FINAL REPORT
WPPC Project # 19906 **Organization Project # 5350-43000-003-008T**

Project title: Manipulation of Pear Fruit Ripening by Control of Ethylene Action
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Objectives:
A primary objective of postharvest management of pears is to prolong storage life by reducing the rate of fruit ripening. The combination of optimum maturity, refrigeration, postharvest chemical treatments and controlled atmosphere (CA) storage slow ripening and reduce physiological disorders and decay. Controlled atmosphere storage in particular reduces ethylene production as well as the capacity of fruit to respond to ethylene, responses that provide the residual effects of CA after fruit is removed from storage. Because of the importance of ethylene in regulating the processes of fruit ripening, practices that interfere with its production and/or action are useful in the commercial storage of pears.

Researchers at North Carolina State University, Dr. Ed Sisler and Dr. Sylvia Blankenship identified a compound that interferes with fruit ethylene metabolism. This compound, 1-methylcyclopropene (MCP), inhibits ethylene perception and shows great potential as a tool for postharvest management of pears. MCP is a volatile compound that can be applied as a gas treatment without drenching fruit. Results with a number of fruit crops have demonstrated MCP treatment can reduce the rate of climacteric fruit ripening as well as development of a number of physiological disorders.

SIGNIFICANT FINDINGS: 2000-2004

- MCP treatment of ‘Bartlett’, ‘Bosc’, ‘Comice’ or ‘d’Anjou’ pears with MCP at concentrations between 0.1 to 1 ppm can delay ripening (softening, acid loss, degreening, volatile production) and development of physiological and pathological disorders. Starch and soluble solids metabolism are not significantly impacted by MCP.

- The duration of ‘Bosc’ responses to MCP have been similar to those of ‘d’Anjou’, ‘Comice’ responses have been intermediate between ‘Bartlett’ and ‘d’Anjou’.

- Factors known to influence the duration of pear responses to MCP applied at harvest include cultivar, MCP concentration, fruit maturity when treated, seasonal variation and post-treatment storage conditions (temperature, atmosphere composition).

- Fruit temperature (32 to 68 ^{o}F) during MCP treatment is not a critical determinant of fruit response.

- Peel degreening is slowed but not prevented by MCP treatment. For both ‘Bartlett’ and ‘d’Anjou’, degreening can occur prior to softening resulting in a false visual assessment of ripeness.

- Production of volatiles typical of ripe pears is delayed following MCP treatment. Volatile production resumes when ethylene production increases.

- Effectiveness of MCP treatment for ‘Bartlett’ decreases with firmness loss ($\leq$ 15 lbs) and as the interval between harvest and treatment increases. For ‘d’Anjou’, treatment delayed 2 weeks or greater may have decreased utility for control of superficial scald.

- Increasing the harvest window for ‘Bartlett’ fruit down to ~15 lbs has been successful when fruit were treated with MCP soon after harvest.

- MCP treatment of commercially packed boxes of ‘Bartlett’ or ‘d’Anjou’ results in typical MCP responses (reduced respiration, ethylene production, slowed loss of quality, delayed onset of decay and development of physiological disorders). However, according to AgroFresh personnel, wet cardboard can absorb MCP and reduce treatment efficiency.

- The duration of MCP responses is dependent on: 1) Storage temperature after treatment. For example, ‘Bartlett’ fruit treated at 0.5 ppm and stored at 68 ^{o}F ripen within one month of treatment. Response duration increases as storage temperature decreases; 2) Storage atmosphere after treatment. Storage in CA prolongs MCP effects. However, the MCP/CA combination can result in ripening delay for both ‘Bartlett’ and ‘d’Anjou’ following removal from CA storage. The delay in ripening after removal from storage decreases with increased storage duration and increased O_2 concentration during storage.

- Effectiveness of MCP treatments applied to partially ripe ‘Bartlett’ or ‘d’Anjou’ fruit is determined by fruit ripeness when MCP is applied. ‘Bartlett’ fruit treated at greater than 13 lbs and 'd’Anjou' greater than 10 lbs have delayed softening compared to controls. Previous storage in CA increases the likelihood of success of ripening delay with a post-storage MCP treatment. The duration of responses to delayed MCP treatment is days to weeks rather than months for fruit treated at harvest and held in cold regular atmosphere (RA) or CA storage.

- MCP treatment applied to 'd’Anjou' pears after > 6 months CA storage does not control development of superficial scald.

