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NAA and Ethylene Regulate Expression of Genes Related to Ethylene Biosynthesis, Perception, and Cell Wall Degradation During Fruit Abscission and Ripening in 'Delicious' Apples

Jianguo Li · Rongcai Yuan

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Abstract Expression of genes for ethylene biosynthesis, ethylene perception, and cell wall degradation in the fruit cortex and abscission zone was examined during fruit abscission and ripening in 'Delicious' apples (*Malus* × *domestica*). An autocatalytic burst of fruit ethylene production and accelerated fruit softening were associated with increased expression of genes related to ethylene biosynthesis (*MdACS* and *MdACO*), whereas reduced expression of ethylene receptor genes (*MdETR* and *MdERS*), increased expression of an ethylene signal transduction gene (*MdCTR1*), and increased expression of genes related to cell wall degradation (*MdPG* and *MdEG*) in the fruit cortex occurred during fruit ripening. Aminoethoxyvinylglycine (AVG) or 1−methylcyclopropene (1-MCP) inhibited fruit ethylene production, suppressed expression of *MdACS1*, *MdACO1*, *MdERS1*, and *MdPG1* in the fruit cortex, and delayed fruit softening, whereas naphthaleneacetic acid (NAA) increased fruit ethylene production, increased expression of *MdACS1*, *MdACO1*, *MdERS1* and *MdPG1* in the fruit cortex, and accelerated fruit softening. Fruit abscission and expression of *MdACS5A*, *MdACS5B*, *MdACO1*, *MdPG2*, and *MdEG1* in the fruit abscission zone were reduced by AVG and 1-MCP. NAA also reduced fruit abscission while reducing expression of *MdPG2* and *MdEG1* only in the fruit abscission zone. The levels of *MdETR1*, *MdETR2*, *MdERS1*, and *MdERS2* transcripts in the fruit abscission zone decreased during fruit abscission and fruit ripening regardless of treatment. The combination of NAA and AVG was more effective in inhibiting expression of *MdPG2* and *MdEG1* in the fruit abscission zone and reducing fruit abscission than was either NAA or AVG used alone.

Keywords Abscission · Ethylene biosynthesis · Ethylene perception · β-1,4-glucanase · *Malus domestica* · Polygalacturonase

J. Li · R. Yuan (✉)
Alson H. Smith, Jr. Agricultural Research and Extension Center, 595 Laurel Grove Road, Winchester, VA 22602, USA
e-mail: yuan@vt.edu

J. Li
South China Agricultural University, Wushan, Guangzhou, People's Republic of China

Introduction

Preharvest apple (*Malus* × *domestica* Borkh.) fruit abscission, which occurs before fruit develop optimum color, maturity, or size, usually causes a serious economic loss. Conversely, picking fruit prior to harvest maturity may lead to poor storability and poor fresh and processed fruit quality. It has been suggested that the interplay between indole-3-acetic acid (IAA) and ethylene plays an important role in the abscission process (Meir and others 2006; Taylor and Whitelaw 2001). The endogenous concentrations of IAA must fall below a certain threshold in the abscission zone to promote abscission because the IAA flux across the abscission zone of flowers, fruit, and leaves appears to determine the sensitivity of those organs to ethylene and the resultant abscission (Osborne 1989; Yuan and others 2001; Meir and others 2006). Applications of naphthaleneacidic acid (NAA) or other synthetic auxins delayed apple fruit abscission even though fruit ethylene production and fruit softening were increased (Smock and Gross 1947; Yuan and Carbaugh 2007). On the contrary, application of ethephon, an ethylene-releasing compound, effectively promoted mature fruit abscission and ripening in apples (Edgerton and Blanpied 1970), whereas aminoethoxyvinylglycine (AVG), an inhibitor of ethylene

biosynthesis, or 1-methylcyclopropene (1-MCP), an inhibitor of ethylene action, reduced fruit ethylene production and delayed mature fruit abscission and ripening (Schupp and Greene 2004; Yuan and Carbaugh 2007). We previously demonstrated that the combination of NAA and AVG or 1-MCP was more effective in delaying preharvest fruit abscission than were NAA, AVG, or 1-MCP alone in apples (Yuan and Carbaugh 2007). The addition of AVG or 1-MCP also overcame the side effect of fruit softening caused by NAA. The molecular mechanism whereby NAA and ethylene affect fruit softening and fruit abscission in apples is not clear.

The biosynthesis of ethylene begins with the production of S-adenosyl methionine (SAM) from the amino acid methionine. The conversions of SAM to 1-aminocyclopropane-1-carboxylate (ACC) and ACC to ethylene are the rate-limiting steps in ethylene biosynthesis, and are catalyzed by ACC synthase (ACS) and ACC oxidase (ACO), respectively (Alexander and Grierson 2002; Wang and others 2002). Genes encoding ACS and ACO are members of multigene families, and their expression is differentially regulated by various developmental, environmental, and hormonal signals (Kende 1993; Wang and others 2002). Auxin stimulates ethylene production by increasing expression of ACS genes (Abel and Theologis 1996; Vandenbussche and Van Der Straeten 2007). After synthesis, ethylene is perceived by a family of membrane-localized receptors that are similar to bacterial two-component histidine kinase receptors (Bleecker and Kende 2000; Wang and others 2002; Klee 2004). In *Arabidopsis*, there are five known ethylene receptors, ETR1, ETR2, ERS1, ERS2, and EIN4 (Wang and others 2002). When ethylene binds to receptors, the ethylene receptors seem to undergo a conformational change and interact with the Raf-like serine/threonine kinase CTR1, a negative regulator of the signal transduction pathway. The signal then passes through a partially elucidated cascade that ultimately influences a myriad of ethylene-associated plant growth and development processes (Bleecker and Kende 2000; Wang and others 2002; Klee 2004).

Concomitant with increased ethylene production is increased expression of genes and activity of enzymes associated with cell wall degradation such as β-1,4-glucanase (cellulase or EG) and polygalacturonase (PG) (Tucker and others 1988; Brown 1997; Bonghi and others 2000; Roberts and others 2002). As a result, the middle lamellae of abscission zone cells dissolve and, ultimately, the organ abscises. Other genes such as pathogenesis-related genes and those involved in secondary metabolism and signal transduction are also upregulated during the abscission process (Roberts and others 2002). By contrast, auxin delays or inhibits leaf or fruit abscission mainly by suppressing expression of PG and EG genes (Tucker and others 1988; Kalaitzis and others 1995; del Campillo and Bennett 1996; Roberts and others 2002).

The purpose of this study was to examine the expression of genes related to ethylene biosynthesis, perception, and cell wall degradation in the fruit abscission zone and fruit cortex in relation to the timing of fruit abscission and fruit softening following treatment with NAA, AVG, 1-MCP or the combination of NAA and AVG in 'Delicious' apples.

Materials and Methods

Plant Material and Treatments

Twenty uniform 'Delicious' apple trees on Mark rootstock were selected in an orchard located at the AHS Agricultural Research and Extension Center, Winchester, Virginia, USA. A randomized complete block design with four replications of five trees each was used. One tree from each block received one of five treatments. Treatments consisted of (1) two applications of water, which served as the control; (2) two applications of a sprayable formulation of 1-MCP (Rohm and Haas Company, Spring House, PA) at 320 mg L^{-1}; (3) two applications of AVG (Retain, Valent BioSciences Corp., Libertyville, IL) at 125 mg L^{-1}; (4) two applications of NAA (Fruitone N, AMVAC Corp., Newport Beach, CA) at 20 mg L^{-1}; and (5) one application of AVG at 125 mg L^{-1} on 6 September and two applications of NAA at 20 mg L^{-1}. The two applications of sprayable 1-MCP, AVG, and NAA as well as the control water were conducted on 6 September (just before fruit abscission started) and on 14 September 2006, respectively. All spray solutions contained Silwet-77 silicone surfactant (Loveland Industries, Loveland, CO) at 0.125% to allow compound dispersion. Solutions were applied to the canopy with a low-pressure hand-wand sprayer until runoff. To avoid contamination during spraying, at least one guard tree was used to separate each of the test trees, and the trees were sprayed with the solutions only when there was a weak or no wind.

Forty fruit were collected from each tree of three replicate blocks on 6 September (just before treatment), 28 September (all compounds were effectively inhibiting fruit abscission), and 20 October 2006 (the effects of NAA and 1-MCP on fruit abscission and fruit ethylene production had vanished), and were immediately separated into cortex and fruit abscission zone. Fruit abscission zones were collected by cutting 1 mm at each side of the abscission fracture plane. For fruit cortex, ten fruit were randomly selected from the 40 fruit of each tree, peeled, cored, and sliced. Harvested tissues were immediately frozen in liquid nitrogen and stored at −80°C for extraction of RNA.

