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Effects of Aminoethoxyvinylglycine (AVG) and 1-Methylcyclopropene (1-MCP) on the Pre-Harvest Drop Rate, Fruit Quality, and Stem-End Splitting in 'Gala' Apples

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Abstract: Preharvest fruit drop is a significant physiological problem that affects numerous commercially significant apple varieties, including 'Gala.' AVG and 1-MCP are two plant growth regulators commonly used to reduce fruit drop by reducing ethylene synthesis and perception, respectively. To optimize yield and market acceptance, a complete investigation of AVG and 1-MCP impacts on fruit drop and fruit quality of 'Gala' apples is required. In this study, four trials were conducted over the course of three years to determine the effects of AVG and 1-MCP on fruit drop and quality at harvest and after cold storage. Our results indicated that applications of AVG at the full-rate (130 mgL^{-1}) three weeks before harvest (WBAH) were more effective at minimizing fruit drop than applications at the half-rate (65 mgL^{-1}) and did not differ significantly from the double rate (260 mgL^{-1}). Additionally, a single application of AVG was as effective in preventing fruit drop as two applications of 1-MCP. We also demonstrated that AVG decreased fruit skin pigmentation when used alone or in conjunction with GA_{4+7} or 1-MCP, while 1-MCP applications had no negative effect on fruit color. Finally, our data showed that when compared to 1-MCP and GA_{4+7}, AVG alone was more effective in preventing stem-end splitting in Gala apples.

Keywords: preharvest drop; ethylene; plant growth regulators; stem end splitting; GA_{4+7}

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1. Introduction

Harvesting at optimal maturity is critical for the successful production of apple (*Malus domestica* Borkh.). Unfortunately, for many important apple cultivars, great economic losses can be caused by excessive preharvest fruit drop (PFD), a phenomenon in which fruits are shed before they reach optimum horticultural maturity [1]. Attempts to avoid PFD by harvesting before the desired maturity often lead to poor storability, reduced marketability, and low yields. Conversely, significant crop value can be achieved when fruits are retained on the tree for several weeks beyond the normal harvest date [2]. Therefore, reducing PFD is of the utmost importance in extending the harvest window and enhancing economic gains in apple production.

It is commonly known that ethylene, a plant hormone involved in many developmental processes in plants, including organ abscission and fruit ripening, is the primary driver of PFD in apples [3]. Apple is a classic climacteric fruit, demonstrating an ethylene burst and an increase in respiration upon ripening [4]. At the abscission zone of the pedicel, ethylene can promote the degradation of the cell wall and intercellular tissues, ultimately resulting in fruit abscission [5,6]. Ethylene production in fruits varies among cultivars, with cultivars with a high inclination to PFD producing more ethylene than cultivars with a low propensity to PFD [7,8]. Li et al. [9] demonstrated, by monitoring ethylene production during ripening, that the PFD rate increases linearly with the rapid growth of ethylene levels in 'Golden Delicious', a PFD-prone cultivar, whereas ethylene levels remain extremely low in 'Fuji', which is not susceptible to PFD. When apple accessions with a low abscission rate

were subjected to exogenous ethylene, the majority exhibited a significant increase in fruit drop [10]. Horticulturists have implemented ethylene control for PFD-prone cultivars to reduce PFD.

Currently, the cultural control of PFD relies mainly on the plant growth regulators (PGRs) that inhibit ethylene biosynthesis, e.g., aminoethoxyvinylglycine (AVG), or signaling, e.g., 1-methylcyclopropene (1-MCP). AVG inhibits the enzyme that catalyzes the rate-limiting step in ethylene biosynthesis, 1-aminocyclopropane-1-carboxylic acid (ACC). The molecule 1-MCP, on the other hand, binds to ethylene receptors, preventing ethylene perception and signal transduction downstream. AVG and 1-MCP are marketed in North America under the brand names ReTain® and Harvista™, respectively. Many studies have documented the effects of AVG and 1-MCP in controlling PFD, and their effectiveness is closely related to application timing and concentrations, but can also be affected by cultivars, years, and locations. Greene [11] demonstrated a linear reduction in PFD in response to AVG application rate up to 90 mgL^{-1} in 'McIntosh', but no significant differences were observed between application timings. When applied one or two weeks before the anticipated harvest date (WBAH), AVG (125 mgL^{-1}) reduced fruit drop in 'Bisbee Delicious' by nearly two-fold compared to the untreated control, and double application had no effect on PFD control [12]. It is also worth noting that the effect of AVG can be enhanced when combined with naphthalene acidic acid (NAA), a synthetic auxin analogue that may downregulate abscission-related genes and reduce abscission zone sensitivity to ethylene [13]. Though 1-MCP primarily inhibits ethylene signaling, it has also been shown to reduce autocatalytic ethylene production, which may contribute to its efficacy [14]. Indeed, one application of 1-MCP was found to increase harvest time by more than two weeks in 'Delicious' cultivars, outperforming AVG in fruit drop control [12,14].

In addition to regulating PFD, AVG and 1-MCP also influence ripening processes and harvest fruit quality. Starch index, fruit peel color, firmness, and total soluble solids are significant maturity indices that determine harvesting time and market acceptance. The starch index, which indicates the proportion of fruit starch that is hydrolyzed to sugar, appears to be highly sensitive to AVG and 1-MCP, as both can significantly delay starch degradation [12,15]. In contrast, the red pigmentation of fruit peel responds differently to AVG and 1-MCP. In 'Bisbee Delicious', Yuan and Li [14] discovered that neither AVG nor 1-MCP affected the red coloration, whereas Greene [11] demonstrated that AVG delays red coloration at the recommended concentration (90 mgL^{-1}) and Yildiz et al. [16] demonstrated that AVG only affects red coloration at high concentrations (e.g., 600 mgL^{-1}). Such an inconsistency may result from differences in site and climatic conditions, which have a substantial effect on the activity of enzymes responsible for the production of anthocyanins, the primary pigments responsible for the development of red color. AVG has also been reported to reduce fruit firmness loss during harvest or cold storage [17,18].

Fruit cracking is a significant physical defect that affects numerous commercially significant apple cultivars, including 'Gala'. In 'Gala', cracking is manifested primarily as stem-end splitting (SES), where splitting occurs at the fruit-stem joint and shoulder area or originates around the stem base [19]. The incidence of fruit cracking varies considerably between years, regions, and even orchards within the same region [20]. Using plant hormones, particularly gibberellins, attempts have also been made to control fruit cracking. Previous research indicated that treatments with GA_{4+7} prevent cuticular cracking in apple, most likely by reducing the irregularity of epidermal cells, which equalizes the stress distribution in the cuticular membrane [21]. Knoche et al. [22] hypothesized that multiple applications of GA_{4+7} could increase the number and size of cells in the epidermal and hypodermal layers, thereby enhancing the mechanical properties of the fruit skin against growth strain. Thus, we hypothesized that application of GA_{4+7} in conjunction with an ethylene inhibitor such as AVG would increase cell elasticity and allow for excessive cell elongation, thereby decreasing the incidence of SES in Gala apples.

It has been observed that the rate of fruit drop and responses to PGRs can be influenced by a number of factors, some of which include crop loads, biotic stresses, and the availability

of nutrients. These factors have a significant amount of variation from year to year and have the potential to accelerate the PFD rate [6]. Although earlier research has documented the effects of ethylene inhibitors on PFD control, very few of those studies investigated responses over the course of multiple consecutive years using application protocols that were comparable. The purpose of this research was to conduct an in-depth analysis of the effects of AVG and 1-MCP on the PFD rate and quality of 'Gala' apples over the course of several years. Additionally, the researchers wanted to investigate the effect that applying GA_{4+7} prior to harvest had on the ability to prevent fruit splitting.