- Ethylene treatments up to 10,000 ppm do not result in full recovery of the ripening capacity in ‘Bartlett’ fruit treated with 0.1 to 0.5 ppm MCP.

- MCP treatment does not increase sensitivity of ‘Bartlett’ or ‘d’Anjou’ fruit to CO_2 during CA storage.

- Storage of MCP treated ‘d’Anjou’ at $\geq$3% O_2 with 0.5% CO_2 may allow fruit to be stored long-term (8-9 months) and ripen after removal from storage.
- Incomplete softening (woody, rubbery texture) that can occur in untreated ‘Bartlett’ and ‘d’Anjou’ stored for extended periods in CA can also occur in MCP-treated fruit.

- Low (30-50 ppb) MCP rates slow ‘Bartlett’ ripening and can prevent ‘d’Anjou’ superficial scald with minimal delay of ripening. However, technical limitations of delivering this low rate in commercial rooms and lot to lot variability in response to low rates are factors requiring further investigation.

- Pre-shipment conditioning of MCP-treated fruit by holding at warm temperatures accelerates ripening. The duration of warm temperature necessary to initiate ripening decreases with storage duration.

- Decay development in fruit inoculated via fresh wounds is not delayed by MCP treatment.

Procedures:
Pears were obtained from commercial orchards. Fruit were held at 68-70 or 33 ^{o}F in air or CA until MCP treatments were conducted. The treatments were applied to fruit in sealed steel or plastic chambers at 70 ^{o}F for 12 h.

MCP was generated from SmartFresh powder provided by Agrofresh, Inc, a subsidiary of the Rohm and Haas Company. Target MCP concentrations, monitored by gas chromatography, were reached within 10-15 minutes after initiation of gas generation.

Fruit firmness was measured using a Mohr Digi-Test instrument (Mohr and Associates, Richland, WA). Titratable acidity (TA) was determined by titrating fresh juice to pH 8.2, and soluble solids content (SSC) was measured with a refractometer (Atago). Fruit respiration and ethylene production were determined using gas chromatography.

Fruit visual assessments. Peel color was rated 1:green to 5:yellow. Superficial and senescent scald were rated as 1:none, 2: 1 to 33%, 3: 34 to 66%, 4: 67 to 100% fruit surface with light brown discoloration, 5: 1 to 33%, 6: 34 to 66%, 7: 67 to 100% fruit surface with dark brown discoloration. Internal breakdown and core browning (browning within the core line) were rated as 1: none, 2: slight, 3: moderate, 4: severe. Scuffing was rated as 1: absent, 2: present. Decay was rated as 1: absent, 2: present. Objective measures of fruit color were performed using a Minolta colorimeter.

Results and discussion:

Bartlett 2003-04

B2. Pre-conditioning of MCP treated fruit. 'Bartlett' pears harvested at 18 lbs were treated the day of harvest at 300 ppb MCP. Fruit were stored at 33 ^{o}F in air or CA (1.5% O_2, 0.5% CO_2) with up to 20 days at 32, 50, 60 or 70 ^{o}F after 2 or 4 (RA), 4 or 6 (CA) months, returned to 32 ^{o}F for 14 days, then held at 70 ^{o}F for 1 or 2 weeks. Initial 2003 results indicate fruit stored in RA did not require preconditioning for ripening to proceed. Additional results will be presented at the research review.

B3. Post-storage MCP treatment. 'Bartlett' pears harvested at 19.4 lbs were stored at 33 ^{o}F in air or CA (1.5% O_2, 0.5% CO_2). Fruit were treated with 300 ppb MCP after 1, 3 or 5 months, then held at 68 ^{o}F and ethylene production monitored. Fruit were held an additional 5 days after ethylene production exceeded 1 ppm. MCP treatment after 1 or more months RA storage did not delay ethylene production or ripening, however, MCP treatment after 1 month CA delayed ethylene production 1 day. All fruit were soft (less than 3 lbs) and yellow when analyzed.

B4. Response of MCP-treated fruit to storage temperature and O_2. 'Bartlett' pears harvested at 18.7 lbs were treated with 300 ppb MCP, then stored at 33, 37 or 41 ^oF in air or CA with 1, 3 or 5% O_2 and 0.5% CO_2 for up to 6 months. After 2 months, MCP treatment and storage in air at 32 or 37 ^oF prevented senescent scald and internal breakdown and fruit softened typically in 7 days. Treated fruit stored at 41 ^oF in air developed senescent scald. An interaction between temperature and O_2 concentration was evident. Treated fruit stored in CA at 33 ^oF did not ripen in 7 days regardless of O_2 concentration. Treated fruit stored at 37 or 41 ^oF ripened if O_2 was 3% or higher, all untreated fruit stored under the same conditions were senescent after 7 days.

