FINAL PROJECT REPORT

Project Title: Apple *ACS3* genotypes and fruit ripening

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Budget:

Organization: USDA, ARS		**Contract Administrator:** Charles Myers, Extramural Agreements Specialist
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Item	**Year 1: 2009-2010**	**Year 2: 2010-2011**
Salaries*	$25,000	26,000
Benefits	9,000	9,000
Wages		
Benefits		
Equipment		
Supplies	11,000	11,000
Travel	1,500	1,500
Miscellaneous	1,500	1,500
Total	48,000	49,000

*0.5 FTE GS9 Postdoctoral Research Associate
Supplies include common molecular biology reagents and fruit from commercial orchards.

Other funding sources

Agency Name: AgroFresh
Amt. awarded: $35,000 for the second year study
Notes: Funds cover 0.5 FTE GS9 Postdoctoral Research Associate

OBJECTIVES

1. Characterize apple fruit ripening characteristics including ethylene evolution for 6-10 cultivars at defined developmental stages.
2. Investigate cultivar-specific fruit softening rate and ethylene regeneration in fruits treated with 1-MCP.
3. Examine expression patterns of *MdACS3* and other ethylene biosynthesis and perception related genes during fruit ripening and in response to 1-MCP treatments (including Harvista on-tree spraying).
4. Explore the potential polymorphism at the *MdACS3* locus for potential functional molecular marker generation, based on gene expression results.

SIGNIFICANT FINDINGS

1. During the 8-week period of apple fruit maturation (from -6 to +2 weeks with physiological maturity as 0 stage), considerable variation in the expression levels of *MdACS3* were observed among 14 elite apple cultivars and breeding parents.

2. Two expression patterns of *MdACS3* were identified in apple fruit peel tissues: pattern A showed higher expression level with progressively-increased patterns, and pattern B exhibited lower expression level with a transient peak.

3. Higher expression of *MdACS3* usually correlated with early ripening cultivars; and lower expression of *MdACS3* usually correlated with late ripening cultivars.

4. The two *MdACS3* expression patterns were also correlated with "on-tree fruit firmness retention" during last 6 weeks before harvest, i.e. the early-ripening cultivars showed larger decrease and late-ripening cultivars had smaller decease in fruit firmness.

5. Postharvest 1-MCP treatment did not suppress *MdACS3* expression level, instead a slight increase was observed for most cultivars; the suppression on *MdACS1* expression was apparent for most cultivars for at least three months after 1-MCP treatment and stored in cold room.

6. Correlation between cultivar-specific *MdACS3* expression patterns and 1-MCP treatment efficacy was weak. However, early-ripening cultivars showed a greater average "fruit firmness retention" (difference in firmness between 1-MCP treated fruit and non-treated controls) than that of late-ripening cultivars.

7. The effect of pre-harvest application of spraying-formula of 1-MCP, i.e. Harvista® on 'Golden Delicious' showed increased firmness and suppressed ethylene production, compared to non-sprayed control. But the effect is still less effective than postharvest 1-MCP treatment.

8. In addition to stimulation of *MdACS3* and suppression of *MdACS1* gene expression (as mentioned above), the suppressed expression of all major ethylene receptor genes by Harvista® was also observed during cold storage.