Determination of Fruit Abscission, Fruit Ethylene Evolution, Fruit Starch Index, and Fruit Firmness

To determine fruit abscission rate, two limbs on each tree were tagged. Fruit on tagged limbs were counted on 6 September 2006 prior to any fruit abscission, and then fruit remaining on tagged limbs were counted weekly until 1 November. To determine fruit ethylene production, six fruit were collected from each tree at 1-2-week intervals beginning on 6 September and ending on 25 October 2006. Fruit were placed in a gas-tight, 3.785-L container and incubated for 3 h. One milliliter of gas sample was withdrawn from the sealed container through the rubber septum affixed to the lid, and the ethylene concentration was measured with a gas chromatograph equipped with an activated alumina column and FID detector (model 3700; Varian, Palo Alto, CA) (Yuan and Carbaugh 2007). At the same time, to determine fruit maturity, ten fruit were sampled from each tree to determine fruit firmness and starch index as described by Yuan and Carbaugh (2007). Briefly, fruit firmness was measured on two sides of each fruit with an Effegi penetrometer (model FT 327; McCormick Fruit Tech, Yakima, WA) with an 11.1-mm tip. Soluble solids concentration (SSC) was measured with an Atago handheld refractometer (model N1; McCormick Fruit Tech) using a composite sample of juice resulting from penetrometer testing of all replicates of each treatment. Each apple fruit was cut in half transversely and the flesh starch index was evaluated by dipping half of each apple in iodine solution for about 15 s. The degree of staining was rated on a scale of 1 to 8, where 1 = staining of the entire cut surface and 8 = absence of staining (Yuan and Carbaugh 2007).

Total RNA Extraction and Real-time Quantitative PCR

Total RNA was extracted from the fruit abscission zone and fruit cortex as described by Wan and Wilkins (1994). DNA was removed from each RNA sample using TURBO DNA-free™ Kit (Ambion Inc., Austin, TX). RT-PCR was performed using primers that spanned an intron in *MdACO* to confirm that each RNA sample was free of genomic DNA contamination (Dal Cin and others 2005).

Two micrograms of total RNA was used to synthesize cDNA in a 20-µl reaction volume using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Real-time quantitative PCR was performed using Power SYBR® Green PCR Master Mix Kit (Applied Biosystems, Foster City, CA) on an Applied Biosystems 7500 Real-Time PCR System according to the manufacturer's instructions. Gene-specific primers were designed for nonconserved areas using Primer Expression 3.0 software (Applied Biosystems) and synthesized by Integrated DNA Technologies (Coralville, IA). The primer sequences are listed in Table 1. Real-time samples were run in triplicate and the reaction volumes were 25 µl. Dissociation curves were run to determine the specificity of the amplification reactions. In addition, the amplified products were sequenced as described by Dal Cin and others (2005). After validation tests, normalization to actin was performed using the $\Delta\Delta C^T$ method described in "Guide to Performing Relative Quantitation of Gene Expression Using Real-time Quantitative PCR" (Applied Biosystems, Foster City, CA).

Table 1 Gene-specific primers used for expression analysis of genes

Gene	Accession No.	Primer left	Primer right
MdActin	CN938023	5′-TGACCGAATGAGCAAGGAAATTACT-3′	5′-TACTCAGCTTTGGCAATCCACATC-3′
MdACS1	L31347	5′-GCCTTCCGGGTTTTCGA-3′	5′-GGCGGCCACAACCATGT-3′
MdACS3	U73816	5′-CCGGTGATGCTTTGCTTGTT-3′	5′-CTCCACCTCAAATCTCTATCAAACC-3′
MdACS5A	AB034992	5′-GCAATGGTGGTCTTTTCGTATG-3′	5′-TTCGAACGTCTGCTCCTTGA-3′
MdACS5B	AB034993	5′-GAATTTTGAGTGTTGGATACCTTCTTT-3′	5′-GAACCAACATCTAAAATCCCATTGT-3′
MdACO1	AB030859	5′-CAGTCGGATGGGACCAGAA-3′	5′-GCTTGGAATTTCAGGCCAGA-3′
MdETR1	AF032448	5′-TTGGCCTGTGAAGAGCAGT-3′	5′-TGCAAACCATGTAGAGCCAT-3′
MdETR2	DQ847145	5′-GTTGTGACGCGGAAAATGC-3′	5′-AATCCAGATGAAACGGCAGTTAC-3′
MdERS1	AY083169	5′-CAACTAGGGATATGCGAC-3′	5′-CACTGGCATCCAAAGACTTC-3′
MdERS2	AB213028	5′-GCTTGTTAAGGTTGGAAGAAATCTG-3′	5′-CGGCATCGTTGAGTGTTACATT-3′
MdCTR1	AY670703	5′-ACAAGATTTTCATGCCGAAC-3′	5′-TATGGACAAGTTTGGAGGCT-3′
MdPG1	L27743	5′-CGCACAACAAATCCATGTCATAT-3′	5′-ACCGTGAGACAGGAAGCTTGA-3′
MdPG2	AB210897	5′-CGGTTCAGCCGACAAGTTG-3′	5′-TACGAGTGAGGAGGAGTAGATGGA-3′
MdEG1	AY350734	5′-ACCAGAACGATGGATTTCCAGAT-3′	5′-GTACGTTGCAGGCTCCGAAT-3′

Statistical Analyses

Statistical analyses included analysis of variance and Duncan's multiple-range test. Statistical Analysis Systems Software for PC (SAS Institute, Cary, NC) was used to analyze the results.

Results

Effect of 1-MCP, AVG, and NAA on Fruit Abscission, Ethylene Production, Firmness, and Starch Index

Fruit abscission did not occur in control trees until 6 September 2006, and cumulative fruit abscission increased dramatically thereafter (Figure 1A, B). Overall, applications of 1-MCP, AVG, and NAA alone effectively reduced preharvest fruit abscission. 1-MCP and AVG + NAA were more effective in controlling preharvest fruit abscission than was AVG or NAA used alone. However, the delay of fruit drop by AVG alone or AVG + NAA was more persistent than that of 1-MCP or NAA. The effect of NAA and 1-MCP on delaying fruit abscission was no longer evident on 10 October (approximately 27 days after the second application of NAA) and on 25 October (about 42 days after second application of 1-MCP), respectively.

Ethylene production of fruit from control trees was very low on 6 September and rapidly increased thereafter (Figure 1C). NAA increased fruit ethylene production, whereas 1-MCP and AVG inhibited fruit ethylene production. However, the inhibitory effect of 1-MCP on fruit ethylene production decreased by 10 October and ceased by 18 October, whereas AVG alone or AVG + NAA completely inhibited fruit ethylene production through 25 October. The rate of decrease in fruit firmness was slowed by AVG alone or AVG + NAA, but increased by NAA alone (Figure 1D). 1-MCP also effectively slowed the rate of decrease in fruit firmness, but its inhibitory effect was no longer evident on 25 October. NAA increased starch degradation as indicated by higher starch ratings, whereas AVG or 1-MCP alone delayed

