



The Impact of Anti-ethylene Compounds Applied Pre- and Postharvest on the Weakly Climacteric, Bicoloured ‘Ambrosia’ Apple (*Malus domestica* Borkh)

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Abstract

Previous research has shown that all major storage disorders associated with the weakly climacteric ‘Ambrosia’ apple (*Malus domestica* Borkh) increased with delayed harvest and maturity. As the fruit grade is dependent on colour development, a moderate rate of anti-ethylene products HarvistaTM (preharvest 1-methylcyclopropene (1-MCP)) and Retain[®] (i.e., aminoethoxyvinylglycine (AVG)) were compared with a control treatment in terms of their impact on colour, maturity and storage (eight months) in this two-year optimum versus delayed harvest study. SmartFreshTM (postharvest 1-MCP) and elevated storage temperatures were added as separate postharvest storage treatments. While results should not be interpreted as a comparison of the two products, HarvistaTM showed greater impact than ReTain[®] on ‘Ambrosia’ maturity for the rates and application timing used in this study. HarvistaTM fruit were higher in starch and acidity relative to control fruit at harvest. Both the HarvistaTM and ReTain[®] treatments impeded colour development relative to the control in Year 1 only—a poor colouration year. Both HarvistaTM and SmartFreshTM fruit had superior firmness and acidity levels compared with control fruit after eight months of controlled atmosphere storage, while ReTain[®] fruit acidity was higher than the control group in only one out of two years. Storage disorders, led by senescent breakdown and core flush, and elevated by delayed harvest, were impacted by treatment in different ways, both positively and negatively, with complex interactions with other factors. Despite the clear connection between maturity and post-storage disorder levels in the previous optimum harvest work, delaying maturity with anti-ethylene compounds applied in the orchard did not result in a higher percentage of disorder-free fruit after storage.

Keywords 1-Methylcyclopropene (1-MCP) · Aminoethoxyvinylglycine (AVG) · HarvistaTM · ReTain[®] · SmartFreshTM · Physiological storage disorders

Introduction

The ‘Ambrosia’ is a bicoloured apple (*Malus domestica* Borkh) that has rapidly gained in popularity as a fresh fruit cultivar in recent years (Cliff and Toivonen 2017). The ‘Ambrosia’ is notable as a low producer of ethylene, the hormone that marks the onset of the ripening process

in climacteric apples. Determining the maturity level of ‘Ambrosia’, which is especially important for fruit destined for long-term storage, can be tricky as this apple does not always display the classic climacteric characteristics observed in many apple cultivars while the starch-iodine index has also been shown to be a poor metric for maturity for the ‘Ambrosia’ apple (Toivonen 2015; Cline 2009). In previous research by DeLong et al. (2016), conducted out of the same lab as this study, a comprehensive ‘Ambrosia’ apple optimum harvest and storage trial was performed over the course of nine weekly harvests at four commercial grower sites over the course of three years. The primary storage disorders observed in ‘Ambrosia’ fruit growing in the region after three months of refrigerated air (RA) (3.5 °C) storage were senescent breakdown, core flush, greasiness and rots. Greasiness was an issue regardless of

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maturity level, ranging from < 10% in immature fruit, but steadily increasing with delayed harvest to > 60% in over-mature fruit. For the remaining three disorders (senescent breakdown, core flush and rots), negligible levels were found in pre-climacteric, immature fruit, while low levels (< 5%) coincided with the first detectable levels of internal ethylene content (IEC). The incidence of these three storage disorders continued to increase steadily with maturity to > 40%, in the case of senescent breakdown and core flush, and approximately 10% for rots (DeLong et al. 2016). Unlike other apple cultivars, such as ‘Honeycrisp’, whose relationship between disorder incidence and maturity is strong, but highly nuanced (Prange et al. 2011; DeLong et al. 2014), the ‘Ambrosia’ maturity-disorder relationship is straightforward: all major disorders increased with the delay of harvest and maturity. However, ‘Ambrosia’ quality is closely connected to colour development and so maturity-related disorder avoidance must be weighed against colour development in the bicoloured ‘Ambrosia’ apple. In the ‘Ambrosia’ optimum harvest work, it was shown that fruit colour was low (< 60%) at maturity levels that coincided with low levels of senescent breakdown, core flush and rots in storage, but it plateaued at > 70% at maturity levels that corresponded with higher disorder levels (DeLong et al. 2016). In this study, we wished to investigate the relationship between colour development, fruit composition and disorder incidence in low-ethylene ‘Ambrosia’ apples after the application of anti-ethylene compounds both pre- and postharvest.

In climacteric fruit, such as apple, the plant hormone ethylene (C_2H_4) is the principal regulator of the ripening process. The synthesis of ethylene is linked to a cascade of biochemical events associated with ripening, including colour and volatile development and changes to fruit firmness, acidity, starch and sugars (Pech et al. 2012). Advancing maturity is associated with an increase in red colour development and specific aroma volatiles as well as a decrease in flesh firmness, acidity and starch. There are two principal anti-ethylene chemicals available to commercial apple growers: aminoethoxyvinylglycine (AVG) and 1-methylcyclopropene (1-MCP). Both AVG, marketed as ReTain® (Valent Canada, Guelph, ON, Canada) in the form of a preharvest application, and 1-MCP, marketed as Harvista™ and SmartFresh™ (AgroFresh Inc., Philadelphia, PA, USA), in the forms of a preharvest and postharvest formulation, respectively, have been shown to reduce ethylene levels and slow maturity development in climacteric fruit (Blankenship and Dole 2003; Lurie 2007; Venburg et al. 2008; Martínez-Romero et al. 2007; Autio and Bramlage 1982; Bramlage et al. 1980). While both AVG and 1-MCP have been shown to depress ethylene levels, they differ in their modes of action. AVG reduces ethylene synthesis through the inhibition of the 1-aminocyclopropane carboxylic acid (ACC) synthase activity, an enzyme responsible for the synthesis of ethylene

precursor ACC (Venburg et al. 2008; Silverman et al. 2004). By contrast, 1-MCP inhibits ethylene perception by blocking ethylene receptor sites and thereby inhibiting the System 2 autocatalytic generation of ethylene (Blankenship and Dole 2003; Lurie 2007). As a result, exogenous ethylene will have a limited impact on fruit treated with 1-MCP, but will still enhance maturity in AVG-treated fruit (Autio and Bramlage 1982). Since ‘Ambrosia’ is a bicoloured fruit whose quality and value are closely connected to colour development, a cautious approach must be taken regarding the rate and timing of preharvest anti-ethylene products. Both the ReTain® and Harvista™ labels suggest that their products may improve colour by allowing for additional hang time on the tree, but simultaneously warn that their use can reduce colouration in bicoloured fruit under poor colouring conditions (AgroFresh 2018; Valent Canada 2020). In addition, as a result of the ‘Ambrosia’ apple’s status as a low-ethylene producer that sometimes lacks the classic climacteric characteristics observed over the course of harvest in other cultivars (Cline 2009), a study detailing the impact of preharvest anti-ethylene compounds on this important cultivar is warranted.