METHODS

1. Physiological characterization of apple fruit maturation/ripening:

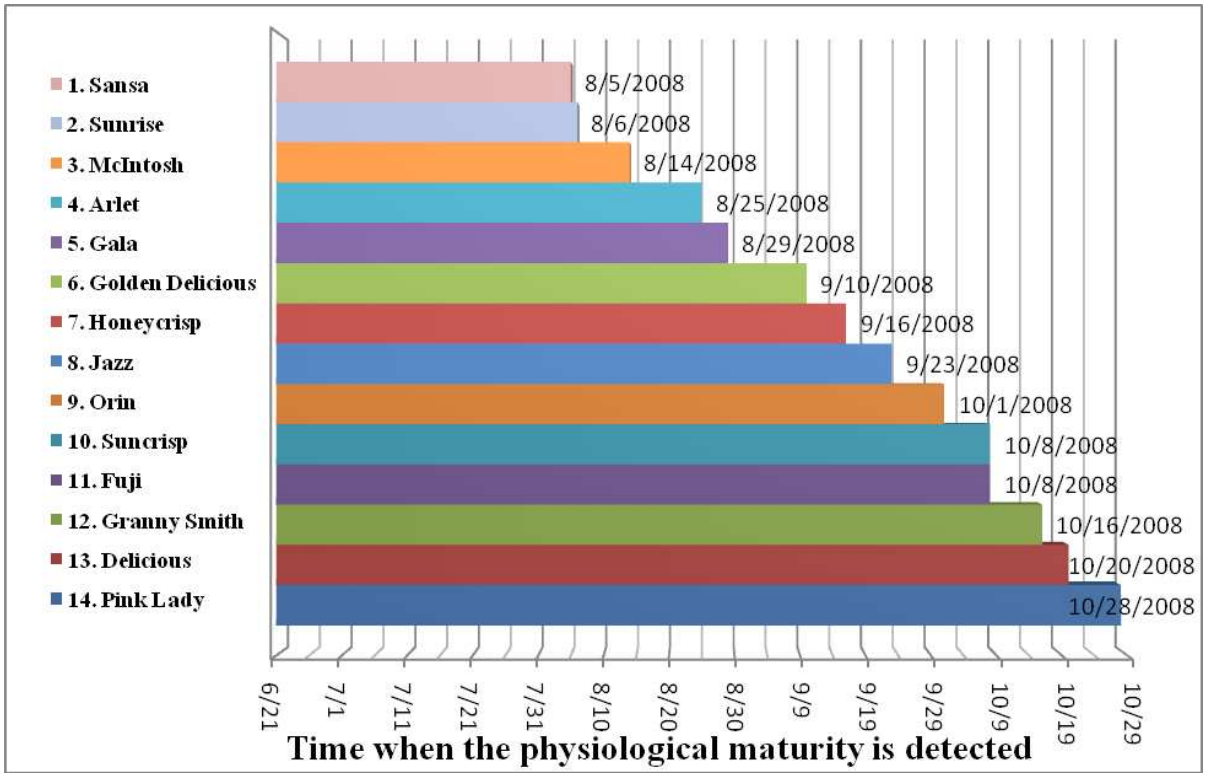
Fruit from 14 apple (*Malus* × *domestica* Borkh.) cultivars, from commercial orchards or experimental orchards in central Washington State, were subjected to systematic characterization of fruit maturation and ripening processes, starting 2 months before projected physiological maturity. Physiological maturity (stage 0) for each cultivar was retrospectively assigned based on the sample with starch staining index close to 3.5 based on 1-6 scale. Fruits harvested at stage 0 will be treated with 1-MCP then stored in air at 33^{o} F.

2. Gene expression analysis for MdACS3 and other genes encoding ethylene receptor and signaling pathway:

Peel tissue were collected and used for total RNA isolation, followed by DNase I digestion, RNA cleanup and cDNA Synthesis. Quantitative Real-Time PCR using SYBR Green I dye were employed for gene expression analysis, including –RT (no reverse transcriptase), no template control (no cDNA) and an actin gene as internal reference genes. Quantitative PCR reaction will be repeated twice with two independent cDNAs. Target gene expression will be normalized to that of the reference actin gene and analyzed by $2^{-\Delta\Delta CT}$ method.

RESULTS AND DISCUSSION

1. A total of 14 cultivars with various ripening date were included in the study. The date at the end of the bar indicates the time physiological maturity is detected for this cultivar. The ripening dates for these cultivars span from early August to late October.



2. Two observed exxpressionpatterns of MdACS3 among 14 cultivar investigated.

Figure 2A. Cultivars with the pattern of high expression level and progressively-increased, including ‘Golden Delicious’, ‘Sunrise’ ‘Honeycrisp’, ‘Jazz’, ‘Gala’, and ‘Arlet’.