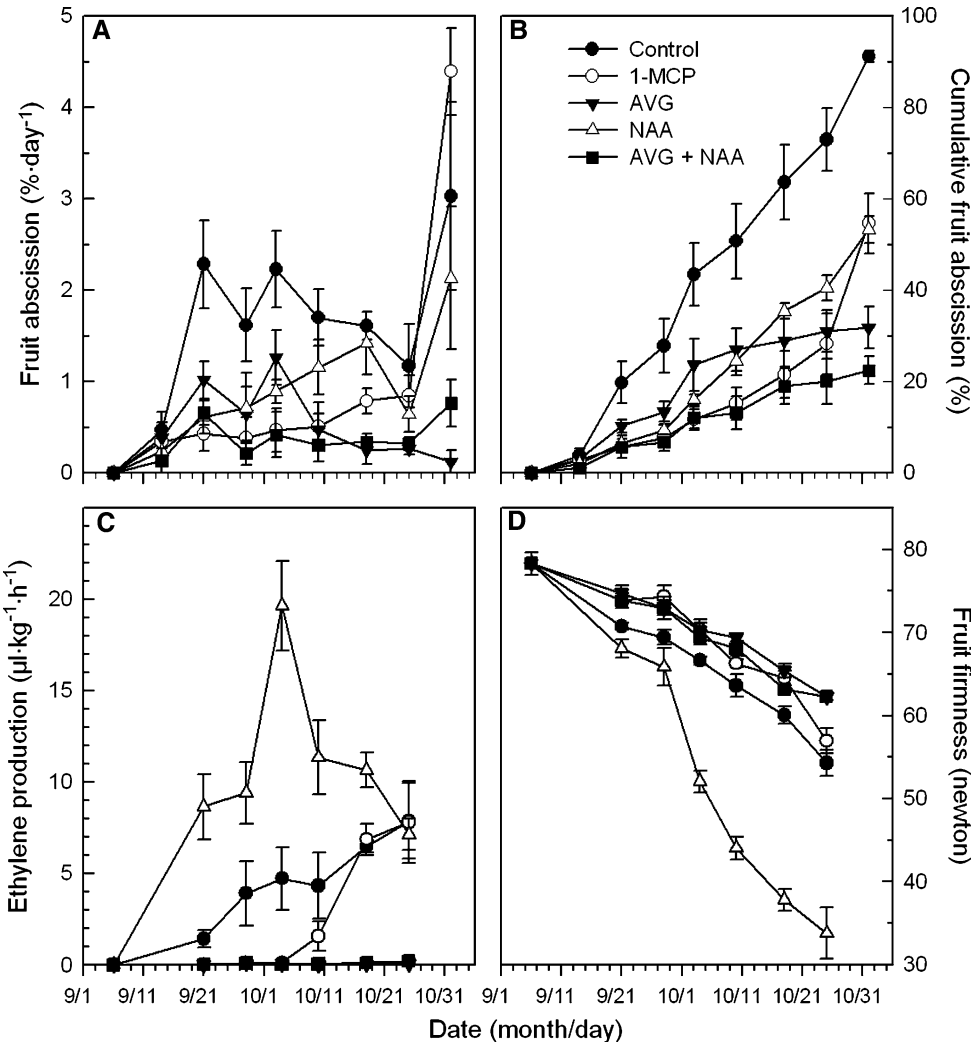

Fig. 1 Effects of 1-MCP at 320 mg L^{-1}, AVG at 125 mg L^{-1}, and NAA at 20 mg L^{-1} on fruit abscission (**A**), cumulative fruit abscission (**B**), fruit ethylene production (**C**), and fruit firmness (**D**) in 'Delicious' apples in 2006. Data are means ± SE [$n = 4$ in (**A**), 4 in (**B**), 4 in (**C**), and 40 in (**D**)]

Table 2 Effects of sprayable 1-MCP at 320 mg L^{-1}, AVG at 125 mg L^{-1}, and NAA at 20 mg L^{-1} on the starch index of 'Delicious' apples (2006)

Treatment	Application time	Starch index (1–8)[y]					
		21 September	28 September	4 October	10 October	18 October	25 October
Control	–	5.1 b[z]	6.1 b	6.8 b	7.0 b	7.3 b	7.8 a
1-MCP	6 September and 14 September	3.9 b	4.6 c	5.4 c	6.4 c	6.8 bc	7.7 ab
AVG	6 September and 14 September	5.1 b	5.5 b	5.4 c	6.0 c	6.4 c	7.3 b
NAA	6 September and 14 September	6.8 a	7.6 a	7.8 a	8.0 a	8.0 a	8.0 a
AVG + NAA	6 September and 14 September	4.9 b	5.5 b	5.8 c	6.1 c	6.7 c	6.8 c

[y] Mean separation within columns by Duncan's multiple range test, $P = 0.05$

[z] Starch rating 1–8, where 1 = staining of the entire cut surface and 8 = absence of staining

starch degradation (Table 2). The inhibitory effect of 1-MCP on starch degradation was no longer evident on 18 October.

Effect of 1-MCP, AVG, and NAA on Expression of Genes Encoding Enzymes Involved in Ethylene Biosynthesis

MdACS1 expression in the cortex of fruit from control trees was low on 6 September and increased more than 27,000-fold from 6 September to 25 October (Figure 2A). The expression of *MdACS1* in the fruit cortex was increased by NAA on 25 October but not on 28 September In contrast, 1-MCP, AVG, or AVG + NAA suppressed *MdACS1* expression in the fruit cortex on 28 September, whereas the inhibitory effect of 1-MCP had diminished by 25 October. *MdACS1* expression in the abscission zone of fruit from control trees significantly decreased from 6 September to 28 September and remained low thereafter (Figure 2B). 1-MCP and AVG alone had no effect on *MdACS1* expression in the fruit abscission zone, whereas NAA and particularly NAA + AVG significantly stimulated its expression in the fruit abscission zone on 28 September. The latter two treatments, however, had no effect on *MdACS1* expression on 25 October.

No expression of *MdACS2* was detected in any sample, even though we designed several sets of *MdACS2*-specific primers (data not shown). *MdACS3* expression in the cortex of fruit from control trees increased from 6 September to 28 September and remained at about the same level thereafter (Figure 2C). *MdACS3* expression in the fruit cortex on 28 September was increased by 1-MCP and particularly NAA but somewhat reduced by AVG or AVG + NAA. On 25 October, all treatments increased *MdACS3* expression, except 1-MCP which was similar to the control. *MdACS3* expression in the fruit abscission zone was not affected by any treatments throughout the experiment, except for a significant increase in the AVG + NAA treatment (Figure 2D).

MdACS5A transcript levels increased approximately eightfold in the cortex of fruit from control trees from 6 September to 25 October, whereas the levels of *MdACS5B* transcripts decreased over that same period (Figure 3A, C). The levels of both *MdACS5A* and *MdACS5B* transcripts were increased by NAA in the fruit cortex but were almost unaffected by 1-MCP, AVG, or AVG + NAA on 28 September, except for a slight increase in the levels of *MdACS5B* transcripts by 1-MCP and AVG + NAA. 1-MCP, AVG, or AVG + NAA reduced the levels of *MdACS5A* and *MdACS5B* transcripts in the fruit cortex on 25 October. The levels of *MdACS5A* and *MdACS5B* transcripts in the abscission zone of fruit from control trees increased from 6 September to 25 October (Figure 3B, D). 1-MCP, AVG, or AVG + NAA reduced the levels of *MdACS5A* and *MdACS5B* transcripts in the fruit abscission zone on 28 September, whereas NAA increased *MdACS5B* expression but had almost no effect on *MdACS5A* expression. On 25 October, only AVG and AVG + NAA inhibited the expression of *MdACS5A* and *MdACS5B* in the fruit abscission zone.

The levels of *MdACO1* transcripts in the cortex of fruit from control trees increased from 6 September to 25 October (Figure 3E). The levels of *MdACO1* transcripts in the fruit cortex were increased by NAA but reduced by 1-MCP, AVG, and AVG + NAA on 28 September. However, 1-MCP enhanced *MdACO1* expression in fruit cortex on 25 October, whereas AVG or AVG + NAA still inhibited *MdACO1* expression. The levels of *MdACO1* transcripts increased in the abscission zone of fruit from control trees from 6 September to 25 October but decreased by 1-MCP, AVG, NAA, and AVG + NAA, except an enhanced expression by NAA on 25 October (Figure 3F).

Effect of 1-MCP, AVG, and NAA on Expression of Genes Encoding Enzymes Involved in Ethylene Perception and Signal Transduction

The levels of *MdETR1* transcripts in the cortex of fruit from control trees decreased from 6 September to 25 October, whereas the levels of *MdETR2* transcripts increased during

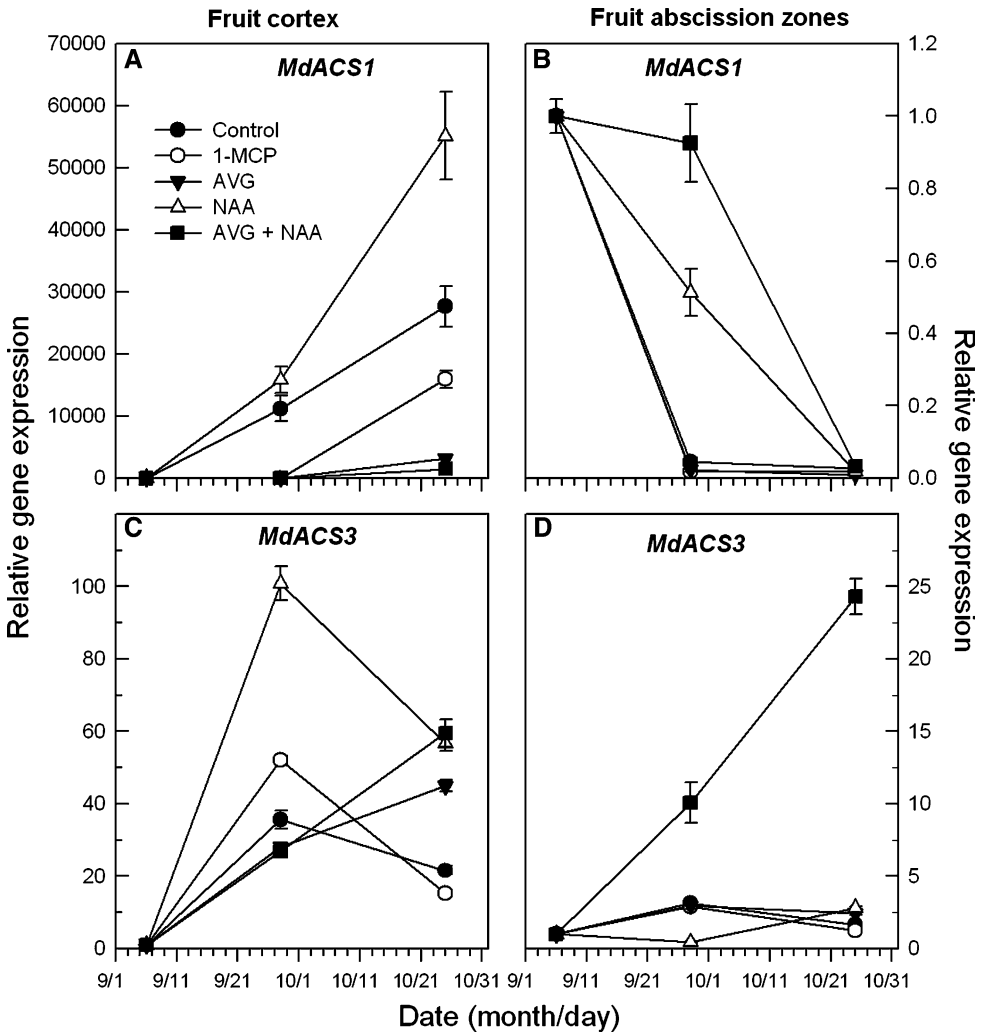

Fig. 2 Real-time quantitative PCR analysis of expression of *MdACS1* and *MdACS3* in fruit cortex (**A, B**) and fruit abscission zone (**B, D**) from 'Delicious' apple trees treated with 1-MCP at 320 mg L^{-1}, AVG at 125 mg L^{-1}, and NAA at 20 mg L^{-1}. The levels of *MdACS1* and *MdACS3* transcripts were normalized using actin. Data are means ± SE ($n = 3$). The values of *MdACS1* and *MdACS3* in the fruit cortex and fruit abscission zone of 'Delicious' apples on 6 September were arbitrarily set to 1

