Strategies for Predicting and Reducing Fruit Ethylene Production to Improve SmartFresh™ Treatment Efficacy and Reliability

Andrew Macnish[1], Rachel Elkins[2], Elizabeth Mitcham[1]

[1]Department of Plant Sciences, University of California, Davis, CA
[2]University of California Cooperative Extension, Lakeport, CA

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INTRODUCTION

1-Methylcyclopropene (1-MCP) is a gaseous inhibitor of ethylene action that delays ripening of European pear fruit. Commercial preparations such as SmartFresh™ are registered for postharvest application inside sealed rooms, containers or tents. We have been evaluating the potential of SmartFresh™ to improve the post-storage quality of 'Bartlett' pears and allow fruit to be shipped to distant markets. While results have been promising, an ongoing challenge is developing robust treatment recommendations that consistently improve fruit quality for all maturity stages and under a range of treatment and storage conditions. We recently established that the accumulation of ethylene in the treatment atmosphere is an important factor that reduces SmartFresh™ efficacy. Ethylene produced by fruit competes with 1-MCP molecules for binding sites in fruit tissues, and at relatively high concentrations can completely negate the benefits of SmartFresh™ treatment. This can be a particular problem for late-season fruit that typically produce higher levels of ethylene at harvest, and may explain the failure of SmartFresh™ treatments to consistently delay ripening of fruit at an advanced maturity stage. During the 2010 season, we showed that SmartFresh™ treatment concentrations of ≥ 3.5 times that of the ethylene concentration that accumulated in the treatment atmosphere were necessary to extend the shelf life of fruit. Thus, we hypothesized that it may be possible to determine the optimal SmartFresh™ treatment concentration based on fruit ethylene production levels. We report here on our progress towards predicting SmartFresh™ efficacy based on fruit ethylene production. We also evaluated alternative strategies (e.g. pre-treatment with ReTain®, an inhibitor of ethylene synthesis) for reducing ethylene competition during SmartFresh™ treatment.

OBJECTIVES

1. Determine the relationship between harvest maturity and ethylene production rates to identify fruit at risk of not responding to SmartFresh™.
2. Determine the efficacy of postharvest ReTain® treatments to reduce fruit ethylene production and enhance SmartFresh™ benefits.

3. Determine the potential of ReTain® field application as an alternative strategy to reduce fruit ethylene production and improve SmartFresh™ efficacy.

MATERIALS AND METHODS

Plant material

In experiment 1, mature green 'Bartlett' fruit were obtained from packinghouses near Sacramento (Greene & Hemly, Inc.) and Lakeport (Scully Packing Co.) in California. Fruit were collected prior to pre-cooling on the day of the first commercial harvest and then every 8-10 days during the season to capture three (early, mid, late) stages of maturity (e.g. 15-20 lbs firmness). Sacramento fruit were obtained on July 26, August 4 and 14, while Lakeport fruit were collected on August 24, September 1 and 9. In experiment 2, 'Bartlett' fruit were harvested from selected trees in an orchard near Lakeport, California at the first commercial harvest date (August 26) and again 12 days later (September 7). In contrast to the first experiment, the fruit for experiment 2 did not pass through a commercial packingline. All fruit were transported to the laboratory on the day of harvest within 1-2 hours at 68°F.

Experiment 1: Predicting the optimal 1-MCP concentration to apply

Upon arrival to the laboratory, fruit were selected for uniform quality and packed into cardboard boxes. The fruit were held at 32°F for 16 hours to equilibrate to treatment temperature. Boxes of fruit were then randomly assigned to open 300 L stainless steel chambers at a loading ratio (110 lbs fruit per 300 L volume) consistent with a marine container. The lids to each chamber were closed and the following 1-MCP (provided as SmartFresh™) treatments were administered:

- Treatment 1: Control fruit were exposed to 0 ppb 1-MCP for 24 hours at 32°F.
- Treatment 2: Fruit were treated with 600 ppb 1-MCP, the current maximum dosage permitted by law, for 24 hours at 32°F.
- Treatment 3: Fruit were treated with 2000 ppb 1-MCP (proposed maximum limit for new label) for 24 hours at 32°F.
- Treatment 4: Once fruit had cooled to 32°F, a random sample was sealed into glass jars (four fruit per 1 gallon jar) for 12-15 hours at 32°F to enable determination of fruit ethylene production. The observed ethylene production rate was used to predict the concentration of ethylene that would accumulate in the chambers during a 24-hour treatment with 1-MCP at 32°F. The optimal 1-MCP concentration (3.5 times that of ethylene) for a 24-hour treatment at 32°F was then calculated based on the predicted ethylene competition.
- Treatment 5: Fruit were exposed to two sequential 24 hour treatments with 600 ppb 1-MCP at 32°F.
- Treatments 6 and 7: We evaluated an alternative mode of 1-MCP delivery - fruit were dipped in 0 or 1 ppm liquid 1-MCP (Harvista™) plus 0.1% NuFilm P

surfactant at 68°F for 1 minute prior to placing in room air (outside of chambers) for 24 hours at 32°F.

Following gaseous and liquid 1-MCP treatments, half of the fruit from each treatment were warmed to 68°F and immediately exposed to 100 ppm ethylene for 24 hours at 68°F. The remaining fruit were stored at 34°F for 5 weeks to simulate a marine shipment to South America. After the ethylene or storage treatment, fruit were maintained at 68°F for shelf life evaluation.

Experiment 2: Potential of ReTain® field application to improve SmartFresh™ efficacy

Two weeks prior to the first commercial harvest, fruit on 'Bartlett' trees were spayed to runoff with 0 or 11.7 oz/acre of ReTain® (active ingredient: aminoethoxyvinylglycine; AVG) in accordance with the manufacturer's instructions. Upon their arrival in the laboratory, the fruit were selected for uniform quality and cooled to 32°F. Fruit were enclosed into stainless steel treatment chambers and treated with 0 or 600 ppb 1-MCP for 24 hours at 32°F as described in experiment 1. Following 1-MCP treatment, fruit were exposed to 100 ppm ethylene or subjected to simulated shipping as described above in experiment 1. Fruit were then maintained at 68°F for evaluation.