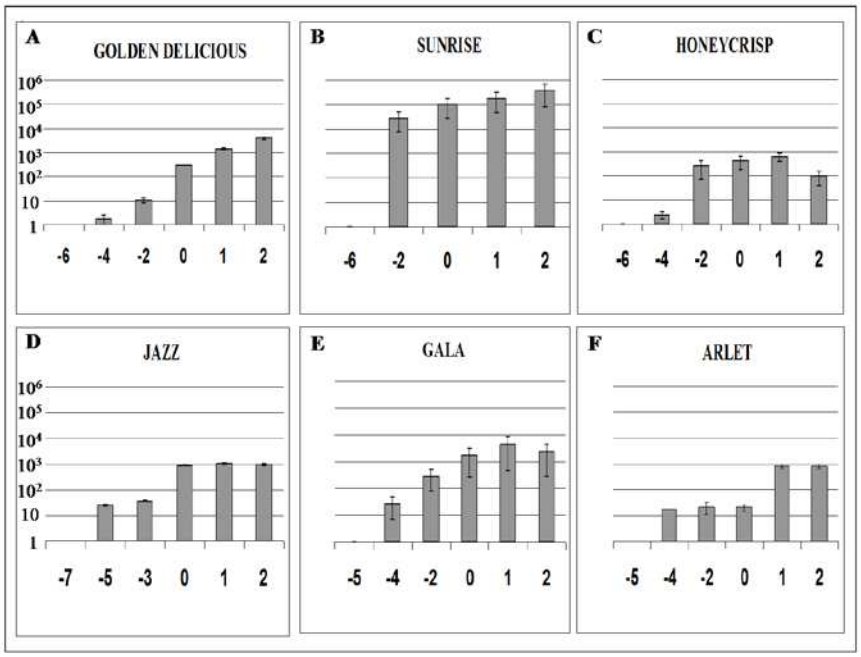
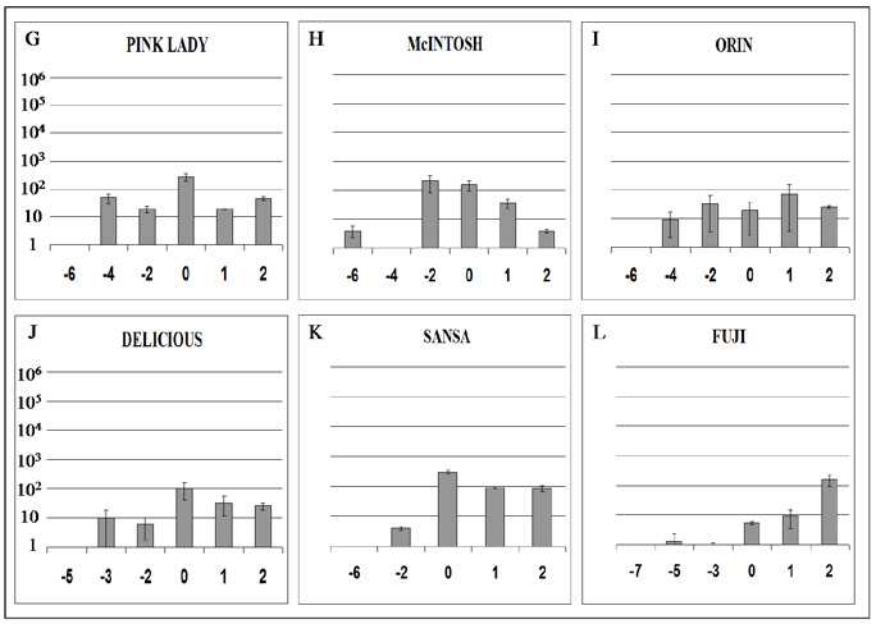


Figure 2B. Cultivars with the pattern of low expression level and transient peak, including ‘Fuji’, ‘Pink Lady’, ‘Orin’, ‘Sansa’, ‘Delicious’ and ‘McIntosh’



3. Figure 3A. The expression level of *MdACS3* was attenuated in fruit 4 weeks after harvest, 1-MCP treatment stimulated *MdACS3* expression.