that period (Figure 4A, C). NAA had no effect on the expression of *MdETR1* and *MdETR2* in the fruit cortex except for an increase in *MdETR1* expression on 25 October. 1-MCP enhanced *MdETR1* expression in the fruit cortex on 28 September, whereas *MdETR2* expression was suppressed by 1-MCP, AVG, and AVG + NAA. On 25 October, all treatments increased *MdETR1* expression in the fruit cortex and, except for AVG or NAA alone, inhibited *MdETR2* expression in this tissue. The expression of *MdETR1* and *MdETR2* in the abscission zone of fruit from control trees decreased from 6 September to 25 October (Figure 4B, D). NAA, 1-MCP, AVG, and AVG + NAA increased the levels of *MdETR1* transcripts in the fruit abscission zone, except for a reduced expression of *MdETR1* by AVG alone on 28 September. NAA increased *MdETR2* expression in the fruit abscission zone, whereas *MdETR2* expression was inhibited by 1-MCP and AVG on 28 September but increased by 1-MCP, AVG, NAA, and NAA + AVG on 25 October.

The levels of *MdERS1* transcripts in the cortex of fruit from control trees increased from 6 September to 25 October, whereas *MdERS2* expression decreased (Figure 5A, C). NAA enhanced the expression of *MdERS1* and *MdERS2* in the fruit cortex at both analysis dates. 1-MCP, AVG, and AVG + NAA inhibited *MdERS1* expression in the fruit cortex on 28 September and 25 October, except 1-MCP, which did not differ from control on 25 October. 1-MCP, AVG, and AVG + NAA slightly decreased the levels of *MdERS2* transcripts in the fruit cortex on 28 September but increased the levels on 25 October. Overall, expression of *MdERS1* and *MdERS2* in the fruit abscission zone decreased from 6 September to 25 October, irrespective of treatment (Figure 5B, D). NAA stimulated expression of *MdERS1* and *MdERS2* in the fruit abscission zone on 28 September but not on 25 October. 1-MCP, AVG, and AVG + NAA reduced expression of *MdERS1* and very slightly also of *MdERS2* in the fruit abscission zone on 28 September but not on 25 October.

Overall, *MdCTR1* expression in the fruit cortex increased from 6 September to 25 October regardless of treatment (Figure 5E). All treatments except 1-MCP increased *MdCTR1* expression in the fruit cortex. The levels of *MdCTR1* transcripts in the abscission zone of fruit

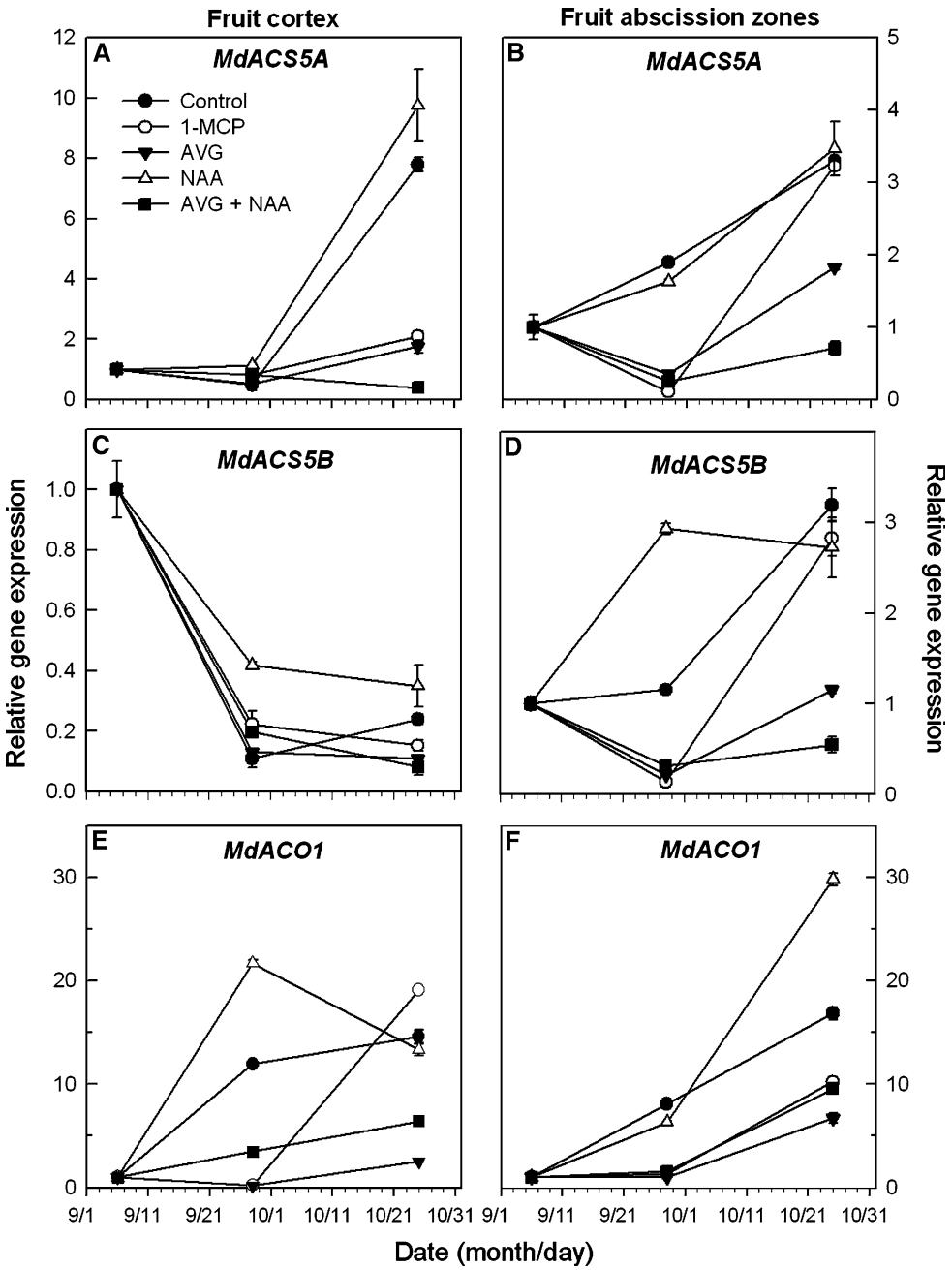

Fig. 3 Real-time quantitative PCR analysis of expression of *MdACS5A*, *MdACS5B*, and *MdACO1* in fruit cortex (**A, C, E**) and the fruit abscission zone (**B, D, F**) from 'Delicious' apple trees treated with 1-MCP at 320 mg L^{-1}, AVG at 125 mg L^{-1}, and NAA at 20 mg L^{-1}. The levels of *MdACS5A*, *MdACS5B*, and *MdACO1* transcripts were normalized using actin. Data are means ± SE ($n = 3$). The values of *MdACS5A*, *MdACS5B*, and *MdACO1* in the fruit cortex and fruit abscission zone of 'Delicious' apples on 6 September were arbitrarily set to 1

from control trees decreased from 6 September to 25 October (Figure 5F). AVG and NAA alone or in combination increased expression of *MdCTR1* on both analysis dates. 1-MCP did not affect the levels of *MdCTR1* transcripts in the fruit abscission zone on 28 September but slightly increased the levels on 25 October.

Effect of 1-MCP, AVG, and NAA on Expression of Genes Encoding Enzymes Involved in Cell Wall Degradation

The levels of *MdPG1* transcripts in the fruit cortex were very low on 6 September, just before fruit ripening started, but increased approximately 4,000-fold from 6 September to 28 September and continued to increase markedly thereafter (Figure 6A). NAA increased *MdPG1* expression in the fruit cortex, whereas the expression of the *MdPG1* gene was inhibited by 1-MCP, AVG, and AVG + NAA on 28 September and by the latter two treatments also on 25 October. The levels of *MdPG1* transcripts in the abscission zone of fruit from control trees also increased from 6 September to 25 October but to a much less extent (Figure 6B). All treatments decreased the levels of *MdPG1* transcripts in the fruit abscission zone.

