

Strategies for predicting and improving SmartFresh™ treatment of pears

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ABSTRACT

The efficacy of 1-MCP on 'Bartlett' pear fruit was determined at three stages of maturity (early, mid, late) from Sacramento and Lakeport packinghouses. Fruit were pre-treated with 0, 600 or 2000 ppb 1-MCP (as SmartFresh™) for 24 hours at 32°F. Additional fruit were pre-treated with an optimal 1-MCP concentration (300 ppb 1-MCP for all harvests except for late-season from Lakeport which was 350 ppb) based on predicted ethylene competition during 1-MCP treatment. The optimal 1-MCP concentrations were calculated based on 3.5 times that of the ethylene concentration. 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. Our results showed that we were able to predict 1-MCP efficacy based on fruit ethylene production at harvest, extending shelf life to 12 to 15 days for non-stored fruit but shelf life varied between 6 and 12 days for fruit stored for 5-weeks before ripening. We also tested if our optimal 1-MCP treatment was effective when applied after one and two weeks of cold storage when fruit would be producing more ethylene. Unfortunately, the optimal treatment based on initial predicted ethylene production at the start of treatment did not work in every case.

We studied the efficacy of liquid 1-MCP, an alternative strategy of gaseous 1-MCP. Fruit were dipped in 0, 250, 500, and 1000 ppb (w/v) liquid 1-MCP for 0, 15, 30, and 60 seconds at 85-95°F (outside working temperature) and held at 68°F overnight. The lowest concentration of 250 ppb with 15 second dip maintained shelf-life at least 12 days from all maturity stages and locations. Liquid 1-MCP showed a lot of promise as a reliable, consistent treatment to reduce the rate of pear fruit ripening after harvest.

INTRODUCTION

1-Methylcyclopropene (1-MCP, SmartFresh™) is an inhibitor of ethylene action that delays ripening of European pear fruit and is commercially applied inside sealed rooms, containers or tents in a gaseous form. It competes with ethylene binding sites in fruit tissues to suppress the ripening process, but becomes less effective when the ethylene production is high. 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 1-MCP treatments to consistently delay ripening of fruit at an advanced maturity stage. On the

other hand, high concentration 1-MCP treatments sometimes outcompetes ethylene such that fruit fails to ripen. Determination of the optimal range of recommended 1-MCP concentrations has been an ongoing challenge to obtain a consistent response, that can vary by season, maturity stage, and fruit ethylene production, to improve fruit quality and extend its shelf life.

We have been evaluating the potential of 1-MCP to improve the post-storage quality of 'Bartlett' pears and allow fruit to be shipped to distant markets. We have been testing to establish a protocol to determine the optimal 1-MCP treatment based on predicting ethylene accumulation in the treatment atmosphere and therefore the 1-MCP concentration needed to obtain a good response. During the 2010 and 2011 season, we showed that 1-MCP 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. In 2012, ethylene production was relatively low compared to previous years, so that we set minimum concentration of 1-MCP at 100 ppm. Unfortunately, this concentration did not have a significant effect on extending fruit shelf-life. Thus, we set a minimum of 300 ppm for 2013 and continued to test our hypothesis that we can determine the optimal 1-MCP treatment concentration based on fruit ethylene production at harvest. We also tested if our optimal 1-MCP treatment was effective when applied after one and two weeks of cold storage when fruit would be producing more ethylene. Finally, we repeated the study of liquid 1-MCP to determine the optimum concentration and dipping duration.

OBJECTIVES

1. Finalize our model to predict ethylene competition and identify optimal SmartFresh™ treatments to reliably extend the shelf life of 'Bartlett' pears under commercial conditions.
2. Investigate the effect of time after harvest on the efficacy of SmartFresh™ treatment.
3. Determine the efficacy of postharvest liquid 1-MCP on fruit from early, mid and late season as alternative strategy to extend the shelf life of 'Bartlett' pears.

MATERIALS AND METHODS

Plant material

Mature green 'Bartlett' pear fruit (110-size) were obtained from packinghouses near Sacramento (Still Water Orchards, Courtland, CA) and Lakeport (Scully Packing Co. LLC, Finely, CA). Fruit were collected prior to pre-cooling near the day of the first commercial harvest and then every 5-8 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 10, 15, and 23, 2013 while Lakeport fruit were collected on August 6, 13, and 20, 2013. All fruit were transported to the laboratory within 1-5 hours on the day of harvest.