B5. Lot variability of MCP response. 'Bartlett' pears from 9 grower lots were treated in bins with 300 ppb MCP within 2 days of harvest. Fruit were stored at 33 ^oF in air (2 or 4 months) or CA (1.5% O_2, 0.5% CO_2) for 3 or 6 months. Fruit from each lot was preconditioned by holding at 70 ^oF for 5 days, returned to 33 ^oF for 14 days, then held at 70 ^oF for up to 7 days. After 2 months RA or 3 months CA, MCP treatment effects were evident for all lots, however, fruit softening and yellowing were acceptable after 7 days ripening.

'd'Anjou' 2002-2003

A1. MCP rate study. 'd'Anjou' pears from 3 grower lots were treated the day after harvest with 0, 30, 140 or 280 ppb MCP, then stored in air or CA (1.5% O_2, 0.5% CO_2) at 33 ^oF. All MCP treatments prevented scald and delayed ripening for fruit from 2 of the 3 lots. For the 3rd lot, 140 ppb was necessary to prevent scald as well as delay ripening.

A2. Pre-conditioning of MCP treated fruit (similar to B2 above). 'd'Anjou' pears harvested at 14.3 lbs were treated the day of harvest at 300 ppb MCP. Fruit were stored at 33 ^oF in air with up to 15 days at 70 ^oF after 2, 4 or 6 months, then returned to 33 ^oF for 2 or 4 weeks. The number of days preconditioning necessary to accelerate ripening decreased with storage duration. After 2, 4 or 6 months, the minimum number of days at 70 ^oF to induce ripening after return to cold for 2 or 4 weeks were 10, 5 and 0, respectively.

A3. Post-storage MCP treatment (similar to B3 above). 'd'Anjou' pears previously stored at 32 ^oF in air or CA will be treated at 1 ppm MCP, then returned to cold storage for up to 4 weeks followed by up to 14 days at 68 ^oF . Results indicated fruit treated with firmness of 9 lbs or higher showed 7 to 14 day delay in softening after MCP treatment. Delayed treatments did not prevent superficial scald but did reduce scuffing and delayed degreening.

'd'Anjou' 2003-2004

A2. Pre-conditioning of MCP-treated fruit. Conducted similarly to B2 above, initial results will be presented at the research review.

A3. MCP and CA O_2 concentration. 'd'Anjou' fruit harvested at 13.4 lbs were treated with 300 ppb MCP then stored at 33 ^oF in air or CA with 1, 3 or 5% O_2 with 0.5% CO_2. Fruit will be evaluated after 3, 6 or 9 months. Initial results will be presented at the research review.

A5. Lot variability of MCP response. Conducted similarly to B5 above, initial results will be presented at the research review.

Summary and Conclusions

Experimental use of MCP applied at harvest has consistently resulted in delayed ripening of European pear cultivars including ‘Bartlett’, ‘Bosc’, ‘Comice’ and ‘d’Anjou’. Typical responses include delayed softening, degreening, acid loss, volatile production and development of disorders including senescent scald, internal breakdown and decay. When applied in sufficient concentration close to harvest, MCP can be an effective means to prevent development of superficial scald. Fruit in these trials has also remained resistant to scuffing over an extended period compared to non-treated fruit. For all ripening parameters and disorders with the exception of superficial scald, effects of MCP eventually subside and ripening and senescence progress. When ripening resumes, these processes accelerate but not necessarily at the same rates. For example, degreening can occur prior to softening and production of ripening-related aromas. For ‘Bartlett’, this results in fruit that appears to be ripe (yellow) but has not fully softened and lacks aroma. For ‘d’Anjou’, degreening and softening can be coincident resulting in a ‘Bartlett’ type of ripening where color is indicative of condition.

Table 1. Effects of 1-MCP treatment on quality and ripening of ‘Bartlett’ pear. Fruit treated with 1-MCP at harvest and stored at 32 ^{o}F in air for 4 months. Color ratings are 1: green, 5: yellow. LSD: least significant difference.