The levels of *MdPG2* transcript in the cortex of fruit from control trees were very low until 28 September, but

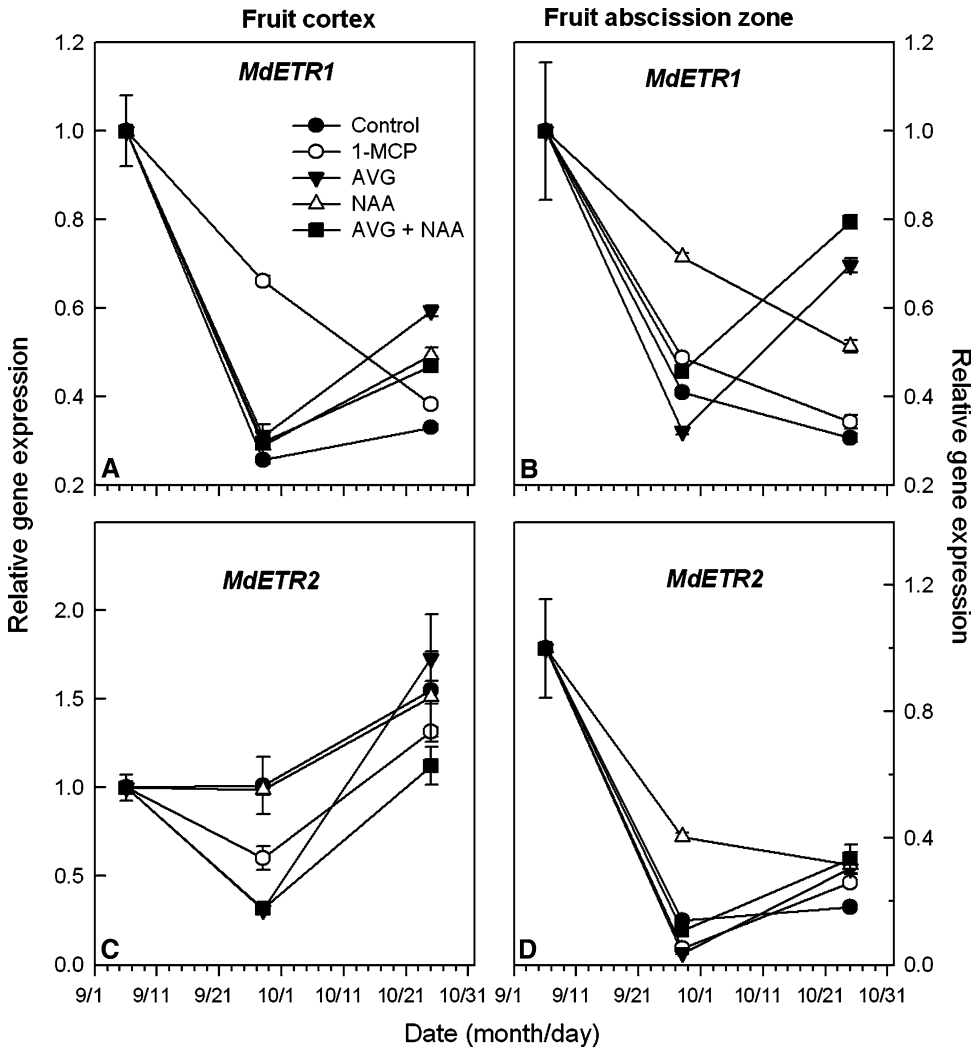

Fig. 4 Real-time quantitative PCR analysis of expression of *MdETR1* and *MdETR2* in the fruit cortex (**A, C**) and fruit abscission zone (**B, D**) from 'Delicious' apple trees treated with 1-MCP at 320 mg L^{-1}, AVG at 125 mg L^{-1}, and NAA at 20 mg L^{-1}. The levels of *MdETR1*and *MdETR2* transcripts were normalized using actin. Data are means ± SE ($n = 3$). The values of *MdETR1* and *MdETR2* in the fruit cortex and fruit abscission zone of 'Delicious' apples on 6 September were arbitrarily set to 1

significantly increased on 25 October (Figure 6C). All treatments reduced expression of *MdPG2* on 25 October. On the other hand, *MdPG2* transcript levels in the fruit abscission zone increased approximately 400-fold in control trees from 6 September to 28 September, and increased more markedly thereafter (Figure 6D). All treatments effectively suppressed *MdPG2* expression in the fruit abscission zone on 28 September but their inhibitory effect decreased on 25 October, except for the combination of AVG and NAA.

The levels of *MdEG1* transcripts in the cortex of fruit from control trees increased from 28 September to 25 October (Figure 6E). 1-MCP and AVG did not have a significant effect on expression of *MdEG1* in the fruit cortex, whereas NAA and particularly AVG + NAA inhibited its expression. The levels of *MdEG1* transcripts in the abscission zone of fruit from control trees increased from 6 September to 25 October (Figure 6F). All treatments suppressed *MdEG1* expression in the fruit abscission zone on 28 September, but only AVG and AVG + NAA inhibited *MdEG1* expression in the fruit abscission zone on 25 October, whereas 1-MCP stimulated its expression.

Discussion

Expression of *ACS*, *ACO*, *ETR*, *ERS*, *CTR*, *PG*, and *EG* Genes in Apple Fruit During Ripening

It has been proposed that two systems of ethylene production operate in higher plants (McMurchie and others 1972; Barry and others 2000). System 1 ethylene production is ethylene autoinhibitory, and is responsible for producing low and basal levels of ethylene in vegetative tissues and in the preclimacteric stage of climacteric fruit. System 2 represents the autocatalytic burst of ethylene production during ripening of climacteric fruit, including apples, and requires the induction of ACS and ACO (Barry and others 2000). In this study, a burst of fruit ethylene production was associated with fruit ripening in control

