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

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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 1-MCP recommendations has been an ongoing challenge to improve fruit quality which varies by season, maturity stages, and the ethylene production.

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 the protocol to determine the optimal 1-MCP treatment based on the prediction of the ethylene accumulation in the treatment atmosphere. During the 2010 and 2011 season, we showed that 1-MCP treatment concentrations of ≥ 3.5 times that of the ethylene production that accumulated in the treatment atmosphere were necessary to extend the shelf life of fruit. Thus, we continued to test our hypothesis for this year whether it may be possible to determine the optimal 1-MCP treatment concentration based on fruit ethylene production levels. Unexpectedly, our results for predicting 1-MCP efficacy based on fruit ethylene production was different from previous years. Liquid 1-MCP showed a lot of promise as a reliable treatment to reduce the rate of pear fruit ripening after harvest.

OBJECTIVES

1. Determine the relationship between harvest maturity and ethylene production rates to identify fruit at risk of not responding to 1-MCP.

2. Determine the efficacy of postharvest liquid 1-MCP (Harvista™) treatments to reduce fruit ethylene production and seek as a potential use in addition to current gaseous 1-MCP benefits.

MATERIALS AND METHODS

Plant material

Mature green ‘Bartlett’ fruit (110-size) were obtained from packinghouses near Sacramento (Greene & Hemly, Inc.) and Lakeport (Scully Packing Co.) in California. Fruit were collected prior to pre-cooling near the day of the first commercial harvest and then every 6-8 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 18, 24, and August 1, 2012 while Lakeport fruit were collected on August 14, 21, and 28, 2012. All fruit were transported to the laboratory on the day of harvest except for those fruit obtained on July 18, 2012 and August 1, 2012 were harvested one day before and delivered within 1-5 hours.

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 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. 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: Potential application of liquid 1-MCP

- Treatment 5: We evaluated an alternative mode of 1-MCP delivery - fruit were dipped in 0, 250, 500, 750, and 1000 ppb (w/v) liquid 1-MCP plus 0.1% NuFilm P surfactant at 85-95°F (outside temperature) for 1 minute.
- Treatment 6: We evaluated the duration of the dip in 250 ppb (w/v) liquid 1-MCP plus 0.1% NuFilm P at 85-95°F (outside temperature). The durations were 0, 15, 30, 45, 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

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 the 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 ripening 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 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 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 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’ 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 both Sacramento and Lakeport packinghouse from all three harvest seasons. Except for the late-season harvest from Lakeport, application of 2000 ppb 1-MCP generally maintained a higher firmness than fruit treated with 600 ppb. This result was different from the 2011 season, for which the treatment of 2000 ppb did not confer additional benefits for fruit over treatment with 600ppb.

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 and 2000 ppb extended the shelf life to 12-15 days and the higher dose of 1-MCP maintained higher firmness for both locations and all harvest stages except for Lakeport mid and late 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 observation, although there was a tendency for 1-MCP-treated fruit to develop full yellow color before reaching eating firmness.