2. Materials and Methods

2.1. Exp. 1: AVG Effects on Fruit Drop and Fruit Quality

This trial was conducted in Middletown, VA, USA (39.027, −78.280) on 6 year-old 'Brookfield Gala'/G.16 trees in 2018. A completely randomized block design (CRB) was used to accommodate five treatments all composed of AVG (ReTain, Valent BioSciences Corporation, Libertyville, IL, USA) and one untreated control, each with six replicate trees, and a minimum of three buffer trees between treated trees. The five AVG treatments comprised three half-rate (65 mgL^{-1}) applications each performed at 3 WBAH (7 August), 1 WBAH (22 August) and 3 + 1 WBAH and two full-rate (130 mgL^{-1}) applications each performed at 3 WBAH and 1 WBAH. All sprays were mixed with 1.0 mL L^{-1} Silwet-77 before application, which were made using a pressurized orchard sprayer.

Three weeks before harvest, a total of about 100 fruits from either side of each tree were selected and tagged. Fruit drop was monitored by counting the numbers of the tagged fruits weekly starting at 1 WBAH until 2 weeks after harvest (WAH). For fruit quality assessment, 20 fruits from each tree were collected at the normal harvest date (28 August) and 2 WAH (10 September). Ten fruits were assessed immediately after collection, and the other 10 fruits were assessed after 3 months of cold storage at 4 °C. Fruit size, weight and firmness were analyzed using a Fruit Texture Analyzer (FTA, QA Supplies, Norfolk, VA, USA). TSS was determined using a Digital Refractometer (Atago 3810, Saitama, Japan). The fruit background color was assessed on blushed and unblushed sides of each fruit using a hand-held Delta Absorbance (DA) meter (Sinteleia, Bologna, Italy), which measures the amount of chlorophyll present in the fruit skin and gives results as the index of absorbance difference (I_{AD}). I_{AD} values decrease as chlorophyll degrades during ripening. The starch pattern index (SPI) was determined using an iodine (KI) stain test according to the Cornell starch–iodine index system [23] on a scale of 1–8. The total titratable acidity (TA) was analyzed in fruit juice using a titrator (Metrohm 848 Titrino plus CH-9100, Herisau, Switzerland), and the pH was measured using a pH meter (Orion Star A210 Series, Thermo Scientific, Waltham, MA, USA).

All statistical analyses were performed with R (Version 4.1, R Core Team, 2021) using a simple least square model ANOVA and Tukey's HSD test for mean comparisons. In fruit quality evaluation, measurements of 10 fruits from each tree were pooled as a replicate.

2.2. Exp. 2: Effects of AVG and 1-MCP on Fruit Drop and Fruit Quality

In 2019, another trial was conducted at the same orchard as in 2018 using two rates of AVG and one rate of 1-MCP (Harvista, AgroFresh, Philadelphia, PA, USA). A CRB design with six replicate trees was used on 6 year-old 'Brookfield Gala'/G.16 trees. The predicted harvest date for this experiment was on 16 August. AVG at full-rate (130 mgL^{-1}) or double rate (260 mgL^{-1}) was applied 3 WBAH (24 July) or 1 WBAH (8 August). In another treatment, AVG at the full-rate was applied 3 and 1 WBAH. 1-MCP (9.4 $mL{\cdot}L^{-1}$) was applied when the starch pattern index (SPI) was 1.5 (1 August) or 3 (7 August). The evaluation of fruit drop and fruit quality was performed in a similar way as described in Exp. 1. In addition, the internal ethylene concentration (IEC) was quantified at harvest using an Agilent 7890A gas chromatograph System (Agilent Technologies, Santa Clara, CA, USA) by injecting a 1 mL gas sample taken from the core cavity of the fruit using an insulin syringe [24].

2.3. Exp. 3: Effects of AVG, 1-MCP and GA_{4+7} on Fruit Drop and Fruit Quality

This experiment was conducted in 2021 on 8 year-old 'Buckeye Gala'/Nic29 in Timberville, VA, USA (38.639, −78.773) with six treatments and an untreated control, each including replicate trees, arranged in a CRB design. Each treatment received the applications containing one or two of the three PGRs: AVG (ReTain, Valent BioSciences Corporation, Libertyville, IL, USA), 1-MCP (Harvista, AgroFresh, Philadelphia, PA, USA), and gibberellic acids (GA_{4+7}) (ProVide, Valent BioSciences Corporation, Libertyville, IL, USA). In two treatments, trees were treated with either AVG (130 mgL^{-1}) alone or AVG (130 mgL^{-1}) and GA_{4+7} (52.8 mgL^{-1}). In two other treatments, trees were treated with either AVG (65 mgL^{-1}) and one application of 1-MCP (9.4 $mL{\cdot}L^{-1}$) or AVG (65 mgL^{-1}) and two applications of 1-MCP (9.4 $mL{\cdot}L^{-1}$), at SPI of 1.5 and 3. Two other treatments were also added to examine the effects of a single (SPI = 1.5) or a double application (SPI = 1.5 and 3) of 1-MCP (9.4 $mL{\cdot}L^{-1}$). All applications containing AVG were mixed with Silwet-77 before spray as described in Exp. 1. In this experiment, the harvest date was anticipated to be 20 August. Fruit drop monitoring, fruit harvest, fruit quality assessments at harvest and after cold storage were performed in a similar way as described in Exp. 1 and Exp. 2. In addition, the stem-end splitting/cracking was also evaluated 2 weeks after the normal harvest date (7 September) by randomly selecting 30 fruits from each tree and counting the fruits with splitting or fissure readily visible at the stem end (Figure S1).

2.4. Exp. 4: Effects of AVGand GA_{4+7} on Fruit Drop and Fruit Quality

This experiment was performed in 2022 on 11-year-old 'Crimson Gala'/M9 at the Alson H. Smith Jr. Agricultural Research and Extension Center, Winchester, VA, USA (39.185, −78.163). This experiment was composed of three treatments and an untreated control, each with three replicate trees. The three treatments included: a) AVG (130 mgL^{-1}) applied 3 WBAH (3 August), b) AVG (130 mgL^{-1}) and GA_{4+7} (52.8 mgL^{-1}) applied at 3 WBAH, c) AVG (130 mgL^{-1}) and three applications of GA_{4+7} (52.8 mgL^{-1}) applied at 3, 2 and 1 WBAH. The anticipated harvest date was 24 August. The fruit drop and ripening characteristics were assessed as described previously. The fruit SES was assessed by randomly selecting 100 fruits from each tree and evaluated as described in Exp. 3. A Pearson's correlation was performed using the cor () function in R (Version 4.1, R Core Team, 2021) to measure the correlations between cracking rate and other ripening traits.

3. Results

3.1. Exp. 1. AVG Effects on Fruit Drop and Fruit Quality at Harvest and after Cold Storage

In the trial of 2018, there was no difference in the rate of fruit drop at 1 WBAH and at harvest (Figure 1), and the rate of fruit drop started to increase remarkably in all treatments one week after harvest. Treatments of full-rate AVG applied 1 and 3 WBAH, and the double application of half-rate AVG produced comparable levels of fruit drop control; all reduced fruit drop by 30–50% relative to the control, and the effects became apparent two weeks after harvest. In contrast, a single application of AVG at half the rate provided less fruit drop control than the aforementioned treatments, and the 3 WBAH application was more effective than the 1 WBAH application, which had almost no effect on fruit drop control.