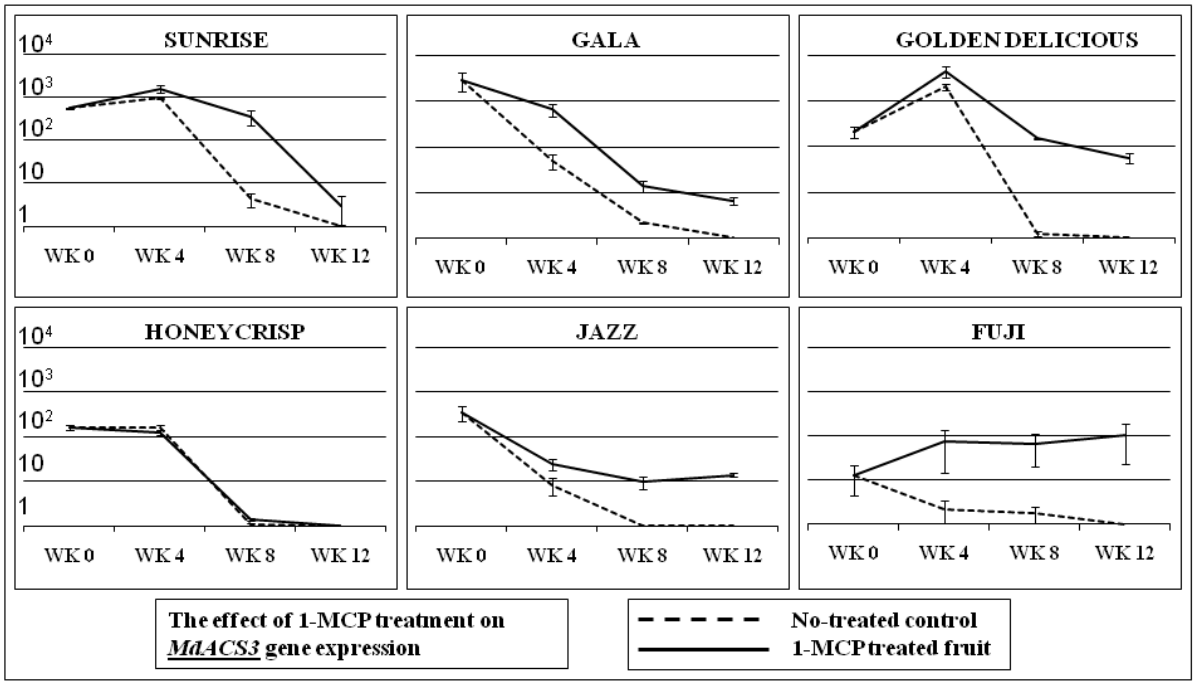
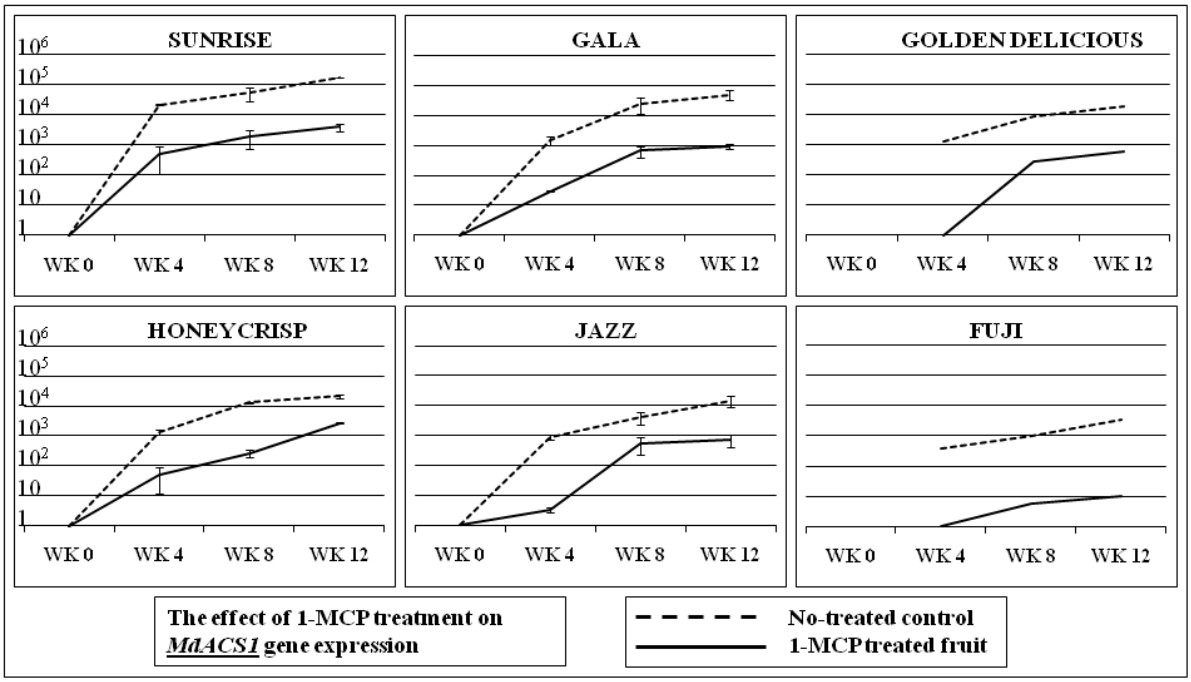


