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Project Title: **Use of SmartFresh™ after harvest to improve fruit quality after long-distance shipment and storage**

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Commodity: 'Bartlett' pear

## Problem and Its Significance

1-Methylcyclopropene (1-MCP) is a gaseous inhibitor of ethylene action that delays ripening of pear fruit. Commercial preparations are registered as SmartFresh™ for postharvest application inside sealed rooms or tents, and as Harvista™ for field application to fruit trees. The Harvista™ formulation is still under development and further work was not pursued during 2010. Instead, we continued to focus on 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. In recent commercial trials conducted by AgroFresh, Inc., SmartFresh™ treatment did not consistently delay fruit ripening unless relatively high 1-MCP concentrations were applied. The accumulation of ethylene in the treatment atmosphere during SmartFresh™ application may be a factor that reduces 1-MCP efficacy. Ethylene produced by fruit is known to compete with 1-MCP molecules for binding sites in fruit tissues. This could be a particular problem for late season pears that typically produce higher levels of ethylene at harvest, and may explain the failure of SmartFresh™ treatments to consistently extend the shelf life of fruit at an advanced maturity stage. Given that commercial treatment rooms are often contaminated with ethylene, there is a need to establish the minimum ethylene concentration that reduces 1-MCP efficacy.

## Objectives

1. Establish the relationship between fruit maturity and the accumulation of ethylene in treatment chambers.
2. Determine the influence of ethylene concentration during SmartFresh™ exposure on the capacity of 1-MCP to delay ripening of fruit of different harvest maturity.

**Materials and Methods**

Plant material

Mature green 'Bartlett' fruit were obtained from commercial packinghouses near Sacramento (Greene & Hemly, Inc.) and Lakeport (Scully Packing Co.) in California. Fruit were collected on the day of the first commercial harvest and then every 8-11 days during the season to capture three (early, mid, late) stages of maturity (e.g. 15-20 lbs firmness). Sacramento fruit were collected on July 21, 28 and August 7, while Lakeport fruit were obtained on August 20, 31 and September 8. Fruit were transported to the laboratory by car at 68 °F within 1-2 hours.

Treatments

Fruit were selected for uniform quality and randomly assigned to treatments. They were packed into cardboard boxes and held at 32 °F for 16 hours to equilibrate to treatment temperature. Fruit were then enclosed into 300 L stainless steel chambers at a loading ratio (110 lbs fruit per 300 L volume) consistent with a marine container. Fruit were exposed to different 1-MCP (provided as SmartFresh™) and ethylene concentrations applied simultaneously for 24 hours at 32 °F (Table 1). Following 1-MCP and ethylene treatments, half of the fruit were warmed to 68 °F and challenged immediately with a 24 hour exposure to 100 ppm ethylene at 68 °F. The other half of the fruit were stored at 34 °F for 5 weeks to simulate a marine shipment to South America. After the ethylene challenge or storage treatment, fruit were maintained at 68 °F for shelf life evaluation.

**Table 1.** 1-MCP and ethylene treatment concentrations.

| Treatment No. | 1-MCP (ppb) | Ethylene (ppb) | 1-MCP:Ethylene |
|---|---|---|---|
| 1 | 0 | 0 | - |
| 2 | 600 | 0 | - |
| 3 | 600 | 12 | 50:1 |
| 4 | 600 | 30 | 20:1 |
| 5 | 600 | 60 | 10:1 |
| 6 | 600 | 600 | 1:1 |

Fruit Evaluations

Flesh firmness and skin color were evaluated at harvest and then every 3 days of ripening at 68 °F. Flesh firmness was measured on opposite sides of each fruit after removing a thin slice of skin using a Gűss FTA penetrometer (Gűss, South Africa) fitted with an 8 mm probe. Skin color was measured objectively using a Minolta Colorimeter CR 300 (Osaka, Japan). The change in color from green to yellow was best represented by the hue angle. The internal ethylene concentration was determined for ten replicate fruit at 32 °F by extracting unbound ethylene inside fruit tissues with gentle vacuum. Ethylene concentrations were quantified using a Carle gas chromatograph fitted with a flame ionization detector. Ethylene concentrations inside closed chambers at the beginning and end of each treatment were measured using the same gas chromatograph. We also measured rates of ethylene production and respiration by fruit at harvest and during ripening using the Carle gas chromatograph and a Horiba gas analyzer, respectively, and results will be reported in a subsequent presentation.

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

Sacramento fruit

Early-, mid- and late-season 'Bartlett' fruit ripened rapidly in response to a 24-hour exposure to 100 ppm ethylene after harvest, reaching an eating firmness of 3 lbs in 6, 3 and 3 days, respectively, at 68 °F (Fig. 1). Pre-treatment with 600 ppb 1-MCP for 24 hours at 32 °F reduced the sensitivity of fruit to this ethylene exposure to varying degrees depending upon the fruit maturity (Fig. 1). For early-season fruit, 1-MCP treatment extended the shelf life (time to eating firmness) by 9 days at 68 °F. For mid- and late-season fruit, 1-MCP treatment was less effective, but still extended shelf life by 3 days. The reduction in fruit firmness (Fig. 1) during ripening was accompanied by the typical yellow coloration of fruit skin (Fig. 2).