In this study, pre- and postharvest anti-ethylene treatments were applied to the low-ethylene producing bicoloured ‘Ambrosia’ apple. The two main objectives were: 1. To determine whether AVG and 1-MCP can delay maturity in ‘Ambrosia’ apples without impacting fruit colour; 2. To determine how preharvest anti-ethylene (AVG and 1-MCP) treatments and postharvest treatments (1-MCP and temperature) impact long-term storage quality and disorder incidence in ‘Ambrosia’ fruit.

Materials and Methods

Grower Sites

Four commercial ‘Ambrosia’ apple (*Malus domestica*) orchard sites were selected in the Annapolis Valley, Nova Scotia, Canada and used in this two-year study conducted from 2020 to 2021. The four orchard sites were spread relatively evenly across a span of approximately 50 km in the eastern-end of the valley. Three of the four orchard sites were the same in both 2020 and 2021, while the fourth site was replaced with a fifth orchard in a nearby location in 2021. General year-to-year weather data were collected from the Environment and Climate Change Canada (ECCC) weather station located in Kentville, NS. None of the sites used were irrigated in either year.

Field Treatments: Preharvest Anti-Ethylene

At each orchard site, individual rows were randomly assigned one of three treatments: 1. Control (no anti-ethylene product applied); 2. ReTain® (15% AVG, Valent Canada, Guelph, ON, Canada) applied via a conventional airblast orchard sprayer at a half rate (412 g/ha), and mixed with a surfactant (Sylgard®, 0.05% v/v, Dow Corning Canada Inc., Georgetown, ON, Canada), two weeks before the optimum harvest date; and 3. Harvista™ (Harvista™ 1.3 SC, 1.3% 1-MCP, AgroFresh Inc., Philadelphia, PA, USA) applied via a modified airblast orchard sprayer at a rate of 9.34 L/ha (recommended apple rate is 5.9–17.7 L/ha) seven days before the optimum harvest date. A low-to-moderate rate and a relatively short preharvest interval were chosen for both anti-ethylene products on account of the bicoloured ‘Ambrosia’ apple’s sensitivity to these products and to minimize their impact on red colour development (Debrouwer 2022; AgroFresh 2018; Valent Canada 2020). A buffer of at least two rows of apple trees was left in between treatment rows. Treatments were applied on the same day across sites. The Harvista™ treatment required the use of a sprayer equipped with a specialized application kit. The predicted optimum harvest date was guided using the index of absorbance difference (I_{AD}) values (i.e., chlorophyll) as measured with a delta-absorbance (DA) meter (Sintéleia χ , Bologna, Italy) (DeLong et al. 2016) and averaged across all four sites in each year.

Harvest Assessment

Two harvests—one optimum and one late—performed nine or 10 days after the optimum harvest, were conducted in both years. In 2020, the optimum and late harvests occurred on October 13 and 23; in 2021, the same occurred on October 12 and 21.¹ In 2020, the fruit index of absorbance (I_{AD}) (i.e., chlorophyll), internal ethylene content (IEC), firmness, soluble solids (SS), titratable acidity (TA) and starch harvest assessment were based on five fruit randomly sampled from each site, treatment and harvest combination. In 2021, the same assessment was based on 10 fruit (with the exception of IEC, which was still based on five fruit). Fruit mass and colour were based on 25 and 30 fruit for each site, treatment and harvest combination in 2020 and 2021, respectively. All assessments were performed on individual fruit and then averaged for each experimental unit consisting of site and treatment combinations. Fruit colour was assessed visually (0 to 100%) as a percentage of red surface area. The assessment was performed by the same trained and highly

experienced individual for all fruit and for both years and was initially broken down by each individual fruit’s sun and shade hemispheres. Fruit mass was measured using a digital scale (CP 4202 S, Sartorius, Germany). A DA meter (Sintéleia χ , Bologna, Italy) was used to measure the I_{AD} on both the sun exposed and shade sides of each fruit, which were then averaged. IEC was measured based on a 1-ml sample extracted from the core of each fruit using a canula and a syringe. Samples were analyzed with a flame ionization detector (FID) via gas chromatography (Shimadzu GC-8A, Tokyo, Japan) using an active alumina column (6'×0.125" OD, F1 60/80, GL Sciences Inc). Hydrogen was used as a carrier gas and nitrogen as a makeup gas. The injector, detector and column were all set to 70 °C. Ethylene peaks and analysis were generated via a PeakSimple chromatography data system (SRI Instruments Inc., USA) calibrated using certified ethylene standards (Praxair Canada Inc., Dartmouth, NS, Canada). For fruit firmness, after the removal of ≈ 3 mm of skin and cortex tissue from the sun and shade-exposed equatorial region using a mandolin, the cortex firmness level was measured using a digital penetrometer (Chatillon DFIS50; Ametek TCI Division, Largo, FL) fitted with a 11.1 mm-diameter tip and a data acquisition program (Fruit Quality Tester; Geo-Met Instruments, New Minas, NS, Canada). Sections of the middle cortex region were removed from both the sun and shade side of each fruit and juiced using a garlic press. A 1-ml sub-sample of the well-mixed juice was used to approximate SS content using a handheld, temperature-compensated digital refractometer (Atago Co., Tokyo, Japan). A 2-ml sub-sample of the same juice was used to measure the TA, expressed as mg equivalents of malic acid per 100 ml of juice, using a Buret/Dispenser (Brinkmann Model 350; Metrohm, Herisau, Switzerland), 0.1 M NaOH and a pH 8.1 end-point as indicated using phenolphthalein. Finally, fruit were first transversely cut in half along the equator, and each resulting hemisphere subsequently sliced again across both the transverse and sagittal planes, to assess for internal disorders (i.e., cavities, core flush, rots, senescent breakdown and vascular breakdown). A starch conversion index assessment was performed on an upper basal cross-section of each bisected apple using a generic starch-iodine index chart as described by Blanpied and Silsby (1992). In this index, a rating of ‘1’ indicates a maximum amount of starch detected while a rating of ‘8’ indicates no starch detected.