At the time of normal harvest (28 August), neither fruit size nor weight were significantly altered by any AVG treatment (Table 1). The only treatment that delayed the loss of fruit firmness was the full-rate AVG application of 3 WBAH. The starch index was significantly lower when half and full-rates of AVG were applied 3 WBAH. The concentration of total soluble solids was significantly reduced across all treatments. Only two treatments, the half-rate AVG applied at 1 WBAH and 1 + 3 WBAH, exhibited a decrease in titratable acid content compared to the control. As indicated by the decrease in chlorophyll, fruit skin coloring was significantly delayed in all treatments with the exception of the half-rate of AVG applied at 1 WBAH.

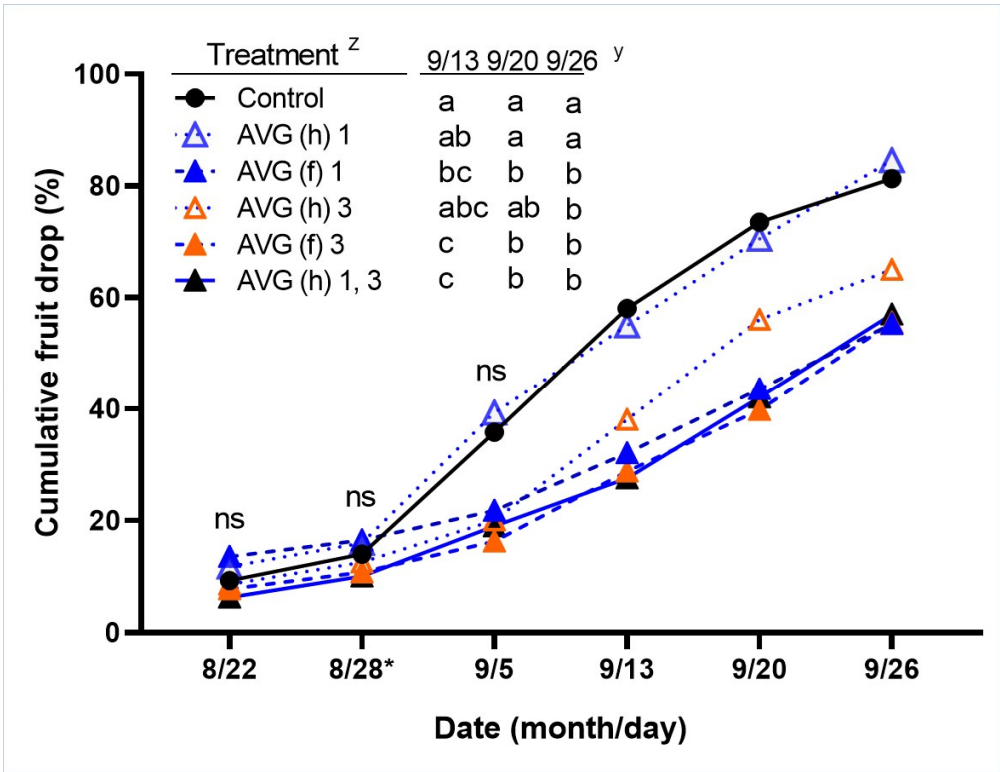

Figure 1. Effects of AVG on preharvest fruit drop of 'Brookfield Gala' apples in 2018. Z, letter h in parentheses represents the half-rate (65 mgL^{-1}), and letter f represents the full-rate (130 mgL^{-1}); numbers in each treatment indicate the numbers of weeks before anticipated harvest (WBAH) when the treatment was conducted. Y, means not sharing any letter are significantly different by the Tukey's HSD test ($p < 0.05$). *, date of harvest.

Table 1. Effects of AVG on fruit quality of 'Brookfield Gala' apples at harvest and after cold storage, 2018, Exp. 1.

Treatment	Diameter (mm)		Weight (g)		Firmness (N)		SPI (1 to 8)		TSS (Brix)		TA (g/L)		pH		Color (I_{AD})	
	H1	H2	H1	H2	H1	H2	H1	H2	H1	H2	H1	H2	H1	H2	H1	H2
At harvest																
Control	64.0 ab	64.3 a	114.7 ab	117.5 a	72.5 b	69.8 a	7.4 a	7.6 a	12.1 a	13.4 a	2.5 a	2.4 a	3.8 b	4.0 a	0.16 c	0.14 d
AVG (h) 1 W	67.7 a	65.9 a	134.7 a	125.3 a	76.0 ab	73.4 a	6.7 ab	7.4 ab	9.2 c	13.0 ab	2.0 b	2.4 a	4.0 a	4.0 a	0.28 bc	0.29 bc
AVG (f) 1 W	64.2 ab	64.6 a	118.3 ab	118.5 a	79.2 ab	72.5 a	5.9 ab	7.7 a	10.3 bc	12.4 ab	2.5 a	2.5 a	4.0 a	4.0 a	0.37 ab	0.25 cd
AVG (h) 3 W	67.1 ab	65.0 a	129.7 ab	120.4 a	80.9 ab	78.3 a	5.1 b	7.2 ab	10.2 bc	12.2 ab	2.5 a	2.3 a	3.9 a	4.0 a	0.55 a	0.48 a
AVG (f) 3 W	62.4 b	64.4 a	106.0 b	117.0 a	88.1 a	80.5 a	5.0 b	6.6 b	10.9 b	12.8 ab	2.5 a	2.4 a	4.0 a	4.0 a	0.48 a	0.42 ab
AVG (h) 1 + 3 W	66.6 ab	67.7 a	126.3 ab	134.2 a	79.2 ab	76.0 a	5.7 ab	7.1 ab	9.3 c	12.0 b	2.0 b	2.2 a	3.9 a	4.0 a	0.39 ab	0.38 ab
After cold storage																
Control	64.8 a	65.1 ab	113.8 a	117.7 ab	52.9 b	49.8 c	8.0 a	8.0 a	8.7 ab	7.0 b	1.4 a	1.1 a	3.7 a	3.8 a	0.10 b	0.09 d
AVG (h) 1 W	66.0 a	65.5 a	124.5 a	120.1 ab	57.4 ab	56.0 b	8.0 a	8.0 a	9.3 a	8.5 ab	1.5 a	1.5 a	3.5 a	3.7 a	0.18 a	0.16 bc
AVG (f) 1 W	63.6 a	64.6 ab	110.0 a	118.3 ab	58.2 a	55.6 b	8.0 a	8.0 a	6.8 c	8.1 ab	1.4 a	1.4 a	3.7 a	4.0 a	0.16 ab	0.15 c
AVG (h) 3 W	66.2 a	66.4 a	123.3 a	124.0 a	58.7 a	54.7 bc	8.0 a	8.0 a	7.7 abc	7.7 ab	1.4 a	1.4 a	3.6 a	3.6 a	0.20 a	0.22 ab
AVG (f) 3 W	63.3 a	61.9 b	106.3 a	101.4 b	59.6 a	61.4 a	8.0 a	8.0 a	6.9 bc	9.2 a	1.5 a	1.3 a	3.3 a	3.5 a	0.23 a	0.26 a
AVG (h) 1 + 3 W	66.3 a	67.2 a	122.4 a	130.4 a	58.2 a	54.2 bc	8.0 a	8.0 a	8.5 abc	7.2 b	1.4 a	1.2 a	3.7 a	3.8 a	0.21 a	0.19 bc

AVG was applied at either half (h, 65 mgL^{-1}) or full (f, 130 mgL^{-1}) rate on 7 August (1 WBAH) or 22 August (3 WBAH), or both. Apples were collected either at the normal harvest date (H1) or two weeks after harvest (H2). W, weeks before anticipated harvest; SPI, starch pattern index (1–8 scale); N, newton; TSS, total soluble solids; TA, titratable acidity. Means ($n = 6$) not sharing any letter are significantly different ($p < 0.05$).