The inclusion of ethylene in the treatment atmosphere modified the efficacy of 1-MCP in a concentration-dependent manner that also varied according to the fruit maturity stage (Figs. 1, 2). In early-season fruit, increasing the initial concentration of ethylene in chambers from 0 ppb to 12 and 30 ppb (SmartFresh™:ethylene ratio of 50:1 and 20:1, respectively) did not interfere with the capacity of 1-MCP to extend shelf life by 9 days (Figs. 1-3). However, inclusion of 60 ppb ethylene (10 SmartFresh™:1 ethylene ratio) in chambers reduced 1-MCP treatment efficacy slightly, such that shelf life was only extended by 6 days over non-treated control fruit. For mid- and late-season fruit, the inclusion of 12, 30 and 60 ppb ethylene in the chamber did not reduce the benefits of 1-MCP although the delay in fruit ripening of 3 days, even without added ethylene, was modest (Figs. 1-3). A simultaneous exposure to 600 ppb ethylene and 600 ppb 1-MCP (1:1 ratio) completely abolished any benefits of SmartFresh™ treatment for fruit at all maturity stages.

Levels of internal ethylene at harvest remained low for early- and mid-season fruit before increasing considerably in the late maturity stage (Table 2). While ethylene was carefully administered to give the desired SmartFresh™:ethylene ratios at the beginning of treatment, additional ethylene arising from fruit production accumulated in chambers (Table 3). The build-up of ethylene inside chambers was low for early-season fruit, in line with their low internal ethylene levels, and higher for late-season fruit that produced higher concentrations of ethylene. Curiously, ethylene accumulation was greatest for mid-season fruit despite their low initial internal ethylene concentration. Nevertheless, there was a general association between a decrease in 1-MCP treatment efficacy for mid- and late-season fruit with an increase in internal ethylene concentrations and/or ethylene accumulation in the treatment chambers.

We observed slightly diminished treatment responses, as compared to the response at harvest, for fruit that were stored at 34 °F for 5 weeks following the 1-MCP and ethylene treatment combinations (Figs. 1, 2). Non-treated early-, mid- and late-season control fruit ripened rapidly in 6, 3 and 3 days, respectively, at 68 °F following removal from cold storage. The benefits of 1-MCP pre-treatment were largely maintained during cold storage for early-season fruit (Figs. 1, 2). For example, treatment with 1-MCP alone extended the post-storage shelf life by 6 days. This increase in shelf life was not diminished by the inclusion of 12 ppb ethylene (50 SmartFresh™:1 ethylene ratio) in the treatment atmosphere. However, the addition of 30 ppb ethylene (20 SmartFresh™:1 ethylene ratio) in treatment chambers reduced treatment efficacy, and the inclusion of 60 and 600 ppb ethylene (10:1 and 1:1 ratio) completely counteracted 1-MCP benefits. Pre-treatment with 1-MCP did not extend the shelf life of mid- and late-season fruit after storage.

Lakeport fruit

Early-, mid- and late-season ‘Bartlett’ fruit from Lakeport ripened uniformly after a 24-hour exposure to 100 ppm ethylene at harvest to reach eating firmness in 6 days at 68 °F (Fig. 5). Pre-treatment with 600 ppb 1-MCP for 24 hours at 32 °F prior to the ethylene challenge extended the shelf life of early-, mid- and late-season fruit by 6, 9 and 6 days, respectively, at 68 °F. As observed for Sacramento fruit, the reduction in fruit firmness (Fig. 5) during ripening was associated with yellow coloration of fruit skin (Fig. 6).

The efficacy of 1-MCP treatment to extend the shelf life of Lakeport pears was also modified by the level of ethylene inside the treatment chambers and the maturity stage of the fruit (Figs. 5, 6). For early-season fruit, increasing the initial concentration of ethylene in the chambers from 0 ppb to 12, 30 and 60 ppb (SmartFresh™:ethylene ratio of 50:1, 20:1 and 10:1, respectively) reduced the efficacy of 1-MCP slightly whereby the shelf life extension was reduced from 6 to 3 days. For mid-season fruit, which responded strongly to 1-MCP, the addition of 12 and 30 ppb ethylene did not reduce 1-MCP efficacy and increased shelf life by 9 days, while 60 ppb ethylene was sufficient to reduce the shelf life extension to 3 days. For late-season fruit, simultaneous exposure to 12 ppb ethylene did not negate 1-MCP benefits, however the addition of 30 and 60 ppb ethylene diminished treatment efficacy and shelf life was only extended by 3 days. The addition of 600 ppb ethylene to the 600 ppb 1-MCP treatment (1:1 ratio) completely obviated the benefits of SmartFresh™ for fruit at all maturity stages, as reported above for Sacramento fruit.