Postharvest Storage Treatment

For each harvest, fruit was harvested in the morning, initial fruit composition measurements were taken the same day in the afternoon, while fruit designated for storage were placed into refrigerated air by late afternoon. Controlled-atmosphere (CA) conditions were established on day three

¹ Only nine days of separation between optimum and late harvest in Year 2.

after harvest. The fruit was divided into five different storage treatments postharvest: the three preharvest anti-ethylene treatments (1. Control, 2. ReTain®, 3. Harvista™) plus two additional postharvest treatments that examined the impact of 1-MCP used postharvest (i.e., SmartFresh™, 0.14% 1-MCP, AgroFresh Inc, Philadelphia, PA, USA) and warmer storage temperatures (i.e., 4. Control + SmartFresh™ (SF) and 5. Control + Warm, respectively), on fruit ethylene, quality and storage disorder incidence. For the ‘Control + Smart-Fresh™’ treatment, 0.54 g of product was dissolved into 3.6 ml of buffer solution, gently agitated until dissolved and then injected into a 4-bushel (140 L) sealed CA cabinet that held the fruit. The chamber was opened and the fruit vented after ≈ 24 h. For the ‘Control + Warm’ treatment, fruit was stored in a separate room at 3.5 °C, the remaining four storage treatments were all stored in the same room at 1 °C. For all five treatments and for each harvest (optimum and late), approximately 50 fruit from each of the four sites and five treatment combinations were placed into a single plastic bushel for a total of 40 bushels of fruit in a given year. For the four treatments stored in the 1 °C room, each bushel of fruit was randomly assigned to a 4-bushel capacity CA cabinet fitted with a top-loading water seal. No individual 4-bushel CA cabinet contained more than one bushel of fruit from the same grower or of the same treatment. This decision reflects both the practice and interests of the local commercial storage industry who do not sort lots of apples based on preharvest anti-ethylene treatment due to logistical challenges. Fruit from the two harvests was placed in separate CA cabinets. For the ‘Control + Warm’ treatment, the four bushels generated from the four sites at each harvest were each placed in their own 4-bushel CA cabinet as above. Oxygen and CO₂ levels in all chambers were monitored and controlled using a GCS-6000 Oxystat 2002 system (Storage Control Systems, Sparta, MI, USA) with the setpoints 2.2 kPa O₂, ≤ 1.0 kPa CO₂. All treatments were held in CA storage for approximately eight months.

Post-Storage Assessments

Both an initial (day 1) and shelf life (day 7 at 20 °C) post-storage assessments were conducted on fruit from all treatments after storage. For the initial assessment, individual fruits from a 10-fruit subset randomly sampled from each treatment were used to assess fruit composition. Fruit colour, mass, I_{AD}, firmness, SS and TA were all measured as described above. The IEC was measured in a five-fruit subset randomly sampled from the 10-fruit subset. A starch assessment was not performed after storage and was assumed to be zero. Finally, each of the 10-fruit were dissected and assessed for both external and internal disorders once the fruit composition parameters were measured. The same complement of measurements was performed on a new

10-fruit subset randomly selected from each treatment on day 7. At the end of the shelf life period, the remaining fruit ($n \approx 30$) from each treatment, which had been held in refrigerated air, were individually sliced open and assessed for storage disorders. It was assumed that storage disorder incidence did not change greatly during the shelf life and incidence numbers were combined.

Statistical Analysis

A statistical analysis was performed according to the statistical design for each fruit composition parameter measured for both the three preharvest treatments and the five postharvest treatments. A multi-factor ANOVA was performed using R software (R Development Core Team 2022). The constant variance and normal distribution assumptions were tested using a residual versus fits plot and a normal quantile–quantile plot, respectively. In instances where one or more of these assumptions were violated, transformed data were used and then a back-transformation was performed. For significant main effects and interactions, treatment means were compared via a Tukey pairwise comparison (emmeans package). Treatment differences were considered significant if $p \leq 0.05$. For all disorder data, a generalized linear model (GLM) using the binomial link function was used followed by an ANOVA in R to determine whether any of the factors in the design, or their interactions, were statistically significant. Pairwise marginal means comparisons (emmeans package) were performed to determine whether any contrasts existed between the levels of the various factors.

Results

Growing Season Conditions

The 2020 and 2021 growing seasons were 3% and 5% above the 10-year average, respectively, in terms of the total amount of heat received, measured using base 5 °C growing degree days (GDD₅). Both years were similar in that they had a warm, dry month of June (Table 1). The 10-year averages for June are 337 GDD₅ and 95 mm of rain. The greatest contrast between the two years was found in the precipitation levels in the latter part of the growing seasons. More specifically, 43%, 72% and 39% more precipitation was received in 2021 relative to 2020 in July, August and September, respectively. The 2020 and 2021 growing seasons for these months had 8% less and 27% more precipitation than the 10-year average, respectively. However, the partial month of October was slightly rainier in 2020 relative to 2021. The number of growing season days with precipitation events in 2021 was also higher than 2020 for all months, with the exception of

Table 1 Base 5 °C growing degree days (GDD₅) (single sine method), precipitation (mm), precipitation events (days with precipitation ≥ 1 mm), solar radiation ($h > 500$ W/m²) and chilling ($h < 8$ °C)

	June		July		August		September		October*	
	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
GDD ₅	378	419	478	438	480	486	323	355	158	156
Precip. (mm)	49	37	115	164	71	122	122	169	54	26
Precip. events	10	11	11	15	9	11	7	14	11	8
Solar hours ($h > 500$ W m ⁻²)	141	145	135	127	159	132	102	86	42	47
Chilling ($h < 8$ °C)	NA	NA	NA	NA	1	1	86	15	107	116

All weather data came from the Environment and Climate Change Canada weather station located at the KRDC

*Partial month data: October 1–22

the partial month of October. Solar radiation levels were inversely correlated with precipitation levels while the number of preharvest chilling hours was higher in 2020 relative to 2021 in the month of September, but 2021 chilling was marginally higher than 2020 in the final two or three weeks before harvest in October (Table 1).