	1 day ripe			**7 day ripe**		
1-MCP ppm	**Firmness lbs**	**TA %**	**Color 1-5**	**Firmness lbs**	**TA %**	**Color 1-5**
0	14.0	0.30	4.7	3.1	0.25	5.0
0.030	14.6	0.38	4.9	2.9	0.35	5.0
0.300	15.6	0.34	3.2	10.3	0.31	4.8
1.0	16.5	0.34	3.7	7.7	0.31	5.0
$LSD_{0.05}$	1.3	0.04	0.7	1.3	0.03	0.3

Treatment with MCP at harvest provides the maximum duration of response for all cultivars tested. MCP-treated fruit will resume ripening given enough time, but identification of a process to reliably predict how long a period is required requires further research. Resumption of ripening is accompanied by increased ethylene production and respiration rate, however, ripening of MCP-treated fruit is not accelerated by exogenous ethylene. Trials with ‘Bartlett’ where MCP-treated fruit were exposed to up to 10,000 ppm ethylene did not demonstrate a reversal of MCP effects. Storage temperature, oxygen concentration, MCP concentration, storage duration, harvest to treatment interval, and fruit ripeness when treated all impact the duration of MCP responses. Manipulation of one or more of these factors can impact MCP responses, but all require a high level of management to be successful. Results to date from trials where application rate, harvest maturity, harvest to treatment interval, and storage environment have been examined indicate manipulation of the duration of MCP responses using one or more of these factors is feasible. What remains to be identified are commercial protocols that are consistently effective and provide predictable estimates of the period over which ripening will occur following the preconditioning treatment.

Table 2. ‘Bartlett’ firmness and incidence of disorders following storage plus 7 days at 68 ^{o}F. Fruit were treated with 300 ppb 1-MCP at harvest then stored in air at 31, 33 or 35 ^{o}F.

			Lbs		**TA % malic**		**C-D0 1-5**		**C-D7 1-5**		**CB**	**Decay**	**SB**	**IB**
Month	**Temp**	**Treat**	Mean	SE	Mean	SE	Mean	SE	Mean	SE	%	%	%	%
0		**Initial**	17.2	0.3	0.323	0.014	1.0	0.0			0	0	0	0
1	**31**	**check**	2.6	0.1	0.263	0.007	1.7	0.1	5.0	0.0	40	0	0	0
		MCP	17.5	0.5	0.354	0.014	1.1	0.1	1.6	0.1	0	0	0	0
	33	**check**	2.4	0.1	0.260	0.014	1.5	0.1	5.0	0.0	60	0	0	0
		MCP	17.9	0.5	0.342	0.010	1.1	0.1	1.7	0.1	0	0	0	0
	35	**check**	2.3	0.2	0.233	0.014	1.8	0.2	5.0	0.0	25	0	0	0
		MCP	15.1	0.5	0.309	0.011	1.4	0.1	2.1	0.1	0	0	0	0
3	**31**	**check**	3.4	0.5	0.246	0.010	3.5	0.2	4.6	0.1	75	30	0	0
		MCP	16.8	0.3	0.322	0.010	2.2	0.2	2.8	0.1	0	0	0	0
	33	**check**	4.5	1.3	0.187	0.004	3.7	0.1	4.2	0.1	0	0	0	0
		MCP	12.8	0.6	0.275	0.005	2.3	0.1	3.3	0.1	0	0	0	0
	35	**check**	.	.	.	.	4.0	0.0	.	.	100	100	90	.
		MCP	8.3	0.7	0.287	0.007	3.4	0.1	4.3	0.1	0	0	0	0
5	**31**	**check**	.	.	.	.	.	.	.	.	.	100	90	.
		MCP	10.2	0.8	0.267	0.006	2.8	0.1	4.9	0.1	0	0	0	0
	33	**check**	.	.	.	.	.	.	.	.	.	10	90	0
		MCP	7.2	0.8	0.244	0.007	4.8	0.1	5.0	0.1	0	20	0	60
	35	**check**	.	.	.	.	.	.	.	.	.	.	100	.
		MCP	9.7	2.5	0.202	0.010	5.0	0.0	5.0	0.0	0	10	0	90

TA: titratable acidity; C-D0: color when removed from storage (day 0); C-D7: color 7 days after removal from storage; CB: core browning; SB: senescent breakdown and scald; IB: internal breakdown; SE: standard error of the mean.

Preconditioning prior to shipment is currently practiced using ethylene to stimulate ripening. Research conducted over the past two seasons indicates periods at higher than typical storage temperatures can result in accelerated recovery of the capacity to ripen in MCP-treated fruit. While these experimental results indicate the possibility of development of commercially useable protocols, more information is needed to assess the consistency of preconditioning protocols between lots and production seasons.

