NS	NS	NS
Control versus 100 mg·L^{-1}	***	NS	*	NS
Control versus Adj.	NS	NS	NS	NS
50 mg·L^{-1} versus 100 mg·L^{-1}	*	NS	NS	*
Adj. versus 50 mg·L^{-1}	***	NS	NS	NS
Adj. versus 100 mg·L^{-1}	*	NS	NS	*

[z]Fruit from all harvests (H1, H2, H3) in 2006 were ripened for 8 and 5 d at 20 °C at harvest and after 3.5 months cold storage, respectively. In 2007, fruit were ripened for 8 (H1) or 6 d (H2) at 20 °C at harvest and for 4 d at 20 °C after 4.5 (H1) or 4 months (H2) cold storage.
***$P < 0.001$; **$p < 0.01$; *$P < 0.05$; NS = nonsignificant. California Department of Food and Agriculture Color Chart (1 = green; 2 = light green; 3 = light yellow; 4 = yellow).
AH = at harvest; 1-MCP = 1-methylcyclopropene; LSD = least significant difference.

developmental signals in which ethylene may not play a role. It is clear, however, that exogenous ethylene and/or treatments that induce ethylene production in fruit attached to the tree such as auxins or wounding induce fruit softening (Clayton et al., 2000; Kondo et al., 1999, 2006; Kondo and Seto, 2004; Kondo and Takano, 2000; Murayama et al., 2006; Yuan and Carbaugh, 2007).

Second, previous studies have shown that contact of the liquid formulation of 1-MCP (AFxRD-038) with the fruit surface plays an important role in treatment efficacy (Choi and Huber, 2008; Choi et al., 2008). In our study, we used a high application volume with 8 and between 9.8 to 10.6 L per tree in 2006 and 2007, respectively, to assure complete fruit coverage. Despite the high volumes, some fruit-to-fruit variation, especially detected in softness and color development during ripening after harvest, remained regardless of the 1-MCP concentration. This suggests that the application system could be further improved. Field applications must account for complex pear tree architecture with trees in many orchards having 150 or more fruits and canopies ranging from 4 to 6 m in height and 2 to 2.5 m width.

In general, no differences in pear fruit maturity on the tree or fruit ripening after harvest were found between the highest and the lowest 1-MCP rates either year of this study, suggesting that the concentrations used might be enough to saturate the majority of the ethylene receptors. However, it might be useful to study whether repetitive applications could improve the 1-MCP effect as a result of 1-MCP binding to new ethylene receptor proteins that might be produced after treatment or rebinding to receptors that might have lost interaction with 1-MCP after a period of time. This and other studies (Elfving et al., 2007; McArtney et al., 2008) demonstrated that the 1-MCP effect diminishes after application, and the shorter intervals between treatment and harvest generally have the highest effect on fruit firmness on the tree (maturity) and ripening control after harvest and storage. Therefore, two or more applications at 5- to 10-d intervals with harvest within 7 d after the last application might improve 1-MCP effects on pear fruit.

In contrast to the mild effects obtained for slowing the maturation on the tree, 1-MCP had a stronger effect in delaying softening during cold storage and ripening after harvest, which suggests that this product might provide benefits in cases in which stresses occur during marketing (rough handling, temperature abuse, and so on). The effect on ripening in 2006 and 2007 was dependent on at least two factors: time between application and harvest and length of storage period. The longer the fruit was held on the tree or in storage at –1 °C after treatment, the less 1-MCP affected fruit ripening. The reasons for the reduced effect are unknown, but some hypotheses can be proposed based on our knowledge of ethylene perception in model plants such as *Arabidopsis* and tomato.

Ethylene response is achieved in plants by a cascade of events in which ethylene receptor proteins interact with ethylene and initiate the signaling process (Kendrick and Chang, 2008). The ethylene receptor proteins have been shown to negatively regulate the ethylene response and are degraded, at least some of them, by ethylene exposure (Chen et al., 2007; Kevany et al., 2007).

Previous work by Kevany et al. (2007) is relevant to the results observed in our study. They found that 1-MCP stabilized *LeETR4* and *LeETR6*, two ethylene receptor proteins associated with ripening in tomato. Once stabilized by 1-MCP, they are not degraded by ethylene as seen when protein abundance analyses are performed within hours of 1-MCP treatment and ethylene exposure.

