HortScience 58(11):1410–1417. 2023. https://doi.org/10.21273/HORTSCI17287-23

# Aminoethoxyvinylglycine Reduces Preharvest Fruit Drop and Fruit Ethylene Evolution in 'Red Delicious' Apple but Affects Fruit Size and Quality Inconsistently

**Anish Malladi and Krittika V. Tonapi**
*Department of Horticulture, University of Georgia, 1111 Miller Plant Sciences, Athens, GA 30602, USA*

**Thomas M. Kon**
*Department of Horticultural Science, North Carolina State University, Mountain Horticultural Crops Research and Extension Center, 455 Research Drive, Mills River, NC 28759, USA*

*Keywords.* abscission, ethylene inhibitor, fruit growth, fruit drop, *Malus ×domestica*, plant growth regulator, ripening

***Abstract.*** **Aminoethoxyvinylglycine (AVG) is widely used in commercial apple (*Malus ×domestica* Borkh.) production to reduce preharvest fruit drop (PFD) and delay ripening for harvest management. Recently, the maximum allowable concentration of AVG was doubled (up to 264 mg·L$^{-1}$). Reports of the relationship between the AVG concentration and fruit growth, size, and quality have been contradictory. We evaluated the relationship between the AVG concentration and PFD, fruit size, fruit quality, and expression of ethylene signaling-related and cell wall modification-related genes. Experiments were conducted in 2019 and 2020 using mature 'Red Delicious' in western North Carolina. The AVG treatments [0 and 132 (AVG-1x) and 264 mg·L$^{-1}$ (AVG-2x)] were applied 3 weeks before the expected harvest. The AVG treatments reduced fruit drop and internal ethylene concentration relative to the control in both years. There was no difference in drop between AVG-1x and AVG-2x applications. Only in 2020 did AVG treatments delay fruit softening and starch hydrolysis and reduce soluble solids concentration. There were no effects on red fruit color development. Fruit size was unaffected by AVG in 2019, but it was reduced in 2020 with the AVG-2x application. AVG reduced ethylene synthesis and altered signaling, evidenced by decreased relative expression of genes related to ethylene signaling (*ARGOS1, ARGOS2*). AVG applications also reduced the expression of *EXPA8;1*, suggesting that reduced cell wall disassembly was associated with a reduction in fruit softening. These results indicate that preharvest applications of 132 mg·L$^{-1}$ AVG effectively reduced PFD via altering ethylene evolution and signaling. Use of a higher AVG concentration was of limited benefit.**

Received for publication 1 Jun 2023. Accepted for publication 14 Sep 2023.
Published online 19 Oct 2023.
This work was supported by the USDA National Institute of Food and Agriculture Hatch Project 7003225 and the Department of Horticulture at the University of Georgia. We thank Caris Jenkins, JD Obermiller, Diana Vercillo, and the technical support staff at the Mountain Horticultural Crops Research and Extension Center for their contributions to this project. Special thanks to Richard Staton at Richmind Co. for hosting a portion of this research. We gratefully acknowledge Dr. Jimmy Larson and Annie Vogel for critical review of this manuscript. Valent USA donated the plant growth regulator used in this study. Mention of a trademark, proprietary product, or vendor does not constitute a guarantee of warranty of the product by the University of Georgia or North Carolina State University and does not imply its approval to the exclusion of other products or vendors that may also be suitable.
T.M.K. is the corresponding author. E-mail: tom_kon@ncsu.edu.


Fruit abscission, or physiological drop of apple (*Malus ×domestica* Borkh.), occurs in multiple phases during growth and development. Preharvest fruit drop (PFD), which occurs with many commercially important apple cultivars, involves fruit abscission before horticultural maturity (stage at which development is optimum for a particular use) (Westwood 1993). PFD may begin several weeks before the expected harvest (Arseneault and Cline 2016). In severe cases, PFD can lead to extensive crop losses of up to 50% (Byers et al. 2005; Greene and Schupp 2004; Stover et al. 2003).

Multiple factors contribute to PFD of apple. Interactions among phytohormones that regulate fruit ripening and abscission, reduction in xylem functionality, and progression of fruit metabolism have been suggested as potential mechanisms contributing to the propensity of a cultivar to display PFD (Arseneault and Cline 2016). Ethylene and auxins have key roles in regulating organ abscission as a promoter and inhibitors, respectively (Taylor and Whitelaw 2001). Specifically, increased ethylene may activate and/or accelerate the production of cell hydrolysis enzymes (Li et al. 2010) at the abscission zone, which is the primary region of organ detachment. Sustained flux of auxins through the abscission zone inhibits the production of cell wall hydrolysis enzymes, maintaining insensitivity to ethylene (Li et al. 2010; Taylor and Whitelaw 2001). The internal ethylene concentration (IEC) is correlated with the extent of PFD (Larson et al. 2023; Li et al. 2010). However, evidence of ethylene-independent mechanisms involved in regulating PFD has also been noted through the analysis of multiple apple genotypes (Sun et al. 2009). Numerous *Malus* genotypes displayed a low internal ethylene concentration (IEC), high PFD, and, conversely, a high IEC and low PFD. Sun et al. (2009) suggested that ethylene production may not be the sole mechanism regulating PFD. Ethylene perception and signaling and its interaction with auxin may further determine the PFD propensity in apple.

The use of plant growth regulators to manage PFD relies on altering the auxin and ethylene content. Naphthaleneacetic acid (NAA) is a synthetic auxin that reduces PFD by decreasing the production of cell hydrolysis enzymes (Li and Yuan 2008). Additionally, application of an inhibitor of ethylene biosynthesis, aminoethoxyvinylglycine (AVG), reduces PFD of several apple cultivars (Bangerth 1978). AVG inhibits the activity of aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS), the enzyme catalyzing conversion of S-adenosyl methionine (SAM) to ACC, the immediate precursor of ethylene. Multiple studies have indicated that preharvest applications of AVG reduce or delay the progression of PFD in several PFD-prone apple cultivars (Arseneault and Cline 2018; Bangerth 1978; Byers et al. 2005; Greene and Schupp 2004; Yildiz et al. 2012; Yuan and Carbaugh 2007). Furthermore, inhibition of ethylene biosynthesis and its perception through combined applications of AVG and 1-methylcyclopropene (1-MCP) enhance fruit retention in 'Arlet' apple (Byers et al. 2005).

Harvesting before optimum maturity can reduce PFD-related losses, but it can lead to inferior fruit quality and associated problems in long-term storage. Furthermore, it often may not be feasible because of limitations in labor availability (Byers et al. 2005). Ethylene evolution increases during climacteric fruit ripening in apple. Because ethylene is an important regulator of progression of ripening, reduction in its synthesis and evolution may delay progression. In fact, the application of AVG reduces fruit softening and starch degradation (Byers et al. 2005; Greene and Schupp 2004; Yuan and Li 2008). In 2016, the concentration range of AVG that can be legally applied was increased (up to 264 mg·L$^{-1}$).

