FINAL REPORT
WTFRC Project # PH-01-116 **Organization Project #** 5350-43000-003-01T

Project Title: Manipulation of Ethylene for Apple Postharvest Management

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Objectives:
1. Determine treatment conditions (concentration, duration, temperature) to maximize responses of PNW apple cultivars to MCP.

2. Determine duration of responses when treated fruit are stored in RA and CA conditions.

3. Determine impact of MCP treatments on fruit physiological disorders and decay, and develop protocols utilizing existing or new technologies to mitigate or complement the impacts of MCP.

Significant findings (1998-2001):
1. MCP treatment reduces apple ethylene production and respiration rate.
2. MCP effects are influenced by treatment concentration and duration.
3. Responses of PNW apple cultivars to MCP are independent of treatment temperatures up to 70 ^{o}F.
4. High (up to 10%) CO_2 during MCP treatment does not reduce treatment responses.
5. High (100–1000 ppm) ethylene during MCP treatment can reduce treatment responses.
6. MCP gas in apples becomes undetectable 6-8 hours after treatment.
7. Application of MCP under CA conditions is effective.
8. MCP treatment slows apple ripening regardless of post-treatment storage temperature up to 70 ^{o}F.
9. CA storage influences fruit response to MCP.
10. Exposure of MCP-treated apples to ethylene does not stimulate fruit ripening.
11. MCP treatment reduces the rates of firmness and titratable acidity loss but has no effect on soluble solids content or starch degradation.
12. MCP effects on delay of softening exceed the residual effects of CA during a 7 day shelf-life period at room temperature.
13. MCP treatment can reduce or prevent development of a number of physiological disorders.
14. MCP treatment delays production of ripening-related aromas.
15. MCP treatment slows degreening and development of yellow peel color.
16. Watercore can be slower to dissipate in apples treated with MCP.
17. MCP treatment does not prevent CO_2 injury.
18. DPA reduces CO_2 sensitivity of MCP-treated fruit.
19. Application of MCP in commercial CA rooms is effective.
20 MCP can expand the range of harvest maturity acceptable for storage.
21. MCP does not prevent decay from pathogens inoculated into fresh wounds.
22. Apple antioxidant and pigment composition is altered following MCP treatment.

23. MCP does not prevent development of Fuji stain.
24 Repeat application of MCP during RA storage does not consistently enhance efficacy compared to a single MCP treatment at harvest or storage of MCP treated fruit in CA.
25 MCP treatment of hot Golden Delicious apples can enhance development of peel injury during cold storage.
26 The current formulation of MCP is not suitable for field application but can be effective as a postharvest dip.

Results and Discussion (1998-2001):

MCP treatment reduces apple fruit ethylene production and respiration rate

For all cultivars tested (including Ginger Gold, Gala, Jonagold, Delicious, Golden Delicious, Honeycrisp, Granny Smith, Braeburn, Cameo, Fuji, and Pink Lady), ethylene production and respiration rate are reduced following application of MCP. When treated apples are stored in air, ethylene and CO_2 production increases over time, with the duration of MCP effects determined by cultivar, fruit maturity at the time of treatment, and post-treatment storage temperature. The duration of reduced ethylene and CO_2 production is longer for MCP treated apples stored in CA.

MCP effects are influenced by treatment concentration and duration

MCP concentrations above 100 ppb are required for consistent efficacy. All trials conducted at the label rate (1 ppm) have successfully achieved maximum response. The use of rates between 100 and 500 ppb has not consistently provided maximum benefit during long-term storage in air. While trials using CA have demonstrated efficacy at less than the label rate of MCP, more research is necessary to validate the consistency of this response.

Responses of PNW apple cultivars to MCP are independent of treatment temperatures up to 70 ^oF

Trials where apples were treated at a range of temperatures from 32 to 70 ^oF indicate no differences in efficacy due to fruit temperature when MCP concentration and treatment duration are sufficient. Further research is necessary to determine if temperatures greater than 70 ^oF influence apple response to MCP.