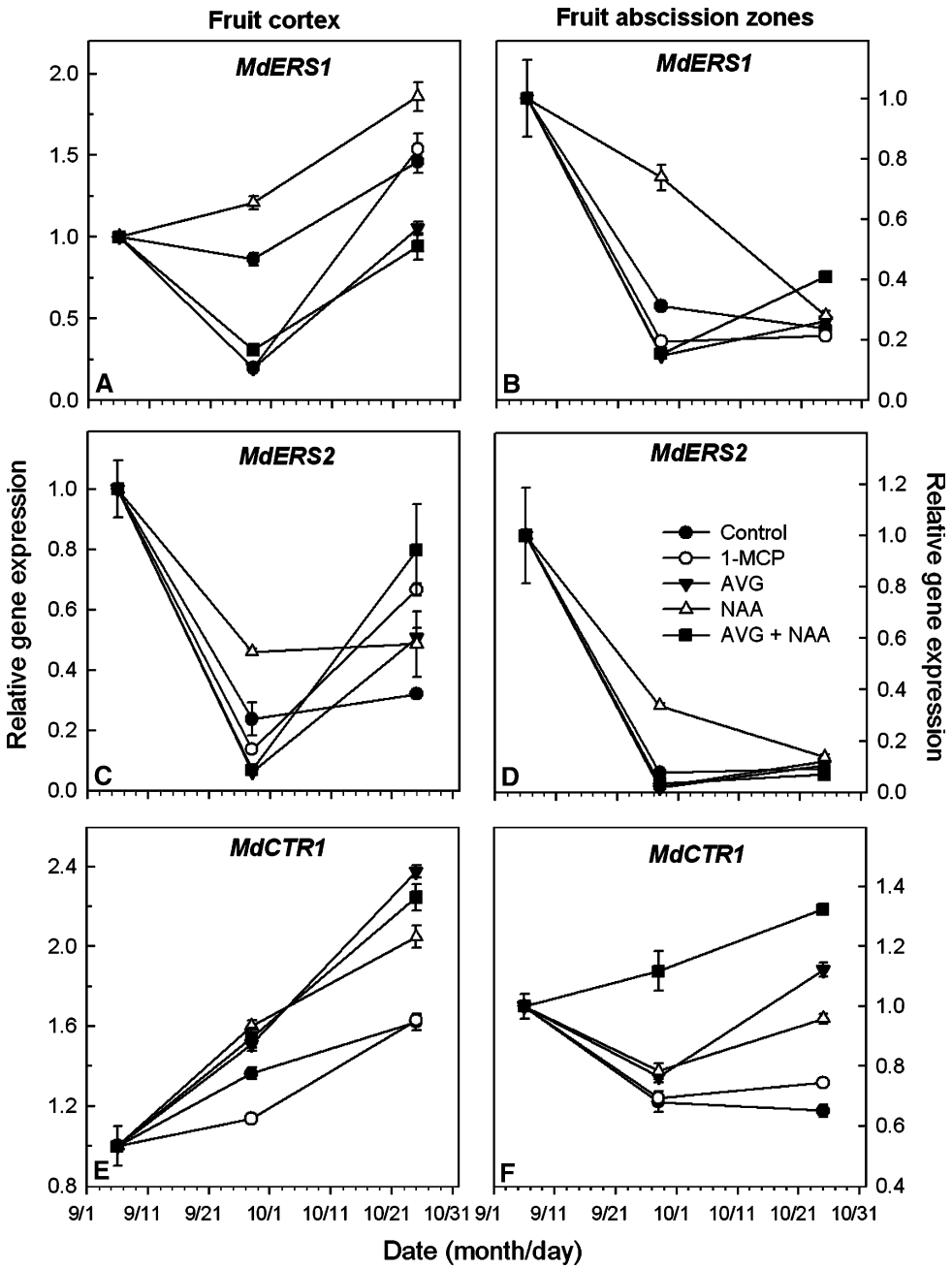

Fig. 5 Real-time quantitative PCR analysis of expression of *MdERS1*, *MdERS2*, and *MdCTR1* in the fruit cortex (**A, C, E**) and fruit abscission zone (**B, D, F**) from 'Delicious' apple trees treated with 1-MCP at 320 mg L^{-1}, AVG at 125 mg L^{-1}, and NAA at 20 mg L^{-1}. The levels of *MdERS1*, *MdERS2*, and *MdCTR1* transcripts were normalized using actin. Data are means ± SE ($n = 3$). The values of *MdERS1*, *MdERS2*, and *MdCTR1* in the fruit cortex and fruit abscission zone of 'Delicious' apples on 6 September were arbitrarily set to 1

'Delicious' apples. In addition, expression of *MdACS1*, *MdACS3*, *MdACS5A*, and *MdACO1* markedly increased in the cortex of fruit from control 'Delicious' apple trees at the onset of fruit ripening, indicating that *MdACS1*, *MdACS3*, *MdACS5A*, and *MdACO1* are related to fruit ripening. This is consistent with previous reports that the levels of *MdACS1*, *MdACS3*, and *MdACO1* transcripts increased during fruit ripening in 'Golden Delicious' apples (Wiersma and others 2007). However, the levels of *MdACS5B* in the fruit cortex decreased during ripening, implying that *MdACS5B* is not related to climacteric ethylene production. Expression of ethylene receptor genes *MdETR1* and *MdERS2* in the cortex of control fruit also decreased during fruit ripening, whereas the levels of *MdETR2* and *MdERS1* transcripts did not change until 25 October when fruit were at the late stage of ripening. This suggests that the sensitivity of the fruit cortex to ethylene increased during fruit ripening in 'Delicious' apples. This is different from previous reports that the levels of the overall ethylene receptor mRNA markedly increased during ripening in tomato (Lashbrook and others 1998; Tieman and others 2000), muskmelons (Sato-Nara and others 1999), and pears (El-Sharkawy and others 2003). This discrepancy could be due to a differential species response. A slight increase in the levels of *MdETR2* and *MdERS1* transcripts at the late stage of fruit ripening might

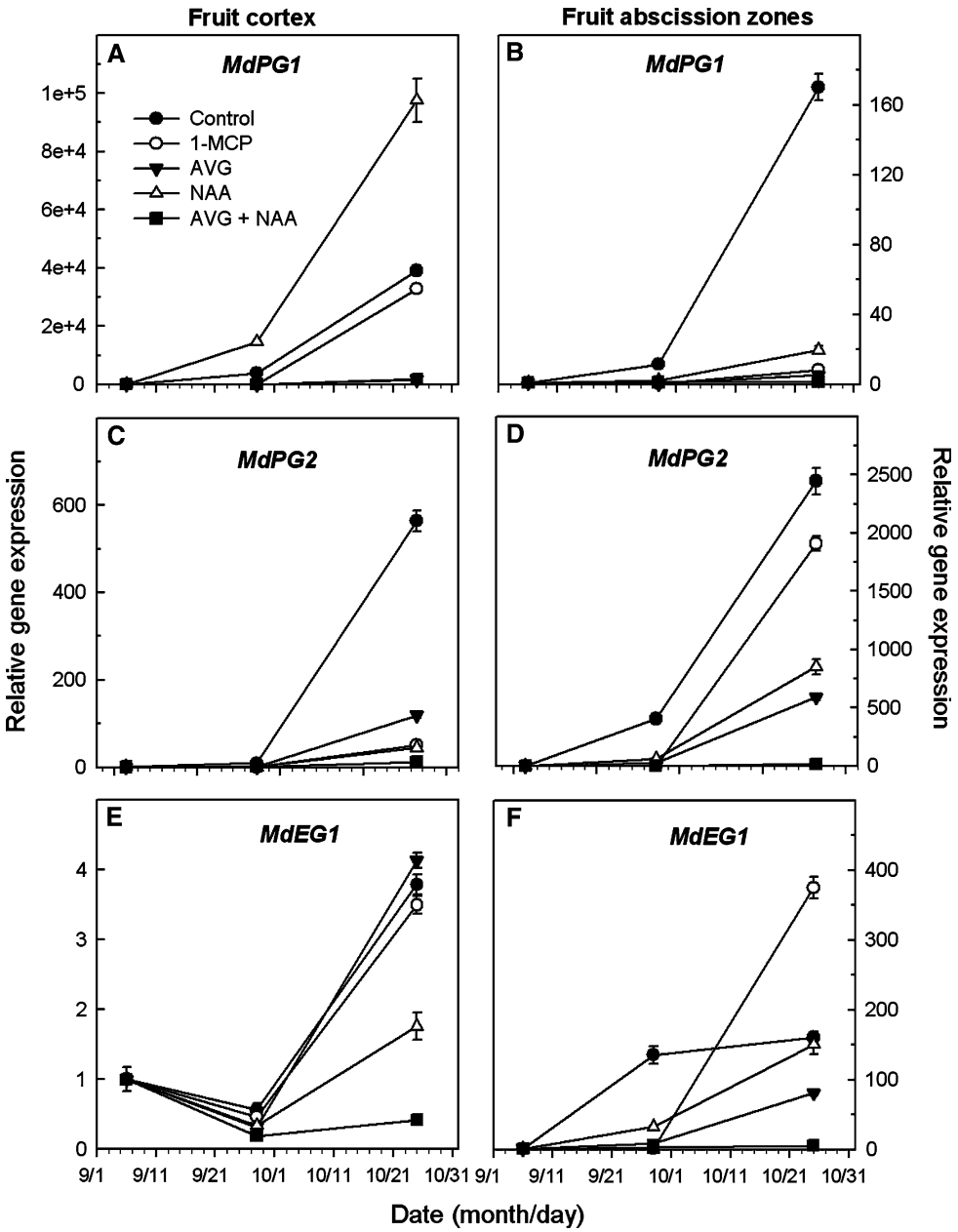

Fig. 6 Real-time quantitative PCR analysis of expression of *MdPG1*, *MdPG2*, *and MdEG1* in the fruit cortex (**A, C, E**) and fruit abscission zone (**B, D, F**) from 'Delicious' apple trees treated with 1-MCP at 320 mg L^{-1}, AVG at 125 mg L^{-1}, and NAA at 20 mg L^{-1}. The levels of *MdPG1*, *MdPG2*, and *MdEG1* transcripts were normalized using actin. Data are means ± SE ($n = 3$). The values of *MdPG1*, *MdPG2*, *and MdEG1* in the fruit cortex and fruit abscission zone of 'Delicious' apples on 6 September were arbitrarily set to 1

be a natural response of plant tissues to increased ethylene biosynthesis (Klee 2004; Dal Cin and others 2006; Kevany and others 2007). *MdCTR1* expression in fruit cortex increased during fruit ripening, indicating that *MdCTR1* may play a regulatory role in ethylene-dependent events. Expression of cell wall degradation-related genes *MdPG1*, *MdPG2*, and *MdEG1* also drastically increased in the cortex of fruit from control trees during fruit ripening, even though the levels of *MdPG2* and *MdEG1* transcripts increased at the late stage of fruit ripening and to a much lesser extent. This might be attributed to the increased ethylene production and increased sensitivity of fruit cortex to ethylene during ripening (Brown 1997; Bonghi and others 2000). Taken together, *MdACS1*, *MdACS3*, *MdACS5A*, *MdACO1*, *MdETR1*, *MdETR2*, *MdERS1*, *MdERS2*, *MdCTR1*, *MdPG1*, *MdPG2*, and *MdEG1* genes in the fruit cortex were ripening-related.