Ethylene-Inhibitor Trial Harvest Assessment

Harvista™ treated fruit had higher TA and more starch (i.e., lower starch index) than Control fruit across both years. Harvista™ fruit did not have higher acidity than ReTain® fruit, but it did have more starch across both years (Table 2). All other treatment differences at harvest had significant interactions with the Year. Of note, Control fruit had superior colour relative to both Harvista™ and ReTain® fruit, but in Year 1 only, the poorer colouration year (Table 2). However, this distinction was only significant for Harvest 1 (i.e., optimum). Colour, SS and the starch index increased with the later harvest (i.e., Harvest 2), while I_{AD}, firmness and TA decreased. However, the colour and SS Harvest trends were only significant in one out of two years. While the IEC trended higher with harvest date, the difference fell short of being significant ($p = 0.08$). Fruit composition was very different across years as a result of growing season differences (Table 1). Overall, Year 2 fruit had higher colour, mass and I_{AD}, but with lower firmness, SS, TA and starch index (Table 2). All three-factor interactions (i.e., Treatment:Harvest:Year) were not significant at harvest.

Post-Storage Fruit Quality Assessment

After eight months of CA storage, firmness was the only fruit composition response variable that showed a significant treatment effect uncomplicated by interactions with the other design factors (i.e., Harvest, Year and Shelf Life). Fruit firmness levels in both the Harvista™ field treatment and the Control + SmartFresh™ postharvest treatment (i.e.,

for June, July, August, September and October (*partial month: Oct 1–22), which is the primary growing season for apple fruit in Nova Scotia, in 2020 and 2021

the two 1-MCP treatments) were higher than found in the remaining three treatments. Fruit treated with ReTain® had the next highest firmness average after the two 1-MCP treatments, but was not significantly different than the Control and Control + Warm treatments (Table 3). While fruit IEC across treatments was complicated by interactions, the Control + SmartFresh™ treatment had significantly lower IEC levels than all other treatments for every factor combination; the Control + Warm treatment was significantly higher than all other treatments for all factor combinations with the exception of Harvest 2 in Year 2. In terms of SS levels, the Control + SmartFresh™ had the highest measured levels and was significantly different than the Control + Warm and ReTain® treatments in Year 1, while the Harvista™ treatment had the highest levels in Year 2 and was significantly different than all other treatments, with the exception of ReTain®. TA levels were higher in all three of the anti-ethylene treatments (i.e., Control + SmartFresh™, Harvista™ and ReTain®) relative to the remaining treatments (i.e., Control and Control + Warm) in Year 1, while only the Control + SmartFresh™ and Harvista™ treatments had a higher TA level relative to the Control fruit, and the Control + SmartFresh™ treatment was greater than ReTain® in Year 2. The Control + SmartFresh™ and Harvista™ treatments averaged the highest I_{AD} levels after storage, but were not statistically different from the remaining treatments (Table 3). The I_{AD}, firmness and TA all declined with Harvest date. I_{AD} levels were higher and SS levels were lower post-storage in Year 2 relative to Year 1. The principle impact of the shelf life was a decrease in TA (Table 3).

Storage Treatment Disorder Assessment

Senescent breakdown (Fig. 1A) and core flush (i.e., core browning) (Fig. 1B) were the dominant two storage disorders in this study while vascular breakdown, rots and cavities were present at lower levels. The five disorders tracked were all higher for the late Harvest 2 fruit relative

Table 2 ‘Ambrosia’ harvest fruit composition parameter averages across preharvest treatment, harvest and year: fruit colour (%), mass (g), chlorophyll (I_{AD}), internal ethylene (C_2H_4) content (IEC), firmness (newtons (N)), soluble solids (SS), titratable acidity (TA) (mg malic acid/100 mL of juice) and starch (Cornell Starch–Iodine Index) (1–8)

Treatment	Colour (%)	Mass (g)	I_{AD}	IEC ($\mu\text{L } C_2H_4 \text{ (kg h)}^{-1}$)	Firm (N)	SS (%)	TA (mg/100 ml)	Starch (1–8)
Control	70	192	0.21	0.59	79.6	12.8	352 ^b	6.0 ^a
Harvista™	68	193	0.24	0.62	79.5	12.7	387 ^a	5.4 ^b
ReTain®	68	199	0.23	0.53	78.5	12.7	360 ^{ab}	5.9 ^a
Harvest								
Harvest 1	66	192	0.27 ^a	0.51	80.6 ^a	12.6	381 ^a	4.8 ^b
Harvest 2	72	197	0.18 ^b	0.66	77.8 ^b	12.9	350 ^b	6.8 ^a
Year								
Year 1	64 ^b	173 ^b	0.20 ^b	0.61	86.0 ^a	13.6 ^a	383 ^a	4.3 ^b
Year 2	74 ^a	214 ^a	0.25 ^a	0.56	72.9 ^b	11.9 ^b	349 ^b	7.2 ^a
Significance								
Treatment	NS	NS	NS	NS	NS	NS	*	*
Harvest	***	NS	***		**	*	**	***
Year	***	***	*	NS	***	***	**	***
Treatment:Harvest	NS	NS	NS	NS	NS	NS	NS	NS
Treatment:Year	* ¹	NS	NS	** ³		* ⁴	NS	NS
Harvest:Year	** ²	NS	NS		NS	* ⁵	NS	** ⁶

Four grower sites acted as the block. Significance is denoted as follows: $p < 0.001$ (***), $p < 0.01$ (**), $p < 0.05$ (*), $p < 0.1$ (.), not significant (NS). A Tukey multiple means comparison was performed across treatments when the Treatment factor was < 0.05 and not impacted by interactions. Averages with similar letter groupings for a given factor and response variable are not significantly different. All high level/superseding significant interactions are detailed in a table footnote

¹Treatment $\times$ Year (Colour): Year 2 > Year 1 for all treatments, but the difference was greater for Harvista and ReTain ($p < 0.001$) than the Control fruit ($p = 0.02$). Control (67.5) > Harvista (61.5) ($p = 0.02$) and Control (67.5) > ReTain (62.8) ($p = 0.05$) in Year 1 only

²Harvest $\times$ Year (Colour): Harvest 2 (70.3) > Harvest 1 (58.0) in Year 1 ($p < 0.001$), but Harvest 2 (74.1) = Harvest 1 (73.2) in Year 2 ($p = 0.72$). Year 2 > Year 1 in for Harvest 1 ($p < 0.001$) and Harvest 2 ($p = 0.03$)

³Treatment $\times$ Year (IEC): Harvista (0.77) > Control (0.41) in Year 2 only

⁴Treatment $\times$ Year (SS): Control (13.9) > ReTain (13.4) in Year 1 only

⁵Harvest $\times$ Year (SS): Harvest 2 (12.2) > Harvest 1 (11.7) in Year 2 only

⁶Harvest $\times$ Year (Starch): Harvest 2 > Harvest 1 in both years; however, the Year 2 difference > Year 1 difference

Table 3 ‘Ambrosia’ post-storage (8 months CA) fruit composition parameter averages across three preharvest Treatments (Control, Harvista™, ReTain®) and two postharvest Treatments (Control + SmartFresh™ (SF), Control + Warm), Year and Shelf Life: chlorophyll (I_{AD}), internal ethylene (C_2H_4) content (IEC), firmness (newtons (N)), soluble solids (SS), titratable acidity (TA) (mg malic acid/100 mL of juice) and starch (Cornell Starch–Iodine) (1–8).