Relationships between the AVG concentration and fruit growth dynamics are unclear. Apples display an expolinear growth pattern in which growth increases through maturity (Bain and Robertson 1951). Applications of

AVG and the resultant decline in PFD can lead to increased fruit size and delayed ripening (Byers et al. 2005; Greene 2005; Greene and Schupp 2004). An increase in fruit size under these conditions is largely ascribed to the extended persistence of fruit on the tree during a period when it displays continued growth. Furthermore, a corresponding decline in the rate of senescence of tissues within the abscission zone and the fruit pedicel allows for sustained fruit growth (Byers et al. 2005). On the contrary, negative effects on fruit size were reported (Wargo et al. 2004; Watkins et al. 1997). Fallahi (2012) observed that AVG reduced fruit weight relative to NAA treatment and attributed the reduction to delayed maturity.

The effects of different application rates of AVG on 'Red Delicious', a cultivar displaying high propensity for PFD in the southeastern United States, particularly under the increased application range, have not been extensively investigated (Unrath et al. 2009). Specifically, the effects of AVG application on fruit growth are not well-documented, particularly in the context of the higher application dosage now allowed in the United States. The goal of this study was to determine the effects of single applications of AVG (AVG-1x) and double applications of AVG (AVG-2x) on fruit size, fruit quality characteristics, and the expression of several genes associated with ethylene signaling and cell wall loosening.

## Materials and Methods

Experiments were conducted in 2019 at a commercial orchard in Flat Rock, NC, USA (lat. 35.308394°N, long. 82.374546°W), and in 2020 at North Caroline State University's Mountain Horticultural Crops Research and Extension Center in Mills River, NC, USA (lat. 35.428079°N, long. 82.563295°W). The experimental site varied across years because of travel restrictions related to the global coronavirus 2019 (COVID-19) pandemic; however, mature 'Red Delicious' trees were used in both years. In 2019, 'Early Red One'/'Mark'/'M. 111' planted to 3.1 × 6.7 m were used. In 2020, we used 'Oregon Spur II'/'M. 111' trees planted with spacing of 2.7 × 6.1 m. Both orchards were trained to a central leader and received plant protectant sprays that adhered to local recommendations throughout the growing season (NC State Extension 2023). In 2019, 12 two-tree plots were used for this study, whereas in 2020, 12 uniform three-tree plots were selected and separated by buffer trees. Treatments were assigned in a randomized completed block design with four replications. Trees were blocked by crop density, which was visually rated before the initiation of the experiment. Within each experimental unit, one tree was designated for fruit drop counts, and one (2019) or two (2020) trees were designated for fruit sampling for fruit quality assessments and gene expression.

The following AVG treatments were evaluated: 0 $mg \cdot L^{-1}$ AVG (untreated control); 132 $mg \cdot L^{-1}$ AVG (AVG-1x) (ReTain®; Valent Biosciences, Libertyville, IL, USA); and 264 $mg \cdot L^{-1}$ AVG (AVG-2x). All chemical treatments were applied in an aqueous solution with 0.1% (volume/volume) organosilicone surfactant (Silwet L-77; Helena Agri-Enterprises, Collierville, TN, USA) on 16 Aug 2019 and 18 Aug 2020. Treatments were applied ~3 weeks before the anticipated harvest with a backpack air blast sprayer (SR 450; Stihl USA Inc., Virginia Beach, VA) calibrated to apply 935 $L \cdot ha^{-1}$. The amounts of spray solution applied to each tree were 1.89 L in 2019 and 1.70 L in 2020. The sprayer was outfitted with a pressure compensation pump to ensure consistent output during its operation.

*Preharvest fruit drop responses to AVG treatments.* Fruit drop counts were initiated 2 weeks before the anticipated harvest, occurred at weekly intervals, and continued for 7 weeks in 2019 and 9 weeks in 2020. The number of abscised fruit were counted and discarded on one tree per plot. Immediately after the final fruit drop count was conducted, all persisting fruit were harvested and counted. To determine PFD patterns over time, the percentage of fruit that abscised was calculated for each week of the experiment.

*Internal ethylene concentration in response to AVG treatments.* Starting at the anticipated week of harvest [0 weeks after expected harvest (WAEH)], 10 to 12 fruit samples were collected from trees designated for fruit quality evaluations. To determine the IEC, 1-mL gas samples were extracted from the core cavity with a syringe fitted with a 0.6-mm × 25.0-mm hypodermic needle. Samples were injected into a gas chromatograph (GC-8A; Shimadzu, Columbia, MD, USA) with a 3.175-mm stainless steel column packed with alumina (Supelco; Bellefonte, PA, USA). All samples were measured within 24 h of harvest.

*Fruit size responses to AVG treatments.* Weekly, a separate 20-fruit sample was collected from each plot to determine relationships between AVG and fruit quality parameters. Fruit diameter and weight were determined with an electronic fruit sorter (Durand-Wayland, Inc., LaGrange, GA, USA) outfitted with a load cell, color and infrared camera system, and full transmittance spectrometer (TrueSort Electronics; Ellips, Eindhoven, Netherlands).

*Fruit quality in response to AVG treatments.* A colorimeter (ColorFlex EZ; Hunter Associates Laboratory, Reston, VA, USA) was used to quantify lightness, chroma, and hue on the green and red fruit peels of each fruit. Fruit firmness was measured with a fruit texture analyzer (GS-20; Güss Manufacturing Ltd. Strand, Cape Town, South Africa). Juice samples were extracted using a potato ricer and tested to determine the soluble solids concentration (SSC) with a digital refractometer (PR-32 alpha; Atago, Bellevue, WA, USA). Fruit were cut in half at the equator, and the cut surface was dipped in an iodine solution. Iodine staining patterns were evaluated in accordance with the Generic Cornell Starch-Iodine Index Chart for apples (scale, 1–8) (Blanpied and Silsby 1992).

*RNA extraction and gene expression analyses by quantitative real-time polymerase chain reaction.* Starting at 2 WAEH in 2020, fruit cortex samples were collected weekly for gene expression analyses from three fruit per plot. Samples were processed and prepared for RNA extraction following the methods described by Larson et al. (2023). Total RNA was extracted from frozen tissue according to the CTAB method (Vashisth et al. 2011). After extraction, RNA quantity and concertation were determined using a spectrophotometer (ND-1000; Thermo Fisher Scientific, Wilmington, DE, USA). cDNA was synthesized in a 20-μL reaction volume using 1 μg of DNase-treated RNA (Vashisth et al. 2011) and diluted six-fold for quantitative real-time polymerase chain reaction (PCR).

Quantitative real-time PCR were performed according to the methods described by Larson et al. (2023). Primer efficiency (Eff) was quantified in LinRegPCR and cycle threshold values (Ct) were determined for each sample (Ramakers et al. 2003; Ruijter et al. 2009). The Relative quantity of expression (RQ) values were calculated using the following equation: $RQ = 1/Eff^{Ct}$. Then, the RQ values of genes of interest were normalized to the expression of the reference genes *ACTIN*, *CACS2*, and *UBC2* (Dash et al. 2013; Jing and Malladi 2022), generating NRQ values. Primer sequences for the genes used in this study have been reported previously (Dash et al. 2013; Jing and Malladi 2022). Genes of interest in this experiment were *ARGOS1*, *ARGOS2*, *COB1*, *EXPA8;1*, *EXPA8;2*, and *EXPA10;1*.