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 chambers were sealed and the following 1-MCP 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: Fruit were treated with the optimal 1-MCP concentration. Optimal concentration was determined as follows: 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 determine fruit ethylene production. Fruit were placed in the jars several hours before the jars were sealed to eliminate any wound ethylene. 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.

Following gaseous 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 ripening evaluation.

Experiment 2: Effect of time after harvest on 1-MCP response

The procedures of fruit selection, random assignment into the cardboard boxes, and the initial cooling condition to equilibrate to treatment temperature were the same as previously described in experiment 1. The fruit were stored for up to two weeks at 32°F and the optimal 1-MCP treatments were administered for 24 hours at 32°F after one day, one week, or two weeks of storage. The optimal 1-MCP concentration was calculated by predicted ethylene production after storage as previously described.

- Treatment 5: Control fruit were exposed to 0 ppb 1-MCP.
- Treatment 6: The optimal 1-MCP concentration (3.5 times that of ethylene) obtained one day after harvest, the same concentration used in treatment 4, was applied after one and two weeks of storage at 32°F if newly predicted concentration was different after one and two weeks of storage.
- Treatment 7: The optimal 1-MCP concentration (3.5 times that of ethylene) was calculated after one week of storage at 32°F and applied.
- Treatment 8: The optimal 1-MCP concentration (3.5 times that of ethylene) was calculated after two weeks of storage at 32°F and applied.

Following gaseous 1-MCP treatments, the fruit from each treatment were maintained at 68°F for ripening evaluation.

Experiment 3: Potential application of liquid 1-MCP

- Treatment 9: We evaluated an alternative mode of 1-MCP delivery - fruit were dipped in 0, 250, 500, or 1000 ppb (w/v) liquid 1-MCP plus 0.1% NuFilm P surfactant at 85-95°F (outside temperature) for 0, 15, 30, and 60 seconds.

Following liquid 1-MCP treatments, all the fruit from each treatment were placed in room air (68°F) overnight, followed by 100 ppm ethylene exposure for 24 hours at 68°F. After the ethylene treatment, fruit were maintained at 68°F for ripening evaluation.

Fruit Evaluations

Ethylene production and respiration

Six fruit were sealed into a 3.8-L glass jar for 0.5 to 1 hour at 68°F, depending on the ripening stage, and headspace samples were drawn with a 10-mL syringe and subjected to a Carle gas chromatograph and a Horiba gas analyzer, for ethylene and CO₂ measurement, respectively. Samples were analyzed every three days during subsequent ripening at 68°F. The initial ethylene production to determine the optimal 1-MCP concentration was measured using a slightly modified protocol with four fruit per jar for 12-15 hours at 32°F. Ethylene and CO₂ concentrations inside closed treatment chambers at the 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 during ripening at 68°F. Flesh firmness was measured using a Güss 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 ripening evaluation. Two replicate boxes containing fruit were used for each treatment. Nine fruit were removed at random from every box at each sampling time for firmness and color evaluation.

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' pear 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 6-9 days at 68°F (Figures 1 and 2). Pre-treatment with 600 ppb 1-MCP for 24 hours at 32°F extended the shelf life (time to eating firmness) of fruit to 18 days for Sacramento and 15-18 days for Lakeport sourced fruit from all three harvest maturities. Except for the mid-season harvest from Lakeport, application of 2000 ppb 1-MCP generally maintained a higher firmness than fruit treated with 600 ppb. This result was similar to those from the 2012 season, for which the treatment of 2000 ppb did not confer additional benefits over treatment with 600ppb for fruit harvested from Sacramento if the goal is to maintain the shelf life for at least 18 days.

For fruit that were stored for 5-weeks at 34°F, control fruit from both locations and all harvest stages ripened in 3 days upon transfer from 34°F to 68°F. Fruit treated with 600 ppb 1-MCP extended the shelf life to 15 to 18 days for Sacramento and 9 to 12 days for Lakeport. Application of 2000 ppb 1-MCP maintained firmness to 18 days for both locations and all harvest stages. In general, the higher dose of 1-MCP maintained higher firmness for both locations and all harvest stages except for Sacramento and Lakeport mid-season harvested fruit.