Internal ethylene at harvest for Lakeport fruit also increased from low levels in early- and mid-season pears to relatively high concentrations in late-season fruit (Table 2). This increase in ethylene production by fruit was translated into a greater accumulation of ethylene within the treatment chambers during SmartFresh™ exposure of late-season fruit as compared to early-season pears (Table 4).

1-MCP treatment effects reported at harvest for Lakeport pears were diminished when green fruit were subsequently stored at 34 °F for 5 weeks and ripened at 68 °F (Figs. 5, 6). Non-treated control fruit at each maturity stage ripened in 6 days at 68 °F following cold storage. Compared to these control fruit, pre-treatment with 1-MCP alone or in combination with ethylene did not extend the shelf life of early- and late-season fruit after storage. However, for mid-season fruit, treatment with 1-MCP alone or in combination with 12 and 30 ppb ethylene (50:1 and 20:1 SmartFresh™:ethylene ratio) continued to extend the post-storage shelf life by 9 days. Inclusion of 60 and 600 ppb ethylene in the treatment atmosphere (10:1 and 1:1 SmartFresh™:ethylene ratio) completely negated the benefits of 1-MCP treatment even for mid-season fruit.

## Conclusions

Our findings highlight the competitive nature of 1-MCP and ethylene for regulating ripening of ‘Bartlett’ pears and, in turn, underscore the importance of monitoring ethylene concentrations in treatment rooms before applying SmartFresh™. We show that a relatively high initial ratio of 1-MCP to ethylene (e.g. 50:1) is required to provide maximum ripening inhibition. Increasing the ratio of 1-MCP to ethylene may be necessary to further improve treatment efficacy, particularly for fruit harvested later in the season that produce higher levels of ethylene. Lowering the 1-MCP to ethylene ratio by adding ethylene to treatment chambers resulted in fruit with intermediate shelf life and this may provide the industry with an opportunity to dial up a range of ripening responses for different shipping and market scenarios. We are planning to develop and evaluate methods to reduce ethylene contamination of treatment areas during the 2011 season as a means to reliably improve 1-MCP treatment efficacy.