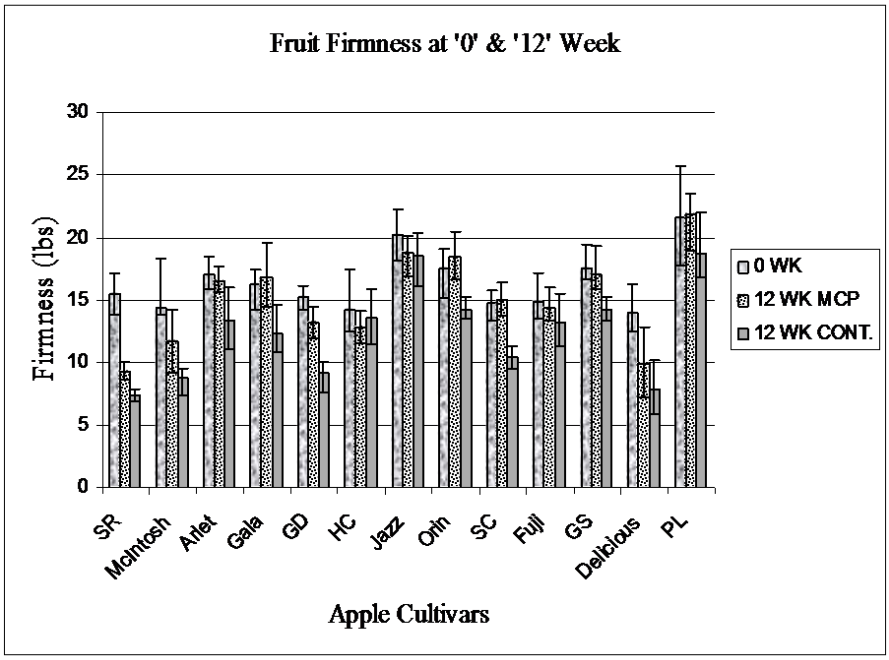
Figure 3B. Treatment with 1-MCP suppressed on *MdACS1* expression for all cultivars.



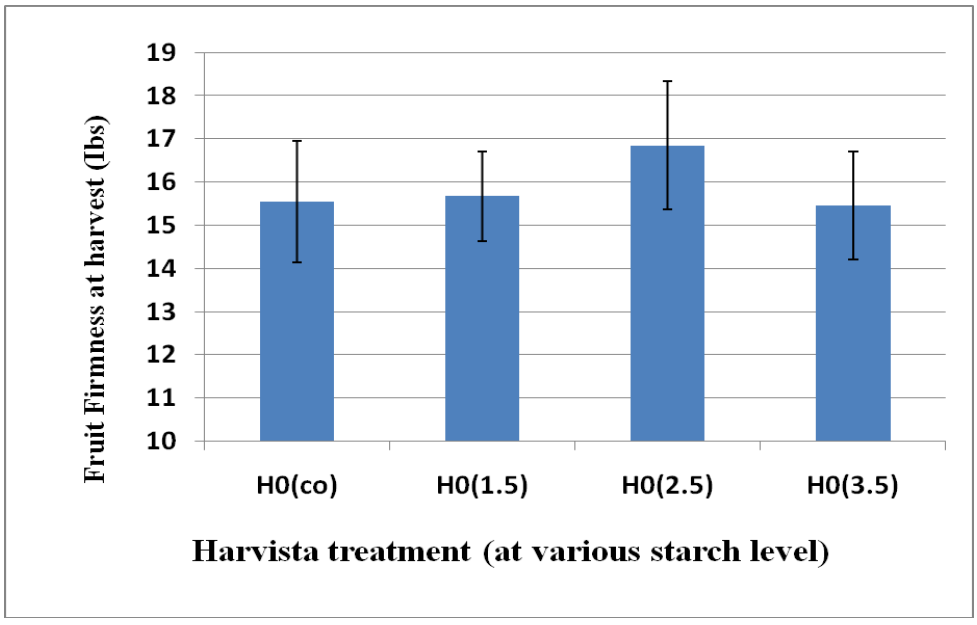
4. The cultivar-specific on-tree firmness loss during the six weeks before physiological maturity usually correlated with fruit ripening season and *MdACS3* expression level. Firmness loss was greater for the early ripening cultivars such as 'Golden Delicious', 'Sunrise' 'Honeycrisp', 'Gala', and smaller decreases were observed for later ripening cultivars such as 'Fuji', 'Pink Lady', , 'Granny Smith', and 'Suncrisp'.