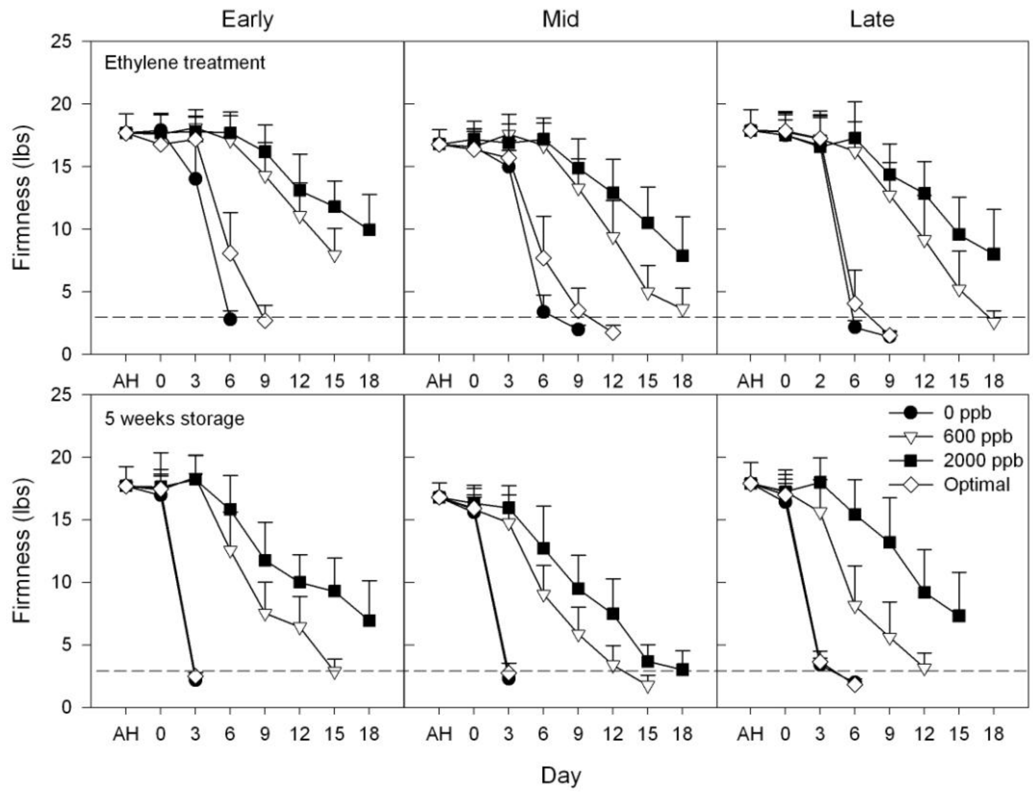

Figure 1. Fruit firmness at harvest (AH) and during ripening at 68°F for 'Bartlett' pears obtained at three stages of maturity (early, mid, late) from a Sacramento packinghouse. Fruit were pre-treated with 0, 600 or 2000 ppb 1-MCP (as SmartFresh^{TM}) for 24 hours at 32°F. Additional fruit were pre-treated with an optimal 1-MCP concentration (100 ppb 1-MCP for all season fruit) 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.