Fruit Evaluations

Internal ethylene concentration

The internal ethylene concentration was determined at 68°F for 12 fruit individually from each harvest upon their arrival in the laboratory. Unbound ethylene was extracted from internal fruit tissues with vacuum. Ethylene concentrations were quantified using a Carle gas chromatograph fitted with a flame ionization detector. An authentic ethylene standard was used to calibrate the gas chromatograph.

Ethylene production and respiration

Once fruit had cooled to 32°F in the laboratory, 12 fruit from each harvest were sealed into glass 1 gallon jars (four fruit per jar) for 12-15 hours at 32°F. The concentration of ethylene and CO_2 that accumulated inside jars was quantified by a Carle gas chromatograph (described above) and a Horiba gas analyzer, respectively. We also measured fruit ethylene production and respiration every 2 days during their subsequent shelf life at 68°F using a slightly modified protocol whereby fruit were sealed in jars (six fruit per jar) for 1-2 hours at 68°F. Ethylene and CO_2 concentrations inside closed chambers at the beginning and end of each treatment were also determined.

Flesh firmness and skin color

Flesh firmness and skin color were evaluated at harvest and then every 3 days of ripening at 68°F. Flesh firmness was measured using a Gűss FTA penetrometer fitted with an 8 mm probe on opposite sides of each fruit after removing a thin slice of skin. Skin color was measured objectively using a Minolta Colorimeter. The change in color from green to yellow was best represented by the hue angle.

Experiment Design

Fruit were arranged in a randomized complete block design during treatment, storage and shelf life evaluation. Four replicate boxes containing fruit were used for each treatment. Six fruit were removed at random from every box at each sampling time for firmness and color evaluation. Data are presented as means ± standard errors.

RESULTS AND DISCUSSION

Experiment 1: Predicting the optimal 1-MCP concentration to apply

1-MCP treatment delayed ethylene-mediated ripening

Early-, mid- and late-season 'Bartlett' fruit ripened rapidly and uniformly in response to a 24-hour exposure to 100 ppm ethylene after harvest, reaching an eating firmness of 3 lbs in 6 days at 68°F (Figures 1, 2). Pre-treatment with 600 ppb 1-MCP for 24 hours at 32°F, the current recommended dose for European pears, reduced the sensitivity of fruit to ethylene to varying degrees depending upon the harvest maturity and growing district. For pears sourced from the Sacramento packinghouse, pre-treatment with 600 ppb 1-MCP extended the shelf life (time to eating firmness) of fruit by 15 days for all three harvest maturity stages (Figure 1). For fruit obtained from the Lakeport packinghouse, 1-MCP treatment extended the shelf life of early-, mid-, and late-season pears by 18, 15 and 12 days, respectively (Figure 2). Increasing the 1-MCP treatment concentration from 600 ppb to 2000 ppb did not confer additional benefits for fruit (Figures 1, 2) and indicates that 600 ppb 1-MCP was a saturating dose. These results differ from our observations from the 2010 season, where treatment with 600 ppb 1-MCP for 24 hours at 32°F only extended the shelf life of Sacramento and Lakeport 'Bartlett' fruit by 3-9 days at 68°F.

The benefits of the different 1-MCP treatments were largely maintained for fruit that were stored for 5 weeks at 34°F (Figures 1, 2). Control (i.e. 0 ppb 1-MCP) fruit at each harvest maturity and from both growing districts also ripened in 6

days upon transfer from 34°F to 68°F in line with matching fruit that were exposed to 100 ppm ethylene after harvest. For all treatments, the reduction in fruit firmness during ripening was accompanied by the typical yellow coloration of fruit skin, although there was a tendency for 1-MCP-treated fruit to develop full yellow color before reaching eating firmness (data not shown).