High (up to 10%) CO_2 during MCP treatment does not reduce treatment responses (Blankenship)

The effect of elevated levels of carbon dioxide present during MCP treatment was evaluated using Delicious apples. This situation could occur if apples were stored in a poorly ventilated room and respiratory carbon dioxide accumulated prior to MCP treatment. Delicious apples at 32^oF were exposed for 24 hours to: air; 1 ppm MCP; or 3, 5 or 10% CO_2 + 1 ppm MCP. Fruit were stored in RA at 32^oF and sampled after 3, 6, or 8 months. All the MCP treatments resulted in increased firmness, reduced respiration, scald prevention and preservation of titratable acidity. After 6 and 8 months, soluble solids were significantly higher in fruit that had received the higher concentrations of CO_2 during 1-MCP treatment. The presence of carbon dioxide up to 10% during 1-MCP treatment will not affect treatment efficacy.

High (100-1000 ppm) ethylene d**uring MCP treatment can reduce treatment responses (Blankenship)**

Ethylene produced by apple fruit stored in a sealed or poorly ventilated room can accumulate to a high (several hundred ppm) concentration. Because ethylene competes with MCP for a receptor site, this experiment was conducted to determine the concentration of ethylene necessary to overcome a 1-ppm MCP treatment. Delicious apples at 32^oF were exposed for 24 hours to: air; 500 ppm ethylene in air; 1, 10, 100 or 1000 ppm ethylene + 1 ppm MCP. Fruit were stored in RA at 32^oF and sampled after 3, 6, or 8 months. Firmness and respiration were affected by the presence of ethylene during 1-MCP treatment. After 3, 6 and 8 months storage, fruit exposed to 500 ppm ethylene only and 1000 ppm ethylene + 1-MCP were significantly softer and had a higher respiration rate than the other treatments, indicating that 1000 ppm ethylene had overcome the 1-MCP. Treatment effects on firmness, respiration and scald between fruit treated with 1, 10 or 100 ppm ethylene plus MCP were similar. Interestingly, fruit treated with 1000 ppm ethylene + MCP had titratable acidity levels equal to the other MCP treatments after 6 months, and titratable acidity of all MCP treatments was higher than untreated controls. The effect of 1 ppm MCP can be negated if treatments are conducted in the presence of 100 – 1000 ppm ethylene. However, it is unlikely that this much ethylene would be present in a storage room at harvest unless there was ethylene coming from an outside source.

MCP gas becomes undetectable 6-8 hours after treatment (Blankenship)
The objective of these experiments was to see how fast MCP diffuses out of individual apples. Delicious, Golden Delicious and Gala apples were treated with 1 ppm MCP for 24 hours at 32°F. Fruit was removed from the treatment container, placed on paper trays in air at 72°F and internal gas samples taken at timed intervals. The concentration of MCP in the container at the end of the treatment was always less than 1 ppm. Initial fruit MCP readings ranged from 0.05 to 0.3 ppm. Apples treated in sealable plastic sandwich bags simulating a packaging barrier to gas diffusion contained slightly higher concentrations of MCP. MCP in the gas extracted from fruit was undetectable after: 340 min for Gala, 375 min for Delicious, and 450 min for Golden Delicious. Plastic bags made very little difference in the diffusion of MCP out of the apples.
MCP becomes undetectable in the internal atmosphere of an apple after 6-8 hours at 72°F.

Application of MCP under CA conditions is effective.
Gala apples were treated with MCP prior to or following establishment of CA (1% O_2, 2% CO_2). Firmness and TA was similar for MCP fruit treated prior to or after CA establishment when fruit were evaluated 2, 4 or 6 months after harvest. MCP applied after 2 months CA was also effective compared to CA alone. This treatment regime may allow more development of aroma during the early season should fruit in CA be packed prior to treatment with MCP. Delaying MCP treatment for 4 months or more did not result in better fruit quality compared to CA alone.