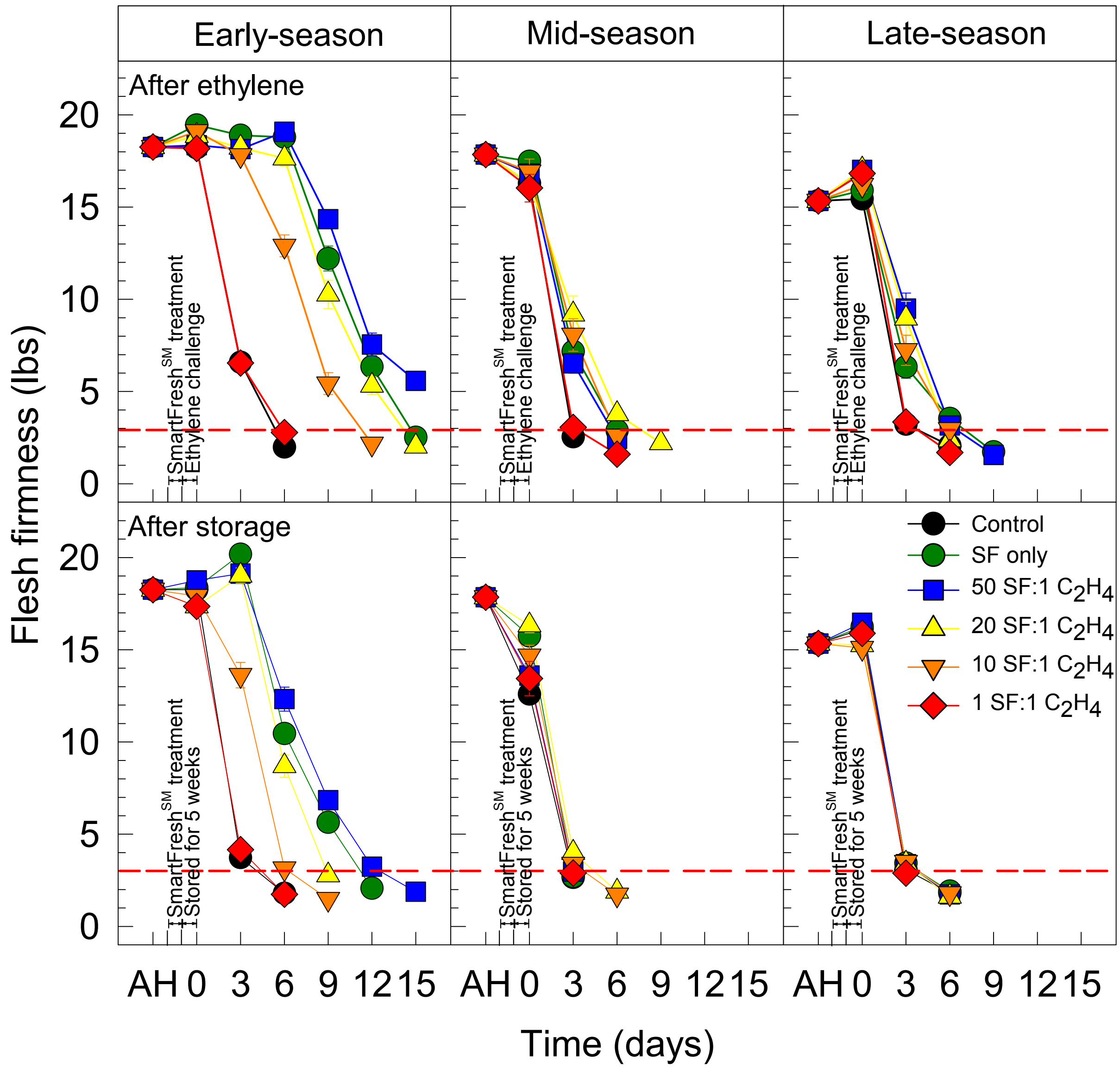


**Figure 1.** Fruit firmness at harvest (AH) and during ripening at 68 °F for 'Bartlett' pears obtained at three stages of maturity from a Sacramento packinghouse. Fruit were pre-treated with 600 ppb 1-MCP (as SmartFresh™) alone or in combination with different concentrations of ethylene (12, 30, 60 and 600 ppb) for 24 hours at 32 °F. Fruit were then challenged with 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.

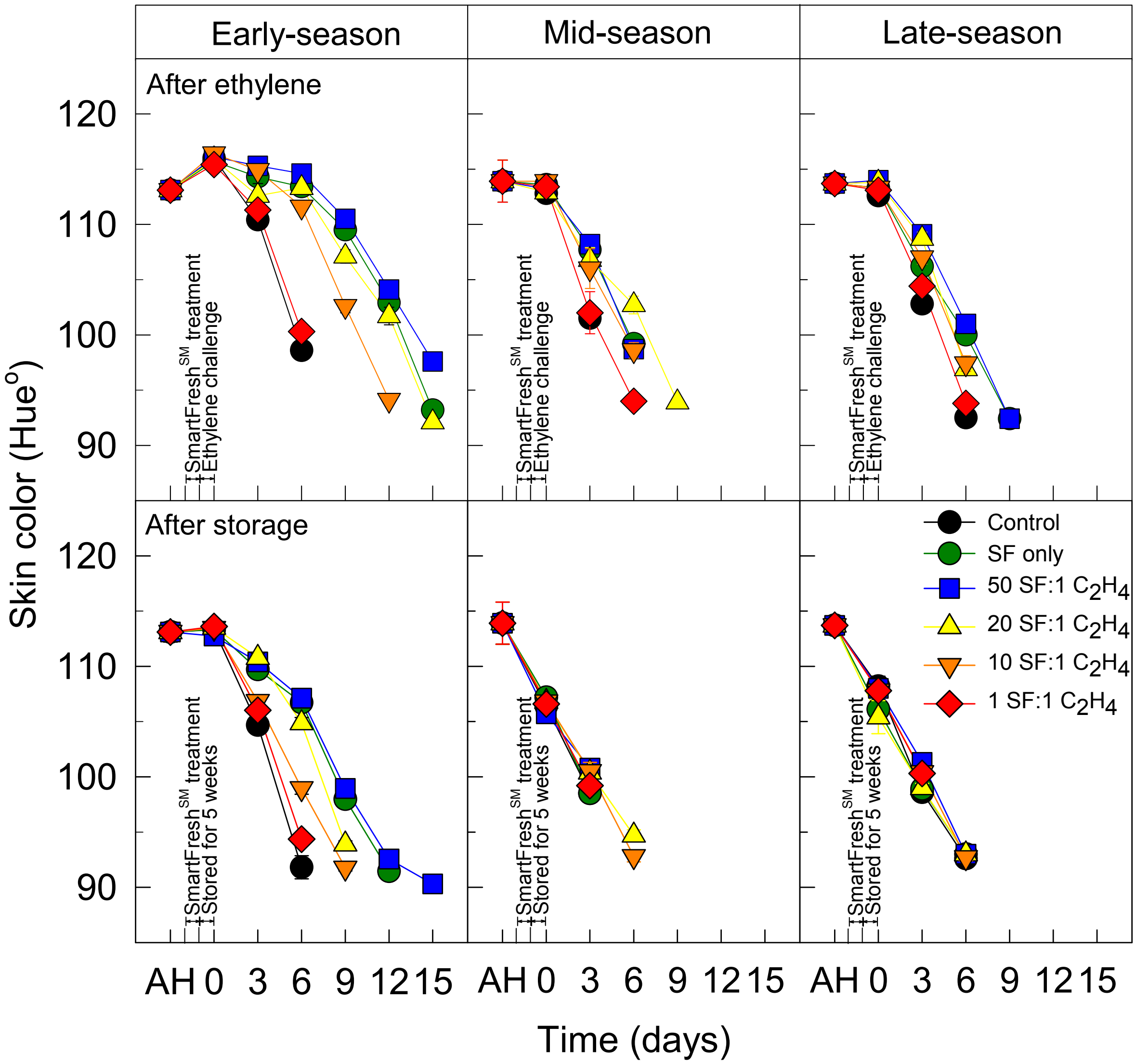


**Figure 2.** Fruit skin color (hue angle decreases as the fruit change from green to yellow) at harvest (AH) and during ripening at 68 °F for 'Bartlett' pears obtained at three stages of maturity from a Sacramento packinghouse. Fruit were pre-treated with 600 ppb 1-MCP (as SmartFresh[TM]) alone or in combination with different concentrations of ethylene (12, 30, 60 and 600 ppb) for 24 hours at 32 °F. Fruit were then challenged with 100 ppm ethylene for 24 hours at 68 °F, or stored for 5 weeks at 34 °F prior to shelf life evaluation.