	I_{AD}	IEC ($\mu\text{L } C_2H_4$ (kg h) $^{-1}$)	Firm (N)	SS (%)	TA (mg/100 ml)
Treatment					
Control	0.17	3.3	54.3 ^b	13.4	194
Control + SF	0.18	0.3	66.3 ^a	13.6	245
Control + Warm	0.15	9.5	52.2 ^b	13.3	208
Harvista™	0.18	1.6	61.5 ^a	13.8	244
ReTain®	0.16	3.1	55.8 ^b	13.6	215
Harvest					
Harvest 1	0.20 ^a	2.2	64.1 ^a	13.6	251 ^a
Harvest 2	0.13 ^b	3.1	51.7 ^b	13.4	190 ^b
Year					
Year 1	0.14 ^b	1.9	57.8	14.0 ^a	217
Year 2	0.19 ^a	0.9	58.1	13.1 ^b	224
Shelf Life					
Day 1 (initial)	0.18	1.3	57.9	13.5	234 ^a
Day 7 (shelf)	0.16	1.4	58.0	13.5	207 ^b
Significance					
Treatment	NS	***	***	***	***
Harvest	***	**	***	**	***
Year	***	***	NS	***	NS
Shelf			NS	NS	***
Treatment:Harvest	NS	***	NS	NS	NS
Treatment:Year	NS	***	NS	*** ³	** ⁴
Treatment:Shelf	NS	**	NS	NS	NS
Harvest:Year	NS	NS	NS		** ⁵
Harvest:Shelf	NS	NS	NS	NS	NS
Year:Shelf	NS	***	NS	NS	NS
Treatment:Harvest:Year	NS	*** ¹	NS	NS	NS
Treatment:Year:Shelf	NS	* ²	NS	NS	NS

Four grower sites acted as the block. Significance is denoted as follows: $p < 0.001$ (***), $p < 0.01$ (**), $p < 0.05$ (*), $p < 0.1$ (.), not significant (NS). A Tukey multiple means comparison was performed across treatments when the Treatment factor was < 0.05 and not impacted by interactions. Averages with similar letter groupings for a given factor and response variable are not significantly different. All high level/super-seeding significant interactions are detailed in a table footnote

¹Treatment $\times$ Harvest $\times$ Year (IEC): ‘Control + SF’ had the lowest average for all four Harvest:Year combinations; ‘Control + Warm’ had the highest average for all Harvest:Year combinations, with the exception of Year 2:Harvest 2. The remaining 3 Treatments varied with Year and Harvest. ‘Control + Warm’ decreased, ‘Harvista’ increased, while ‘Control’ and ‘Control + SF’ IEC was unchanged across Harvests both Years; ‘ReTain’ fruit IEC increased with Harvest in Year 2, but not Year 1. Year 1 $>$ Year 2 for ‘Control’, ‘Control + Warm’ and ‘ReTain’ Treatments; the same was true for ‘Harvista’ fruit for Harvest 2 fruit only. ‘Control + SF’ was unchanged across years

²Treatment $\times$ Year $\times$ Shelf (IEC): ‘Control + Warm’ was higher than all other treatments in Year 1 (Day 1 & Day 7), higher than all but the ReTain treatment in Year 2, Day 1 and only higher than ‘Control + SF’ in Year 2, Day 7. ‘Control + SF’ was lower than all other treatments in Year 1 (Day 1 & Day 7), but was only lower than the ‘Control + Warm’ fruit in Year 2 (Day 1 & Day 7)

³Treatment $\times$ Year (SS): ‘Control + SF’ $>$ (‘Control + Warm’ & ‘ReTain’) in Year 1. Harvista $>$ (Control & ‘Control + Warm’ & ‘Control + SF’), ReTain $>$ ‘Control + Warm’ in Year 2

⁴Treatment $\times$ Year (TA): (‘Harvista’ & ‘Retain’ & ‘Control + SF’) $>$ (‘Control’ & ‘Control + Warm’) in Year 1, (‘Harvista & ‘Control + SF’) $>$ ‘Control’ and ‘Control + SF’ $>$ ‘ReTain’ in Year 2

⁵Harvest $\times$ Year (TA): Year 2 $>$ Year 1 for Harvest 2 only

Fig. 1 The most prominent two ‘Ambrosia’ apple storage disorders found within this study included **A** senescent breakdown (i.e., presenting as diffuse browning)—a browning of the cortex tissue exterior to the core and vascular bundles that extends to the epidermis; **B** core flush—browning and discoloration is localized around the core and interior to the vascular bundles

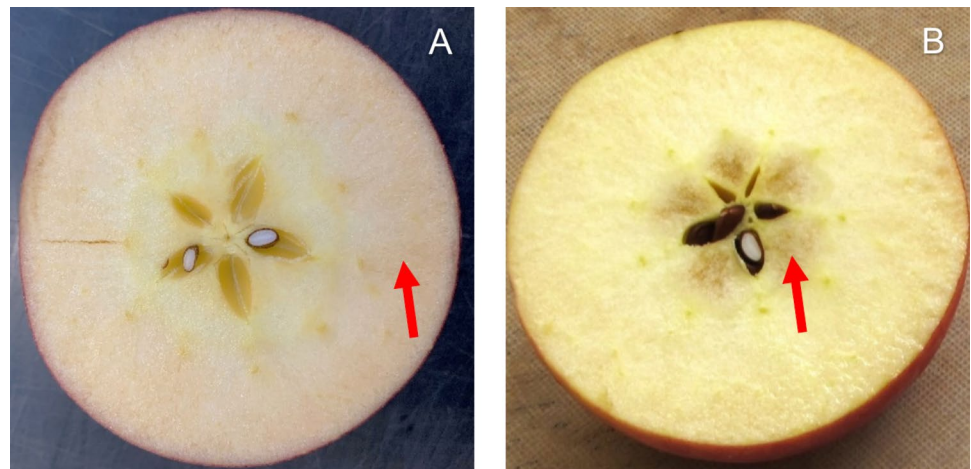


Table 4 ‘Ambrosia’ post-storage (8 months CA) fruit storage disorder incidence and marketable fruit (%) across three preharvest Treatments (Control, Harvista™, ReTain®) and two postharvest Treatments (Control+SmartFresh™ (SF), Control+Warm), Harvest and Year