	Apple Cultivar	Firmness at -4 week (lbs)	Firmness at +2 week (lbs)	Firmness loss (lbs)
Early ↑	Sunrise	20.3 ± 0.9	12.1 ± 0.6	8.2
	McIntosh	23.7 ± 4.0	14.7 ± 1.1	9.0
	Arlet	20.6 ± 0.9	15.7 ± 0.9	4.9
	Gala	22.1 ± 1.4	14.6 ± 1.6	7.5
	Golden Delicious	19.6 ± 1.3	14.5 ± 0.9	5.1
	Honeycrisp	17.7 ± 1.5 (-5 week)	13.3 ± 1.5	4.4
	Jazz	26.0 ± 0.9 (-5 week)	17.8 ± 1.1	8.2
	Orin	21.8 ± 1.7	17.0 ± 1.2	4.8
	Suncrisp	18.6 ± 1.3 (-5 week)	16.0 ± 0.8	2.6
	Fuji	18.1 ± 1.0 (-5 week)	15.4 ± 1.2	2.7
	Granny Smith	19.5 ± 1.4 (-5 week)	16.8 ± 1.4	2.7
	Delicious	16.0 ± 0.7 (-5 week)	12.8 ± 1.1	3.2
Late ↓	Pink Lady	23.8 ± 2.1 (-5 week)	20.9 ± 1.4	2.9

5. No clear relationship between 1-MCP treatment efficacy and *MdACS3* expression pattern was observed (Figure 4B), suggesting efficacy is dependent on factors other than *MdACS3* expression alone. However, early-ripening cultivars had higher average firmness retention (3.1 pounds) than the late-ripening group (1.5 pounds).

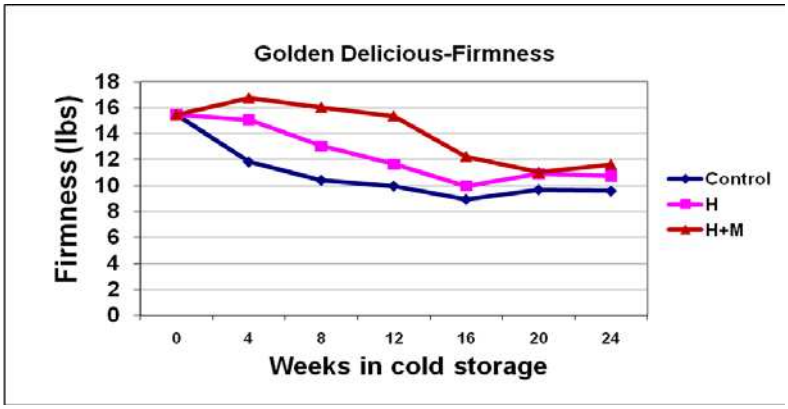


6. The timing of Harvista® treatment indicated that the best timing is at the starch level of 2.5, presumably when the ethylene receptor genes expression initiated.