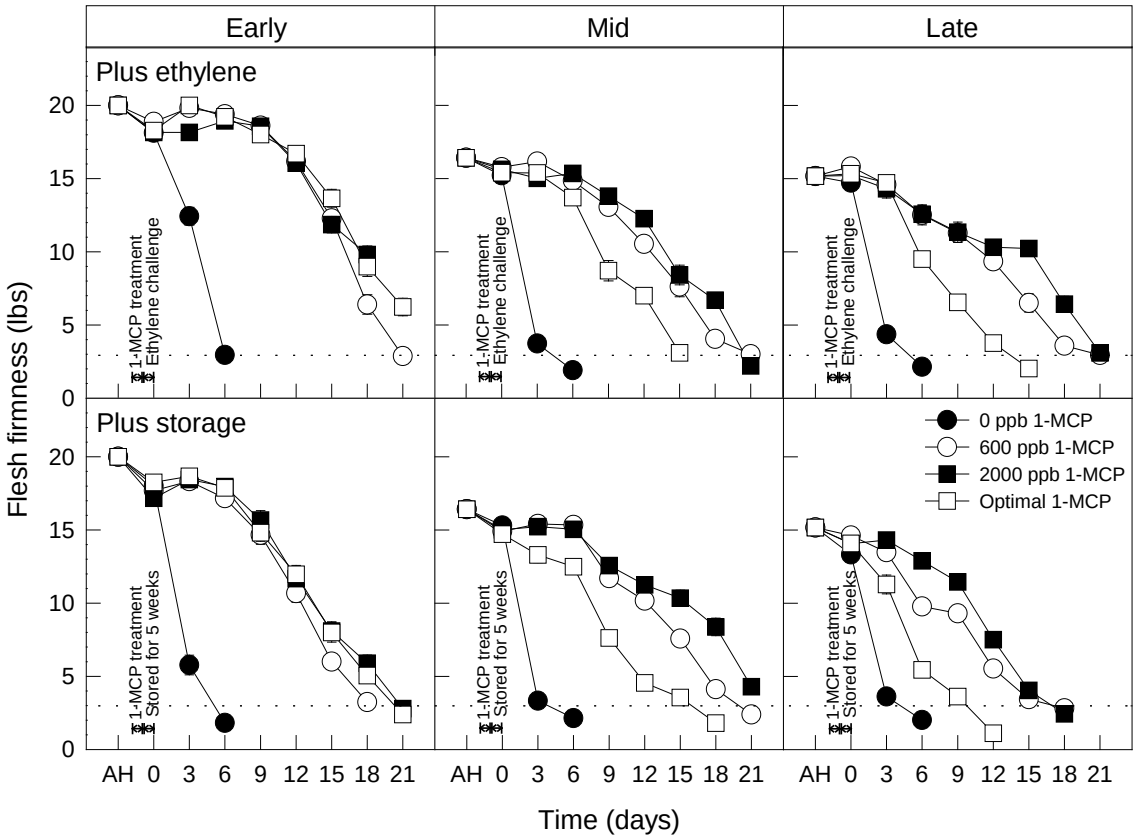

Figure 1. Fruit firmness at harvest (AH) and during ripening at 68°F for 'Bartlett' pears obtained at three stages of maturity (early, mid, late) from a Sacramento packinghouse. Fruit were pre-treated with 0, 600 or 2000 ppb 1-MCP (as SmartFresh^{TM}) for 24 hours at 32°F. Additional fruit were pre-treated with an optimal 1-MCP concentration (900, 300 and 200 ppb 1-MCP for early-, mid- and late-season fruit, respectively) based on predicted ethylene competition during 1-MCP treatment. Fruit were then exposed to 100 ppm ethylene for 24 hours at 68°F, or stored for 5 weeks at 34°F prior to shelf life evaluation. The dashed horizontal line represents an eating firmness of 3 lbs.

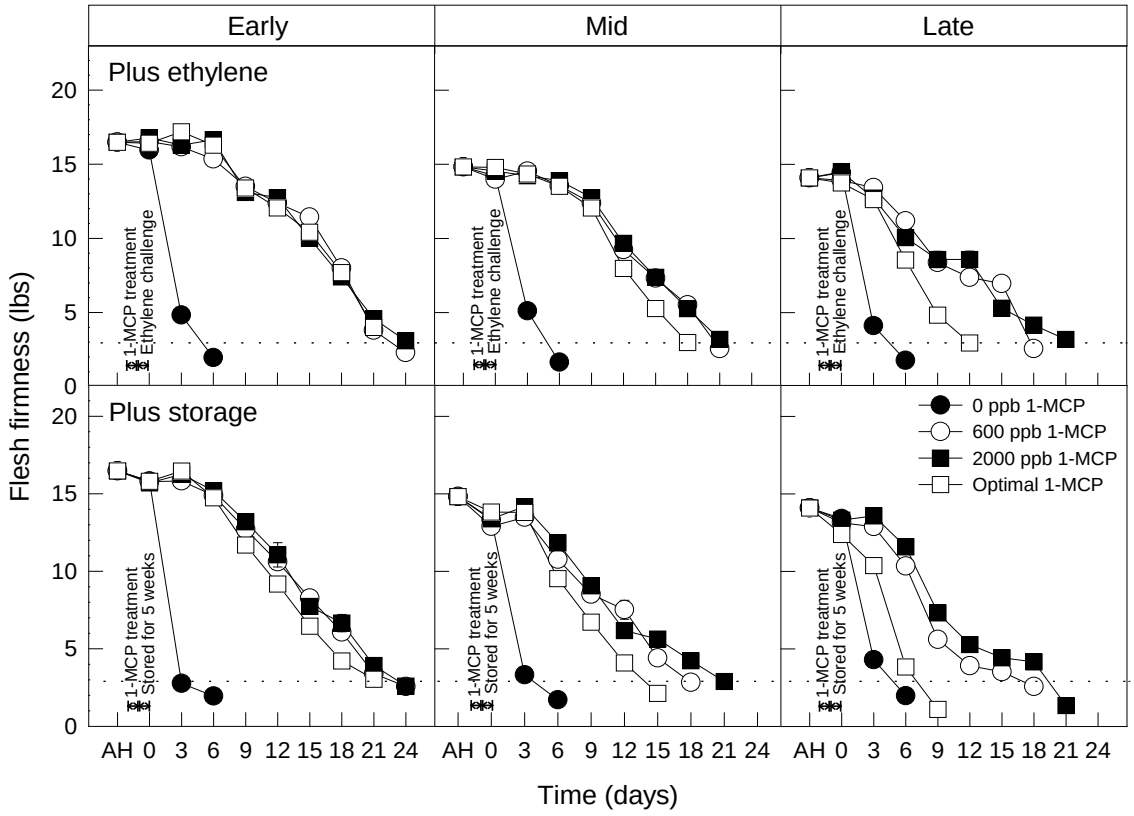

Figure 2. Fruit firmness at harvest (AH) and during ripening at 68°F for 'Bartlett' pears obtained at three stages of maturity (early, mid, late) from a Lakeport packinghouse. Fruit were pre-treated with 0, 600 or 2000 ppb 1-MCP (as SmartFresh^{TM}) for 24 hours at 32°F. Additional fruit were pre-treated with an optimal 1-MCP concentration (400, 300 and 300 ppb 1-MCP for early-, mid- and late-season fruit, respectively) based on predicted ethylene competition during 1-MCP treatment. Fruit were then exposed to 100 ppm ethylene for 24 hours at 68°F, or stored for 5 weeks at 34°F prior to shelf life evaluation. The dashed horizontal line represents an eating firmness of 3 lbs.