MCP treatment slows apple ripening regardless of post-treatment storage temperatures up to at least 70 °F.
Apples treated with MCP ripen slower at storage temperatures from 32 to 70 °F. While ripening is slowed over this temperature range, the duration of MCP responses decreases with increased storage temperature. Other factors that determine the duration of MCP responses when treated fruit are stored at warmer temperatures are cultivar and fruit maturity at the time of MCP treatment. While a single MCP treatment at harvest does not substitute for proper temperature management during storage, MCP treated apples ripen slower at warm temperatures and can be expected to retain quality longer during transport and marketing when temperature mishandling occurs.

CA storage influences fruit response to MCP
Application of MCP then storage in CA consistently results in better fruit quality after mid or long-term storage than the use of either technology alone. The MCP/CA response has been observed for all cultivars we have evaluated. Initial research also indicates some potential for CA O_2 concentration and temperature to be higher if apples have previously been treated with MCP. More research is needed to define the range over which these parameters can be altered for various cultivars.

Exposure of MCP-treated apples to ethylene does not stimulate fruit ripening

MCP effects result from irreversible binding to the ethylene receptor in plant tissue. While one of the consequences of MCP binding is reduced ethylene production, exposure to exogenous ethylene is not effective because MCP remains bound blocking access of ethylene to the same site. The diminution of MCP effects over time may be related to generation of new receptors; however, in trials with Gala, exposing MCP treated fruit to ethylene did not overcome MCP effects over a 7 month period.

MCP treatment reduces the rates of firmness and titratable acidity loss but has no effect on soluble solids content or starch degradation

Effects of MCP on fruit quality have been most consistent for reduced firmness loss. In most of our trials, fruit treated with MCP also had higher titratable acidity relative to non-treated controls. These responses are maximized when fruit are treated at a maturity currently recognized as appropriate for long-term CA storage, and then stored in CA. The progression of starch degradation and associated increase in soluble solids content are not altered by MCP treatment.

MCP effects on delay of softening are pronounced during a 7 day shelf-life period at room temperature after removal from storage

Temperature mishandling during transport and retail marketing is a factor limiting fruit quality at retail. Fruit with high quality at the time of shipment can lose several pounds firmness prior to consumer purchase, and poor quality is a factor hindering repeat sales. In studies where quality has been evaluated after 1 or 7 days at room temperature, MCP-treated fruit consistently loses less firmness during the 7 day shelf life period compared to non-treated fruit previously stored in air or CA. This attribute of MCP addresses a problem that has been difficult to solve due to the loss of product control once packed fruit leaves the warehouse. Results for Delicious in particular have been very good, indicating a potential for significant improvement in consistency of quality for this cultivar at retail.

MCP treatment can reduce or prevent development of a number of apple fruit physiological disorders

Incidence of several apple fruit physiological disorders can be significantly reduced or prevented by application of MCP. MCP can prevent development of superficial scald if treatment occurs soon after harvest. This effect results at least in part from reduced production of -farnesene, a compound in apple peel associated with scald development. For Granny Smith and Delicious, delaying MCP treatment 2-4 weeks significantly reduces scald control. Success for control of core flush, soft scald and peel greasiness is also dependent on the duration between harvest and MCP treatment, as well as fruit maturity at the time of treatment. These disorders are more difficult to control in fruit with advanced maturity, particularly control of greasiness in Gala and Jonagold. MCP treatment also reduces development of bitter pit in Braeburn, cracking of Ginger Gold, and internal and external (calyx end) browning of Pink Lady stored in RA or CA.