For all treatments, the reduction in fruit firmness during ripening was accompanied by the typical yellow coloration of fruit skin (data not shown) which was consistent with the previous year's observations, although there was a tendency for 1-MCP-treated fruit to develop full yellow color before reaching eating firmness.

Predicting ethylene competition during 1-MCP treatment

In 2011, optimal 1-MCP concentration (ppb) for pear fruit treatments was calculated based on the predicted ethylene concentration (ppb) that would accumulate in the treatment atmosphere multiplied by 3.5. This prediction model is being tested to allow the applicator to consistently extend the shelf life of fruit without locking up the ripening capacity regardless of the harvest maturity and growing district. In 2012, the predicted ethylene production was significantly low (Table 1) and the calculated optimal 1-MCP concentrations were far less than 100 ppb. Since the calculated optimal 1-MCP concentration was too low, we applied 100 ppb as a minimum dose. This optimal 1-MCP treatment was not very effective perhaps due to leakage and absorption of 1-MCP

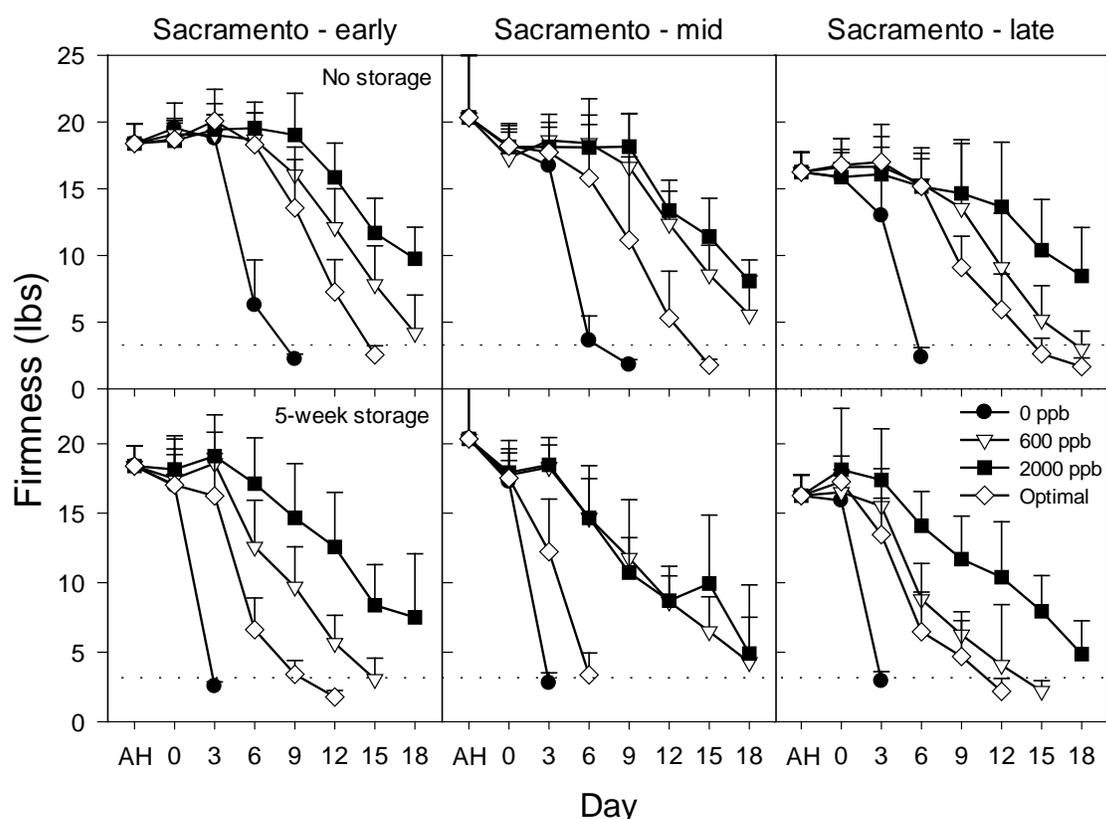


Figure 1. Fruit firmness at harvest (AH) and during ripening at 68°F for 'Bartlett' pears obtained at three stages of maturity (early, mid, late) from a Sacramento packinghouse. Fruit were pre-treated with 0, 600 or 2000 ppb 1-MCP (as SmartFresh™) for 24 hours at 32°F. Additional fruit were pre-treated with an optimal 1-MCP concentration (300 ppb 1-MCP for all harvests in 2013) 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. Data are presented as means ± standard errors.