*Statistical analyses.* All data were analyzed using statistical software (JMP Pro version 15; SAS, Cary, NC, USA). Data were analyzed using a mixed model with the AVG treatment levels as a fixed factor and block as a random factor. Mean separation was performed using Tukey's honestly significant difference (HSD) test when the treatment factor was significant. For gene expression data, NRQ values were transformed using $\log_2$ transformation before statistical analyses. Plots were prepared in Sigmaplot 14.0 (Systat Software Inc., Palo Alto, CA, USA), and the figures were prepared using Inkscape 1.0 (Inkscape USA).

## Results

*Preharvest fruit drop responses to AVG treatments.* Extensive fruit drop was noted in control fruit in both years of the study, although the onset of drop was delayed in 2020 compared with that in 2019 (Fig. 1). In 2019, the highest extent of fruit drop was noted at 0 and 1 WAEH. In 2020, fruit drop could be observed from 2 WAEH, reaching a peak at 3 WAEH, and subsequently declining. The AVG treatments reduced the degree of PFD. In 2019, the AVG-1x treatment resulted in lower fruit drop (up to 17-fold) than that of the control at 0 and 1 WAEH. From 3 to 5 WAEH, AVG-1x displayed up to four-fold higher fruit drop than the control. AVG-2x treatments resulted in greatly reduced fruit drop at 0 and 1 WAEH in 2019 (up to 17-fold). At 5 WAEH, AVG-2x displayed three-fold greater fruit drop than in the

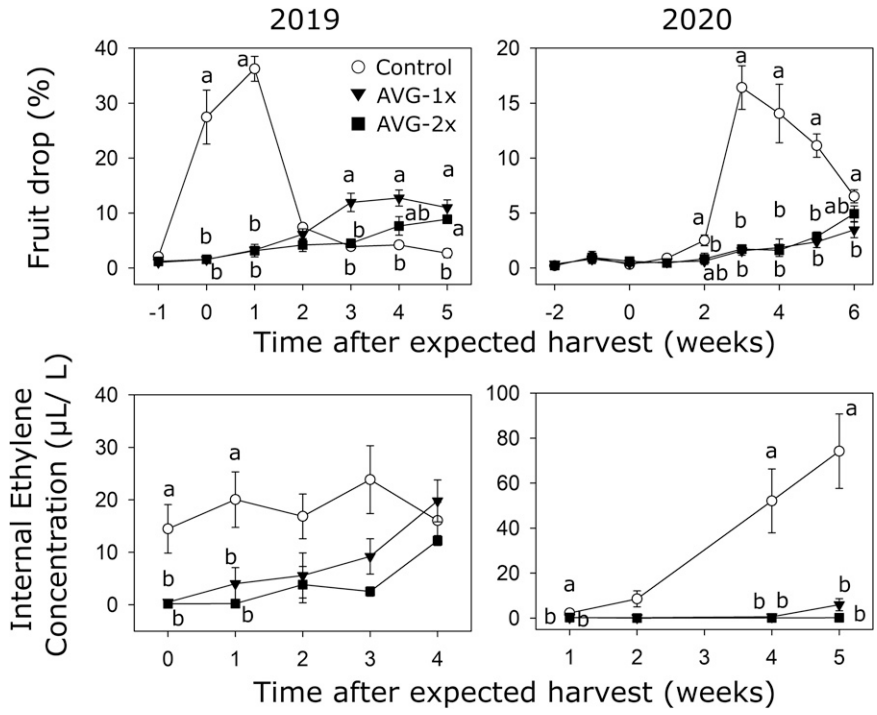


Fig. 1. Preharvest fruit drop (%) and internal ethylene concentration of 'Red Delicious' (*Malus ×domestica* Borkh.) fruit in response to aminoethoxyvinylglycine (AVG) treatments in 2019 and 2020. Zero weeks after expected harvest indicates the time of the anticipated harvest. The percent fruit drop at each time was calculated by dividing the number of fruit that abscised each week by the total number of fruit. Means and *SE* are presented ($n$ = 4). Similar letters above the symbols indicate that means are not significantly different within a given time point. AVG-1x = application rate at 132 mg·L$^{-1}$; AVG-2x = application rate of 264 mg·L$^{-1}$.

control. In 2020, AVG-1x significantly reduced fruit drop during the period when peak fruit drop occurred in the control (2–6 WAEH; by up to 10-fold). AVG-2x treatments resulted in reduced fruit drop from 3 to 5 WAEH (up to 9.5-fold). AVG-2x treatments did not result in further reduction in fruit drop in comparison with the AVG-1x, except at 3 WAEH in 2019.

*Internal ethylene concentration in response to AVG treatments.* The IEC was not substantially altered in control fruit between 0 and 4 WAEH in 2019 (Fig. 1). The AVG treatments resulted in substantially reduced IEC (up to 86-fold) at 0 and 1 WAEH, but not at later stages. In 2020, the IEC gradually increased in control fruit between 1 and 5 WAEH. The AVG treatments reduced IEC by up to 8-fold at 1 WAEH, and by more than 400-fold at 4 and 5 WAEH

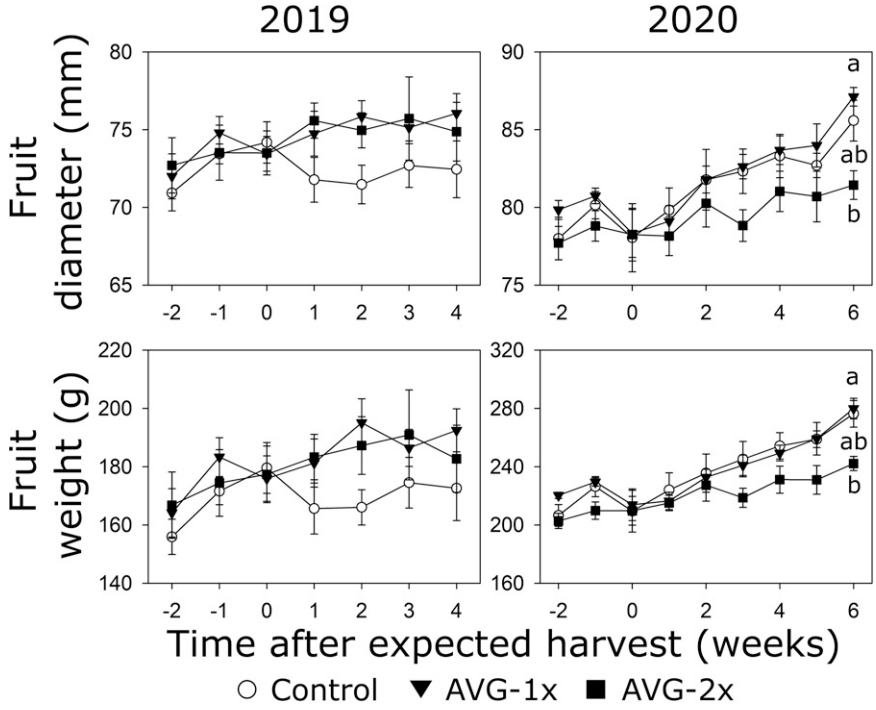


Fig. 2. Effects of aminoethoxyvinylglycine (AVG) application on fruit size of 'Red Delicious' (*Malus ×domestica* Borkh.). Means and *SE* are presented ($n$ = 4). Similar letters above the symbols indicate that means are not significantly different within a given time point (Tukey's honestly significant difference). AVG-1x = 132 mg·L$^{-1}$; AVG-2x = 264 mg·L$^{-1}$.