	Cavity (%)	Core flush (%)	Rot (%)	Senescent breakdown (%)	V. breakdown (%)	Disorder-free (%)
Treatment						
Control	1.1	6.3	1.8	20.9	3.5	67.2
Control + SF	0.0	3.2	0.0	12.1	0.0	75.3
Control + Warm	0.0	12.5	3.1	8.1	1.5	67.2
Harvista™	0.0	13.1	2.5	10.3	3.3	66.1
ReTain®	0.0	9.1	0.0	10.6	0.0	71.3
Harvest						
Harvest 1	0.0	5.9	0.2	4.6	0.0 ^b	81.4 ^a
Harvest 2	0.1	10.7	0.6	27.1	6.6 ^a	57.6 ^b
Year						
Year 1	1.3	5.6	3.5	14.1	NA ⁶	69.6
Year 2	0.0	11.2	0.0	9.9	3.3	69.4
Significance						
Treatment	NS	***	***	***	NS	NS
Harvest	NS	***	***	***	***	***
Year	***	**	***	***	NA	NS
Treatment:Harvest	* ¹	**	NS	***	NS	NS
Treatment:Year		***	*	***	NA	
Harvest:Year	* ²	*	NS	***	NA	
Treatment:Harvest:Year	NS	*** ³	* ⁴	*** ⁵	NA	NS

Four grower sites acted as the block. Significance is denoted as follows: $p < 0.001$ (***), $p < 0.01$ (**), $p < 0.05$ (*), $p < 0.1$ (.), not significant (NS). A Tukey multiple means comparison was made across treatments when the Treatment factor was < 0.05 and uncomplicated by interactions. Averages with similar letter groupings for a given harvest are not significantly different. All high level/superseding significant interactions are detailed in a table footnote

¹Treatment × Harvest (Cavity): posthoc tukey multiple means comparison showed NS

²Harvest × Year (Cavity): posthoc tukey multiple means comparison showed NS

³Treatment × Harvest × Year (Coreflush): see supplementary Table 1

⁴Treatment × Harvest × Year (Rot): see supplementary Table 2

⁵Treatment × Harvest × Year (Senescent breakdown): see supplementary Table 3

⁶Analysis was restricted to Year 2 due to 0 incidence in Year 1

to the optimum Harvest 1 fruit. However, with the exception of vascular breakdown, this overall trend was complicated by significant interactions with the Treatment and Year factors (Table 4). The pre-harvest (Control, ReTain® and Harvista™) and the additional postharvest (Control + Warm and Control + SmartFresh™) treatments had no significant impact on the minor cavities and vascular breakdown disorders. The Control + SmartFresh™ treatment averaged the lowest core flush incidence, while the Control + Warm and Harvista™ treatments were typically higher, but this was dependent on the Year and Harvest factors (Tables 4, S1). The highest level of rot was found in the Year 1, Harvest 2 Control + Warm (16.0%) and Control (11.5%) treatments, while the majority of the remaining Treatment, Year and Harvest combinations were under 3% (Tables 4, S2). The Control + Warm treatment averaged the lowest senescent breakdown level, while the Control fruit was the highest, but significance was dependent on interactions with the Year and Harvest factors (Tables 4, S3). Finally, while the percentage of disorder-free fruit dropped from greater than 80% to less than 60% between harvests, there were no Treatment differences (Table 4).

Discussion

Year and Harvest Effects

The different growing conditions found across the two years, primarily on account of a wetter growing season in Year 2 (Table 1), resulted in contradictions between the two years in terms of fruit composition and indication of maturity. These findings also demonstrate how no one metric is a good indicator of maturity as all can be influenced by a range of factors that may differ and so are not necessarily in step. Year 2 fruit appeared more mature than Year 1 fruit in terms of greater colour, size and lower firmness, acidity and starch levels (i.e., higher Starch-Iodine Index levels). However, Year 2 fruit appeared less mature than Year 1 fruit in terms of higher epidermal chlorophyll content (i.e., I_{AD}) and low SS levels (Table 2). A combination of a cooler July (30–60 days after full bloom), a period of fruit cell division and maximal calcium uptake, followed by elevated temperature and precipitation for the remainder of the growing season, a period of cell expansion, in Year 2 relative to Year 1 (Table 1), likely explain the lower fruit firmness in Year 2. Cooler temperatures during cell division would both limit the number of cells and calcium uptake, a necessary component for cell integrity, while elevated temperatures and precipitation during the remainder of the season would accentuate the cell expansion phase, also observed as larger fruit (Table 2), thereby increasing the need for resources and further compromising cell integrity (Lachapelle et al. 2013)

With the link between increased precipitation, cloud cover and decreased solar radiation reflected in the weather data (Table 1), reduced photosynthate production, along with dilution in the fruit and possible competition with increased vegetation, likely explain the depressed fruit starch, SS and TA levels in Year 2 relative to Year 1 (Wang et al. 2019; Rumayor-Rodriguez and Bravo-Lozano 1991). It is not known what caused the poor colouration in Year 1 relative to Year 2. Year 2 had only minimally higher levels of solar hours and chilling in the weeks leading up to harvest (October data only; Table 1). Crop load, negatively correlated with colour development (Wright et al. 2006; Lu and Xu 2021), was not accounted for and could be a factor in the colour difference between years; however, this explanation is speculative and incongruous with the fruit sugar levels, which were lower in Year 2 relative to Year 1.