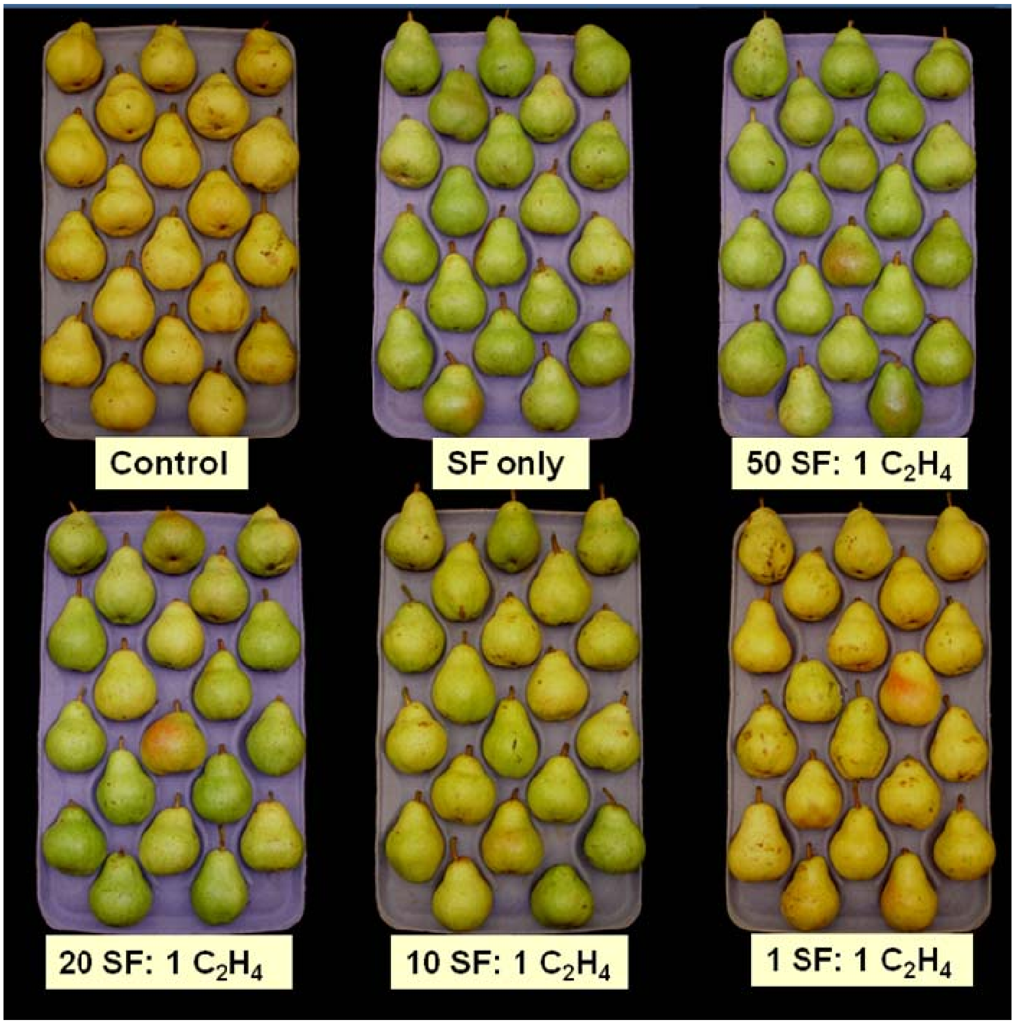


**Figure 3.** Photographs of early-season 'Bartlett' pears from a packinghouse near Sacramento, CA on day 9 of ripening at 68 °F. Fruit were treated at harvest with SmartFresh™ only or in combination with ethylene for 24 hours at 32 °F. All fruit were then challenged with 100 ppm ethylene for 24 hours at 68 °F to test treatment efficacy.