Predicting ethylene competition during 1-MCP treatment

A key objective of our work was to determine the optimal 1-MCP concentration to apply based on the predicted concentration of ethylene that would accumulate in treatment chambers. In theory this optimal treatment could be relied on to consistently extend the shelf life of fruit without locking up the ripening capacity regardless of the harvest maturity and growing district. We sealed pre-cooled fruit in glass jars at 32°F and measured the accumulation of ethylene over time. We then estimated the concentration of ethylene that likewise would accumulate in the treatment atmosphere. Using this as a guide, we calculated the appropriate 1-MCP treatment concentration based on our previous finding from the 2010 season that effective 1-MCP concentrations were 3.5 times that of the ethylene that accumulated in the chamber.

Our ability to accurately predict ethylene competition and hence the optimal 1-MCP treatment concentration varied considerably. While the buildup of ethylene in the 1-MCP treatment atmosphere increased steadily with advancing harvest maturity, this relationship was not always captured by our ethylene production prediction protocol (Table 1). We also found there was no clear association between the internal ethylene concentrations within fruit at harvest with eventual chamber concentrations (Table 1). For early-season Sacramento and Lakeport fruit, we greatly over-estimated the buildup of ethylene in chambers (Table 1). As a consequence, the 'optimal' 1-MCP concentrations that we applied (900 and 400 ppb for Sacramento and Lakeport fruit, respectively) were saturating and similarly as effective as treatment with 600 and 2000 ppb 1-MCP reported above (Figures 1, 2, 3). For mid-season Sacramento and Lakeport fruit, we slightly over-estimated the accumulation of ethylene in the treatment atmosphere (Table 1) and our optimal treatment of 300 ppb 1-MCP gave an intermediate response in extending shelf life by 9 and 12 days, respectively (Figures 1, 2). While we slightly under-estimated the buildup of ethylene in treatment chambers for late-season Sacramento fruit (Table 1), treatment with the optimal 200 ppb 1-MCP still resulted in an intermediate extension of shelf life of 9 days relative to non-treated control fruit (Figure 1). For late-season Lakeport fruit, we again slightly over-estimated ethylene production (Table 1) and treatment with the optimal 300 ppb 1-MCP extended shelf life by 6 days as compared to control fruit (Figure 2).

Table 1. Internal ethylene concentration (IEC) within non-treated control 'Bartlett' fruit at harvest at 68°F and the predicted and actual concentrations of ethylene produced by fruit that accumulated in 300 L chambers during a 24-hour treatment with 1-MCP at 32°F. 1-MCP was applied at a concentration of 3.5 times that of the predicted ethylene competition. Fruit were obtained at early, mid-, and late-season maturity from a Sacramento and Lakeport packinghouse.

Harvest maturity	IEC at harvest (ppb)	Predicted ethylene concentration in chambers (ppb)	Calculated optimal 1-MCP concentration (ppb)	Actual ethylene concentration in chambers (ppb)
Sacramento				
Early	36	250	900	33
Mid	127	90	300	34
Late	75	36	200	53
Lake				
Early	17	110	400	12
Mid	18	63	300	46
Late	251	80	300	61

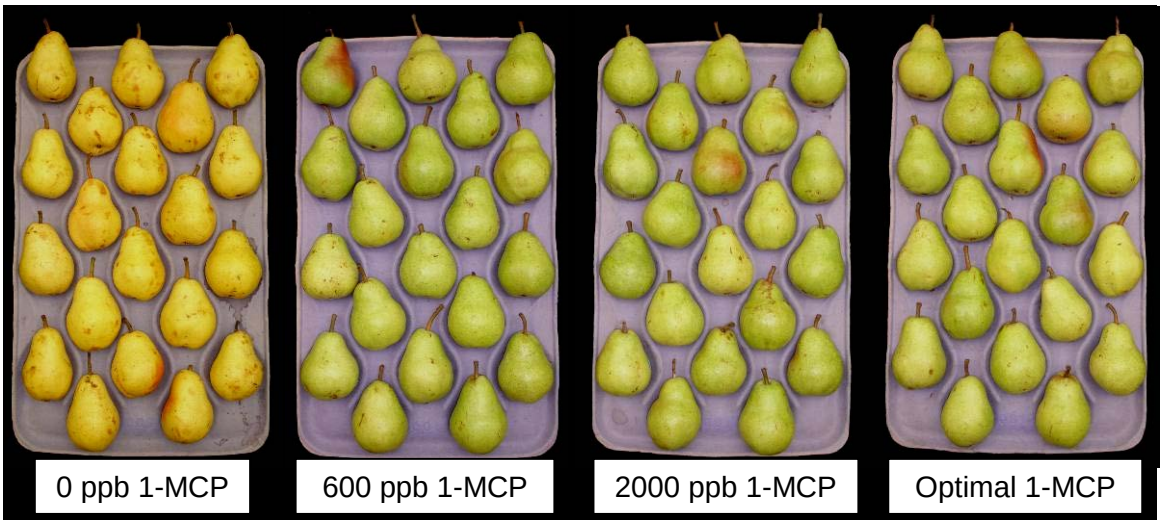

Figure 3. Photographs of early-season 'Bartlett' fruit from Lakeport on day 6 of shelf life at 68°F. Fruit were pre-treated with 0, 600 or 200 ppb 1-MCP for 24 hours at 32°F. Additional fruit were pre-treated with 400 ppb 1-MCP, an optimal concentration based on predicted ethylene competition during 1-MCP treatment. All fruit were subsequently exposed to 100 ppm ethylene for 24 hours at 68°F prior to shelf life.

Despite encountering some challenges in predicting ethylene competition, the use of our lower optimized 1-MCP treatment concentrations (e.g. 200-400 ppb) were still successful in extending the shelf life of 'Bartlett' fruit from the 2011 season by 6-12 days at 68°F, even after 5 weeks of cold storage. With some refinement to protocol, we believe there is potential to predict the efficacy of 1-MCP treatment based on fruit ethylene production prior to treatment. This approach could be particularly useful given the observed variation in ethylene production and response for fruit of different maturity stages, growing districts, and seasons. For example, treatment of fruit from the 2010 season with 600 ppb 1-MCP for 24 hours at 32°F often resulted in partial ripening inhibition (see our previous report), while for the 2011 season this treatment strongly inhibited ripening. 1-MCP treatment efficacy still appears to be a function of the ratio of 1-MCP and ethylene concentrations in the treatment atmosphere, and we are continuing to analyze data from the current season in an effort to better understand this relationship.