There were minor, disorder-specific differences across years that were dependent on interactions with the Harvest and Treatment factors. For example, core flush and senescent breakdown were higher and lower, respectively, in Year 2 relative to Year 1, for specific Treatment and Harvest combinations (Table 4). The interplay of these two most prominent disorders resulted in there being no difference in the percentage of marketable, disorder-free fruit across years (Table 4). While both core flush and senescent breakdown in ‘Ambrosia’ were shown to be positively correlated with maturity (DeLong et al. 2016), and the trend was reflected in this study, albeit with interactions (Table 4), it is not known why the trends of these two disorders diverged in opposite directions across the two years. As core flush has been linked with calcium deficiencies (Sidhu et al. 2023), the elevated precipitation and lower solar radiation in Year 2 relative to Year 1 (Table 1) resulted in larger fruit (Table 2), and although unmeasured, likely increased vegetation, a greater sink than fruit, and a lower passive uptake of calcium. Calcium is a macronutrient integral to maintaining cell wall structural integrity in fruit; it is also an important secondary messenger, linked to reduced cell wall degradation activity as well as reduced ethylene and its impact on ripening in climacteric fruit. Calcium deficiency in fruit has been associated with reduced fruit firmness, quality and storage life and increased water loss and disorder incidence (Jaime-Guerrero et al. 2024). Reduced fruit calcium levels could be an explanatory factor for increased core flush incidence in Year 2. It is also a matter of speculation why senescent breakdown—another disorder associated with advanced maturity—trended higher in Year 1 compared with Year 2. Year 1 fruit appeared less mature than Year 2 fruit by many indices (TA, starch, firmness). However, many of these parameters were likely affected by fruit size (Table 2) and a lower dry matter content in the wetter Year 2. Year 1 fruit indicated a higher maturity than Year 2 in terms of its epidermal chlorophyll content, a parameter possibly less

impacted by water availability and fruit size (Table 2). In addition, chilling during the growing season, which was nearly 40% more in Year 1 relative to Year 2 when combining September and October values (Table 1), has been associated with diffuse browning—classified as senescent breakdown in this study—in several apple cultivars. While this relationship remains complex and sometimes contradictory, the disorder has been found to be preceded by an increase in cell permeability, likely the result of a loss in cell wall integrity (Argenta et al. 2022a; Tong et al. 2016; Meheriuk et al. 1994; Sidhu et al. 2023).

Anti-Ethylene Treatments, Colour Development and Fruit Composition

The market value of the bi-coloured ‘Ambrosia’ apple is tightly connected to its characteristic red blush (Toivonen et al. 2019a; Cline 2009). Both the Harvista™ 1.3 SC and ReTain® labels suggest that the use of their anti-ethylene products will allow additional time for colour development, but with the caveat that their product may also impact or delay colour development, especially when applied at a higher rate on bi-coloured apples or under difficult colouring conditions (AgroFresh 2018; Valent Canada 2020). Both AVG and preharvest 1-MCP have been observed to reduce colour development in apples, due to the positive relationship between ethylene and anthocyanin accumulation, though this effect is dependent on cultivar, rate and conditions (Al Shoffe et al. 2024; do Amarante et al. 2022, 2010; Boyacı 2022). The moderate rates and late timing of the applications used in this study for both products were an attempt to balance the intended suppressed maturity development, while minimizing the potential negative impact on fruit colour development. The results were mixed as colour development was impacted in the poorer colouration year (i.e., Year 1), but not in Year 2 (Table 3). This finding confirms a cautious approach should be taken with the use of anti-ethylene products in the field in order to avoid a negative impact on colour development in bicoloured ‘Ambrosia’.

The impact of the Harvista™ treatment on ‘Ambrosia’ fruit maturity, as measured by various fruit composition measures, was greater than that observed using ReTain® for the rates and application timings used in this study (Tables 2 and 3). However, it is important to emphasize that this result should not be interpreted as a direct comparison of the two products. The efficacy of both the Harvista™ and ReTain® products in reducing ethylene and maturity is dependent on the rate, timing, cultivar, environmental conditions, state of maturity and the different modes of actions of these two compounds. The results of this study could have been different if a higher rate of ReTain® had been used, though this likely would have exacerbated the negative impact on colour in the poorer colouration year. Several studies exist

comparing the use of both Harvista™ and ReTain® with untreated controls with a range of outcomes (Doerflinger et al. 2017; Al Shoffe et al. 2024; Argenta et al. 2022b; Lafer 2006). For example, in contrast to the current study, in a similar study on RubyFrost® apples that used comparable rates and the same timing as those used in this study, trees treated with ReTain® were less mature than those treated with Harvista™ by several metrics (Al Shoffe et al. 2024).

In this ‘Ambrosia’ study, the Harvista™ treatment fruit had higher TA and more starch than the control at harvest in both years of the study. While starch and TA were the main fruit composition parameters impacted by the field application of anti-ethylene products at harvest (Table 2), the greatest impact after storage was found in the firmness variable (Table 3). Both the Harvista™ and the postharvest Control + SmartFresh™ treatment showed higher firmness levels after storage relative to all other treatments. The impact of 1-MCP on post-storage apple firmness is in line with other studies (Cliff and Toivonen 2017; Małachowska and Tomala 2022). Given that the firmness was not impacted by Harvista™ at harvest in this study (Table 2), this result suggests that the impact of Harvista™ on firmness was more on account of its carryover effect in storage, similar to the use of SmartFresh™, as opposed to its impact in the orchard. Studies conducted by Valent BioSciences showed ‘Ambrosia’ apples to be among the cultivars that are more sensitive to ReTain® and that while our rate was in line with their cultivar-specific recommendation for ‘Ambrosia’ (i.e., 412–824 g/ha), the timing was later than recommended (three to four weeks before optimum harvest) (Valent BioSciences 2021). The low impact of the ReTain® treatment in this study may have been the result of a late application. The I_{AD} value at the first harvest, which had to be estimated two weeks before the harvest date for our ReTain® application, averaged 0.27 across the two years. Work performed by DeLong et al. (2016) suggested the optimum harvest window for ‘Ambrosia’ storage correlated with an I_{AD} range of 0.47 to 0.28 in the same region. The fact that the Harvista™ treatment produced a greater effect in ‘Ambrosia’ when applied at an even later stage of development than the ReTain® treatment (i.e., one week before Harvest 1 for Harvista™) likely speaks to the two different modes of action employed by these anti-ethylene compounds. In addition, because the combination of treatments while in CA storage likely reduced the efficacy of the ReTain® treatment, which would have been vulnerable to the ethylene produced by other fruit, including that from the Control fruit, but would have been less of a factor for the Harvista™ fruit. While it is recommended that fruit treated with ReTain® be separated from untreated fruit in storage (Debrouwer 2022), this is often not done in the commercial setting due to practical considerations. Despite this, there is evidence that ReTain® can influence metabolism and maturity in the presence of

control fruit and exogenous ethylene in storage after negating the possible impact of ReTain® on harvest maturity. In a study by Wright and Prange (2023), Bartlett pears treated with ReTain® only 24 h before harvest and stored with control fruit were greener and showed superior sugar retention after 4 months in CA storage.