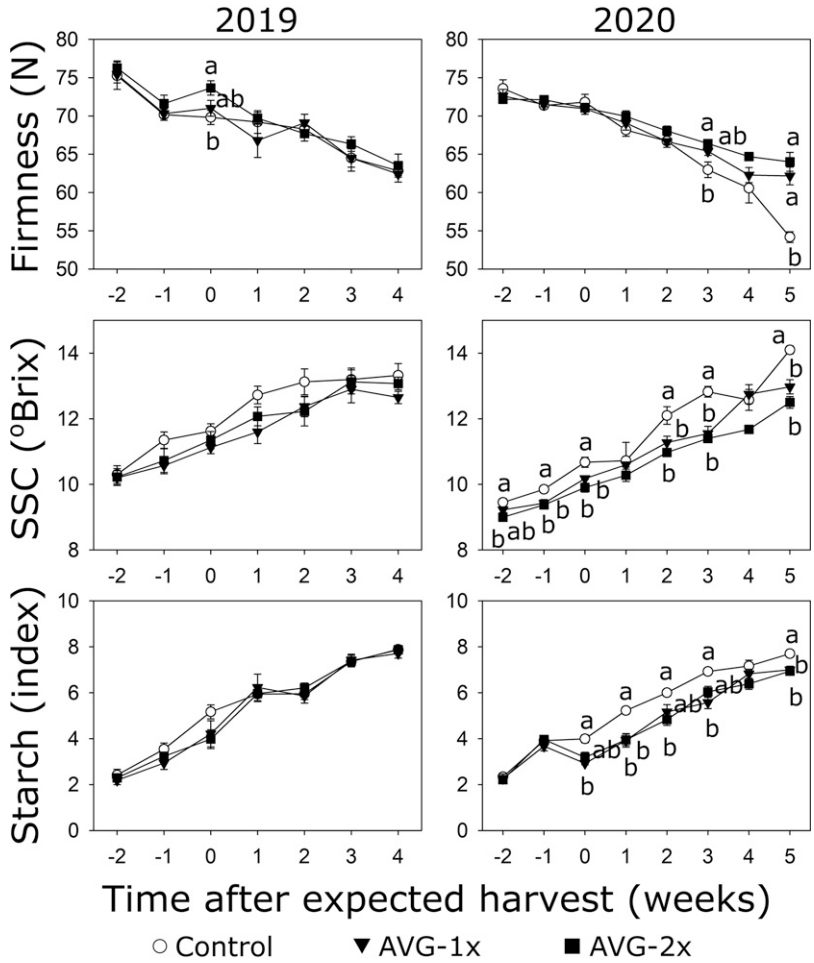


Fig. 3. Effects of aminoethoxyvinylglycine (AVG) application on fruit quality of 'Red Delicious' (*Malus ×domestica* Borkh.) in 2019 and 2020. Means and *SE* are presented ($n = 4$). Similar letters above the symbols indicate that means are not significantly different within a given time point (Tukey's honestly significant difference). AVG-1x = 132 mg·L$^{-1}$; AVG-2x = 264 mg·L$^{-1}$.

relative to the control. An increasing AVG dosage did not result in further reduction in IEC during either year.

*Fruit size responses to AVG treatments.* In 2019, fruit size (diameter and weight) continued to increase from −2 to 0 WAEH in the control (Fig. 2). The AVG treatments did not significantly affect fruit size in 2019. In 2020, fruit diameter and weight steadily increased in the control fruit by 9.7% and 34%, respectively, between −2 and 6 WAEH. A similar trend was noted with AVG treatments, particularly AVG-1x. The AVG-2x treatment resulted in lower fruit diameter and weight than the AVG-1x treatment at 6 WAEH by ∼6.5% and 13.5%, respectively.

*Fruit quality in response to AVG treatments.* In 2019, fruit firmness declined progressively in all treatments. The AVG-2x treatment resulted in greater (5.5%) fruit firmness than the control at 0 WAEH (Fig. 3). In 2020, fruit firmness declined gradually between −2 and 4 WAEH, and then sharply by 5 WAEH. The AVG-2x treatment resulted in approximately 5% higher fruit firmness than the control fruit at 3 WAEH. At 5 WAEH, both AVG treatments resulted in greater fruit firmness than that in the control fruit by up to 18%.

In 2019, fruit SSC gradually increased in the control fruit during the period of evaluation (Fig. 3), but the AVG had no effect on fruit SSC. In 2020, the AVG-1x treatment resulted in reduced SSC during most of fruit development, except at −2, 1, and 4 WAEH. The AVG-2x treatment resulted in lower fruit SSC at all stages, except at 1 and 4 WAEH. The starch pattern index consistently increased during both years of this study. In 2019, the AVG treatments did not significantly alter the starch index. In 2020, the AVG-1x treatments reduced the starch index at 0, 1, 3 and 5 WAEH, whereas the AVG-2x treatment reduced it at 1, 2, and 5 WAEH compared with the control. The AVG dosage did not influence fruit SSC or starch index.

In 2019, fruit color parameters, lightness, and hue decreased, whereas chroma increased over the period of evaluation from −1 to 4 WAEH (Fig. 4). Neither of the AVG treatments significantly altered fruit color characteristics in 2019. In 2020, lightness decreased over the evaluation period, hue was not appreciably altered, and chroma gradually decreased from 1 WAEH. The AVG treatments had minimal and inconsistent effects on fruit color. The two AVG treatments decreased hue at 0 WAEH by up to 2.7°. At 3 WAEH, the AVG-2x treatment displayed higher chroma values than the control by up to 1.5 units.

*Expression of ethylene signaling-related and cell wall modification-related genes in response to AVG treatments.* Transcript abundance of two genes coding for proteins involved in ethylene signaling, *ARGOS1* (*AUXIN REGULATED GENE INVOLVED IN ORGAN SIZE1*) and *ARGOS2*, was substantially reduced in response to AVG treatments (Fig. 5). *ARGOS1* transcript abundance increased by up to 3.2-fold in the control fruit between 2 and 5 WAEH. It was reduced in response to AVG-1x treatments at 3 and 4 WAEH by up to three-fold, and by AVG-2x treatments by up to four-fold at 3, 4, and 6 WAEH. *ARGOS2* transcript abundance also increased in the control fruit by up to 3.7-fold between 2 and 5 WAEH. The AVG-1x treatment resulted in a decrease in *ARGOS2* transcript abundance by up to five-fold from 2 to 5 WAEH, whereas the AVG-2x treatment reduced *ARGOS2* transcript abundance by up to six-fold between 2 and 6 WAEH.