Alternative strategies to improve 1-MCP efficacy

We investigated alternative strategies to improve the efficacy of 1-MCP treatments, including the option to retreat fruit with 1-MCP. We found that exposure to two sequential 24-hour treatments with 600 ppb 1-MCP at 32°F was equally effective as a single 24-hour application in delaying ethylene-mediated ripening of 'Bartlett' pears (Figures 4, 5). Given that exposure to 600 ppb 1-MCP

was a saturating dose during the 2011 season, it is not surprising that repeated exposures to 1-MCP at this concentration offered no additional benefits.

We also evaluated the potential of a liquid 1-MCP formulation, Harvista™, to provide an effective and more convenient mode of postharvest application to 'Bartlett' pears than current gaseous treatments. We previously tested the efficacy of this formulation as a field treatment. In the current season, we found that a 1 minute postharvest dip in 1 ppm liquid 1-MCP at 68°F was similarly as effective as a 24-hour exposure to 600 ppb 1-MCP gas at 32°F (Figures 6, 7). For example, the liquid 1-MCP treatment extended the shelf life of early-, mid-, and late-season Sacramento and Lakeport fruit by 12-18 days at 68°F immediately after harvest. The benefits of this alternative 1-MCP treatment were also largely maintained for fruit that were stored for 5 weeks at 34°F.

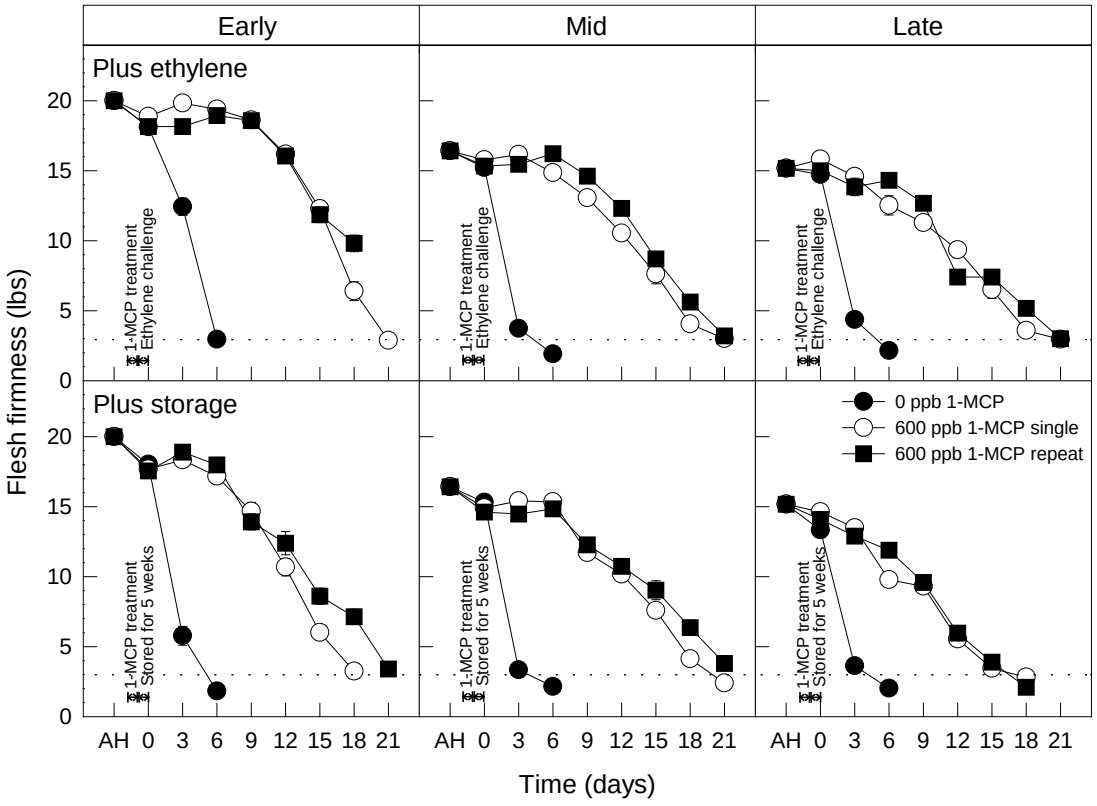

Figure 4. Fruit firmness at harvest (AH) and during ripening at 68°F for 'Bartlett' pears obtained at three stages of maturity (early, mid, late) from a Sacramento packinghouse. Fruit were pre-treated with 0 or 600 ppb 1-MCP (as SmartFresh^{TM}) applied as single or two sequential (repeated) 24-hour exposures at 32°F. Fruit were then exposed to 100 ppm ethylene for 24 hours at 68°F, or stored for 5 weeks at 34°F prior to shelf life evaluation. The dashed horizontal line represents an eating firmness of 3 lbs.