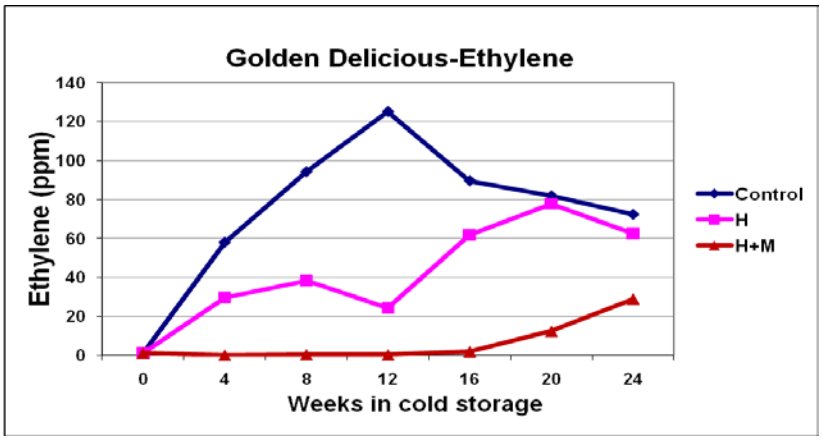


Harvista® was applied at the different time according to starch level (1.5, 2.5 and 3.5; co represents the no-applied control); then the fruit were harvested at the same time when physiological maturity has acquired.

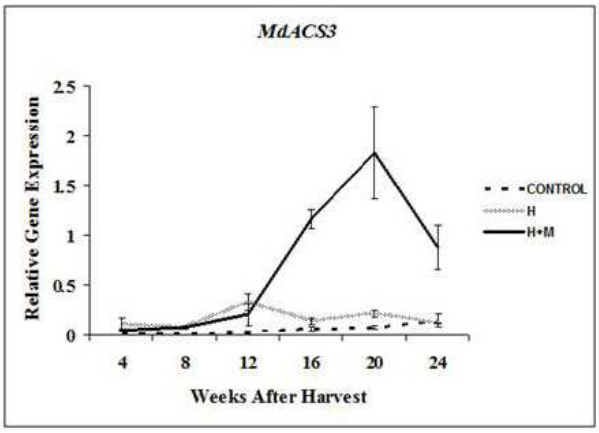
7. The post-harvest firmness change after Harvista® treatment at starch level of 2.5.