Transcript abundance of *COB1 (COBRA1)*, which codes for a protein associated with cellulose synthesis and assembly, and oriented cell expansion, was not substantially altered over the duration of the analyses and was unaffected by the AVG treatments. Transcript abundance of three genes coding for α-expansins was investigated. Transcript abundance of *EXPA8;1*

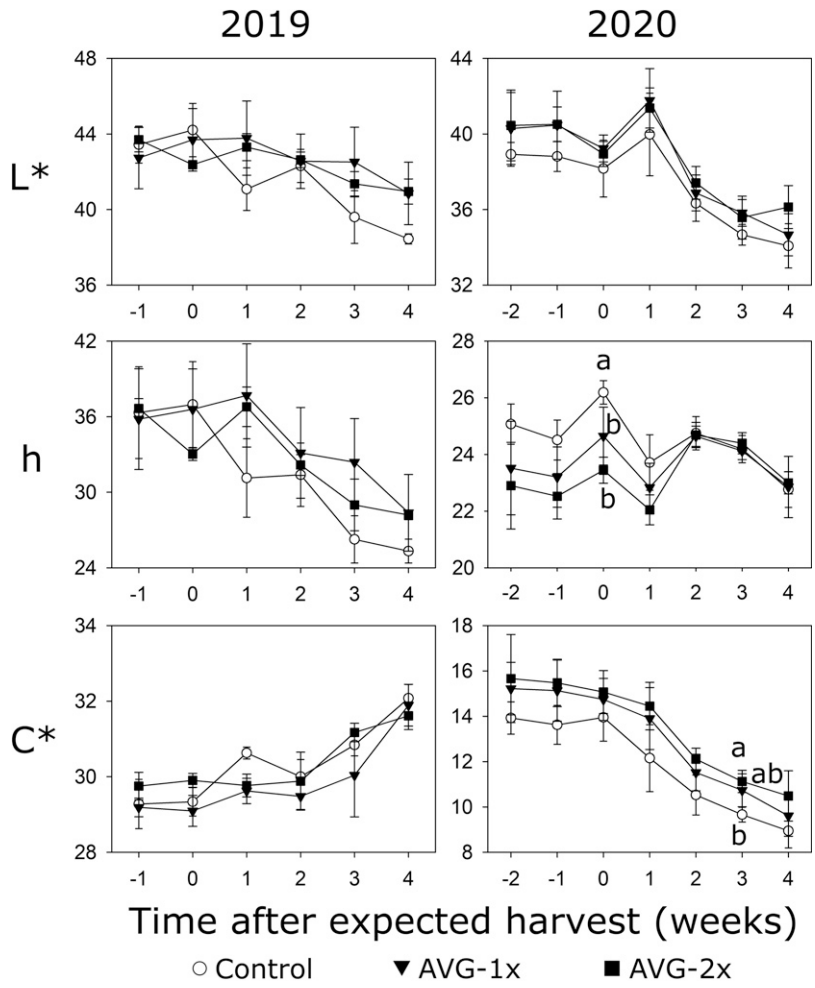


Fig. 4. Effects of aminoethoxyvinylglycine (AVG) application on fruit color of 'Red Delicious' (*Malus* ×*domestica* Borkh.). Means and *SE* are presented ($n = 4$). Similar letters above the symbols indicate that means are not significantly different within a given time point (Tukey's honestly significant difference). AVG-1x = 132 mg·L$^{-1}$; AVG-2x = 264 mg·L$^{-1}$. L* = lightness; h = hue; C* = chroma.

increased in the control by approximately 1.7-fold between 2 and 3 WAEH. The AVG-1x treatment reduced its abundance by up to 2.5-fold at 3 and 4 WAEH, and the AVG-2x treatment reduced its expression by up to 4.7-fold between 2 and 4 WAEH. Transcript abundance of *EXPA8;2* increased greatly (by up to 18-fold) in the control between 2 and 4 WAEH. AVG treatments did not significantly reduce its abundance, except for a six-fold reduction at 3 WAEH by the AVG-2x treatment. *EXPA10;1* abundance was highly variable during the period of evaluation and was not significantly affected by AVG treatments.

## Discussion

PFD extended over several weeks in the current study. In 2019, high levels of PFD were noted in the control treatments at approximately the anticipated harvest time; however, in 2020, PFD extended over several weeks at later stages, specifically over 3 to 5 WAEH. These data are consistent with those of previous reports that indicated that PFD occurs over multiple weeks following the anticipated harvest date (Arseneault and Cline 2016). Considerable variation in the apple PFD can be observed from year-to-year. Unrath et al. (2009) evaluated natural PFD in the same orchard for an 11-year period. Depending on the year, cumulative fruit drop ranged from 2% to 33% if harvest was delayed only 1 week after the normal harvest date (Unrath et al. 2009). We used two different orchards in this research because of travel restrictions related to the global pandemic. Although both orchards were mature 'Red Delicious', the scion strain and rootstocks differed across years. It is possible that these factors may have contributed to the difference in PFD patterns across years. However, observed air temperatures during the period of study may have contributed to this variation (Table 1). In 2019, the average and maximum daily air temperature in September was higher than that in 2020 (3.0 °C and 5.7 °C, respectively). The sampling date for 0 WAEH occurred on 5 Sep and 9 Sep in 2019 and 2020, respectively. Poapst et al. (1959) observed a positive association between the timing of fruit abscission and the decline of starch in 'McIntosh'. Notably, this relationship was influenced by the average daily temperature. Additional research is warranted to gain a better understanding of the interactions between environmental factors, such as air temperature, and PFD drop patterns.

During both years of this study of 'Red Delicious' apple, the application of AVG significantly reduced PFD, thereby confirming previous reports (Byers et al. 2005; Greene 2005; Schupp and Greene 2004). However, unlike in several previous studies (Greene and Schupp 2004; Schupp and Greene 2004), increasing the dose of AVG application did not result in a consistent additional reduction in PFD, indicating that AVG-1x applications were likely sufficient to reduce PFD occurrence in 'Red Delicious' apple in the southeastern United States.

