THE INFLUENCE OF AMINOETHOXYVINYLGLYCINE AND ETHEPHON ON OBJECTIVE AND SENSORY QUALITY OF 'DELICIOUS' APPLES AND APPLE JUICE AT HARVEST AND AFTER STORAGE

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INTRODUCTION

Bioregulators can influence many important physiological processes in tree fruits when applied before harvest. Two bioregulators, aminoethoxyvinylglycine (AVG, ReTain®, Valent BioSciences, Walnut Creek, Calif.) and ethephon (ETH, Ethrel®, Bayer Crop Science, Research Triangle Park, N.C.) have been used to manipulate both tree and fruit physiological behavior (Looney, 2004). Control of vegetative growth and regulation of flowering (Elfving and Cline, 1990; Elfving et al., 2003), preharvest drop (Greene et al., 2000; Stover et al., 2003), fruit maturity (Byers, 1997; Greene, 2002; Layne et al., 2002; Stover et al., 2003; Wang and Dilley, 2001), fruit size, shape, color, firmness and postharvest quality (Andrews, 2001; Beaudry and Jayanty, 2003; Drake et al., 2002, 2003, 2005; Halder-Doll and Bangerth, 1987; Larrigaudiere et al., 1996; Watkins and Rosenberger, 1999; Wang and Dilley, 2001; Williams, 1980) have all been manipulated with the use of bioregulators. While many reports document effects on fruit quality, little information on bioregulator effects on sensory perception of fruit quality by consumers is available. One study suggests that a key factor that stimulates sales of apples is flavor (Washington Apple Commission, 2002).

Ethylene is thought to be the naturally-occurring hormone that triggers fruit ripening (Blanpied, 1972). AVG inhibits ethylene biosynthesis by the fruit, thereby retarding the ripening process (Bangerth, 1978). ETH releases ethylene upon uptake by plant tissues (Yang, 1969); applying ETH to apple trees shortly before harvest can induce ethylene biosynthesis and early ripening of fruit (Edgerton and Blanpied, 1968). However, pre-harvest ETH treatment also stimulates the climacteric behavior of the fruit, thereby reducing its capacity for long-term storage. Could the ethylene biosynthesis-inhibiting properties of AVG be exploited to allow brief bursts of ethylene from pre-harvest exogenous applications of ETH to temporarily stimulate ripening processes in apples without inducing the uncontrolled biosynthesis of ethylene in apple fruit and the accompanying loss of storability and quality? Combinations of AVG and ETH have been shown to permit better fruit color development in 'McIntosh' apple while controlling fruit ripening and maintaining fruit storability (Wang and Dilley, 2001).

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The objectives of this project were: 1) to evaluate the effects of AVG and ETH applied either separately or sequentially on objective measures of fruit quality, 2) to document any effects of these bioregulators on human perceptions of sensory qualities of fresh apples or apple juice, and 3) to determine whether any bioregulator-induced improvements in sensory perception could be obtained while minimizing negative effects on storability or the incidence of fruit disorders.

MATERIALS AND METHODS

This study was conducted over three crop seasons using mature 'Delicious' (Scarletspur strain) apple trees on MM.111 rootstock. Trees were located in a commercial orchard in East Wenatchee, WA and were planted in 1979 at a spacing of 3.3 x 6.6 m.

Trial 1 (2000)
Applications of AVG were made to single-tree replicates in 5 randomized complete blocks using a hydraulic hand-gun sprayer (sprayed to run-off). Trees were sprayed at four weeks before first (commercial) harvest (4 WBH) with 125 mg active ingredient/liter (mg•L^{-1}) AVG supplemented with 0.1% v/v SilWet® surfactant (Kalo, Overland Park, KA). Control trees were not sprayed with either bioregulator or surfactant.

Trial 2 (2000)
Applications of ETH were made to single-tree replicates in 6 randomized complete blocks using a hydraulic hand-gun sprayer (sprayed to run-off). Trees were sprayed at either one or two weeks before first (commercial) harvest (1WBH; 2WBH) with either 150 or 300 mg•L^{-1} ETH. All sprays were supplemented with 0.1% v/v Regulaid® surfactant (Kalo, Overland Park, KA). Control trees were not sprayed with either bioregulator or surfactant.

Trial 3 (2001)
Five randomized complete blocks containing 6 double-tree replicates each were selected from another part of the orchard that had not been previously treated with either AVG or ETH. Five double-tree replicates per block (10 trees per block) were sprayed 4 WBH with 125 mg•L^{-1} AVG plus 0.1% v/v Sylgard 309® organosilicone surfactant (Wilbur-Ellis, Fresno, CA) to run-off using a hydraulic hand-gun sprayer. The sixth double-tree replicate in each block served as an unsprayed control. Four double-tree replicates previously treated with AVG were individually sprayed to run-off using a hydraulic hand-gun sprayer with one of the following combinations of ETH and timing: ETH 150 mg•L^{-1} at either 2 WBH or 1 WBH or ETH 300 mg•L^{-1} at either 2 WBH or 1 WBH. All ETH sprays were supplemented with 0.1% v/v Regulaid® surfactant. Control trees were not sprayed with either bioregulator or surfactant.