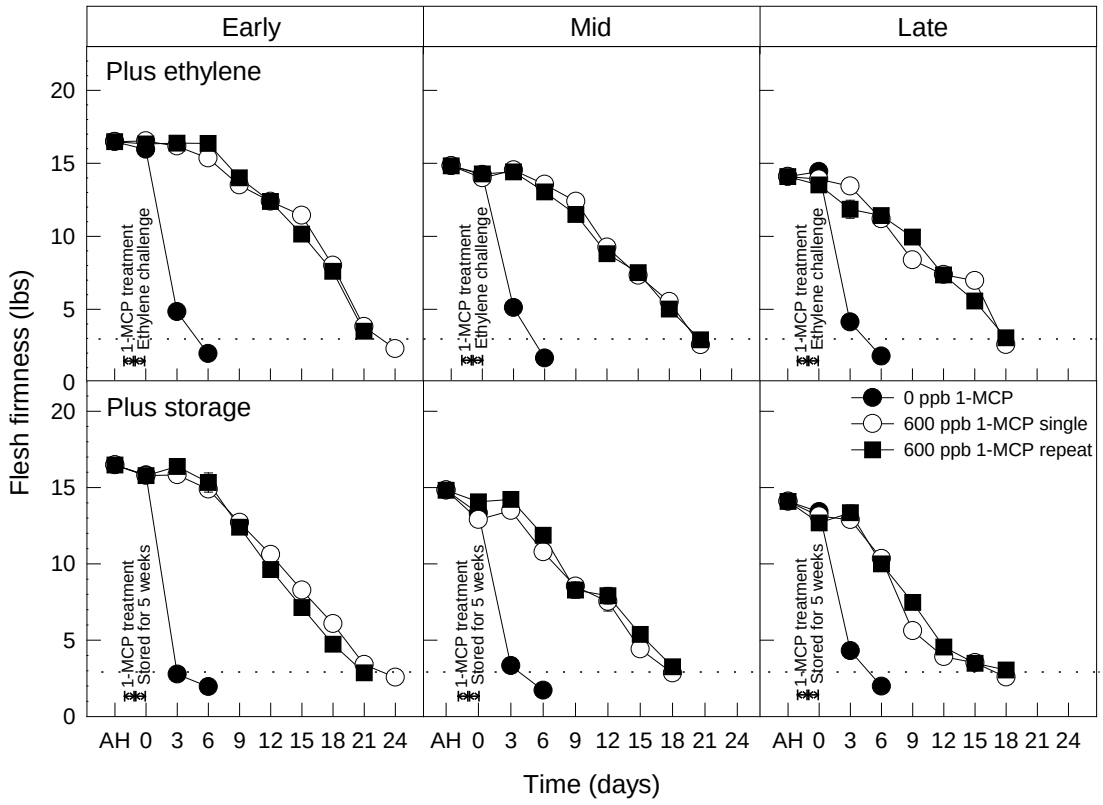

Figure 5. Fruit firmness at harvest (AH) and during ripening at 68°F for 'Bartlett' pears obtained at three stages of maturity (early, mid, late) from a Lakeport packinghouse. Fruit were pre-treated with 0 or 600 ppb 1-MCP (as SmartFresh^{TM}) applied as single or two sequential (repeated) 24-hour exposures at 32°F. Fruit were then exposed to 100 ppm ethylene for 24 hours at 68°F, or stored for 5 weeks at 34°F prior to shelf life evaluation. The dashed horizontal line represents an eating firmness of 3 lbs.

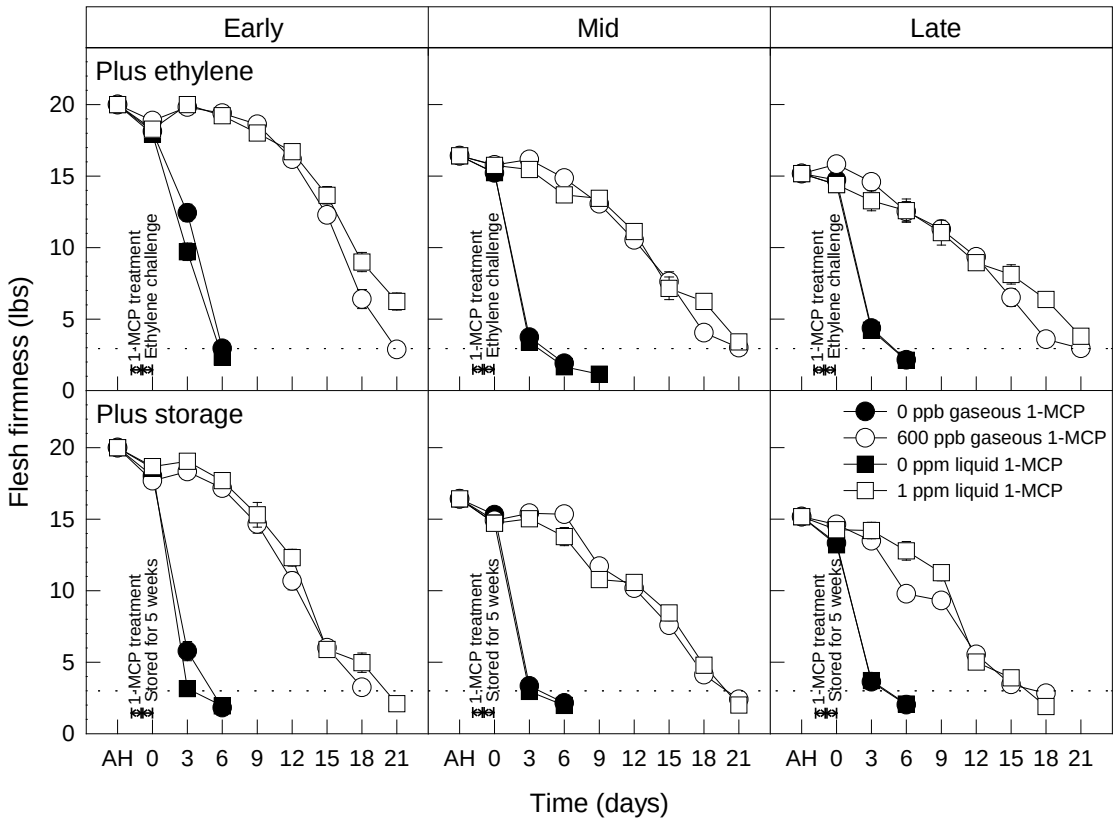

Figure 6. Fruit firmness at harvest (AH) and during ripening at 68°F for 'Bartlett' pears obtained at three stages of maturity (early, mid, late) from a Sacramento packinghouse. Fruit were pre-treated with 0 or 600 ppb gaseous 1-MCP (as SmartFresh^{TM}) for 24 hours at 32°F. Additional fruit were dipped in 0 or 1 ppm liquid 1-MCP for 1 minute at 68°F prior to maintenance at 32°F for 24 hours. All fruit were then exposed to 100 ppm ethylene for 24 hours at 68°F, or stored for 5 weeks at 34°F prior to shelf life evaluation. The dashed horizontal line represents an eating firmness of 3 lbs.