Table 3. Firmness of ‘d’Anjou’ pear fruit following preconditioning at various temperatures. Fruit were treated at harvest with 300 ppb 1-MCP, then stored at 32 ^{o}F in air for 4 months. Pears were then stored for 5 days at condition temperatures, then returned to 32 ^{o}F for 2 or 4 weeks. Fruit were then held at 68 ^{o}F for 10 or 20 days.

	Firmness lbs				
Conditioning temperature °F	**after 5 days conditioning**	**+2W 32 °F + 10D 68 °F**	**+ 2W 32 °F + 20D 68 °F**	**+ 4W 32 °F + 10D 68 °F**	**+ 4W32 °F + 20D 68 °F**
32	12.5	11	7.5	10.7	5
41	13.5	10	5	6.2	3.8
50	11	5	2.8	3.3	2.2
59	12	5	1.7	3.2	-*
$LSD_{0.05}$	ns	1.5	1.8	1.4	1.3

*Non-marketable fruit due to senescent breakdown and decay.

Table 4. Firmness of 'Bartlett' pears following 2 months storage plus 7 days at 68 °F. Fruit were treated at harvest with 300 ppb MCP, then stored in air or CA at temperatures and atmospheres indicated. Values are means (n=20), where IB (internal breakdown) or S.Scald (senescent scald) are indicated >75% of

Temp F	O_2/CO_2	check	MCP
32	RA	1.9	5.8
	1/0.5	2.4	15.6
	3/0.5	2.0	16.6
	5/0.5	1.7	13.4
37	RA	IB	2.5
	1/0.5	IB	13.7
	3/0.5	IB	5.8
	5/0.5	IB	8.9
41	RA	S.Scald	S.Scald
	1/0.5	IB	10.5
	3/0.5	IB	3.4
	5/0.5	IB	2.3

Development of decay has been consistently delayed in our trials. While it is evident that MCP applied to field run fruit can delay development of decay, the key word is delay. In all of our trials, marketability of MCP-treated fruit is eventually limited in part by decay. Measures to control decay will continue to be necessary for fruit that is stored after treatment. Results to date indicate similar challenges for use of TBZ, that being the causal organism typically changes from *Botrytis* (grey mold) to *Penicllium* (blue mold) when TBZ has been used. Based on work with the yeast *Cryptoccus laurentii,* MCP appears compatible with this and presumably similar biocontrol agents.

Experiments conducted in the fall of 2003 included application of MCP in research scale CA rooms at the Stemilt RCA facility. Fruit in bins were treated and responses similar to those of small scale laboratory applications have been observed. While these results demonstrate efficacy of MCP for application to fruit in bins, a number of MCP trials in large CA rooms conducted over the past 2 years have failed. There are a number of possible explanations for these failures and identifying what factor or factors are responsible for non-performance when MCP is applied in large commercial rooms requires further research.

MCP is a powerful tool for manipulation of the fruit ripening process. Its availability to the research community allows investigations of fruit development and ripening to be conducted that previously were not possible. However, pear quality at the point of consumption requires a soft, juicy texture, typical aroma, with an attractive appearance. The challenge to use MCP commercially is to 1) have efficacy, and 2) have predictability of ripening. Based on our experience to date, both of these challenges appear to be achievable but may require a level of management beyond what is currently practiced by the fresh pear industry.

Publication:

Argenta, L.C., Fan, X., and Mattheis, J.P. Influence of 1-methylcyclopropene on ripening, storage life and volatile production by 'd'Anjou' cv. pear fruit. J. Agric. Food Chem. 51:3858–3864. 2003.

Project title: Manipulation of Pear Fruit Ripening by Control of Ethylene Action
PI: James P. Mattheis
Project duration:: 2002-2003
Project total (2 years): $54,238

Year	Year 1 (2002)	**Year 2 (2003)**
Total	$26,075	**$28,163**
Current year breakdown		
Salaries[1]	$17,904	**$19,664**
Operations (lab supplies, fruit)	$ 2,800	**$ 2,600**
Employee benefits	$ 5,371	**$ 5,899**
Total	$26,075	**$28,163**

[1]GS-9 biological science technician, 0.5 FTE

This project was started under ARS Project #5350-43000-003-06T in 2000. Due to administrative contract changes it is now under ARS Project #5350-43000-003-08T. Funding for this project has also been received from AgroFresh, Inc., $25,000 per year.