The size and weight of fruits harvested two weeks after the normal harvest date (13 September) were unaffected by any treatment. The treated fruits were still firmer than the untreated control, but the differences were not statistically significant. There was a significant reduction in starch pattern index (SPI) and total soluble solids (TSS) when the full AVG rate was applied 3 WBAH and when the half-rate was applied twice. Only at the normal harvest date (H1) were titratable acidity (TA) and pH altered; TA was lower than the control in two half-rate treatments of AVG administered at 1 WBAH and 3 + 1 WBAH, and pH was somewhat higher in all treatments. Compared to the control, AVG

treatments generally increased the IAD values on both harvest dates, especially in the 3 WBAH applications of both full- and half-rate. Higher IAD values indicate delayed fruit coloration, as IAD measures the chlorophyll content, which decreases during ripening and the red color development.

After 3 months of cold storage, the differences between the control and the treatments generally became less significant (Table 1). Overall, the treatment effects on fruit firmness, size, weight, SPI, TA and pH values were insignificant. Nearly all AVG treatments significantly delayed the peel coloration.

3.2. Exp. 2. Effects of AVG and 1-MCP on Fruit Drop and Fruit Quality at Harvest and after Cold Storage

The overall fruit drop rate in 2019 was lower relative to the trial in 2018 and remained nearly unchanged during the first two weeks after harvest. AVG and 1-MCP reduced the fruit drop moderately, and none of the treatments showed a significant effect (Figure 2). Relatively, the best fruit drop control was found in the double rate of AVG applied at 3 WBAH and the double application of 1-MCP, both of which reduced fruit drop by more than 60% during the first two weeks after harvest.

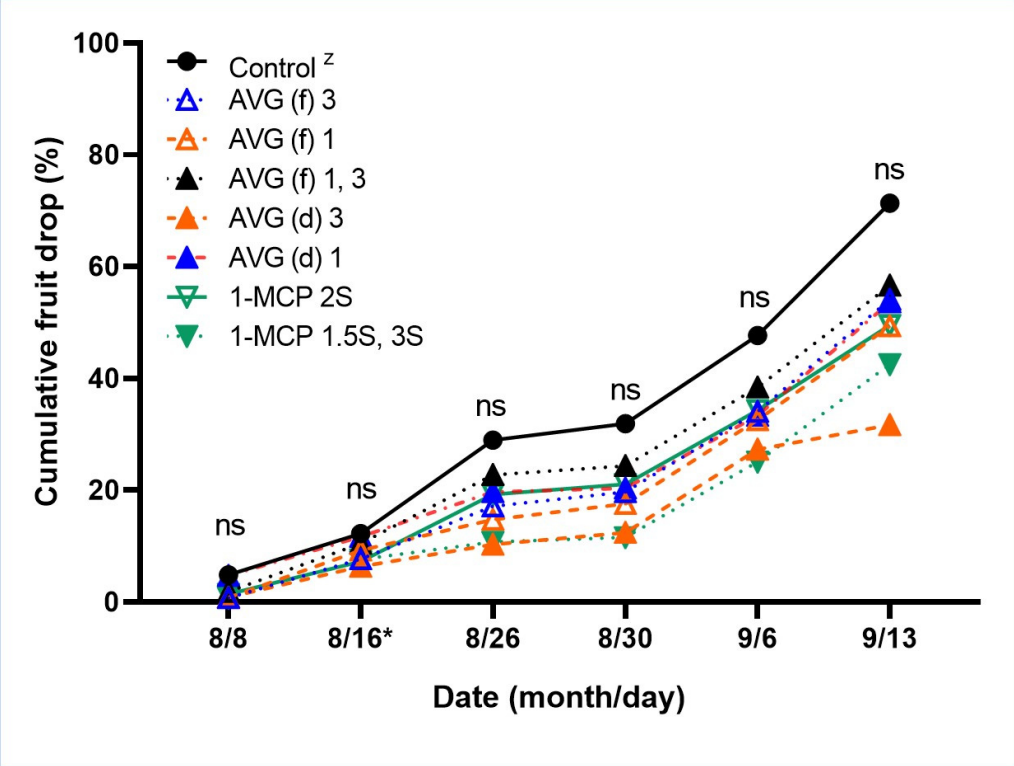

Figure 2. Effects of AVG and 1-MCP on preharvest fruit drop of 'Brookfield Gala' apples in 2019. Z, AVG was applied at full-rate (f, 130 mgL^{-1}) or double rate (d, 260 mgL^{-1}); 1-MCP was applied at a starch pattern index (SPI) = 2; d, double of 1-MCP at SPI = 1.5 and 3; numbers in each treatment indicate the numbers of weeks before anticipated harvest (WBAH) when the treatment was conducted; *, date of harvest.

Fruit size and weight were largely unaffected by AVG and 1-MCP at the normal harvest date (16 August) compared to the control, except for the treatment of AVG at full-rate applied 1 WBAH, which significantly reduced both (Table 2). In general, both AVG and 1-MCP increased fruit firmness, but AVG had a greater effect, with significant increases observed in treatments of full-rate at 1 WBAH and double rate at 3 WBAH. Fruit color development was generally inhibited by AVG, with significant retardation observed in treatments with the full-rate and double rate at 3 WBAH. 1-MCP also delayed coloration,

but to a lesser extent than AVG, and none of the effects were statistically significant. Likewise, starch hydrolysis was significantly slowed in the AVG treatments of full-rate and double rate applied at 3 WBAH. AVG and 1-MCP both reduced internal ethylene levels, but only three of the five AVG treatments and none of the 1-MCP treatments resulted in a significant reduction. Two weeks later (30 August), the fruit firmness of all treatments and the control decreased to the same degree. Except for the full-rate of AVG at 1 WBAH and 1-MCP applied at 2 SPI, the majority of treatments resulted in a significant delay in skin color development. All treatments resulted in a significant increase in internal ethylene levels, with none significantly different from the control.

Table 2. Effects of AVG and 1-MCP on fruit quality of 'Brookfield Gala' apples at harvest and after cold storage, 2019, Exp. 2.