During both years of this study, periods of high PFD were associated with higher IEC in the apple fruit, particularly in the control treatment. Reduction in PFD in response to AVG, irrespective of the dosage, was associated with reduced IEC in both years. Similarly, multiple previous studies have identified an association between ethylene synthesis/evolution from the apple fruit and PFD (Arseneault and Cline 2016; Larson et al. 2023; Li et al. 2010). The IEC may explain the different patterns in drop between 2019 and 2020. For untreated fruit, in 2019, the IEC was 20-fold higher at 1 WAEH compared with that in 2020. Fruit were more likely susceptible to drop in 2019 than in 2020. Again, elevated temperatures proximal to the 2019 anticipated harvest date may

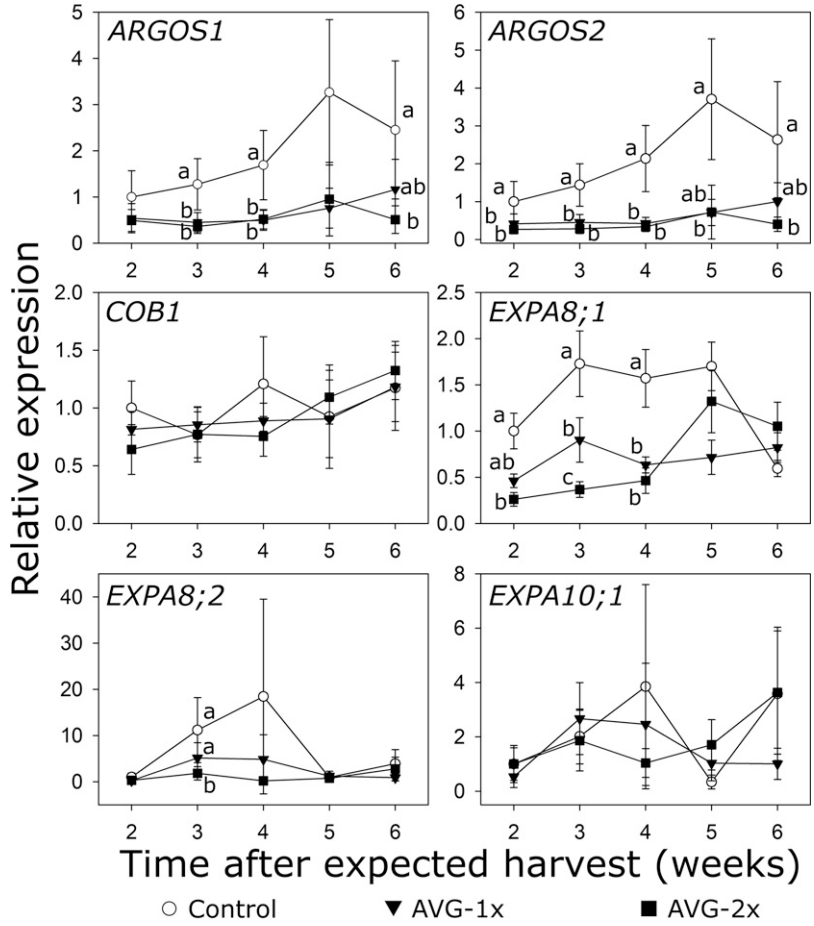


Fig. 5. Effects of aminoethoxyvinylglycine (AVG) application on relative gene expression of 'Red Delicious' (*Malus ×domestica* Borkh.) in 2020. Means and *SE* are presented ($n = 4$). Similar letters above the symbols indicate that means are not significantly different within a given time point (Tukey's honestly significant difference). AVG-1x = 132 mg·L$^{-1}$; AVG-2x = 264 mg·L$^{-1}$. Expression of a given gene is normalized to its expression in the control at 2 weeks after expected harvest. *ARGOS = AUXIN REGULATED GENE INVOLVED IN ORGAN SIZE; COB1 = COBRA1; EXPA = α-EXPANSIN.*

partly explain the observed increase in PFD and IEC. A comparison of two apple cultivars with differing PFD proclivities indicated that greater fruit ethylene evolution in 'Golden Delicious' was associated with higher PFD in comparison with 'Fuji' (Li et al. 2010).

Li et al. (2010) found that the higher ethylene evolution in 'Golden Delicious' was associated with higher expression of several genes associated with its biosynthesis such as *ACS1*, *ACS3*, and *ACS5A*, as well as *ACC OXIDASE 1* (*ACO1*) in the fruit cortex. Application of AVG reduced ethylene evolution and PFD and was associated with the decreased expression of multiple *ACS* genes and *ACO1* (Yuan and Li 2008). Hence, it is likely that similar reductions in *ACS* and *ACO* transcript abundance and corresponding activity in response to AVG applications reduced IEC during our study in 'Red Delicious'. In the current study, expressions of *ARGOS1* and *ARGOS2* increased during 2 to 6 WAEH, particularly in the control treatment, and was significantly reduced in response to AVG applications. ARGOS is a negative regulator of ethylene signaling and functions in the reduction of organ sensitivity to ethylene (Rai et al. 2015; Shi et al. 2015). Transcript abundance of *ARGOS* family genes is responsive to ethylene (Rai et al. 2015; Shi et al. 2015). Thus, decreases in the transcript abundance of *ARGOS1* and *ARGOS2* in response to AVG treatments are likely associated with lower ethylene biosynthesis. Furthermore, because ethylene signaling is required to elicit changes in *ARGOS* expression (Rai et al. 2015), these data indicate that reductions in ethylene evolution by AVG applications also result in altered ethylene signaling. Therefore, these data indicate that a reduction in ethylene evolution by AVG also altered ethylene signaling and downstream responses.

A sustained increase in fruit weight is an important advantage of PFD reduction via PGR applications (Byers et al. 2005). Retention of fruit on the tree may allow for continued cell expansion with the primary mechanism supporting

Table 1. Summary of average daily air temperature data for select months in 2019 and 2020 in Mills River, NC, USA.[i,ii]

| Month | Average air temperature[iii] (°C) | Maximum air temperature (°C) | Minimum air temperature (°C) |
|---|---|---|---|
| | | *2019* | |
| Aug | 21.6 | 28.1 | 16.8 |
| Sep | 21.1 | 29.1 | 14.9 |
| Oct | 14.9 | 21.1 | 9.5 |
| | | *2020* | |
| Aug | 21.6 | 26.8 | 17.8 |
| Sep | 18.1 | 23.4 | 14.2 |
| Oct | 14.2 | 20.9 | 9.0 |

[i] Data courtesy of State Climate Office of North Carolina, North Carolina State University. Cardinal (data retrieval interface) available at https://products.climate.ncsu.edu/cardinal/request. Accessed 11 Sep 2023.

[ii] Weather station (FLET) located at 35.42721°N, 82.55888°W.

[iii] Means reported are the daily average air temperature across each month of interest. Selected months correspond with the period of study in 2019 and 2020.

fruit growth during this period, thereby sustaining the continued increase in fruit size. However, during both years of this study, fruit size was not significantly improved by AVG treatments. Furthermore, AVG-2x applications appeared to result in a lower fruit size than the AVG-1x treatment, potentially suggesting an inhibitory effect of high AVG concentrations.