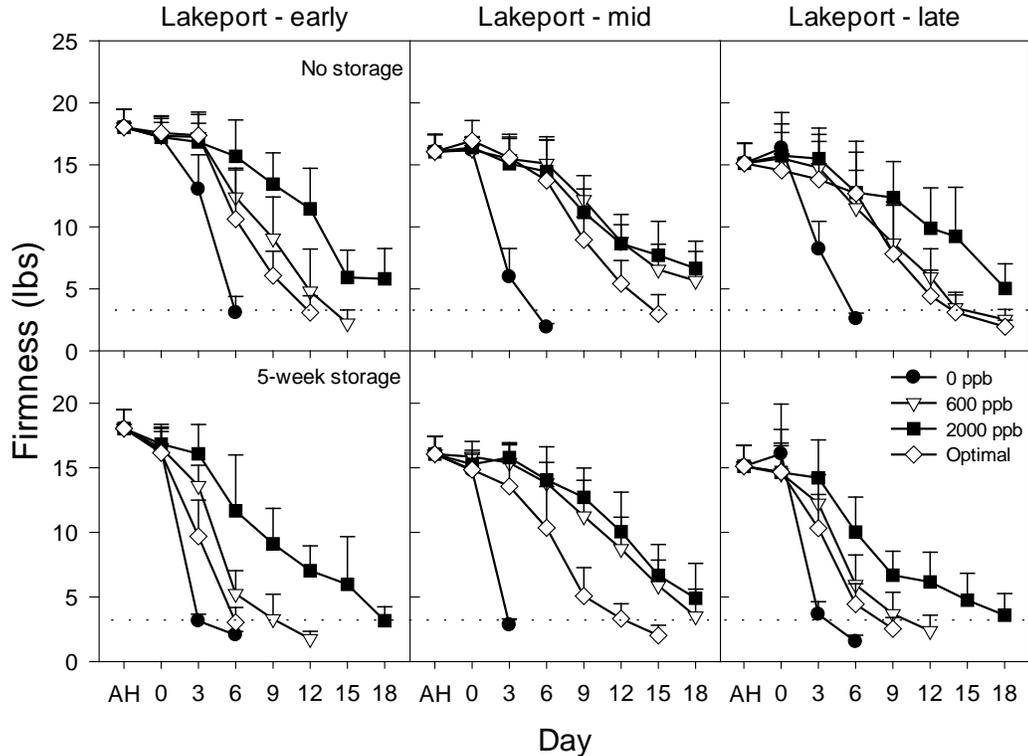


Figure 2. Fruit firmness at harvest (AH) and during ripening at 68°F for ‘Bartlett’ pears obtained at three stages of maturity (early, mid, late) from a Lakeport packinghouse. Fruit were pre-treated with 0, 600 or 2000 ppb 1-MCP (as SmartFresh™) for 24 hours at 32°F. Additional fruit were pre-treated with an optimal 1-MCP concentration (300, 300 and 350 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. Data are presented as means ± standard errors.

by cardboard boxes); therefore, we increased the minimum concentration to 300 ppb for this 2013.

This year’s predicted ethylene production was similarly low as in 2012, except for the fruit harvested from Lakeport late-season which had the highest predicted ethylene concentration. The actual ethylene concentration in the chambers was higher than that from 2012 and was twice as high as the predicted ethylene concentration except for Lakeport late-season fruit.

Fruit treated with the predicted concentration of 1-MCP (optimal) had extended shelf life to 12-15 days for both locations and all harvests (Figure 1). The optimal concentration of 350 ppb applied to fruit harvested from Lakeport late-season maintained a similar firmness as the 600 ppb treatment. For 5-week stored fruit, the optimal 1-MCP

treatment reached ripened firmness after 9-12 days except for fruit harvested from Sacramento mid-season and Lakeport early-season which only extended the days to ripeness to 6 days from 3 days (Figure 2).