MCP treatment delays production of ripening-related aromas

Apple fruit ripening is characterized in part by production of many volatile compounds that contribute to aroma. Synthesis of many compounds that impart fruity, ripe aroma is regulated by ethylene. Treatment with MCP has a rapid and pronounced impact on production of these compounds due to the lack of ethylene action. However, production of other volatiles that contribute green, fresh characters is not affected by MCP. The magnitude of MCP-induced inhibition of volatile production is similar to that resulting from prolonged CA storage, however, MCP effects are detectable soon after treatment. The capacity to produce ripe aroma recovers over time in fruit treated with MCP stored in air. MCP treated fruit stored in CA are less likely to resume ripe volatile production, particularly after long-term storage. Although reduced volatile production has been observed for all cultivars evaluated after MCP treatment, the impact on quality is most notable in cultivars where ripe aroma has a relatively larger role in contributing to overall fruit quality (i.e. Gala, Golden Delicious) and less in others (i.e. Granny Smith). It is important to consider the impact of MCP on volatile production in relation to the other effects of MCP on fruit quality. Fruit treated with MCP consistently have better firmness and titratable acidity compared to non-treated fruit. The lack of characteristic ripe aroma in MCP treated fruit early in the storage season is a factor that may impact marketability. However, as the season progresses, comparisons between fruit treated with MCP and non-treated fruit stored in air or CA become based less on aroma due to differences in other quality attributes, particularly firmness. Non-treated fruit stored in air have riper aroma but may be soft, and non-treated fruit stored in CA also have reduced ripe aroma. While the impact of MCP on volatile production has been well characterized, how this impact affects fruit marketability remains to be determined.

MCP treatment slows degreening and development of yellow peel color

Chlorophyll degradation in apple peel is regulated in part by ethylene. In apples treated with MCP, loss of green ground color is delayed but not prevented. The length of delay is dependent on color and fruit maturity at the time of treatment. Effects on peel color have been observed regardless of cultivar. For Granny Smith, slowing degreening is a positive effect, while for other cultivars green ground color may limit marketability. For all cultivars, this effect of MCP is transient and degreening occurs over time. For most cultivars, including Gala and Golden Delicious, development of yellow peel color in MCP-treated fruit proceeds at a faster rate than firmness and titratable acidity loss. This difference eventually results in fruit that appear ripe based on peel color but still have excellent internal quality.

Watercore can be slower to dissipate in apples treated with MCP

Dissipation of watercore is a metabolically active process. As MCP slows fruit metabolism in part by reducing respiration rate, the duration over which watercore may be present has been extended in many of our trials. This effect is most apparent in MCP treated apples stored in air as CA alone also tends to slow watercore dissipation. MCP effects on watercore have not been as consistent as effects on other aspects of fruit quality but we have observed this result frequently and consider it to be an issue worthy of consideration for marketing of susceptible cultivars. As MCP decreases the rate of fruit ripening at elevated temperatures, it is possible that conditioning regimes at higher storage temperatures to accelerate loss of watercore can be developed that minimize loss of fruit quality.

MCP treatment does not prevent CO_2 injury

Development of internal CO_2 injury during storage of Braeburn and Fuji apples is not controlled by prior treatment with MCP. While this type of injury is most common during CA storage, Braeburn apples treated with MCP appear to be more prone to injury even when stored in air. Currently used CA storage protocols for these cultivars utilize a delay in establishment of CA after harvest with or without elevated storage temperature during that period. This is followed by control of CO_2 to 1% or below during CA. Research to date indicates this protocol should continue to be used and MCP treatment should be delayed until shortly before establishment of CA. For Braeburn stored in air, delaying MCP treatment until the end of the preconditioning period also may contribute to reducing the risk of subsequent injury during storage. We have not observed CO_2 injury in Fuji apples treated with MCP then stored in air, even when MCP is applied within 24 hours of harvest. CA storage of MCP treated Fuji and Golden Delicious apples has also resulted in sporadic peel CO_2 injury, indicating the continued necessity of CO_2 control during CA storage of MCP treated fruit.

DPA reduces CO_2 sensitivity of MCP-treated fruit

The use of DPA has been demonstrated to significantly reduce the incidence of internal CO_2 disorders in Braeburn and Fuji apples during CA. Similar results have been observed when Fuji apples treated with MCP were subsequently drenched with DPA. This treatment allows rapid establishment of CA following MCP and DPA treatments, however, CO_2 concentration during CA should still be managed.