Treatment	Diameter (mm)		Weight (g)		Firmness (N)		Color (I_{AD})		SPI (1–8)		IEC (ppm)	
	H1	H2	H1	H2	H1	H2	H1	H2	H1	H2	H1	H2
At harvest												
Control	75.8 a	77.5 a	195.9 ab	215.1 a	73.6 c	62.7 a	0.12 b	0.03 b	6.2 a	7.2 a	3.3 a	11.0 a
AVG (f) 1 W	71.3 b	74.3 a	170.8 b	194.5 a	85.6 a	65.4 a	0.26 ab	0.06 ab	4.5 ab	6.8 ab	2.3 ab	6.5 a
A VG (f) 3 W	73.6 ab	74.2 a	183.6 ab	193.3 a	81.6 abc	67.1 a	0.47 a	0.11 a	3.9 b	5.8 b	1.2 b	6.1 a
AVG (d) 1 W	73.6 ab	75.4 a	188.1 ab	207.1 a	79.1 abc	66.3 a	0.35 ab	0.10 a	5.7 ab	6.3 ab	1.6 b	6.0 a
AVG (d) 3 W	73.0 ab	76.2 a	182.8 ab	208.4 a	85.4 ab	66.7 a	0.54 a	0.12 a	3.5 b	6.0 ab	2.3 ab	6.4 a
AVG (f) 1, 3 W	74.2 ab	74.3 a	187.1 ab	191.5 a	73.8 c	63.6 a	0.41 ab	0.11 a	4.2 ab	6.7 ab	1.2 b	6.4 a
1-MCP 1.5 + 3 SPI	75.6 a	74.7 a	193.5 ab	195.3 a	76.5 bc	62.7 a	0.28 ab	0.11 a	5.1 ab	6.7 ab	1.8 ab	11. a
1-MCP 2 SPI	75.6 a	75.1 a	198.9 a	203.3 a	78.2 abc	67.6 a	0.25 ab	0.09 ab	4.8 ab	6.2 ab	2.5 ab	9.8 a
After cold storage												
Control	75.3 a	74.1 a	193.1 a	185.1 a	56.9 a	48.9 a	0.06 b	0.02 a	8.00 a	8.0 a	81.4 a	127.7 a
AVG (f) 1 W	70.1 b	74.8 a	162.1 b	176.2 a	62.7 a	47.1 a	0.16 ab	0.03 a	7.70 a	8.0 a	100.0 a	85.7 a
AVG (f) 3 W	72.3 ab	76.6 a	172.6 ab	194.2 a	60.5 a	47.1 a	0.19 ab	0.03 a	7.86 a	8.0 a	84.1 a	80.0 a
AVG (d) 1 W	73.0 ab	77.1 a	177.1 ab	197.0 a	59.1 a	43.3 a	0.12 ab	0.03 a	8.00 a	8.0 a	74.0 a	91.8 a
AVG (d) 3 W	73.4 ab	74.6 a	179.2 ab	181.4 a	60.9 a	48.9 a	0.20 a	0.03 a	7.66 a	8.0 a	77.9 a	106.8 a
AVG (f) 1 + 3 W	74.7 a	74.1 a	190.5 ab	177.6 a	60.9 a	47.1 a	0.20 ab	0.04 a	7.80 a	8.0 a	66.5 a	152.5 a
1-MCP 1.5 + 3 SPI	75.5 a	71.3 a	192.3 a	166.7 a	57.4 a	50.7 a	0.14 ab	0.04 a	7.86 a	8.0 a	69.6 a	127.7 a
1-MCP 2 SPI	74.2 ab	71.8 a	188.3 ab	170.4 a	59.6 a	49.8 a	0.13 ab	0.03 a	7.86 a	8.0 a	91.8 a	94.1 a

AVG was applied at either a full (f, 130 mgL^{-1}) or double (d, 260 mgL^{-1}) rate on 26 July, (3 WBAH) or 9 August (1 WBAH). 1-MCP were applied at 9.45 $mL{\cdot}L^{-1}$ on 1 August (1.5 SPI) and 12 August (3 SPI), or 7 August (2 SPI). Apples were collected either at normal harvest date (H1) or two weeks after harvest (H2). W, weeks before anticipated harvest; SPI, starch pattern index (1–8 scale); N, newton; TA, titratable acidity; TSS, total soluble solids; IEC, internal ethylene content. Means (n = 6) not sharing any letter are significantly different ($p < 0.05$).

After 3 months of cold storage, fruit firmness decreased in all treatments, and no treatment effect was significant (Table 2). Fruit color further developed, and all the treatments showed some levels of delay from first harvest, but not from the second harvest. Starch hydrolysis was nearly or fully completed from first harvest and second harvest, respectively. The internal ethylene levels increased dramatically in all treatments from both harvests, in which none showed significant effect.

3.3. Exp. 3. Effects of AVG, 1-MCP and GA_{4+7} on Fruit Drop, Fruit Quality and Stem End Splitting

A week before harvest, no fruit loss was observed. At the normal harvest date (23 August), there was no significant difference between the control and any treatment. At 1 WAH, the only difference in fruit drop was observed between the control and the treatment of 1-MCP applied at SPI 3 (Figure 3). At 2 WAH, all treatments reduced fruit drop significantly, with the exception of AVG (half) + 1-MCP (SPI 3). Trees treated with AVG (full-rate) at 3 WBAH and those treated with 1-MCP (SPI 1.5 and 3) exhibited the lowest fruit drop percentage.

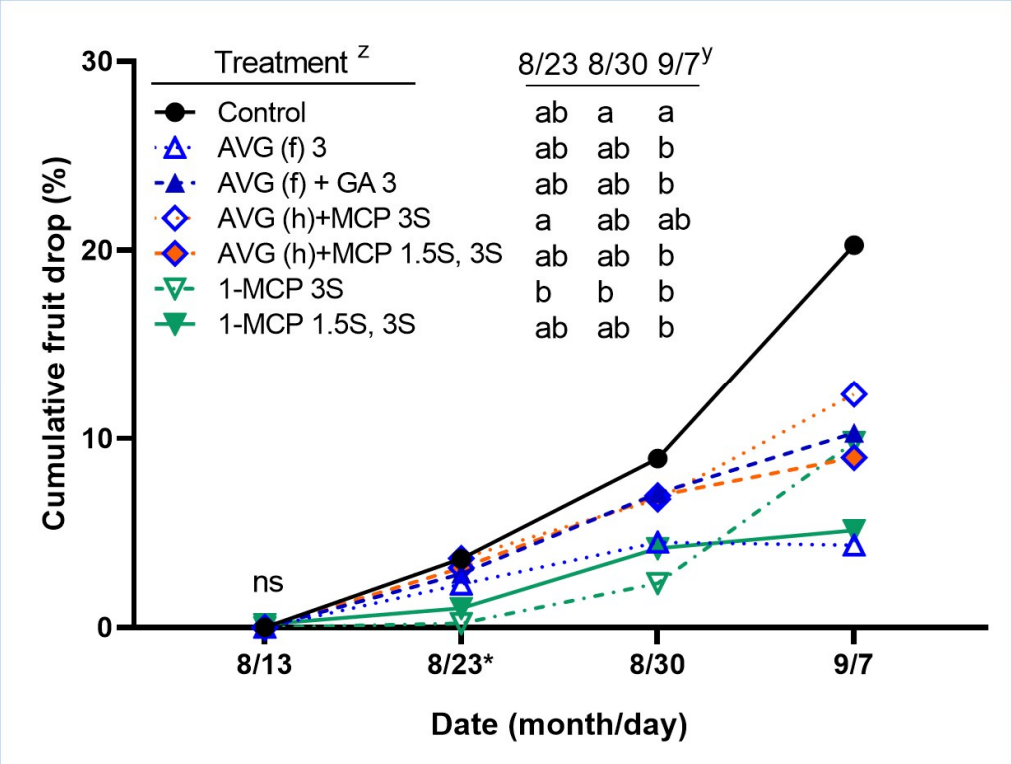

Figure 3. Effects of AVG and 1-MCP on preharvest fruit drop of 'Buckeye Gala' apples ($n = 6$). Z, h represents half-rate (65 mgL^{-1}) of AVG; f, full-rate (130 mgL^{-1}) of AVG; GA, GA_{4+7}; s, single application of 1-MCP at starch pattern index (SPI) = 1.5 and/or 3. Numbers in each treatment indicate the numbers of weeks before anticipated harvest (WBAH) when the treatment was conducted. Y, means not sharing any letter are significantly different by the Tukey's HSD test ($p < 0.05$). *, date of harvest.

Fruit size, weight, and firmness did not differ between treated and untreated trees at the normal harvest date (23 August) (Table 3). In contrast, all treatments, with the exception of 1-MCP applied at SPI 3, significantly reduced peel coloration. In general, AVG treatment had a stronger effect in delaying coloration than 1-MCP treatments, especially the AVG-only application. Full-rate AVG alone and in combination with GA_{4+7} reduced total soluble solids by 12.9 and 14.3%, respectively, but other treatments had no effect. All treatments suppressed starch hydrolysis, with the half-rate of AVG combined with double application of 1-MCP having the greatest suppressive effect. Internal ethylene levels were dramatically reduced in all treatments when compared to the control, with no difference observed between treatments.