Table 1. The predicted and actual concentrations of ethylene produced by ‘Bartlett’ pear 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 3.5 times that of the predicted ethylene concentration. 300 ppb 1-MCP was applied when the predicted 1-MCP concentrations were below 300 ppb. Fruit were obtained at early, mid-, and late-season maturity from Sacramento and Lakeport packinghouses. Numbers in brackets are from 2012.

Harvest maturity	Predicted ethylene concentration (ppb)	Optimal 1-MCP concentration (ppb)	Actual ethylene concentration (ppb)
<u>Sacramento</u>			
Early	9 [8]	300 [100]	38 [9]
Mid	15 [8]	300 [100]	33 [20]
Late	17 [20]	300 [100]	37 [56]
<u>Lakeport</u>			
Early	29 [13]	300 [100]	80 [52]
Mid	24 [67]	300 [246]	50 [50]
Late	100 [12]	350 [100]	131 [41]

Our approach of applying a minimum of 3000 ppb when the calculated optimal 1-MCP concentration was below 300ppb was effective for this 2013. AgroFresh Inc.’s recommendation (verbal communication) to use a minimal dose of 300 ppb to assure a minimal effective treatment dose to delay ripening was consistent with our findings.

Experiment 2: Effect of time after harvest on 1-MCP response

We tested the effectiveness of optimal 1-MCP concentration after one and two weeks of storage at 32°F with fruit harvested from Lakeport. The optimal 1-MCP concentration was determined based on the predicted ethylene concentration multiplied by 3.5, as described previously. The calculated optimal 1-MCP concentration after one week of storage was similar to that at harvest early in the season, but increased by approximately 100 ppb toward the end of the season (Table 2). After two weeks of storage, the predicted ethylene concentration increased significantly for mid- and late-season harvested fruit, therefore, the optimal 1-MCP concentration also increased.

The optimal treatments of 300-455 ppb 1-MCP only delayed full ripeness up to 6 to 9 days which was similar to untreated control fruit (Figure 3). Interestingly, 3.7 ppm 1-

MCP treatment only extended shelf life by 3 days compared to control in mid-season harvested fruit. When 11.4 ppm 1-MCP was applied, which is about 19 times the current registered dose, the fruit extended shelf life by 14 days after two weeks of storage in late-season harvested fruit.

The result shows that the prediction for optimal 1-MCP concentration to compete with ethylene during treatment when the fruit has been stored for more than one week was less accurate than at harvest. A different model may be needed. This suggests that for now, 1-MCP should be applied as early as possible after harvest but more likely within 3 days.

Table 2. The predicted and actual concentrations of ethylene produced by 'Bartlett' fruit that accumulated in 300 L chambers during a 24-hour treatment with 1-MCP at 32°F. 1-MCP was applied after one and two weeks of storage at 32°F at a concentration 3.5 times that of the predicted ethylene concentration. 300 ppb 1-MCP was applied when the predicted ethylene concentrations were below 300 ppb. Fruit were obtained at early, mid-, and late-season maturity from Lakeport packinghouse.

Harvest maturity	Delay (week)	Predicted ethylene concentration (ppb)	Optimal 1-MCP concentration (ppb)	Actual ethylene concentration (ppb)
Early	0	29	300	80
	1	66	300	39
	2	130	455	161
Mid	0	24	300	50
	1	127	445	208
	2	1075	3766	19201
Late	0	100	350	131
	1	124	435	600
	2	3271	11448	4495