Fruit quality characteristics were inconsistently affected by AVG treatments across the two years of this study. In 2019, the fruit quality characteristics were not affected by AVG treatments, except fruit firmness was higher in response to the AVG-2x treatment than the control at the anticipated harvest date. Again, these limited responses in 2019 may be partly explained by elevated temperatures after AVG application. High temperatures after AVG applications resulted in inconsistent PFD and maturity management across multiple studies (Kon et al. 2023; Stover et al. 2003; Stover and Greene 2005). In 2020, fruit firmness, SSC, and the starch index were affected by AVG applications, particularly during later stages of the study. In 2020, fruit firmness substantially declined in the control between 4 and 5 WAEH, and this decline was attenuated by AVG applications, similar to the reports of previous studies (Bangerth 1978; Byers et al. 2005; Greene and Schupp 2004; Schupp and Greene 2004). These data indicate that AVG applications delayed fruit softening. Furthermore, the extent of starch degradation was reduced, indicating that AVG applications delayed progression of ripening and loss of fruit quality in 'Red Delicious'. The decline in starch degradation observed during this study is consistent with that reported by previous studies (Byers et al. 2005; Greene and Schupp 2004; Yuan and Li 2008). The response of fruit SSC to AVG treatments has not been consistent. Fruit SSC increased slightly with AVG treatments (Byers et al. 2005; Schupp and Greene 2004) or was unaltered (Yuan and Li 2008). During the current study (2020), SSC decreased slightly in response to AVG, suggesting delay in ripening. Similarly, previous reports indicated that fruit color development is not consistently affected by AVG applications. Although several studies reported a slight reduction in the proportion of red color (Byers et al. 2005; Schupp and Greene 2004), others indicated little or no effects of AVG on color development (Greene and Schupp 2004; Yuan and Li 2008). During the current study of 'Red Delicious', AVG applications did not consistently affect fruit color. Together, these data indicated that loss of fruit quality related to fruit softening was reduced, and that ripening was delayed by AVG applications in 'Red Delicious' only in 2020, but that other quality characteristics were largely unaltered.

Among several genes evaluated, expression of *EXPA8;1* was reduced by more than two-fold in response to AVG applications. This was particularly evident between 2 and 4 WAEH. Expansins (*EXPs*) are proteins that nonenzymatically modify noncovalent bonding among cell wall polysaccharides, resulting in cell wall loosening (Cosgrove 2015). They have multiple roles in facilitating cell expansion, growth and development, and stress responses. Ripening during fruit development is associated with multiple physiological and biochemical changes, which include increased fruit softening associated with cell wall loosening and disassembly. In strawberry (*Fragaria* × *ananassa*) fruit, the loss of fruit firmness during ripening has been associated with greater expression of several *EXPs* and higher extension activity (Harrison et al. 2001). A comparison of genotypes with differences in fruit softening characteristics indicated that cultivars with lower fruit firmness displayed greater expression of several *EXP* genes (Dotto et al. 2006). In tomato (*Solanum lycopersicum*), *LeEXP1* is specifically expressed in ripening fruit and is ethylene-inducible (Rose et al. 1997). In apple, several *EXPs* displayed higher expression in ripening fruit or during postharvest storage in association with reduction in fruit firmness (Dash et al. 2013; Mann et al. 2008; Wakasa et al. 2003). Higher expression of *EXPA8;1* in the control treatment compared with that in the AVG treatments, during the current study is consistent with lower fruit firmness in these fruit. Reductions in ethylene evolution and signaling are likely associated with the reduction in *EXPA8;1* transcript abundance, potentially leading to reduced expansin activity and reduced fruit softening. Alternatively, reduced expression of *EXPA8;1* with AVG treatments may explain, in part, the observed reduction in fruit size with 2x-AVG in 2020. It is possible that reduced expansin activity caused by high rates of AVG may negatively influence cell enlargement rates and subsequent fruit size. Additionally, transcript abundance of another *EXP*, *EXPA8;2*, was also transiently higher by six-fold than that of the control fruit with the AVG-2x treatment at 3 WAEH. Together, these may constitute part of a delayed cell wall disassembly program in response to AVG treatments. Consistently, AVG applications also result in the reduced expression of two *POLYGALACTURONASE* genes, *PG1* and *PG2*, the products of which are also associated with cell wall disassembly (Yuan and Li 2008).

## Conclusion

These study data indicate that AVG is effective for reducing PFD in 'Red Delicious'. The AVG treatment reduced ethylene evolution and signaling, as noted by a decrease in *ARGOS1* and *ARGOS2* expression. The reduction in PFD through the application of AVG did not increase fruit size. The AVG applications reduced fruit softening and delayed ripening during one year of the study, but it did not greatly alter other fruit quality characteristics. *EXPA8;1* may function as a component of an ethylene-responsive program associated with fruit softening. Across both years, AVG-1x was sufficient to reduce PFD. A higher dose of AVG does not appear to result in greater benefits for 'Red Delicious' in the southeastern United States.

### References Cited


Arseneault MH, Cline JA. 2016. A review of apple preharvest fruit drop and practices for horticultural management. Scientia Hortic. 211:40–52. https://doi.org/10.1016/j.scienta.2016.08.002.

Arseneault MH, Cline JA. 2018. AVG, NAA, boron, and magnesium influence preharvest fruit drop and fruit quality of 'Honeycrisp' apples. Can J Plant Sci. 98:741–752. https://doi.org/10.1139/cjps-2017-0170.

Bain JM, Robertson RN. 1951. The physiology of growth in apple fruits. I. Cell size, cell number, and fruit development. Aust J Sci Res. 4:75–91. https://doi.org/10.1071/bi9510075.

Bangerth F. 1978. The effect of a substituted amino acid on ethylene biosynthesis, respiration, ripening, and preharvest drop of apple fruits. J Am Soc Hortic Sci. 103:401–404. https://doi.org/10.21273/JASHS.103.3.401.

Blanpied GD, Silsby KJ. 1992. Predicting harvest date windows for apples. Cornell Coop. Ext. Info. Bul. 221. https://hdl.handle.net/1813/3299. [accessed 21 Dec 2022].

Byers RE, Carbaugh DH, Combs LD. 2005. Ethylene inhibitors delay fruit drop, maturity, and increase fruit size of 'Arlet' apples. HortScience. 40:2061–2065. https://doi.org/10.21273/HORTSCI.40.7.2061.

Cosgrove DJ. 2015. Plant expansins: Diversity and interactions with plant cell walls. Curr Opin Plant Biol. 25:162–172. https://doi.org/10.1016/j.pbi.2015.05.014.

Dash M, Johnson LK, Malladi A. 2013. Reduction of fruit load affects early fruit growth in apple by enhancing carbohydrate availability, altering the expression of cell production-related genes, and increasing cell production. J Am Soc Hortic Sci. 138:253–262. https://doi.org/10.21273/JASHS.138.4.253.

Dotto MC, Martínez GA, Civello PM. 2006. Expression of expansin genes in strawberry varieties with contrasting fruit firmness. Plant Physiol Biochem. 44:301–307. https://doi.org/10.1016/j.plaphy.2006.06.008.

Fallahi E. 2012. Influence of 1-aminoethoxyvinylglycine hydrochloride and α-naphthalene acetic acid on fruit retention, quality, evolved ethylene, and respiration in apples. Int J Plant Prod. 1:53–61.

Greene DW. 2005. Time of aminoethoxyvinylglycine application influences preharvest drop and fruit quality of 'McIntosh' apples. HortScience. 40:2056–2060. https://doi.org/10.21273/HORTSCI.40.7.2056.

Greene DW, Schupp JR. 2004. Effect of aminoethoxyvinylglycine (AVG) on preharvest drop, fruit quality, and maturation of 'McIntosh' apples. II. effect of timing and concentration relationships and spray volume. HortScience. 39:1036–1041. https://doi.org/10.21273/HORTSCI.39.5.1036.