If the receptor proteins are stabilized by 1-MCP, why do the effects on ripening after harvest vary with the time between application and harvest and after cold storage? Our results suggest at least some potential explanations. First, it is unknown whether the stabilization of the receptor proteins is permanent, but if it is not permanent, that would allow ethylene responses to recover as was shown in our study. Second, it might be possible that ethylene receptor proteins are permanently stabilized by 1-MCP, and the recovery of fruit response to ethylene and ripening is the result of changes downstream of ethylene perception or through production of new ethylene receptor proteins after 1-MCP treatment or a combination. The biochemistry of ethylene action is very plastic; thus, the abundance of these elements can evolve after 1-MCP treatment, changing the

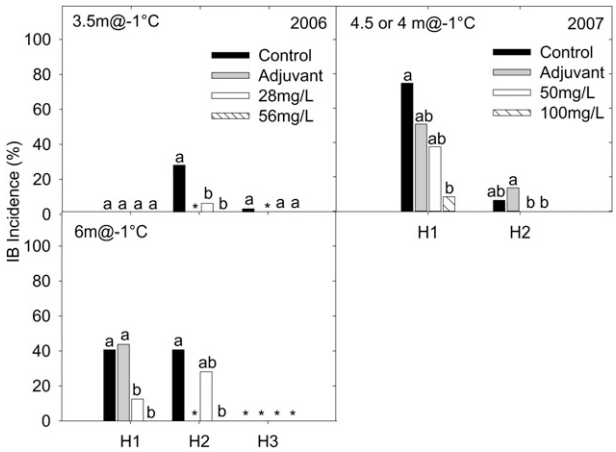

Fig. 5. Pear fruit internal breakdown (IB) incidence after cold storage and ripening. In 2006, fruit were evaluated after 3.5 months or 6 months at –1 °C plus 5 d ripening at 20 °C for all harvest times. In 2007, fruit were evaluated after 4.5 months (H1) or 4 months (H2) at –1 °C plus 4 d ripening at 20 °C. Different letters within each evaluation time represent statistical differences using least significant difference ($P < 0.05$). *Treatment not evaluated.

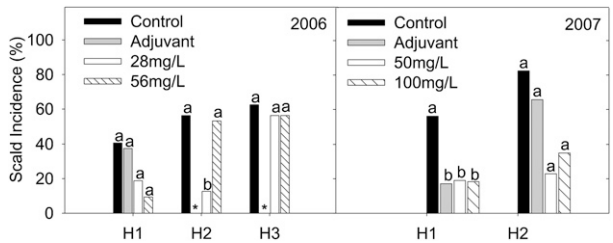

Fig. 6. Pear fruit scald incidence after cold storage and ripening. In 2006, fruit were evaluated after 3.5 months at –1 °C plus 5 d ripening at 20 °C for all harvest times. In 2007, fruit were evaluated after 4.5 (H1) or 4 months (H2) at –1 °C plus 4 d ripening at 20 °C. Different letters within each evaluation time represent statistical differences using least significant difference ($P < 0.05$). *Treatment not evaluated.

strength of the signaling. For example, it has been suggested that EIN3 and EIL1, transcription factors located downstream of ethylene perception, might be essential for rapid and highly sensitive responses to environmental stresses or development (Kendrick and Chang, 2008). Five and six ethylene receptor proteins have been identified in *Arabidopsis* and tomato, respectively, and at least five additional proteins have been shown thus far to participate in the ethylene action signaling process in *Arabidopsis.* Therefore, many possibilities of interaction, receptor turnover, signaling strength, and differential expression patterns could contribute to regulate ethylene action (Kendrick and Chang, 2008) and recovery in 1-MCP-treated fruit.

1-MCP significantly reduced internal breakdown at many evaluation times. Internal breakdown or browning affects 'Bartlett' pears at later stages than storage scald and has been associated with a limited availability or depletion of energy (Pedreshi et al., 2009; Veltman et al., 2003) caused by long periods of cold storage in which energy is used to sustain respiration and secondary metabolism. In this experiment, 1-MCP reduced respiration rates (data not shown) in a similar pattern to the reduction in ethylene production; thus, it likely provided metabolic savings for the fruit, allowing longer postharvest life.

Conclusions

Field application of 1-MCP on 'Bartlett' pear trees was as effective as NAA to reduce premature fruit drop during some evaluation times. All the 1-MCP concentrations produced similar results in terms of maturity and ripening control. Field applications slowed fruit softening on the tree slightly when compared with the untreated fruit, but 1-MCP did not show statistical differences when compared with the adjuvant only treatment. The greatest effect of field-applied 1-MCP on 'Bartlett' pears was on ripening immediately after harvest, and this effect decreased after fruit were stored at –1 °C for more than 3.5 months. The effect of 1-MCP was greatest when the fruit were harvested soon after treatment. Additional work is needed to assure the field application is optimized and to further understand how pear fruit regain their ability to respond to ethylene and ripen.

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