Application of MCP in commercial CA rooms is effective
Pilot scale tests of MCP have indicated application of MCP under commercial conditions is effective. We have cooperated in trials conducted at the RCA facility at the Stemilt Growers main plant in Wenatchee as well as in a 1250 bin CA room at the Stemilt hill plant. In all cases, rooms were loaded with bins of apples and MCP treatments applied with the door sealed. During several of these trials a system for sampling room atmosphere was set up prior to loading. Air samples removed during treatment showed MCP gas rapidly equilibrated throughout the rooms. In all cases, measurable differences in fruit quality between MCP treated fruit and non-treated controls were apparent and similar to those observed under small scale lab experiments.

MCP can expand the range of harvest maturity acceptable for storage
Trials conducted using Delicious, Gala and Golden Delicious indicate the range of maturity over which apples can be successfully stored can be expanded if MCP is applied after harvest. Conservative interpretation of the results indicates a shift where maturity now acceptable for mid-term CA could go long-term, short-term to mid-term, and RA either to short-term CA or an extended period in RA. Apples harvested within current guidelines for these storage periods that are treated with MPC typically have superior quality after storage compared to non-MCP-treated fruit. Further research is necessary to further expand this data base to provide ranges for various indices of maturity for these and additional cultivars.

MCP does not prevent decay from pathogens inoculated into fresh wounds
Incidence of decay has been evaluated in all of the MCP trials we have conducted to date. Typically, the number of apples developing decay is very low regardless of treatment, and no consistent differences between MCP treated and non-treated fruit have been apparent. The low incidence of decay may be due in part to the lack of opportunity for fresh wounds to be inoculated with spores of decay organisms. This aspect of postharvest decay can occur in the packinghouse via use of a recirculating drench prior to storage. However, decay of apples wounded then inoculated with P. expansum is not controlled by MCP. Assuming a portion of decay originating during storage results from inoculation of wounds via drenching, the use of MCP without a drench could result in less decay due to the absence of an opportunity for inoculation.

Apple Anti-oxidant and Pigment Composition are altered following MCP treatment
Antioxidant compounds are important apple components that enhance resistance to development of physiological disorders and contribute to fruit nutritive value. Studies with Golden Delicious apples indicate lipid soluble anti-oxidant activity was highest in fruit treated with MCP and stored in CA, and the activity of water-soluble anti-oxidants increased in the order of CA, RA, MCP/RA and MCP/CA. Total phenolic compounds were highest for MCP/CA, followed by CA, then RA and MCP/RA were similar. Analysis of Delicious apple peel indicates MCP inhibits the degradation of compounds responsible for red color including idaein and other flavonoid antioxidants. In peel from apples with a muddy, brown-red color, chlorogenic acid is higher than in non-muddy MCP-treated fruit. MCP treatment results in reduced peel ß-carotene and increased lutein content. Degradation of chlorophyll a and b are enhanced in MCP-treated fruit stored in RA but not CA.

MCP does not prevent development of Fuji stain

Studies using field-run Fuji apples from orchards prone to stain development, or apples receiving a UV light treatment to induce stain indicate application of MCP prior to storage is not sufficient to prevent stain. Previous results indicate CA storage can reduce stain incidence, and MCP treated fruit stored in CA also have less stain compared to non-treated controls stored in air. Delayed cooling has in some trials contributed to reductions in stain development, but MCP treatment followed by delayed cooling does not enhance the effect of delayed cooling for stain reduction. Further research is needed to clarify if ethylene has a role in Fuji stain development.

Repeat application of MCP during cold storage in air does not consistently enhance efficacy compared to a single MCP treatment at harvest or storage of MCP treated fruit in CA
Repeat application of MCP during RA storage of Gala and Delicious apples was conducted at 33 ^{o}F using 1 ppm MCP. Gala apples were retreated at 1, 2 or 3 month intervals and evaluated after 7 months plus 7 days at 70 ^{o}F. Apples retreated with MCP had higher firmness (~ 1 lb) compared to a single treatment at harvest, however, apples treated with MCP then stored in CA (1% O_2, 2% CO_2) had higher firmness (~2 lb) and TA (~0.09%). Retreatment of Delicious apples every 2 or 3 months in RA did not result in improved fruit quality compared to a single treatment at harvest. Storage of MCP treated apples in CA appears to be a better means to enhance fruit quality compared to repeat applications of MCP when fruit is stored at low temperatures.