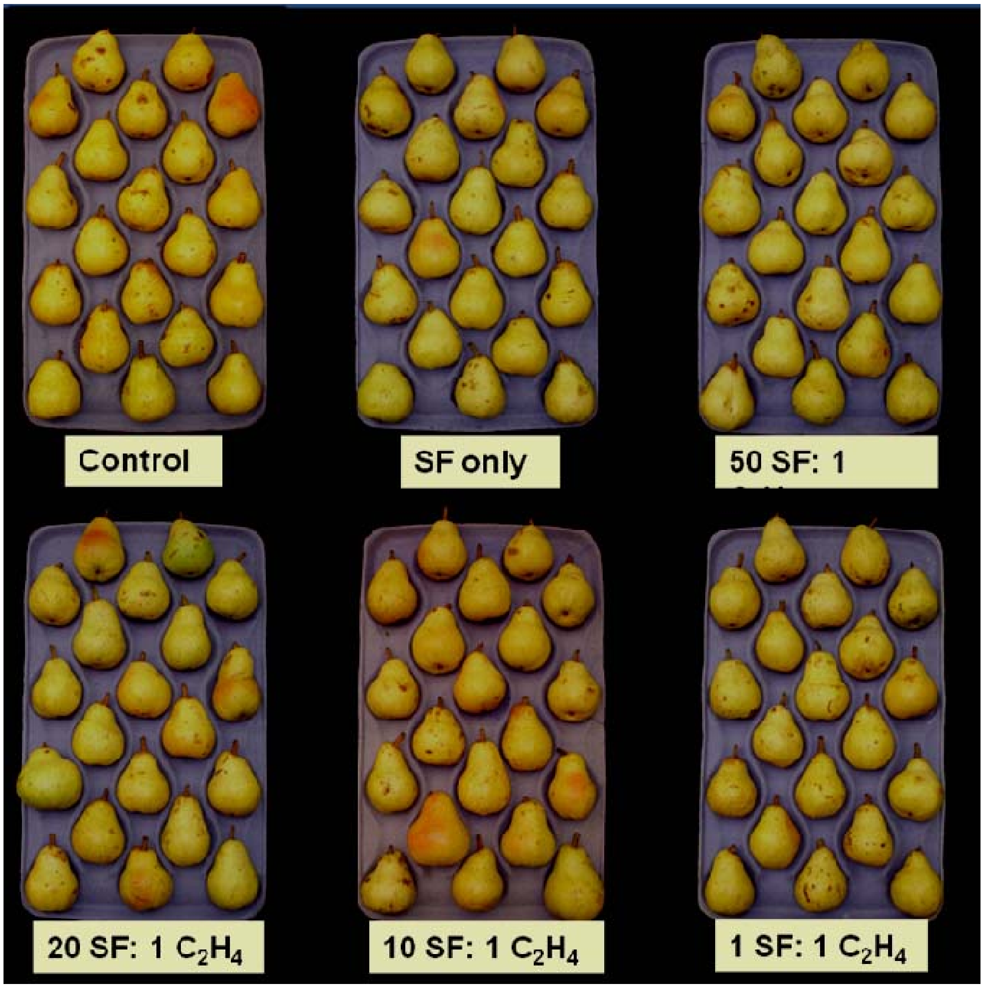


**Figure 4.** Photographs of mid-season 'Bartlett' pears from a packinghouse near Sacramento, CA on day 9 of ripening at 68 °F. Fruit were treated at harvest with SmartFresh$^{TM}$ only or in combination with ethylene for 24 hours at 32 °F. All fruit were then challenged with 100 ppm ethylene for 24 hours at 68 °F to test treatment efficacy.

**Table 2.** Levels of internal ethylene (ppm) at harvest for 'Bartlett' pears obtained at three maturity stages from packinghouses near Sacramento and Lakeport, CA. Fruit were cooled to 32 °F for 16 hours prior to measurement.

| | **Internal ethylene concentration (ppm)** | |
|---|---|---|
| **Maturity stage** | **Sacramento** | **Lakeport** |
| Early-season | 0.04 ± 0.01 | 0.07 ± 0.01 |
| Mid-season | 0.16 ± 0.02 | 0.10 ± 0.02 |
| Late-season | 3.98 ± 1.18 | 1.99 ± 1.13 |

**Table 3.** Ethylene concentrations inside treatment chambers at the beginning and end of 24-hour 1-MCP and ethylene combination treatments at 32 °F. Chambers contained fruit obtained at different maturity stages from a packinghouse near Sacramento, CA.

| **Treatment** | **Ethylene concentration (ppm)** | | | | | |
|---|---|---|---|---|---|---|
| | **Early-season** | | **Mid-season** | | **Late-season** | |
| | **Beginning** | **End** | **Beginning** | **End** | **Beginning** | **End** |
| Control | 0.000 | 0.172 | 0.006 | 1.086 | 0.007 | 0.258 |
| SF alone | 0.000 | 0.056 | 0.006 | 4.200 | 0.007 | 0.224 |
| 50 SF:1$C_2H_4$ | 0.017 | 0.086 | 0.015 | 3.638 | 0.019 | 0.480 |
| 20 SF:1$C_2H_4$ | 0.034 | 0.108 | 0.036 | 8.858 | 0.036 | 0.547 |
| 10 SF:1$C_2H_4$ | 0.072 | 0.173 | 0.064 | 4.540 | 0.067 | 0.688 |
| 1 SF:1$C_2H_4$ | 0.625 | 1.455 | 0.690 | 4.058 | 0.650 | 1.573 |