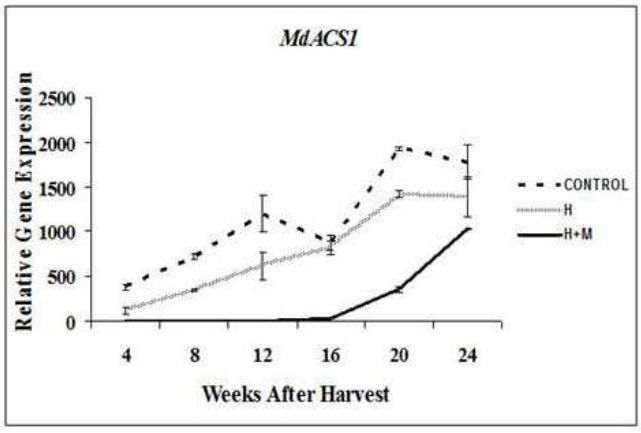


8. Suppression of ethylene evolution by spraying Harvista® during postharvest storage.

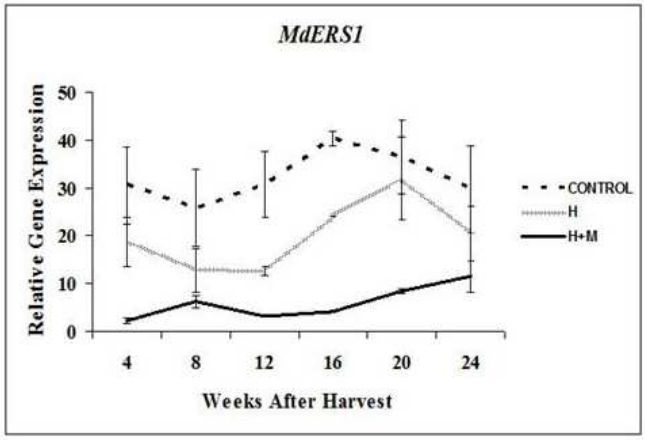


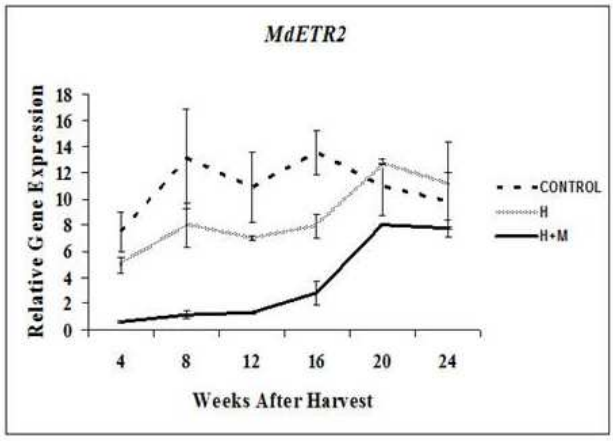
9. Suprresion of ethylene biosynthesis gene expression compared with non-sprayed control.

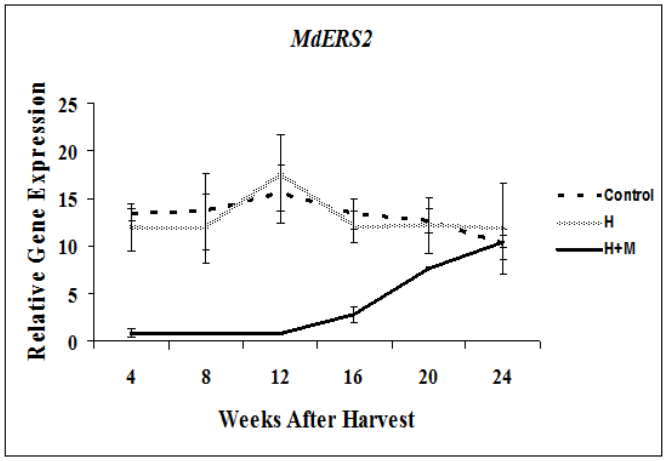




10. All major ethylene receptor genes showed down-regualted expression during postharvest storage upon the application of Harvista®.







EXECUTIVE SUMMARY

Apple fruit ripening is a tightly-regulated genetic process, and ethylene is known to play a pivotal role in regulating apple fruit ripening. While many studies on ethylene's roles focused on the ethylene biosynthesis upon climacteric ripening at or after harvest, little information is available on the roles of a pre-climacteric ethylene biosynthesis gene, i.e. *MdACS3*. In particular, how this low-level expressed, early-activated ethylene biosynthesis gene is related to the timing and strength of the major *MdACS1* gene is unknown. The relationship between *MdACS1* and *MdACS3* is important for understanding apple ripening patterns, developing strategy of fruit quality management and molecular tools for breeding practices.

We utilized 14 cultivars/breeding parents to investigate the expression patterns of *MdACS3* during apple fruit maturation, ripening and postharvest storage. The projected ripening season of these apple cultivars range from August to November in central Washington State. Our results indicated that *MdACS3* has significant influence on apple fruit ripening season (or the timing of *MdACS1* gene activation), fruit firmness and postharvest storability including 1-MCP treatment efficacy. Our results, for the first time clearly demonstrate the relationship between these two genes, i.e. *MdACS3* stimulates and triggers the activation of *MdACS1* genes. Based on the differential responses to 1-MCP treatment, it can be concluded that *MdACS3* expression is system 1 ethylene biosynthesis gene and *MdACS1* the system 2 ethylene biosynthesis gene. Furthermore we also tested a reported *MdACS3* gene-specific marker based on the allelotypes of *MdACS3a* allele. However, our results indicated there is no good association with our observed expression patterns. A further refined marker is needed.

Second part of the project focused on the effect of a new spraying formula of 1-MCP, i.e. Harvista®, its pre-harvest application and its effect on postharvest storability. Our result indicated that application of Harvista® at proper pre-harvest maturity (starch level of an average of 2.5) showed obvious effect on suppressing *MdACS1* gene expression, and fruit firmness retention during storage, although less effective than postharvest 1-MCP treatment. The suppressing effects on all major ethylene receptor genes were also observed.

Overall this is a systematic investigation on ethylene pathway which critically impacts apple fruit ripening, quality and storability. Our results elucidated the relationship between *MdACS3* and *MdACS1* genes, as well as its roles on apple fruit ripening season and on-tree firmness retention. These results set the foundation to develop a DNA marker for predicting the ripening season and fruit storability, for potentially selecting individuals in breeding population with desired ripening time.