Fruit size, weight, and firmness were comparable between the treatments and the control for fruits harvested 2 weeks after the normal harvest date (7 September) (Table 3). Except for the one combined with 1-MCP (SPI 3), all treatments containing AVG at full and half-rate delayed fruit coloration significantly. In contrast, the 1-MCP treatments, whether used once or twice, had no effect on fruit coloration. No treatment had an effect on the total soluble solids. The SPIs in two full-rate AVG treatments and two 1-MCP (SPI 1.5 and 3) treatments with or without AVG were significantly lower. Surprisingly, no significant difference in ethylene levels was found between the control and treatments.

Table 3. Effects of AVG and 1-MCP on the fruit quality of 'Buckeye Gala' apples at harvest and after cold storage, 2021, Exp. 3.

Treatment	Diameter (mm)		Weight (g)		Firmness (N)		Color (I_{AD})		TSS (Brix)		SPI (1–8)		IEC (PPM)		SES (%)	
	H1	H2	H1	H2	H1	H2	H1	H2	H1	H2	H1	H2	H1	H2	H1	H2
After Harvest																
Control	60.1 ab	62.7 ab	158.7 ab	163.2 ab	73.8 a	61.8 a	0.19 c	0.120 c	13.9 a	13.5 ab	7.1 a	7.5 a	10.3 a	2.1 ab		33.3 a
AVG (f) 3 W	59.9 ab	60.9 ab	154.2 ab	163.2 ab	82.9 a	75.4 a	0.66 a	0.52 a	12.1 b	11.5 b	4.0 cd	5.1 b	1.3 b	1.3 ab		0 d
AVG (f) + GA 3 W	60.7 ab	62.4 ab	158.7 ab	176.9 ab	74.6 a	72.6 a	0.59 ab	0.37 ab	11.9 b	12.5 b	4.8 bcd	5.7 b	1.5 b	1.2 ab		6.66 cd
AVG (h) 3 W, 1-MCP 1.5 SPI	60.9 a	63.5 a	167.8 a	185.9 a	78.7 a	70.1 a	0.58 ab	0.24 bc	12.6 ab	13.3 ab	5.1 bc	6.9 a	1.3 b	2.6 ab		20.0 abc
AVG (h) 3 W, 1-MCP 1.5 + 3 SPI	58.1 ab	60.4 b	136.0 b	154.2 b	79.8 a	66.4 a	0.72 a	0.52 a	12.9 ab	12.2 b	4.0 d	4.7 b	0.84 b	0.7 b		0 d
1-MCP 3 SPI	59.6 ab	62.9 ab	149.6 ab	167.8 ab	74.5 a	60.4 a	0.39 bc	0.26 bc	13.9 a	15.9 a	5.8 b	6.9 a	4.7 b	7.3 a		30.0 ab
1-MCP 1.5+3 SPI	57.6 b	61.2 ab	136.0 b	163.2 b	88.3 a	79.2 a	0.62 a	0.33 abc	14.1 a	14.3 ab	4.2 cd	5.6 b	2.4 b	2.5 ab		13.3 bcd
After cold storage																
Control	57.6 abc		131.5 ab		57.9 b		0.16 c		13.9 ab		8.0 a					
AVG (f) 3 W	56.2 bc		122.4 b		62.0 ab		0.43 a		12.9 b		7.9 a					
AVG (f) + GA 3 W	58.6 abc		154.2 a		60.8 b		0.35 ab		13.6 ab		8.0 a					
AVG (h) 3 W, 1-MCP 1.5 SPI	60.2 a		154.2 a		58.5 b		0.26 abc		17.4 a		8.0 a					
AVG (h) 3 W, 1-MCP 1.5 + 3 SPI	55.4 c		113.3 b		70.3 a		0.43 a		14.0 ab		8.0 a					
1-MCP 3 SPI	58.7 ab		140.6 ab		63.4 ab		0.25 bc		14.9 ab		8.0 a					
1-MCP 1.5 + 3 SPI	56.9 bc		127.0 ab		70.3 a		0.35 ab		15.3 ab		7.9 a					

AVG was applied at either half (h, 65 mgL^{-1}) or full (f, 130 mgL^{-1}) rate on 30 July (3 W) with or without GA (52.8 mgL^{-1}), and 1-MCP (9.4 $mL{\cdot}L^{-1}$) was applied on 5 August (1.5 s) for single spray (s) or with an additional application on 13 August (3 s) for double spray. W, weeks before harvest; SPI, starch pattern index (1–8 scale); N, newton; TSS, total soluble solids; IEC, internal ethylene content; SES, stem end splitting. Means (n = 6) not sharing any letter are significantly different ($p < 0.05$).

Stem-end splitting (SES) was evaluated for all fruits harvested two weeks after the normal harvest date (7 September). Compared to the control, the percentage of SES was significantly lower in all treatments with the exception of those in which a single application of 1-MCP alone or in combination with a half-rate of AVG was used. No fruit cracking was observed on trees treated with a full-rate of AVG or a half-rate of AVG in conjunction with two applications of 1-MCP.

After cold storage, only fruits collected at the normal harvest time were evaluated for quality (Table 3). None of the treatment effects on fruit size or weight were statistically significant. In general, fruits from the treatments were still firmer than those from the control, but the treatments of double application of 1-MCP, alone or in combination with half-rate AVG (3 WBAH) maintained the greatest fruit firmness, which was significantly greater than the control. All treatments inhibited peel color development, with the exception of half-rate AVG with 1-MCP or 1-MCP-alone treatment. There were no significant differences in total soluble solids or internal ethylene levels.

3.4. Exp. 4. Effects of AVG and GA_{4+7} on Fruit Drop, Fruit Quality and Stem End Splitting

In this experiment, GA_{4+7} was combined with AVG to determine its effectiveness in controlling SES. AVG applied at 3 WBAH alone or in combination with a single application of GA_{4+7} provided a comparable control of fruit drop, delaying the fruit drop by approximately 13 days (Figure 4). Increased GA_{4+7} application by a factor of three reduced the effect of AVG on fruit drop control to a level comparable to the control.