Ethylene-dependent Expression of *ACS*, *ACO*, *ETR*, *ERS*, *CTR*, *PG*, and *EG* Genes in Apple Fruit During Ripening

It has been documented that AVG inhibits ethylene biosynthesis by inhibiting ACS enzyme activity (Boller and others 1979), and 1-MCP counteracts ethylene action through blocking ethylene receptors (Sisler and others 1996). On the other hand, auxin stimulates ethylene production by enhancing ACS expression in various plant

species (Abel and Theologis 1996; Arguseso and others 2007; Vandenbussche and Van Der Straeten 2007). The use of AVG, 1-MCP, and NAA has allowed us to understand which genes in fruit are regulated by ethylene during fruit ripening. Our results from this study demonstrated that fruit ethylene production was decreased by AVG and 1-MCP. As a result of low ethylene production caused by AVG and 1-MCP, the levels of *MdACS1* and *MdACO1* transcripts in the fruit cortex were reduced, but the levels of *MdACS3* in the fruit cortex were not affected. These results suggest that *MdACS1* and *MdACO1* are ethylene-dependent and may account for the burst of fruit ethylene production in control apples during ripening, whereas *MdACS3* is developmentally dependent and is not related to climacteric ethylene production during ripening. These findings are in agreement with previous reports that *MdACS1* and *MdACO1* are involved in the climacteric ethylene production in apples (Harada and others 2000; Dandekar and others 2004; Wiersma and others 2007; Li and Yuan 2008, Unpublished manuscript). Like *MdACS3*, *MdACS5A* and *MdACS5B* were also developmentally dependent and were not related to climacteric ethylene production during ripening because AVG did not affect the levels of *MdACS5B* transcripts in the fruit cortex, and there was no significant difference in the levels of *MdACS5A* in the fruit cortex between AVG and 1-MCP on 25 October when fruit ethylene production was still inhibited by AVG but not by 1-MCP. On the other hand, NAA stimulated ethylene production and expression *of MdACS1*, *MdACS3*, *MdACS5A*, *MdACS5B*, and *MdACO1* in the fruit cortex, but only NAA-enhanced expression of *MdACS1* and *MdACO1* was suppressed by AVG, suggesting that NAA enhances fruit ethylene production by increasing expression of *MdACS1* and *MdACO1* but not by affecting *MdACS3*, *MdACS5A*, and *MdACS5B*.

Overall, 1-MCP and AVG did not consistently affect the levels of *MdETR1* and *MdERS2* transcripts in the fruit cortex during ripening, but they did decrease the levels of *MdETR2* and *MdERS1* when they effectively inhibited ethylene production. This suggests that *MdETR1* and *MdERS2* are ethylene-independent, whereas *MdETR2* and *MdERS1* are ethylene-dependent. This suggestion is supported by the results that *MdERS1* expression in the fruit cortex was increased by NAA but the increase was suppressed by AVG. Neither AVG nor NAA affected expression of *MdCTR1*, *MdPG2*, or *MdEG1* in the fruit cortex even though NAA increased fruit ethylene production and fruit softening and AVG reduced fruit ethylene production and fruit softening. These results indicate that *MdCTR1*, *MdPG2*, and *MdEG1* in the fruit cortex may be ethylene-independent and are unlikely to be involved in fruit softening in 'Delicious' apples. On the other hand, *MdPG1* expression was enhanced by NAA but reduced by AVG, 1-MCP, and NAA + AVG, which slowed the loss of fruit firmness, indicating that *MdPG1* is ethylene-dependent and is related to apple fruit softening. This is consistent with previous reports that expression of *MdPG1*, but not *MdPG2* and *MdEG1*, is involved in apple fruit softening during ripening (Wakasa and others 2006; Li and Yuan 2008, Unpublished manuscript).

Expression of *ACS*, *ACO*, *ETR*, *ERS*, *CTR*, *PG*, and *EG* Genes in Apple Fruit Abscission Zone With or Without 1-MCP, AVG, and NAA

Our results herein clearly showed that fruit abscission was accompanied by increased fruit ethylene production in control 'Delicious' apple trees, whereas AVG and 1-MCP reduced fruit ethylene production and fruit abscission. These results suggest that ethylene is involved in fruit abscission in 'Delicious' apples. NAA also reduced fruit abscission even though it enhanced fruit ethylene production and fruit softening, indicating that NAA plays a different role in fruit abscission and fruit softening. Moreover, AVG + NAA resulted in much lower fruit abscission than did either AVG or NAA alone, although there was no significant difference in fruit ethylene production between AVG and AVG + NAA, suggesting that there is a synergistic effect between NAA and AVG in controlling fruit abscission.

Expression of *MdACS5A*, *MdACS5B*, and *MdACO1* in the abscission zone of fruit from control 'Delicious' apple trees increased after abscission began, whereas expression of *MdACS1* and *MdACS3* either decreased or did not change significantly. In addition, application of 1-MCP, AVG, or AVG + NAA either increased or did not affect expression of *MdACS1* and *MdACS3* in the fruit abscission zone even though they reduced fruit abscission. These results suggest that *MdACS1* and *MdACS3* in the fruit abscission zone are ethylene-independent and are unlikely to be involved in fruit abscission. However, expression of *MdACS5A*, *MdACS5B*, and *MdACO1* in the fruit abscission zone was suppressed by 1-MCP, AVG, or AVG + NAA when these treatments effectively reduced fruit abscission on 28 September, but the expression of these genes was increased when these treatments had less or no effect on fruit abscission on 25 October. This implies that 1-MCP, AVG, and AVG + NAA delayed fruit abscission by inhibiting the expression of the *MdACS5A*, *MdACS5B*, and *MdACO1* genes via inhibiting ethylene biosynthesis or action in the fruit abscission zone in 'Delicious' apples. This is consistent with our previous reports that expression of *MdACS5A* and *MdACO1* genes in the fruit abscission zone increased in 'Golden Delicious' apples, which have a serious preharvest fruit abscission problem, but 'Fuji' apples showed no preharvest fruit abscission (Li and Yuan 2008, Unpublished manuscript). NAA, however, might

reduce fruit abscission not through inhibiting ethylene biosynthesis in the fruit abscission zone because NAA-enhanced *MdACS5B* expression and had almost no effect on expression of *MdACS5A* and *MdACO1* in fruit abscission zone.

Ethylene receptors negatively regulate ethylene responses and there is an inverse relationship between receptor levels and ethylene sensitivity of a tissue (Hua and Meyerowitz 1998; Tieman and others 2000; Klee 2004). Our results showed that, overall, the levels of *MdETR1*, *MdETR2*, *MdERS1*, and *MdERS2* transcripts decreased in the fruit abscission zone during ripening and fruit abscission regardless of treatment. The levels of *MdCTR1* transcripts in the abscission zone of control fruit also decreased at the same time. These results suggest that the sensitivity of fruit abscission zone tissues to ethylene increases during fruit ripening. However, this is different from our previous report that the levels of *MdETR2* and *MdERS2* transcripts in the fruit abscission zone increased during fruit abscission and ripening in 'Golden Delicious' apples (Li and Yuan 2008, Unpublished manuscript). This discrepancy could be due to a differential cultivar response. Expression of *MdETR2*, *MdERS1*, and *MdERS2* in the fruit abscission zone was suppressed by 1-MCP and AVG but increased by NAA in 'Delicious' apples. This may also be a natural response to ethylene biosynthesis increased by NAA or decreased by 1-MCP or AVG (Klee 2004). Alternatively, NAA may decrease the sensitivity of the fruit abscission zone to ethylene by increasing the expression of genes encoding ethylene receptors, and thus reduce fruit abscission in apples. Further work will be necessary to determine the relationship between the levels of ethylene receptor proteins in the abscission zone and abscission itself.

Our results revealed that a drastic increase in the levels of *MdPG2* and *MdEG1* transcripts in the fruit abscission zone was associated with increased fruit abscission in control 'Delicious' apple trees. By contrast, AVG and 1-MCP, which inhibited fruit ethylene production, decreased the levels of *MdPG2* and *MdEG1* transcripts in the fruit abscission zone, and reduced fruit abscission. These results suggest that *MdPG2* and *MdEG1* in the fruit abscission zone are regulated by ethylene and are involved in fruit abscission in 'Delicious' apples. This is consistent with previous reports that the increase in the activities of PG and cellulase is associated with increased fruit abscission (Tonutti and others 1995; Bonghi and others 2000; Hong and others 2000). However, expression of *MdPG1* in the fruit abscission zone was effectively suppressed by 1-MCP and AVG on both sampling dates, even though 1-MCP no longer had an effect on fruit abscission on 25 October, suggesting that *MdPG1* expression may not be associated with fruit abscission. NAA also inhibited expression of *MdPG2* and *MdEG1* in the fruit abscission zone and decreased fruit abscission in 'Delicious' apples. However, unlike AVG and 1-MCP, NAA as an auxin can inhibit abscission by directly suppressing accumulation of cellulase genes in the tomato flower abscission zone (del Campillo and Bennett 1996) and PG in the abscission zone of tomato flower and leaves (Kalaitzis and others 1995). Therefore, the synergistic effect of NAA + AVG in inhibiting expression of *MdPG2* and *MdEG1* in the fruit abscission zone might be ascribed to the stronger inhibitory effect of NAA + AVG on fruit abscission than when either NAA or AVG was used alone in 'Delicious' apples.