It is not a surprise that there were complex interactions between the various factors involved in this trial and the IEC. Firstly, ‘Ambrosia’ fruit has low-ethylene levels compared with many other apple cultivars, and it does not always show the classic rise and drop in ethylene that most climacteric fruit do. Some fruit require a trigger, such as low temperatures, to induce a transition from System 1 to System 2 of ethylene production (Pech et al. 2012). In some years, this low temperature trigger may occur in the field while in others it may begin with the onset of cold storage. Secondly, interpreting a low-ethylene reading in a climacteric fruit can have two meanings, each at the opposite end of the maturity spectrum, e.g., an immature fruit and an overmature fruit may both show low IEC levels. Finally, the relationship between 1-MCP, ethylene and respiration is also complex. While 1-MCP typically reduces both ethylene and respiration in the long-term (weeks, months), it has been shown that this ripening hormone site blocker can actually increase both ethylene and respiration in the short term (days) before reducing it (Prange et al. 2012). This could explain why the fruit from Harvista™ trees appeared to have an elevated IEC at harvest relative to the Control, at least in Year 2 (Table 3).

Anti-ethylene Compounds and Storage Disorders

Several studies have made a connection between the use of anti-ethylene compounds and storage disorders. One of the most common examples is the link between the use of anti-ethylene compounds, such as AVG and 1-MCP, and the reduction in superficial scald incidence, a disorder associated with the production of ethylene and α -farnesene (Lurie and Watkins 2012; Rupasinghe et al. 2000; do Amarante et al. 2010). Preharvest applications of both anti-ethylene compounds have also been linked to elevated apple bitter pit incidence in storage (Al Shoffe et al. 2021; do Amarante et al. 2010), which is an intuitive relationship given that bitter pit development in storage is exacerbated by lower maturity levels (DeLong et al. 2014). Reports of a relationship between the use of anti-ethylene compounds and an elevated incidence of CO₂ injury are also prevalent (Lafer 2006; Al Shoffe et al. 2021; DeEll et al. 2003; Watkins and Nock 2012, 2013). The aforementioned relationships should be viewed as generalizations and not as universal principles. A multitude of studies exists linking anti-ethylene compounds to a range of apple storage disorders, besides those listed above, sometimes positively and sometimes negatively, including soft scald, core flush, fungal rots, radial stem-end

flesh breakdown, soggy breakdown, as well as senescent breakdown and watercore (Lafer 2006; Lee et al. 2016; Al Shoffe et al. 2021; Argenta et al. 2022b; Watkins and Nock 2012; Doerflinger et al. 2017). Finding unanimity on the impact of a particular anti-ethylene compound and a specific disorder is elusive. These relationships often depend on other factors, such as disorder pressure, genetics, timing, environmental conditions and maturity. Other complicating factors include the fact that certain disorders may manifest differently in different apple cultivars resulting in a misidentification or disagreement in the terminology used to describe a specific disorder. A final factor for consideration in identifying the impact of an anti-ethylene compound on storage disorders is whether the impact is simply on account of a change in maturity, in the case of anti-ethylene products applied preharvest, or if it is the result of a change in fruit metabolism in storage, which could result from either the pre- or postharvest application of anti-ethylene compounds.

In the present study, the two leading ‘Ambrosia’ storage disorders were senescent breakdown and core flush (Fig. 1A, B, respectively), which is in keeping with the findings of DeLong et al. (2016). It should be noted that, in other regions, such as the North American west coast, other studies have described soft scald and soggy breakdown as prominent ‘Ambrosia’ apple storage disorders (Toivonen 2019; Toivonen et al. 2019b; Lu and Toivonen 2022). These two disorders, which are distinct from core flush and senescent breakdown, were rarely observed in the present and previous studies (DeLong et al. 2016). Climate differences likely account for these differences. For example, soft scald in ‘Ambrosia’ fruit has been linked to low rainfall (Lu and Toivonen 2022), something that is rarely an issue in the Canadian Maritimes.

Senescent breakdown, which presented as a form of diffuse browning in this study, has been described as a form of chilling injury (Sidhu et al. 2023). While the treatment differences in the senescent breakdown incidence levels were complicated by interactions (Tables 4, S3), the finding that the Control + Warm treatment, a storage regimen that advanced maturity by several metrics (Table 3), averaged the lowest incidence of senescent breakdown, overall, supports this characterization. As for core flush, other studies have found that SmartFresh™ and lower internal ethylene reduce the incidence in apples (Fan et al. 1999; Ehsani-Moghaddam and DeEll 2009; Watkins et al. 2000). While this was reflected in the fact that the fruit in the SmartFresh™ treatment had the lowest average incidence of core flush fruit, this finding was, again, complicated by interactions (Tables 4, S1). Overall, although multiple disorders observed in this study showed significant anti-ethylene treatment effects, both positive and negative, they were invariably complicated by significant interactions with other factors and the overall level of disorder-free fruit was not impacted

by anti-ethylene treatment for ‘Ambrosia’ fruit in this study. This finding is in agreement with a review on apple internal browning disorder by Sidhu et al. (2023) that found the preharvest use of AVG and the pre- and postharvest use of 1-MCP resulted in varied and contradictory results, often specific to cultivar, maturity, growing and storage conditions, over a range of studies.

Conclusions

The findings from this study demonstrate that ‘Ambrosia’ fruit treated with a preharvest application of AVG (i.e., ReTain®) and 1-MCP (i.e., Harvista™) delayed maturity. Treated fruit were less mature than untreated Control fruit by various metrics both at harvest and after eight months of CA storage. While this study is not intended or designed to directly compare the two anti-ethylene products, each with a unique mode of action, the impact of Harvista™ was greater than ReTain® for the rates and timing used in this study. Increasing the rate or number of applications of ReTain® could result in a different outcome. This study showed that both preharvest Harvista™ and ReTain® anti-ethylene treatments negatively impacted colour development in ‘Ambrosia’ apples in a poor colouration year, but had no impact in a good colouration year. Determining the environmental conditions required to avoid an impact on colour requires further study. Anti-ethylene compounds have both positive and negative relationships with specific ‘Ambrosia’ apple disorders that involve complex relationships with other factors such as harvest timing and growing conditions. While previous work showed all prominent ‘Ambrosia’ storage disorders observed in the region increased with delayed harvest and fruit maturity, reducing maturity with the application of late-season, preharvest anti-ethylene compounds did not have the desired effect of reducing the overall incidence of ‘Ambrosia’ apple storage disorders in the region.

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Declarations

Conflict of interest None of the authors have relevant financial or non-financial interests that could pose a conflict of interest to disclose. Data can be made available upon reasonable request. All authors made substantial contributions to this manuscript.

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