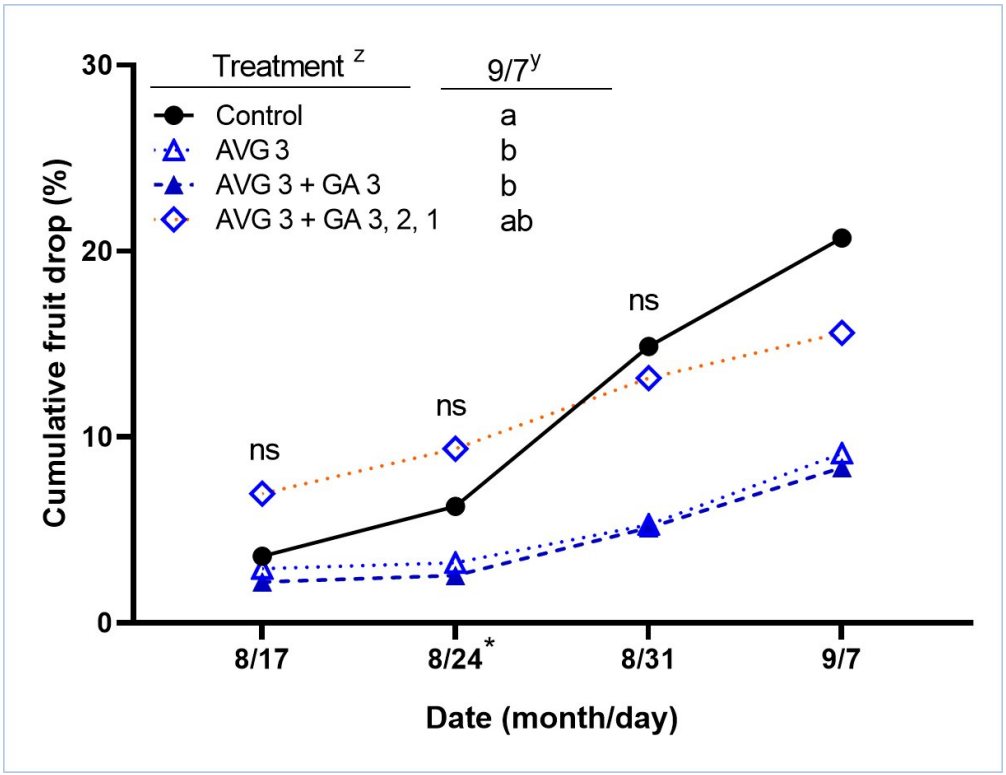

Figure 4. Effects of AVG and GA_{4+7} on fruit drop of 'Crimson Gala' apples (n = 6). Z, numbers indicate the numbers of weeks before harvest; GA, GA_{4+7}. Y means not sharing any letters are significantly different by the Tukey's HSD test ($p < 0.05$). *, date of harvest.

At the normal harvest date (24 August), no treatment affected the fruit size or weight (Table 4). All of the treatments significantly outperformed the control in terms of fruit firmness. The treatments delayed both coloration and starch hydrolysis, while total soluble solids were unaffected. Internal ethylene concentrations were significantly diminished across all treatments. The SES rate was significantly reduced when AVG was applied alone at 3 WBAH. Combining AVG with a single application of GA_{4+7} and a triple application of GA_{4+7} resulted in only a slight and moderate reduction in SES, respectively. Two weeks after the normal harvest time (7 September), fruit ripening accelerated slightly, and the differences between the maturity indices of the control and treatments remained comparable to those at the normal harvest time.

Table 4. Effects of AVG and GA_{4+7} on fruit quality of 'Crimson Gala' apples at harvest, 2021, Exp. 4.

Treatment	Diameter (mm)		Weight (g)		Firmness (N)		Color (I_{AD})		TSS (Brix)		SPI (1–8)		IEC (ppm)		SES (%)
	H1	H2	H1	H2	H1	H2	H1	H2	H1	H2	H1	H2	H1	H2	H2
Control	59.6 a	60.4 a	140.6 a	154.2 a	64.9 b	59.6 b	0.13 b	0.10 b	13.0 a	12.8 a	7.6 a	7.7 a	3.51 a	1.69 a	9.45 a
AVG 3 W	59.1 a	60.1 a	136.0 a	154.2 a	79.1 a	74.7 a	0.52 a	0.40 a	12.3 a	12.2 a	4.1 b	5.5 c	0.96 b	1.37 a	1.08 b
AVG 3 W, GA 3 W	58.9 a	59.4 a	140.6 a	140.6 a	78.7 a	72.9 a	0.63 a	0.34 a	11.8 a	12.1 a	3.8 b	6.6 b	0.16 b	2.28 a	7.51 a
AVG 3 W, GA 3 + 2+1 W	57.9 a	60.7 a	131.5 a	149.6 a	78.7 a	68.9 a	0.56 a	0.27 ab	12.2 a	12.1 a	5.3 b	6.8 ab	0.97 b	1.38 a	4.34 ab

AVG (130 mgL^{-1}) was applied on 30 July (3 W) with or without GA, which was applied on 3 August (3 WBAH), 10 (2 WBAH) and 17 (1 WBAH). W, weeks before anticipated harvest; SPI, starch pattern index (1–8 scale); N, newton; TSS, total soluble solids; IEC, internal ethylene content; SES, stem-end-splitting. Means (n = 6) not sharing any letters are significantly different ($p < 0.05$).

To determine the strength of the linear relationship between the SES rate and other maturity indices, Pearson's correlation coefficients were calculated among all measured traits (Table 5). The fruit SES correlated positively with fruit drop, size, total soluble solids, and SPI, but negatively with fruit firmness and color (I_{AD}).

Table 5. Pearson's correlation between fruit morphology traits at harvest in 'Crimson Gala' apples, 2021, Exp. 4.

Variables	Fruit Drop	Splitting	Firmness	Diameter	Weight	Color	TSS	Starch	Ethylene
Fruit drop									
Splitting	**0.54**								
Firmness	**−0.40**	**−0.32**							
Diameter	**0.45**	**0.46**	−0.29						
Weight	0.24	0.27	−0.11	**0.88**					
Color	**−0.55**	**−0.67**	**0.32**	**−0.66**	**−0.44**				
Brix	0.30	**0.50**	−0.20	0.25	0.09	**−0.44**			
Starch	**0.60**	**0.68**	**−0.38**	**0.64**	**0.42**	**−0.79**	**0.41**		
Ethylene	−0.081	0.27	−0.19	0.00	−0.06	−0.09	0.03	0.25	

A positive correlation is represented in red, and negative correlation in blue, with the shades indicating the strength of correlation. Numbers in bold indicate significant correlation coefficient ($p < 0.05$).

4. Discussion

Due to its direct impact on the final yield, preharvest fruit drop (PFD) is a major concern in apple production. AVG and 1-MCP have been widely used to prevent fruit drop because of their ability to inhibit ethylene biosynthesis and action, respectively. The effectiveness of these two PGRs depends heavily on application timing and concentrations. In Exp. 1 of this study, a half-rate application of AVG at 3 WBAH provided better PFD control than the application at 1 WBAH (Figure 1). This improvement from the early application may be the result of the additional time given for the AVG effect to manifest. Greene [11] indicated that the onset of action time for AVG in 'McIntosh' apples is between 10 and 14 days. The relative inefficacy of an early application was also reflected in the double application (3 and 1 WBAH), which demonstrated no improvement over the application at 3 WBAH. This result is consistent with the findings of Yuan and Carbaugh [12], who discovered that double application of AVG at full-rate at 1 and 3 WBAH has the same effect as a single application at 3 WBAH. However, there were no significant differences between early and late applications when AVG was applied at the maximum rate, indicating that a higher concentration of AVG may compensate for the late application. Although previous studies have shown a linear relationship between AVG concentration and fruit drop reduction [25–27], we found no significant difference in fruit drop between the full-rate and double rate of AVG (Figure 2). This lack of response to the change in AVG concentrations observed in Exp. 2 may be due to the light crop load in 2019.

Unlike AVG, 1-MCP applications should be coordinated with the starch pattern index (SPI). The double application of 1-MCP improved fruit drop control moderately compared to the single application (Figures 2 and 3), confirming the findings of Yuan and Li [14] on 'Golden Delicious' apples. The combination of 1-MCP and AVG had no additive effect compared to their use separately (Figures 2 and 3). In previous studies on the combined use of 1-MCP and AVG, inconsistent outcomes have been observed. Yuan and Li [14] reported in "Golden Delicious" that the combined application of AVG and 1-MCP, applied at a 1 WBAH did not produce a greater effect than either agent alone. By contrast, Byers et al. [28] demonstrated a significant synergistic interaction between AVG and 1-MCP when applied to 'Arlet' apple at 4 WBAH. An early application, particularly for AVG, was generally more effective than late applications, which may explain the discrepancy between these two studies.