Harrison EP, McQueen-Mason SJ, Manning K. 2001. Expression of six expansin genes in relation to extension activity in developing strawberry fruit. J Expt Bot. 52:1437–1446. https://doi.org/10.1093/jexbot/52.360.1437.

Jing S, Malladi A. 2022. Identification of potential regulators of cell production and early fruit growth in apple (*Malus* ×*domestica* Borkh.). Scientia Hortic. 297:110939. https://doi.org/10.1016/j.scienta.2022.110939.

Kon TM, Clavet CD, Clarke GG. 2023. Organic aminoethyoxyvinylglycine is an effective alternative for reducing apple preharvest drop. HortScience. 58:733–738. https://doi.org/10.21273/HORTSCI17115-23.

Larson JE, Malladi A, Kon TM. 2023. Xylem functionality is not a direct indicator of apple preharvest fruit drop. J Am Soc Hortic Sci. 148:117–125. https://doi.org/10.21273/JASHS05302-23.

Li J, Yuan R. 2008. NAA and ethylene regulate expression of genes related to ethylene biosynthesis, perception, and cell wall degradation during fruit abscission and ripening in 'Delicious' apples. J Plant Growth Regul. 27:283–295. https://doi.org/10.1007/s00344-008-9055-6.

Li J, Zhu H, Yuan R. 2010. Profiling the expression of genes related to ethylene biosynthesis, ethylene perception, and cell wall degradation during fruit abscission and fruit ripening in apple. J Am Soc Hortic Sci. 135:391–401. https://doi.org/10.21273/JASHS.135.5.391.

Mann HS, Alton JJ, Kim S, Tong CBS. 2008. Differential expression of cell-wall – modifying genes and novel cDNAs in apple fruit during storage. J Am Soc Hortic Sci. 133:152–157. https://doi.org/10.21273/JASHS.133.1.152.

NC State Extension. 2023. Integrated orchard management guide for commercial apples in the southeast. NC State Extension Publication, AG-572. https://content.ces.ncsu.edu/integrated-orchard-management-guide-for-commercial-apples-in-the-southeast. [accessed 11 Sep 2023].

Poapst PA, Ward GM, Phillips WR. 1959. Maturation of McIntosh apples in relation to starch loss and abscission. Can J Plant Sci. 39:257–263. https://doi.org/10.4141/cjps59-037.

Rai MI, Wang X, Thibault DM, Kim HJ, Bombyk MM, Binder BM, Shakeel SN, Schaller GE. 2015. The *ARGOS* gene family functions in a negative feedback loop to desensitize plants to ethylene. BMC Plant Biol. 15:157. https://doi.org/10.1186/s12870-015-0554-x.

Ramakers C, Ruijter JM, Deprez RHL, Moorman AF. 2003. Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. Neurosci Lett. 339 1 62 66 https://doi.org/10.1016/S0304-3940(02)01423-4.

Rose JC, Lee HH, Bennett AB. 1997. Expression of a divergent expansin gene is fruit-specific and ripening-regulated. Proc Natl Acad Sci USA. 94:5955–5960. https://doi.org/10.1073/pnas.94.11.5955.

Ruijter JM, Ramakers C, Hoogaars WMH, Karlen Y, Bakker O, Van den Hoff MJB, Moorman A. 2009. Amplification efficiency: Linking baseline and bias in the analysis of quantitative PCR data. Nucleic Acids Res. 37 6 e45 https://doi.org/10.1093/nar/gkp045.

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. https://doi.org/10.21273/HORTSCI.39.5.1030.

Shi J, Habben JE, Archibald RL, Drummond BJ, Chamberlin MA, Williams RW, Lafitte RH, Weers BP. 2015. Overexpression of ARGOS genes modifies plant sensitivity to ethylene, leading to improved drought tolerance in both arabidopsis and maize. Plant Physiol. 169:266–282. https://doi.org/10.1104/pp.15.00780.

Stover E, Fargione MJ, Watkins CB, Iungerman KA. 2003. Harvest management of marshall 'McIntosh' apples: Effects of AVG, NAA, ethephon, and summer pruning on preharvest drop and fruit quality. HortScience. 38:1093–1099. https://doi.org/10.21273/HORTSCI.38.6.1093.

Stover EW, Greene DW. 2005. Environmental effects on the performance of foliar applied plant growth regulators: A review focusing on tree fruits. HortTechnology. 15:214–221. https://doi.org/10.21273/HORTTECH.15.2.0214.

Sun L, Bukovac MJ, Forsline PL, van Nocker S. 2009. Natural variation in fruit abscission-related traits in apple (*Malus*). Euphytica. 165:55–67. https://doi.org/10.1007/s10681-008-9754-x.

Taylor JE, Whitelaw CA. 2001. Signals in abscission. New Phytol. 151:323–339. https://doi.org/10.1046/j.0028-646x.2001.00194.x.

Unrath CR, Obermiller JD, Green A, McArtney SJ. 2009. The effects of aminoethoxyvinylglycine and naphthaleneacetic acid treatments on abscission and firmness of 'Scarletspur Delicious' apples at normal and delayed harvests. HortTechnology. 19:620–625. https://doi.org/10.21273/HORTSCI.19.3.620.

Vashisth T, Johnson LK, Malladi A. 2011. An efficient RNA isolation procedure and identification of reference genes for normalization of gene expression in blueberry. Plant Cell Rep. 30:2167–2176. https://doi.org/10.1007/s00299-011-1121-z.

Wakasa Y, Hatsuyama Y, Takahashi A, Sato T, Niizeki M, Harada T. 2003. Divergent expression of six expansin genes during apple fruit ontogeny. Eur J Hortic Sci. 68:253–259.

Wargo JM, Merwin IA, Watkins CB. 2004. Nitrogen fertilization, midsummer trunk girdling, and AVG treatments affect maturity and quality of 'Jonagold' apples. HortScience. 39:493–500. https://doi.org/10.21273/HORTSCI.39.3.493.

Watkins CB, Stover E, Halsey J, Torrice CJ. 1997. ReTain—Experiences with 'McIntosh' and 'Jonagold' in New York. Proc. Apple Harvesting, Handling, and Storage Workshop, Ithaca, NY, USA. NRAES 112:19–25.

Westwood MN. 1993. Crop maturity, p 300–315. In: Westwood MN (eds). Temperate-zone pomology: Physiology and culture (3rd ed). Timber Press, Inc., Portland, OR, USA.

Yildiz K, Ozturk B, Ozkan Y. 2012. Effects of aminoethoxyvinylglycine (AVG) on preharvest fruit drop, fruit maturity, and quality of 'Red Chief' apple. Scientia Hortic. 144:121–124. https://doi.org/10.1016/j.scienta.2012.07.005.

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. https://doi.org/10.21273/HORTSCI.42.1.101.

Yuan R, Li J. 2008. Effect of sprayable 1-MCP, AVG, and NAA on ethylene biosynthesis, preharvest fruit drop, fruit maturity, and quality of 'Delicious' apples. HortScience. 43:1454–1460. https://doi.org/10.21273/HORTSCI.43.5.1454.