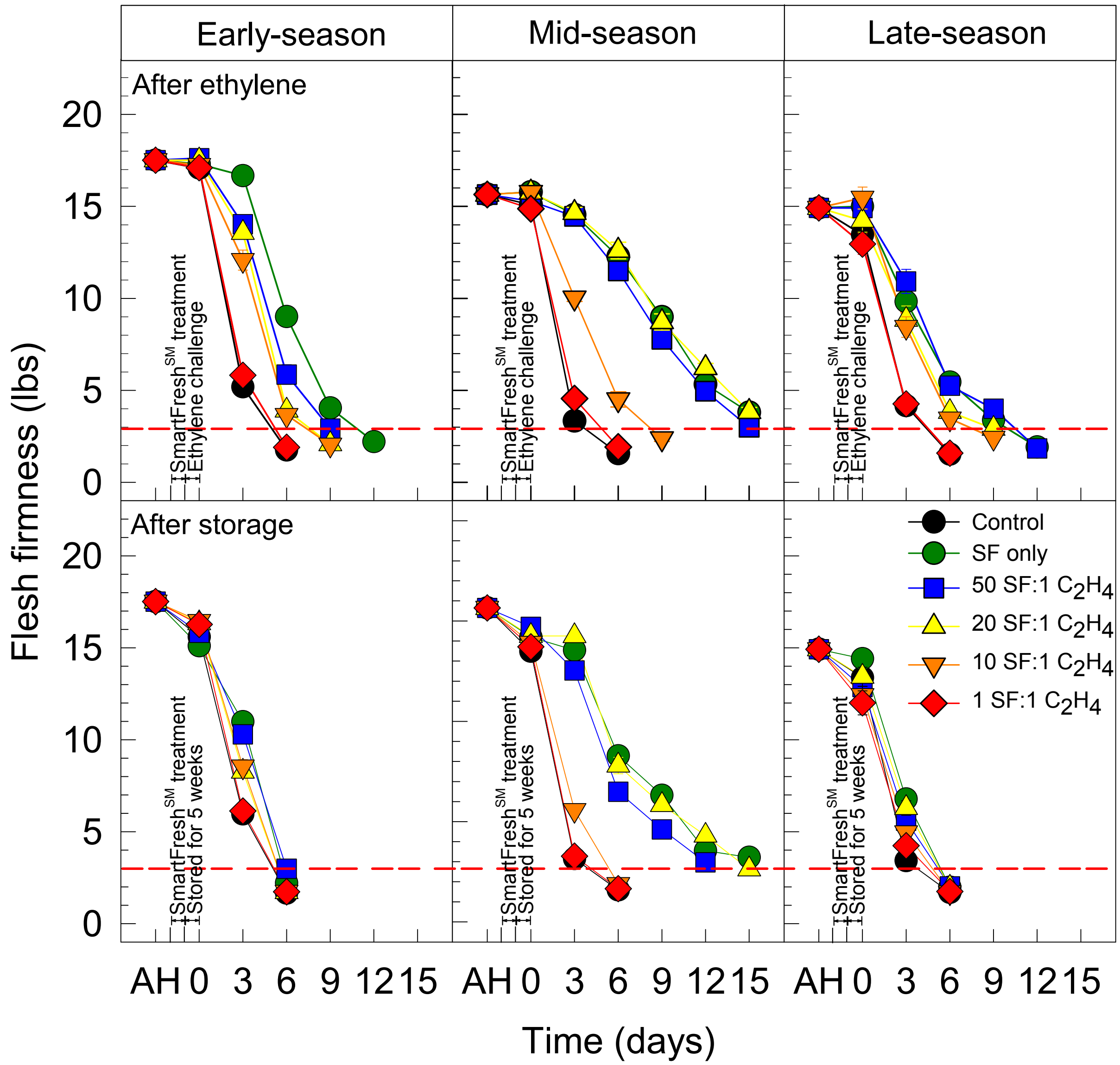


**Figure 5.** Fruit firmness at harvest (AH) and during ripening at 68 °F for 'Bartlett' pears obtained at three stages of maturity from a Lakeport packinghouse. Fruit were pre-treated with 600 ppb 1-MCP (as SmartFresh$^{TM}$) alone or in combination with different concentrations of ethylene (12, 30, 60 and 600 ppb) for 24 hours at 32 °F. Fruit were then challenged with 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.