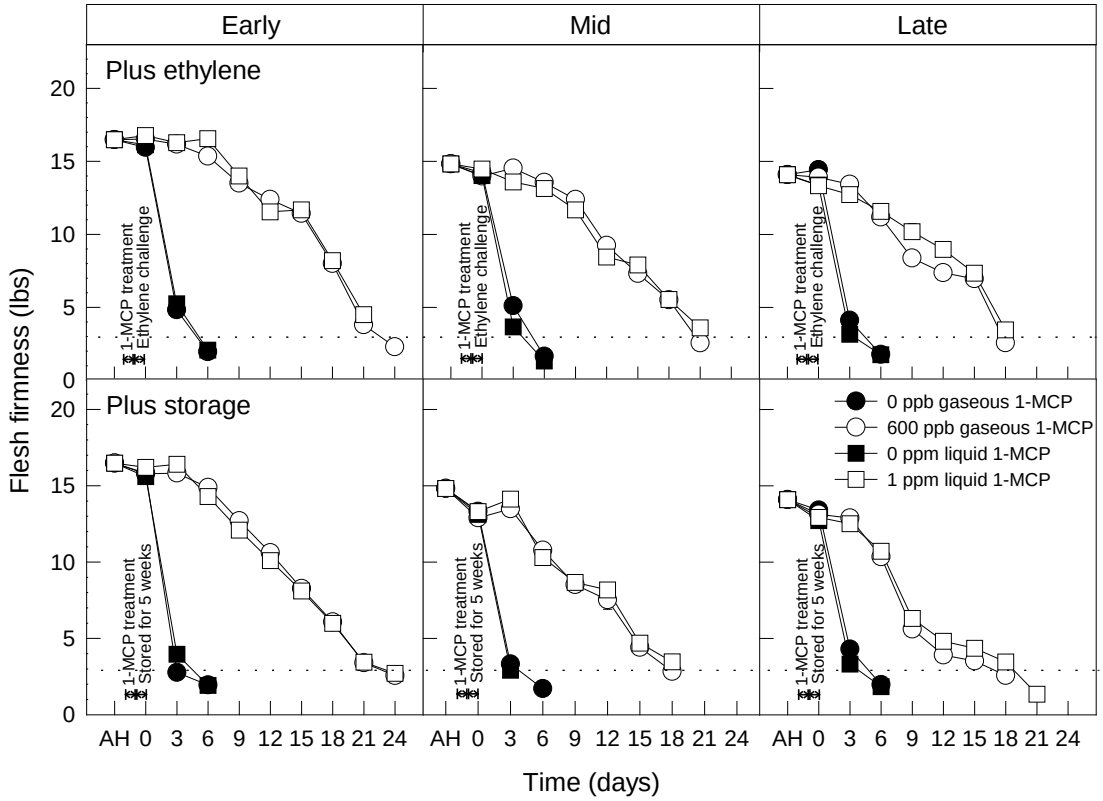

Figure 7. Fruit firmness at harvest (AH) and during ripening at 68°F for 'Bartlett' pears obtained at three stages of maturity (early, mid, late) from a Lakeport packinghouse. Fruit were pre-treated with 0 or 600 ppb gaseous 1-MCP (as SmartFresh™) for 24 hours at 32°F. Additional fruit were dipped in 0 or 1 ppm liquid 1-MCP for 1 minute at 68°F prior to maintenance at 32°F for 24 hours. All fruit were then exposed to 100 ppm ethylene for 24 hours at 68°F, or stored for 5 weeks at 34°F prior to shelf life evaluation. The dashed horizontal line represents an eating firmness of 3 lbs.

Experiment 2: Potential of ReTain® field application to improve SmartFresh™ efficacy

Our final objective was to determine the potential of AVG (provided as ReTain®), applied to 'Bartlett' pears as a field spray, to reduce fruit ethylene production rates and thereby enhance 1-MCP treatment efficacy. We pursued this approach because fruit harvested during the 2010 season produced relatively high concentrations of ethylene that interfered with 1-MCP efficacy. For the 2011 season, we found that applying AVG to pears 2 weeks before the first commercial harvest reduced rates of ethylene production at harvest by 29% and 68% for early- and late-season fruit, respectively. The lower rates of ethylene production by AVG-treated fruit translated into reduced ethylene competition in the 1-MCP treatment atmosphere. Given that treatment with 600 ppb 1-MCP for 24 hours at 32°F was already highly effective in delaying ethylene-mediated ripening during the 2011 season, there was no benefit of pre-treating fruit with

AVG, at least for fruit picked early in the season (Figure 8). However, the combination of a field application of AVG with a postharvest 1-MCP treatment did extend the shelf life of late-season fruit by an additional 3 days relative to fruit treated with 1-MCP alone (Figures 8, 9). While the benefits of the AVG pre-treatment were modest in terms of enhancing 1-MCP efficacy, it may be more effective in seasons where fruit produce higher rates of ethylene at harvest.