In conclusion, *MdACS1*, *MdACO1*, *MdETR2*, *MdERS1*, and *MdPG1* in the fruit cortex are ethylene-dependent and are related to fruit softening during fruit ripening, whereas *MdACS3*, *MdACS5A*, *MdACS5B*, *MdETR1*, *MdERS2*, *MdCTR1*, *MdPG2*, and *MdEG1* in the fruit cortex are developmentally dependent in 'Delicious' apples. AVG, 1-MCP, AVG + NAA inhibited the expression of *MdACS1*, *MdACO1*, *MdETR2*, *MdERS1*, and *MdPG1* in the fruit cortex by inhibiting ethylene biosynthesis or action, thereby slowing the loss of fruit firmness and fruit softening, whereas NAA increased the expression of these genes via increasing ethylene biosynthesis and thus enhanced fruit softening. *MdACS5A*, *MdACS5B*, *MdACO1*, *MdETR2*, *MdERS1* and *MdERS2*, *MdPG2*, and *MdEG1* in the fruit abscission zone are regulated by ethylene and are related to fruit abscission in 'Delicious' apples. NAA inhibited abscission by directly suppressing accumulation of *MdPG2* and *MdEG1* genes in the fruit abscission zone in 'Delicious' apples. The combination of NAA and AVG more effectively reduced fruit abscission than did either compound used alone due to their synergistic effects in suppressing expression of *MdPG2* and *MdEG1* in the fruit abscission zone.

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References

Abel S, Theologis A (1996) Early genes and auxin action. Plant Physiol 111:9–17

Alexander L, Grierson D (2002) Ethylene biosynthesis and action in tomato: a model for climacteric fruit ripening. J Exp Bot 53:2039–2055

Argueso CT, Hansen M, Kieber JJ (2007) Regulation of ethylene biosynthesis. J Plant Growth Regul 26:92–105

Barry CS, Llop-Tous MT, Grierson D (2000) The regulation of 1-aminocyclopropane-1-carboxylic acid synthase gene expression during the transition from system-1 to system-2 ethylene synthesis in tomato. Plant Physiol 123:979–986

Bleecker AB, Kende H (2000) Ethylene: a gaseous signal molecule in plants. Annu Rev Cell Dev Biol 16:1–40

Boller T, Herner RC, Kende H (1979) Assay for the enzymatic formation of an ethylene precursor 1-aminocyclopropane-1-carborylic acid. Planta 145:293–303

Bonghi C, Tonutti P, Ramina A (2000) Biochemical and molecular aspects of fruitlet abscission. Plant Growth Regul 31:35–42

Brown KM (1997) Ethylene and abscission. Physiol Plant 100:567–576

Dal Cin V, Danesin M, Boschetti A, Dorigoni A, Ramina A (2005) Ethylene biosynthesis and perception in apple fruitlet abscission (*Malus domestica* L. Borck). J Exp Bot 56:2995–3005

Dal Cin V, Rizzini FM, Botton A, Tonutti P (2006) The ethylene biosynthetic and signal transduction pathways are differently affected by 1-MCP in apple and peach fruit. Postharvest Biol Technol 42:125–133

Dandekar AM, Teo G, Defilippi BG, Uratsu SL, Passey AJ, Kader AA, Stow JR, Colgan RJ, James DJ (2004) Effect of down-regulation of ethylene biosynthesis on fruit flavor complex in apple fruit. Transgenic Res 13:373–384

del Campillo E, Bennett AB (1996) Pedicel breakstrength and cellulase gene expression during tomato flower abscission. Plant Physiol 111:813–820

Edgerton LJ, Blanpied GD (1970) Interaction of succinic acid 2,2-dimethyl hydrazide, 2-chloroethylphosphonic acid and auxins on maturity, quality and abscission of apples. J Am Soc Hortic Sci 95:664–666

El-Sharkawy I, Jones B, Li Z, Lelievre J, Pech JC, Latche A (2003) Isolation and characterization of four ethylene perception elements and their expression during ripening in pears with/without cold requirement. J Exp Bot 54:1615–1625

Harada T, Sunako T, Wakasa Y, Soejima J, Satoh T, Niizeki M (2000) An allele of the 1-aminocyclopropane-1-carboxylate synthase gene (*Md-ACS1*) accounts for the low level of ethylene production in climacteric fruits of some apple cultivars. Theor Appl Genet 101:742–746

Hong SB, Sexton R, Tucker ML (2000) Analysis of gene promoters for two tomato polygalacturonases expressed in abscission zones and the stigma. Plant Physiol 123:869–881

Hua J, Meyerowitz EM (1998) Ethylene responses are negatively regulated by a receptor gene family in *Arabidopsis thaliana*. Cell 94:261–271

Kalaitzis P, Koehler SM, Tucker ML (1995) Cloning of a tomato polygalacturonase expressed in abscission. Plant Mol Biol 28:647–656

Kende H (1993) Ethylene biosynthesis. Annu Rev Plant Physiol Plant Mol Biol 44:283–307

Kevany BM, Tieman DM, Taylor MG, Dal Cin V, Klee HJ (2007) Ethylene receptor degradation controls the timing of ripening in tomato fruit. Plant J 51:458–467

Klee HJ (2004) Ethylene signal transduction. Moving beyond Arabidopsis. Plant Physiol 135:660–667

Lashbrook CC, Tieman DM, Klee HJ (1998) Differential regulation of the tomato *ETR* gene family throughout plant development. Plant J 15:243–252

McMurchie EJ, McGlasson WB, Eaks IL (1972) Treatment of fruit with propane gives information about the biogenesis of ethylene. Nature 237:235–236

Meir S, Hunter DA, Chen JC, Halaly V, Reid MS (2006) Molecular changes occurring during acquisition of abscission competence following lowering auxin depletion in *Mirabilis jalapa*. Plant Physiol 141:1604–1616

Osborne DJ (1989) Abscission. CRC Crit Rev Plant Sci 8:103–129

Roberts JA, Elliott KA, Gonzalez-Carranza ZH (2002) Abscission, dehiscence, and other cell separation process. Annu Rev Plant Biol 53:131–158

Sato-Nara K, Yuhashi K, Higashi K, Hosoya K, Kubota M, Ezura H (1999) Stage- and tissue-specific expression of ethylene receptor homologue genes during fruit development in muskmelon. Plant Physiol 119:321–329

Schupp JR, Greene DW (2004) Effect of aminoethoxyvinylglycine (AVG) on preharvest drop, fruit quality, and maturation of 'McIntosh' apples. I. Concentration and timing of dilute applications of AVG. HortScience 39:1030–1035

Sisler EC, Dupille E, Serek M (1996) Effects of 1-methylcyclopropene and methylcyclopropene on ethylene binding and ethylene action on cut carnation. Plant Growth Regul 18:79–86

Smock RM, Gross CR (1947) The effect of some hormone materials on the respiration and softening rates of apples. Proc Am Soc Hortic Sci 49:67–77

Taylor JE, Whitelaw CA (2001) Signals in abscission. New Phytol 151:323–339

Tieman DV, Taylor MG, Ciardi JA, Klee HJ (2000) The tomato ethylene receptors NR and LeETR4 are negative regulators of ethylene response and exhibit functional compensation within a multigene family. Proc Natl Acad Sci USA 97:5663–5668

Tonutti P, Cass LG, Christoffersen RE (1995) The expression of cellulase gene family members during induced avocado fruit abscission and ripening. Plant Cell Environ 18:709–703

Tucker ML, Sexton R, Campillo ED, Lewis LN (1988) Bean abscission cellulase. Plant Physiol 88:1257–1262

Vandenbussche F, Van Der Straeten D (2007) One for all and all for one: Cross-talk of multiple signals controlling the plant phenotype. J. Plant Growth Regul 26:178–187

Wakasa Y, Kudo H, Ishikawa R, Akada S, Senda M, Niizeki M, Harada T (2006) Low expression of an endopolygalacturonase gene in apple fruit with long-term storage potential. Postharvest Biol Technol 39:193–198

Wan CY, Wilkins TA (1994) A modified hot borate method significantly enhances the yield of high-quality RNA from cotton (*Gossypium hirsutum* L.). Anal Biochem 223:7–12

Wang KLC, Li H, Ecker JR (2002) Ethylene biosynthesis and signaling networks. Plant Cell 14:S131–S151

Wiersma PA, Zhang H, Lu C, Quail A, Toivonen PMA (2007) Survey of the expression of genes for ethylene synthesis and perception during maturation and ripening of 'Sunrise' and 'Golden Delicious' apple fruit. Postharvest Biol Technol 44:204–211

Yuan R, Carbaugh DH (2007) Effects of NAA, AVG, and 1-MCP on ethylene biosynthesis, preharvest fruit drop, fruit maturity, and quality of 'Golden Supreme' and 'Golden Delicious' apples. HortScience 42:101–105

Yuan R, Hartmond U, Kender WJ (2001) Physiological factors affecting response of mature 'Valencia' oranges to CMN-pyrazole. II. Endogenous concentrations of indole-3-acetic acid, abscisic acid, and ethylene. J Am Soc Hortic Sci 126:420–426