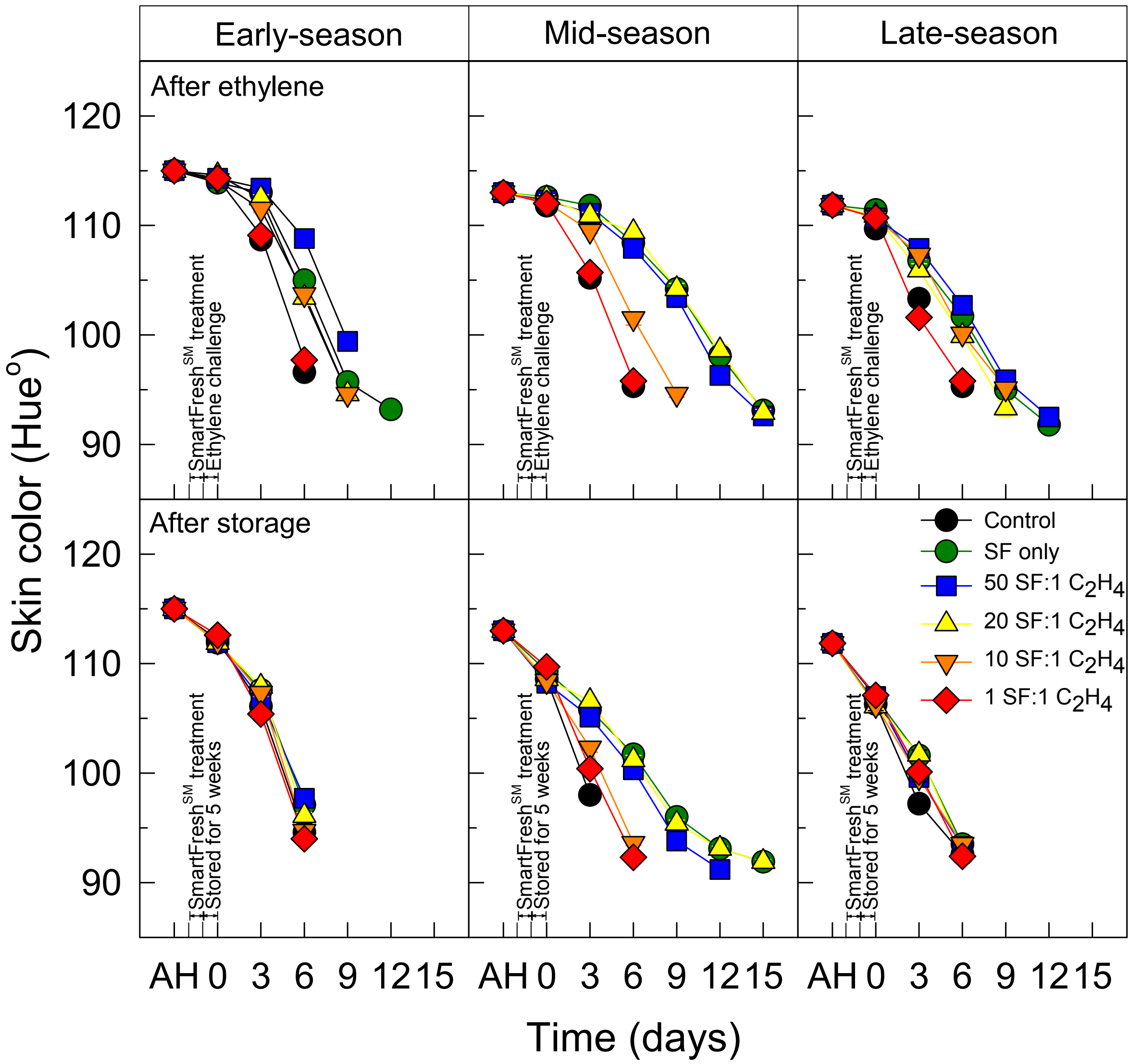


**Figure 6.** Fruit skin color (hue angle decreases as the fruit change from green to yellow) at harvest (AH) and during ripening at 68 °F for 'Bartlett' pears obtained at three stages of maturity from a Lakeport packinghouse. Fruit were pre-treated with 600 ppb 1-MCP (as SmartFresh[TM]) alone or in combination with different concentrations of ethylene (12, 30, 60 and 600 ppb) for 24 hours at 32 °F. Fruit were then challenged with 100 ppm ethylene for 24 hours at 68 °F, or stored for 5 weeks at 34 °F prior to shelf life evaluation.

**Table 4.** Ethylene concentrations (ppm) inside treatment chambers at the beginning and end of 24-hour 1-MCP and ethylene combination treatments at 32 °F. Chambers contained fruit obtained at different maturity stages from a packinghouse near Lakeport, CA.

| Treatment | Early-season | | Mid-season | | Late-season | |
|---|---|---|---|---|---|---|
| | **Beginning** | **End** | **Beginning** | **End** | **Beginning** | **End** |
| Control | 0.000 | 0.084 | 0.000 | 0.072 | 0.009 | 0.256 |
| SF alone | 0.000 | 0.038 | 0.000 | 0.071 | 0.009 | 0.418 |
| 50 SF:1$C_2H_4$ | 0.011 | 0.082 | 0.013 | 0.101 | 0.020 | 0.727 |
| 20 SF:1$C_2H_4$ | 0.028 | 0.148 | 0.034 | 0.090 | 0.037 | 0.175 |
| 10 SF:1$C_2H_4$ | 0.057 | 0.101 | 0.058 | 0.180 | 0.068 | 0.649 |
| 1 SF:1$C_2H_4$ | 0.489 | 0.800 | 0.621 | 1.095 | 0.632 | 1.135 |