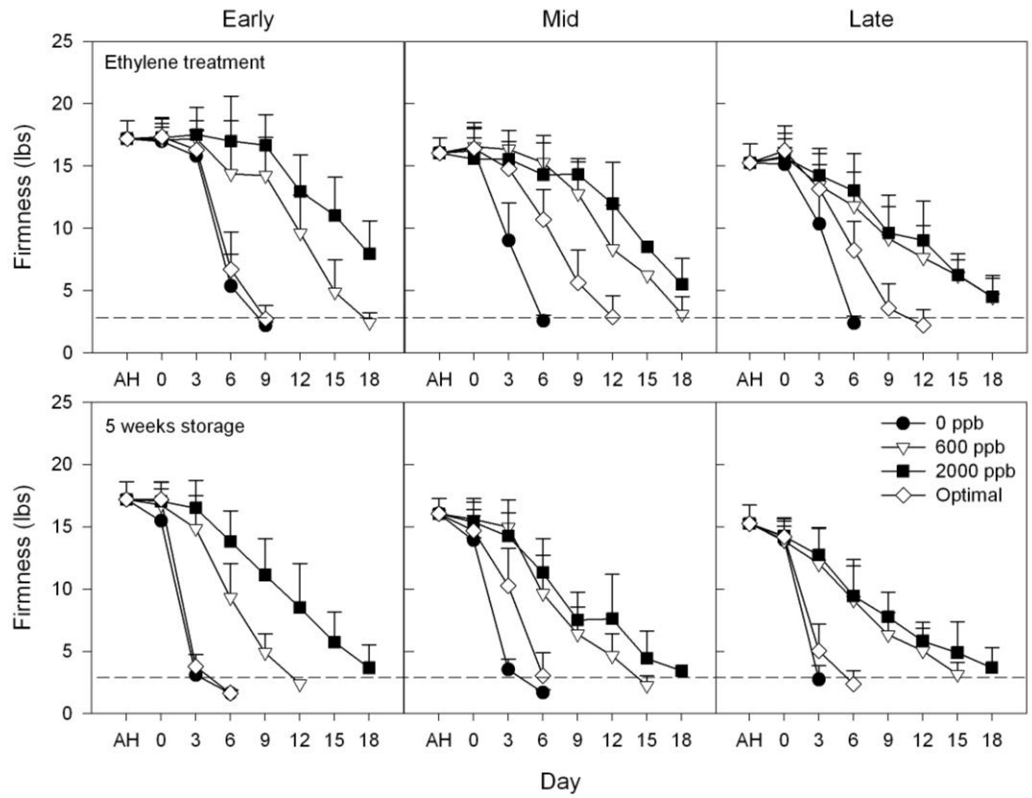

Figure 2. Fruit firmness at harvest (AH) and during ripening at 68°F for 'Bartlett' pears obtained at three stages of maturity (early, mid, late) from a Lakeport packinghouse. Fruit were pre-treated with 0, 600 or 2000 ppb 1-MCP (as SmartFresh^{TM}) for 24 hours at 32°F. Additional fruit were pre-treated with an optimal 1-MCP concentration (100, 246 and 100 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.

Predicting ethylene competition during 1-MCP treatment

In 2011, optimal 1-MCP concentration (ppb) was calculated based on the predicted ethylene production (ppb) that would accumulate in the treatment atmosphere multiplied by 3.5. This theory 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. The previous year's experiment showed that optimal 1-MCP treatment was able to extend shelf life by at least 9 days including 5-weeks stored fruit from all locations and harvest seasons, and was as effective as the 600 ppb treatment.

This year's result was quite different from the previous year. In general, the predicted ethylene production was significantly lower than 2011 (Table 1) and the calculated optimal 1-MCP was far less than 100 ppb. Only the fruit harvested from Lakeport in mid-season had a higher ethylene production that was more similar to the previous year. Since the calculated optimal 1-MCP concentration was too low, we applied 100 ppb as a minimum dose. Interestingly, the actual ethylene production in the chambers was similar to that from 2011.

For Sacramento fruit, only early- and mid-season harvested fruit exposed to the predicted concentration of 1-MCP (optimal) showed extended shelf life by three days relative to control fruit; however, the 5-week stored fruit did not show any effect from the optimal concentration (Figure 1). For Lakeport fruit, there was no effect on early-harvested fruit (Figure 2), but ripening was delayed by 3-6 days compared to control fruit on mid- and late-season harvested fruit, including 5-week stored fruit.

Table 1. 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 at a concentration 3.5 times that of the predicted ethylene competition. 100 ppb 1-MCP was applied when the predicted ethylene concentrations was below 100 ppb. Fruit were obtained at early, mid-, and late-season maturity from a Sacramento and Lakeport packinghouse. Numbers in parenthesis are from 2011.

Harvest maturity	Predicted ethylene concentration in chambers (ppb)	Calculated optimal 1-MCP concentration (ppb)	Actual ethylene concentration in chambers (ppb)
Sacramento			
Early	8 (250)	100 (900)	9 (33)
Mid	8 (90)	100 (300)	20 (34)
Late	20 (36)	100 (200)	56 (53)
Lake			
Early	13 (110)	100 (400)	52 (12)
Mid	67 (63)	246 (300)	50 (46)
Late	12 (80)	100 (300)	41 (61)

The approach of applying optimal 1-MCP may need to be changed if the predicted ethylene production is lower, such as to set the minimal dose when the calculated 1-MCP concentration is below 300 ppb. Based on AgroFresh Inc. recommendation (verbal communication), the minimal dose will be 300 ppb in order to assure a minimal effective treatment dose to delay ripening. However, treatment at 600 ppb resulted in partial ripening in 2010 and strong inhibition of ripening in 2011. The successful optimal treatments which varied between 200-400 ppb in 2011 and the Lakeport mid-season result (246 ppb) suggest that the desired optimal range for 1-MCP concentration maybe in this range when ethylene production is not too high. 1-MCP treatment efficacy appears to be in competition with ethylene action, however, the initial predicted ethylene production may be affected by the year, having lower or high summer temperatures, as well as the maturity stage at the time of the harvest.

Alternative strategies to improve 1-MCP efficacy – application of liquid 1-MCP

In 2011, 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 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 and also largely maintained fruit that were stored for 5-weeks at 34°F. Since the 1 ppm liquid 1-MCP application was so effective, we decided to test using lower concentrations for fruit harvested from Sacramento (3 maturities) and Lakeport early-season. Based these results, we found that 250 ppb was nearly as effective as 1ppm and therefore was used in further analysis, changing the dipping duration for Lakeport mid- and late-season harvested fruit.