MCP treatment of hot Golden Delicious apples can enhance development of peel injury during cold storage
Golden Delicious apples subjected to a 30 minute heat treatment in 120 ^{o}F water were then treated with MCP. After two months in air at 33 ^{o}F, MCP treated apples had developed peel injury while non-MCP treated apples had no injury. Symptoms were similar to that observed in early MCP trials on field run Golden Delicious, indicating the potential for injury in Golden Delicious apples from MCP treatment. More research is necessary to determine if injury is the result of fruit temperature at the time of treatment, the duration of heating prior to MCP treatment or if heat source (water, dry heat) is significant. Initial studies using UV/visible light stress prior to MCP treatment have been inconclusive.

The current formulation of MCP is not suitable for field application but can be effective as a postharvest dip
Trials conducted on bearing Delicious trees in the field and blooming Delicious trees in the greenhouse indicate the current formulation of MCP applied preharvest or to trees during bloom has no detectable impact on fruit development or longevity of apple flowers. MCP applied by dipping apples in a water solution is effective but a higher concentration (10 fold) is required to induce responses equivalent to MCP application via a gas treatment.

Overall Summary
Recognition that MCP inhibits ethylene action in plant tissue has provided a means to further characterize the role of ethylene in plant development. For apple fruit, research utilizing MCP has confirmed ethylene action is required for normal ripening and that inhibition of ethylene activity greatly extends the duration of the ripening process. The role of ethylene in development of many apple physiological disorders including superficial and soft scald, core flush and greasiness has become more evident, as has the potential to use MCP to control these disorders. The slowing of apple metabolism impacts pigments and watercore potentially slowing changes in peel color and extending the period of ratable watercore. Delayed production of ripe aroma is a rapidly detectable response to MCP, however, for most cultivars this issue is perhaps not market limiting. Combining MCP with refrigeration and CA results in further control of the ripening process beyond what is achievable using these technologies alone. Susceptibility to CO_2 injury appears to be extended

following MCP treatment indicating acclimation in apple fruit after harvest that results in reduced CO_2 sensitivity is regulated at least in part by ethylene.

Commercialization of MCP (trade name SmartFresh®) will provide an additional tool for the apple industry to manage fruit quality during storage. Its use can be an enhancement, alternative, and/or a new means to manage quality. Results from storage in air or CA can be enhanced by use of MCP. The broader range of acceptable maturity for storage may allow more fruit to be harvested with cosmetics for top grades. MCP can be an alternative method for chemical scald control that also has less residue. Use of MCP provides a means to control ripening should fruit be mishandled during shipping and marketing, a period over which no current control can be employed by packers. These desirable attributes MCP use will need to be balanced by other impacts, delayed aroma, increased potential for CO_2 related disorders, slower color changes and metabolism of watercore are a few. While much is know regarding what to expect from MCP, it would be surprising if widespread commercial use does not identify something as yet undescribed. Research to address known issues associated with the use of MCP and to explore new areas should be expected to continue as there are many questions that remain.

Budget:
Manipulation of Ethylene for Apple Postharvest Management
James Mattheis
Project duration: 1998-2001
Project total – 4 years: **$352,551**

Year	1998	1999	2000	2001
Total	$29,269	$64,550	$132,420	$126,312

Current year breakdown:

Salaries	$26,075	$48,875	$ 93,492	$ 94,430
Benefits	$ 1,994	$13,875	$ 19,428	$ 18,382
Wages	$	$	$ 6,000	$ 6,000
Supplies	$ 1,200	$ 1,800	$ 13,500	$ 7,500
Total	$29,269	$64,550	$132,420	$126,312

Salaries include a full time post-doctoral research associate and a full time research technician.
Supply funds used primarily for purchase of fruit and consumable supplies.
Wages include support for student worker in S. Blankenship laboratory.