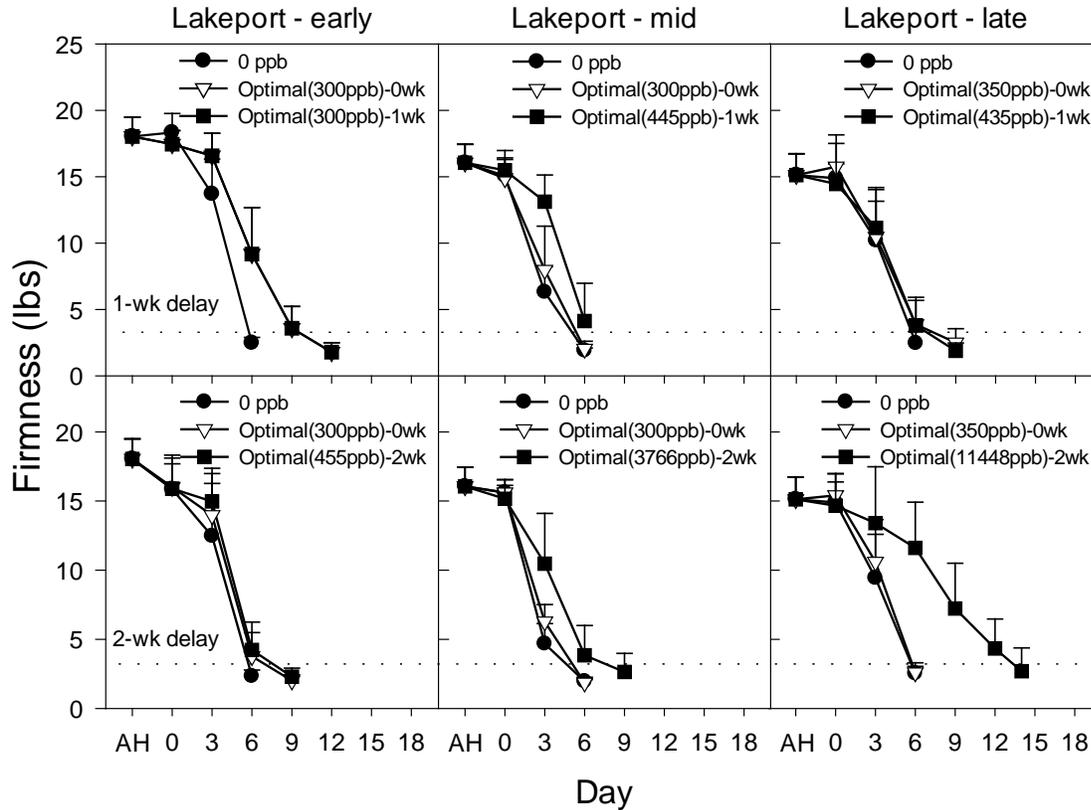


Figure 3. Fruit firmness at harvest (AH) and during ripening at 68°F for ‘Bartlett’ pears obtained at three stages of maturity (early, mid, late) from a Lakeport packinghouse. After harvest, fruit were stored at 32°F for up to two weeks. Fruit were then pre-treated with an optimal 1-MCP concentration after one and two weeks of storage. The optimal 1-MCP concentration was calculated based on predicted ethylene concentration during 1-MCP treatment for 24 hours at 32°F. The optimal 1-MCP concentration after harvest (300, 300 and 350 ppb 1-MCP for early-, mid- and late-season fruit, respectively) was also applied after one and two weeks of storage. Fruit were then warmed at 68°F for shelf life evaluation. The dashed horizontal line represents an eating firmness of 3 lbs. Data are presented as means \pm standard errors.

Experiment 3: Potential application of liquid 1-MCP

Alternative strategies to improve 1-MCP efficacy

In 2012, we evaluated the potential of a liquid 1-MCP formulation, to provide an effective and more convenient mode of postharvest application to ‘Bartlett’ pears than current gaseous treatments. We found that a concentration of 250 ppb was nearly as effective as 1000 ppb (1 ppm) and a dipping duration of only 15 seconds was able to extend shelf life to 12-15 days. Since the fruit response to 1-MCP varies year to year, we tested a similar range of concentrations and duration for 2013.

Fruit treated with the lowest concentration of 250 ppb with 60 seconds were able to maintain firmness at least 18 days which was similar to our findings from 2012 (Figure 4). The treatments over 500 ppb extended the shelf life to a minimum 15 days for all harvests and maturity stages. The lowest concentration of 250 ppb with 15-second dip had a lowest firmness, but was not full ripe until at least 12 days from all maturity stages and locations. In general, the longest dipping duration maintained higher firmness. Interestingly, when lower concentration of 250 ppb was used, fruit dipped for 30 and 60 seconds were firmer than 500 ppb for 15 seconds.

We accomplished all the dipping treatments within an hour from the time creating the 1-MCP solution. We have not tested how long the active ingredient remains in solution and this needs to be tested in the future study.