Measurement of internal ethylene indicated that 1-MCP reduced ethylene levels in the fruits at the normal harvest date to a similar extent as AVG (Exp. 3). 1-MCP inhibits ethylene perception through binding to the ethylene receptors, which consequently results in the reduction of autocatalytic ethylene production, and eventually ethylene levels [29]. Gene expression analysis of harvested apples indicated that 1-MCP can also inhibit the expression of several genes in the ethylene biosynthetic pathway, such as

1-aminocyclopropane carboxylase (ACC) synthase (ACS) and ACC oxidase (ACO), in addition to downregulating a range of genes related to the ethylene perception [30].

As the apple ripening process is primarily driven by ethylene, attempts to control PFD by reducing ethylene synthesis or perception would necessarily delay fruit maturity. In this study, both AVG and 1-MCP affected several attributes of fruit quality, depending on the application regimes. An early application (3 WBAH) of AVG delayed the ripening processes, such as fruit softening, skin coloration, and starch degradation, more effectively than late applications (1 WBAH) (Exp. 1 and 2). This result agrees with the findings of Greene [11], in which applications at 4–6 WBAH delayed ripening more than later applications at 1–2 WBAH. In accordance with previous findings [15,27,31], our results also showed that early applications of AVG did not significantly affect fruit weight and size, indicating that these two traits are relatively ethylene-independent. Additionally, AVG had no effect on acidity decline in fruits (Exp. 1), but it significantly reduced total soluble solids (Exp. 3), more so than 1-MCP, which had almost no effect on TSS.

A well-developed red color in most apple cultivars, including 'Gala', is commercially important, and color changes on apple peel are the most obvious signal for fruit ripening. The red color of fruit peels is caused by the accumulation of anthocyanins, which is regulated in part by endogenous ethylene [32,33]. Exp. 2 and 3 suggested that 1-MCP had a smaller effect on retarding 'Gala' fruit red color development than AVG (Tables 2 and 3). This finding is consistent with that of 'Cripps Pink' apples, where even a very high concentration of 1-MCP (300 mgL^{-1}) had less of an effect on the rate of coloration than the recommended dose of AVG [34]. The differences in how AVG and 1-MCP affect coloration could be due to their different modes of action. Jasmonic acid (JA), in addition to ethylene, is an important regulator of color development in apple fruits [35] and has been shown to be synergistic with ethylene [36]. Conceivably, AVG-induced ethylene reduction may also lessen the effect of JA on coloration, whereas 1-MCP only represses ethylene perception, having less impact on the synergy between ethylene and JA in promoting coloration. It is also important to note that the negative effects of AVG on fruit coloration were more related to the time of AVG application than to the AVG rate. This was evident in all experiments, where a full-rate application of AVG at 3 WBAH significantly reduced fruit coloration more than a half-rate (Exp. 1) or a double-rate (Exp. 2) of AVG applied at 1 WBAH. The combination of AVG and GA_{4+7} did not mitigate the detrimental effect of AVG on fruit coloration. Anthocyanin accumulation is closely linked to ethylene production during fruit ripening. It has been found that MdEIL1, a key component of the ethylene signaling pathway, binds directly to the *MdMYB1* promoter to induce expression, which in turn promotes anthocyanin accumulation and fruit coloration in apples. Furthermore, MdMYB1 interacts with the promoter of ethylene response factor 3 (MdERF3), a key regulator of ethylene biosynthesis [37].

The 'Gala' apple is among several cultivars that are susceptible to stem-end splitting (SES). Only limited success has been achieved in preventing SES through cultural measures such as nutrient spraying, fertilization, irrigation and pruning. GA_{4+7} has been generally recognized to promote cell elongation and increase cell wall elasticity [38,39], and the application of GA_{4+7} may thus potentially help mitigate fruit splitting. In this study, the addition of GA_{4+7} to AVG showed no effect in reducing the incidence of fruit splitting (Tables 3 and 4). Previous studies indicated that reductions in skin cracking can be achieved through repetitive application of GA_{4+7} beginning at petal fall [22,40]. Similar results were obtained on 'Pink Lady' apples where GA_{4+7} was applied more than 60 days after petal fall, in combination with 6-benzyladenine (BA), a PGR known to increase the rate of cell division [41]. In these studies, the epidermal planar cell density was increased, which confers stronger cuticle structure and higher peel flexibility [42]. However, conclusions drawn from these studies may not apply to the incidence of SES in 'Gala', as Opara et al. [20] proposed that SES is a similar, but a distinct physiological phenomenon of skin cracking compared to that observed in 'Pink Lady'. The authors reported that irrigation during the fruit expansion stage is significantly correlated with the incidence of SES in 'Gala',

implying that irregular water intake is the leading cause of expansion strain that exceeds the strength limit of pericarp, as well as the integrity limit of exocarp, to a lesser extent [20]. In the present study, we showed that fruit splitting was significantly inhibited by the AVG-only application (Tables 3 and 4) and to a lesser extent by 1-MCP. However, it required two applications of 1-MCP to achieve the effect of one AVG treatment. Interestingly, the combined treatment of VAG and GA_{4+7} did not show any additive effect compared to the AVG-only. In contrary, GA_{4+7} negated the positive effect of AVG when applied multiple times before harvest. The correlation analysis of fruit splitting and fruit quality traits indicated that SES was the most correlated with the starch index and peel coloration (Table 5), the two direct indicators of maturity. These results are consistent with the observation of Fallahi et al. [43], who documented that 'Pacific Gala' apple fruits from trees on rootstock with advanced maturity tend to produce fruits with a lower firmness and higher SES rate, reinforcing the relationship between SES in 'Gala' and fruit maturity.

Apple fruits are prone to physiological disorders and quality deterioration during storage, and the rate of the physiological changes is associated with the maturity status at harvest [44]. In this study, both AVG and 1-MCP generally retarded the postharvest fruit ripening, especially fruit softening and coloration, and the extent of the retardation was relative to their effects on ripening delay at harvest. Fruits with high firmness at harvest tended to remain relatively firm during storage, confirming the notion that postharvest fruit firmness is highly correlated with the fruit firmness at harvest [45]. Postharvest fruit softening is generally undesirable, as it reduces the fruit shelf life and market acceptance. A previous investigation has indicated that preharvest treatments with AVG helps retain fruit firmness during cold storage [46]. Our data also indicated that the double application of 1-MCP retained fruit firmness after cold storage more effectively than AVG (Exp. 3). The gaseous 1-MCP has been widely used postharvest to maintain fruit quality during storage. Preharvest use of 1-MCP was also shown to positively affect fruit quality attributes, but to a lesser extent than postharvest applications [47]. In contrast to fruit firmness, the treatment of AVG and 1-MCP only had slight effect on acidity levels, total soluble solids and starch degradation during cold storage.

In conclusion, the four trials we conducted on 'Gala' apples over three years revealed that both AVG and 1-MCP efficiently decreased fruit drop in 'Gala' apples, with early AVG applications providing superior fruit drop control than later applications. In addition, the combined application of AVG and 1-MCP had no synergistic effect on fruit drop control or fruit quality. Furthermore, we demonstrated that AVG and 1-MCP treatments had no effect on fruit size or weight, but slowed ripening in several ways, in terms of fruit firmness, skin color formation, and starch degradation. Compared to 1-MCP, AVG treatments tend to produce fruits with less pronounced peel coloring and more firmness. A single treatment of AVG (full-rate), applied at 3 WBAH, was more effective at preventing fruit splitting than either a single application of 1-MCP or a combination of AVG and GA_{4+7}.

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