All treatments extended the shelf life to 18 days for all harvests from Sacramento and early-season Lakeport fruit (Figure 3). The lowest concentration of 250 ppb had a lower firmness than fruit treated at the higher concentrations, but maintained the shelf life similarly by 18 days. The changes in dipping duration did not seem to have a significant effect. The 60-second treated fruit only extended shelf life by three additional days compared to 15-second treated fruit.

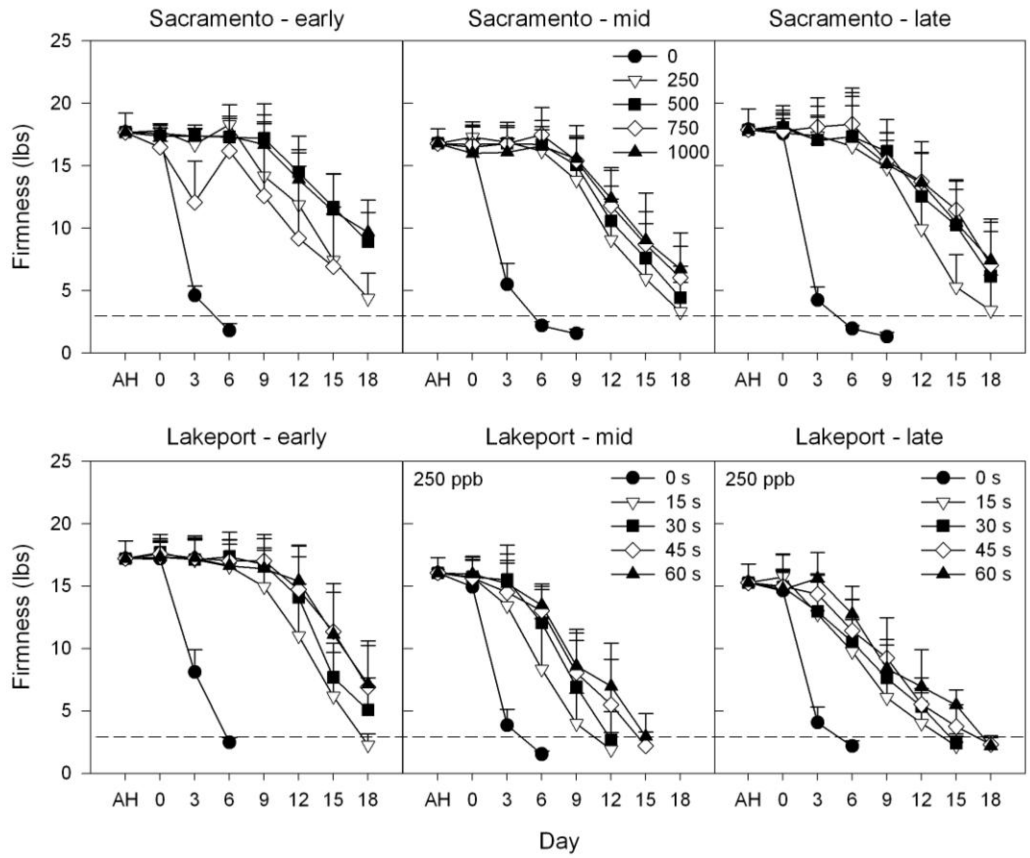

Figure 3. Fruit firmness at harvest (AH) and during ripening at 68°F for 'Bartlett' pears obtained at three stages of maturity (early, mid, late) from a Sacramento and Lakeport packinghouse. Fruit harvested from Sacramento (all three stages) and early-season Lakeport were dipped in 0, 250, 500, 750, and 1000 ppb (w/v) liquid 1-MCP for 1 minute and fruit harvested from mid- and late-season Lakeport were treated with 250 ppb for 0, 15, 30, 45, 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. Data are presented as means ± standard errors.

CONCLUSIONS

Our findings were consistent with previous findings that 1-MCP treatment had a significant effect in maintaining pear shelf life. Application of optimized 1-MCP concentration based on the prediction of ethylene production did not give a good indication when the initial ethylene production was extremely low. Since ethylene production rates vary by harvest seasons, our method can be altered to set a minimal 1-MCP concentration for a better protocol.

We also found that liquid 1-MCP treatments were highly effective in extending the shelf life of fruit, comparable to gaseous treatment. We plan to continue testing 1-MCP liquid preparations for their potential as postharvest applications.