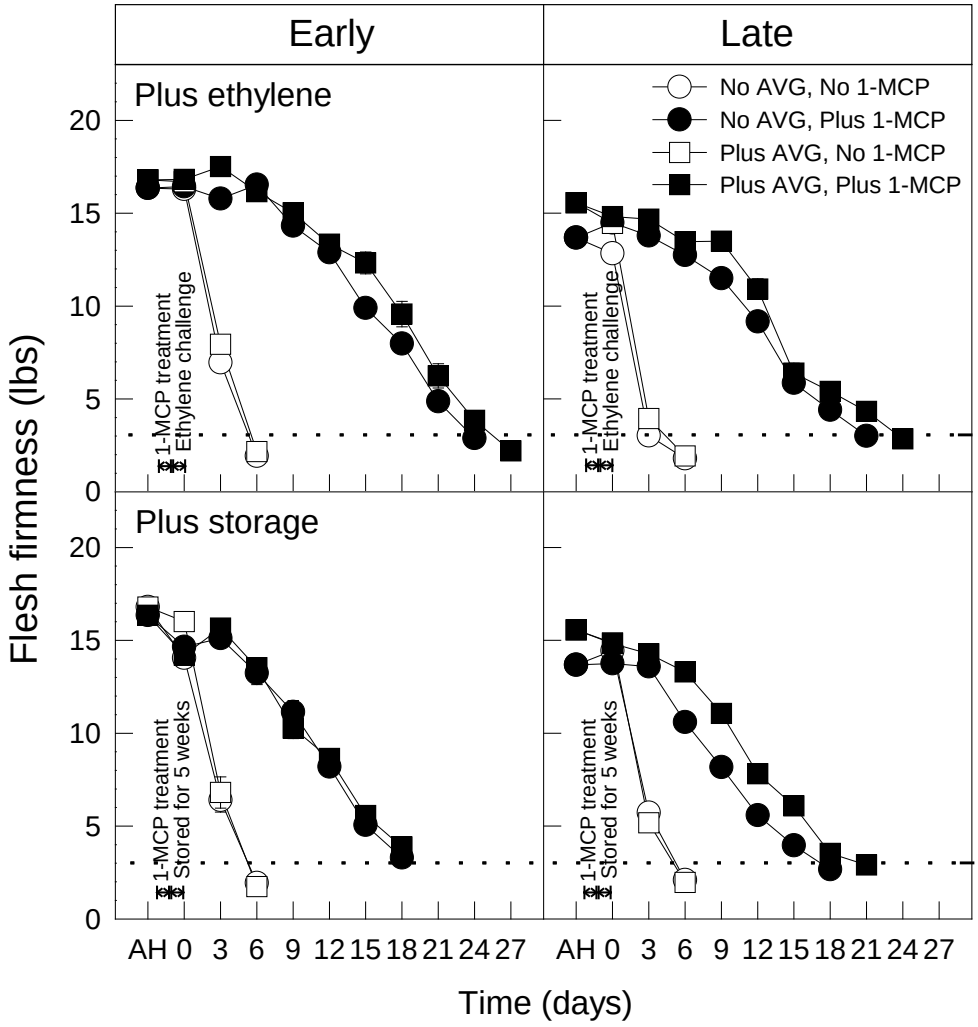

Figure 8. Fruit firmness at harvest (AH) and during ripening at 68°F for 'Bartlett' pears harvested at two stages of maturity (early, late) from a Lakeport orchard. Fruit on trees were spayed to runoff with 0 or 11.7 oz/acre of ReTain® (active ingredient AVG) 2 weeks prior to the first harvest. Fruit were treated with 0 or 600 ppb 1-MCP (as SmartFresh™) for 24 hours at 32°F and then exposed to 100 ppm ethylene for 24 hours at 68°F, or stored for 5 weeks at 34°F prior to shelf life evaluation. The dashed line represents an eating firmness of 3 lbs.

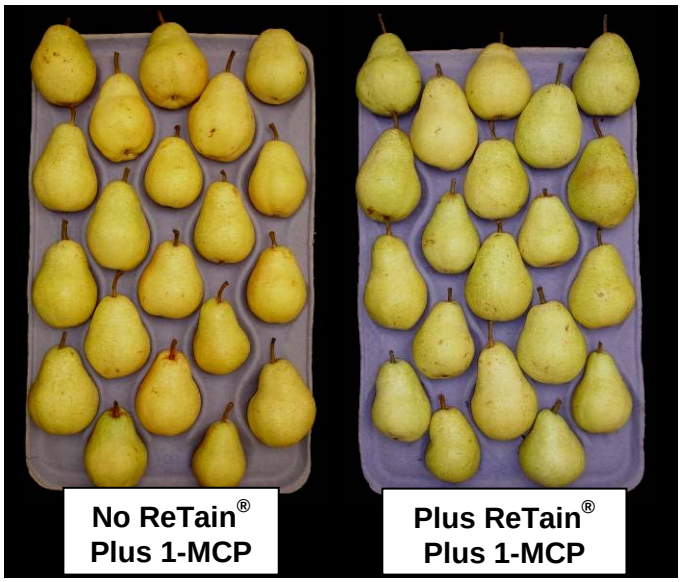

Figure 9. Photographs of late-season 'Bartlett' pears from a Lakeport orchard on day 12 of shelf life at 68°F. Fruit on trees were spayed to runoff with 0 or 11.7 oz/acre of ReTain® (active ingredient AVG) 4 weeks prior to harvest. Fruit were then treated with 600 ppb 1-MCP (as SmartFresh™) for 24 hours at 32°F. All fruit were subsequently exposed to 100 ppm ethylene for 24 hours at 68°F prior to shelf life.

CONCLUSIONS

Our findings continue to highlight the competitive nature of 1-MCP and ethylene in regulating ripening of 'Bartlett' pears, and the critical need to develop a method for predicting this ethylene competition before applying SmartFresh™. We showed that measuring fruit ethylene production before SmartFresh™ treatment gave a reasonable indication of ethylene competition during 1-MCP exposure. This approach allowed us to deliver optimized 1-MCP treatment concentrations. However, further research is required to test this method across multiple harvest seasons where ethylene production rates and response can vary considerably. We also found that postharvest liquid 1-MCP treatments were highly effective in extending the shelf life of fruit, and this potentially represents a more practical mode of application that circumvents some of the ethylene competition issues associated with the gaseous treatment. We plan to continue testing 1-MCP liquid preparations for their potential as field and/or postharvest applications.