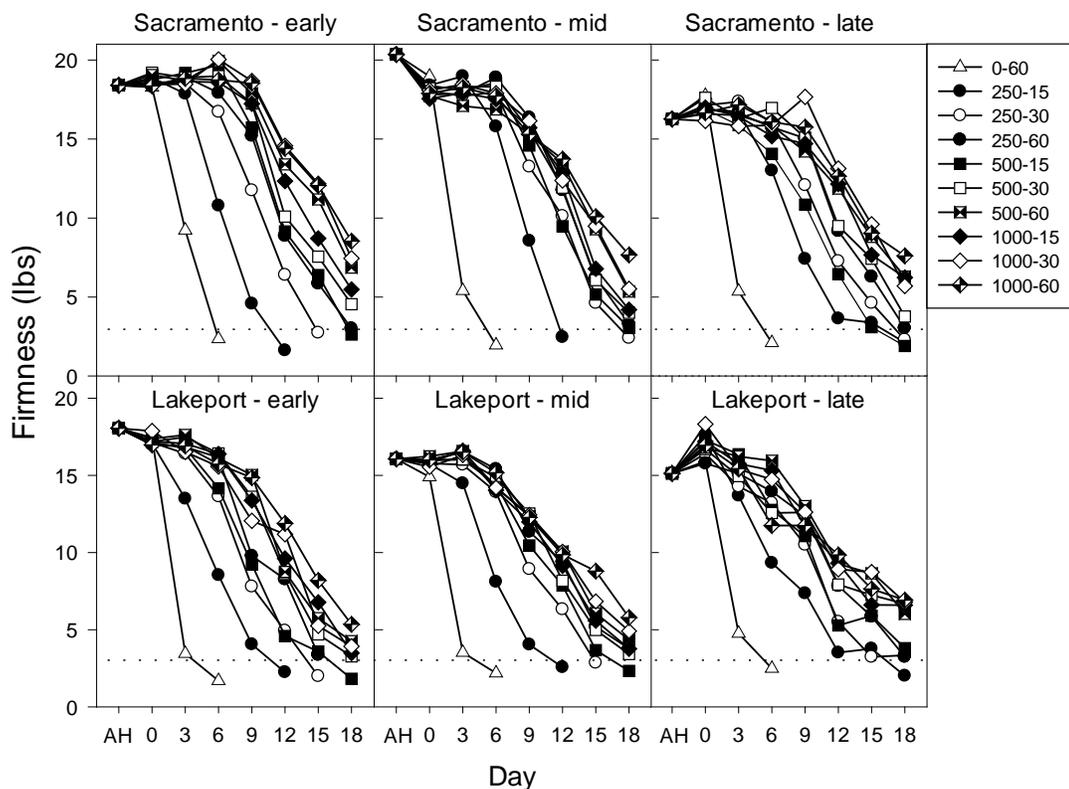


Figure 4. Fruit firmness at harvest (AH) and during ripening at 68°F for ‘Bartlett’ pears obtained at three stages of maturity (early, mid, late) from Sacramento and Lakeport packinghouses. Fruit were dipped in 0, 250, 500, and 1000 ppb (w/v) liquid 1-MCP for 0, 15, 30, and 60 seconds at 85-95°F (outside working temperature) and held at 68°F overnight. All fruit were then exposed to 100 ppm ethylene for 24 hours at 68°F prior to shelf life evaluation. The dashed horizontal line represents an eating firmness of 3 lbs.

CONCLUSIONS

Our findings were consistent with previous findings that 1-MCP treatment had a significant effect in extending pear shelf life. Application of optimized 1-MCP concentration based on the prediction of ethylene concentration that would accumulate in the treatment chamber was able to work well even when the initial fruit ethylene production was extremely low if we set a minimal 1-MCP concentration of 300 ppb for treatment. Fruit treated with the optimal concentration reached full ripeness after 12-15 days compared to 6-9 days for untreated fruit. Delaying 1-MCP application by one week or more after harvest is not recommended due to increased ethylene production and reduced efficacy of our prediction model.

We found that liquid 1-MCP treatments were highly effective in extending the shelf life of pear fruit, comparable to gaseous treatment. The 1-MCP concentrations and the dipping durations need to be tested in different seasons when initial ethylene production is higher to determine the full potential for postharvest applications. The liquid formulation may eliminate concerns about ethylene accumulation during treatment and subsequently reduced treatment efficacy. The liquid formulation is not yet a registered treatment.