Trial 4 (2002)
Seven randomized complete blocks containing six 4-tree replicates each were selected from a part of the orchard that had not been previously treated with either AVG or ETH. Three 4-tree replicates per block (12 trees per block) were sprayed 4 WBH with 125 mg•L^{-1} AVG plus 0.1% v/v Sylgard 309® organosilicone surfactant to run-off using a hydraulic hand-gun sprayer. One 4-tree replicate in each block served as an unsprayed control. The other two 4-tree replicates previously treated with AVG were individually sprayed with either ETH 150 mg•L^{-1} at 2 WBH or with ETH 75 mg•L^{-1} at 2 WBH and again at 1 WBH to run-off. The remaining two untreated 4-tree replicates were treated with ETH at either 75 or 150 mg•L^{-1} as described above. All ETH

sprays were supplemented with 0.1% v/v Regulaid® surfactant. Control trees were not sprayed with either bioregulator or surfactant.

Apples were harvested on two dates each year of the study corresponding to first commercial harvest and commercial harvest + 2 weeks (**Trial 1:** 20 Sept. and 4 Oct., 2000; **Trial 2:** 18 Sept and 2 Oct, 2000; **Trial 3:** 10 Sept. and 24 Sept., 2001; **Trial 4:** 23 Sept and 7 Oct, 2002). On each harvest date, 90 apples were harvested at random from each replicate.   Several objective fruit quality parameters, including mean sample fruit weight, flesh firmness, flesh starch content, peel and flesh color, soluble solids concentration (SSC) and titratable acidity (TA) were determined immediately on 10 fruit and five fruit were used to determine internal ethylene concentration (IEC) from each replicate. Forty fruit (20 each storage period) were placed in either regular atmosphere (RA) for 60 days, or controlled atmosphere storage (CA), 1.0% O_2 and 1.5% CO_2 at1 °C, for 120, 150, or 180 days. After each storage period, fruit quality parameters were determined immediately on 10 fruit. Post-storage quality factors evaluated were firmness, peel and flesh color, SSC, TA, IEC and visible disorders (scald, watercore, internal breakdown).

At each harvest, twenty apples selected at random from each treatment and replication were held in refrigerated storage (<1 month) prior to sensory analysis of expressed juice. Twenty additional apples from each treatment and replication were packed and sent (3 days non-refrigerated transit) to North Carolina State University for sensory analysis of the whole fruit. Upon receipt, apples were held in refrigerated storage until use (<7 days).

Starch index was determined visually using a rating scale of 1 to 6 (Washington Apple Maturity Handbook, 1993). Firmness was determined at two locations per fruit with the Ta-XT2 Texture Analyzer (Texture Technologies, Scarsdale, NY) equipped with a 11.1 mm probe. SSC and TA were determined from an aliquot of expressed juice from a cross-sectional slice from each of the same 10 fruit.   SSC was determined with a model 10450 refractometer (American Optical, Buffalo, NY) equipped with a sucrose scale and calibrated with distilled H_2O at 20°C TA was measured with a Radiometer titrator, model TTT85 (Radiometer, Copenhagen, Denmark). Acids were titrated to pH 8.2 with 0.1 N NaOH and expressed as percent malic acid. Peel and flesh color was determined with the ColorFlex model 45/0 (Hunter Labs, Reston, VA) using the Hunter L*, a*, b* system and calculated hue angle (h°) values (Hunter and Harold, 1987). IEC was determined on 5 fruit for each replication, treatment, and storage period using a Hewlett Packard (Avondale, PA) model 5880A GC/FID equipped with a Porapak Q column. Fruit disorders were visually assessed by laboratory personnel and expressed as the percentage of each fruit sample showing the disorder. All data were analyzed using the General Linear Models (GLM) procedure of the Statistical Analysis System (SAS) program package (SAS Institute, Cary, NC). Where appropriate, means were separated by Tukey's test ($P \leq 0.05$).

Juice preparation: For each treatment and replication, 10 randomly selected apples were washed, surface dried, sliced and juiced using a Omega Juicer, model 4000 (Omega Products, Inc., Harrisburg, PA) at ambient temperature. Juice was centrifuged in an IEC Centra-7 (International Eq., Co., Needham Heights, MA) at 523 rad/s and then filtered through an odor-free coffee filter to remove all insoluble solids.   The juice from each group within a treatment was combined and stored at 5.6 °C for immediate flavor panel evaluation.

Sensory analysis, Juice: Sensory testing was conducted on apple juice samples in a laboratory environment by a panel of 25 judges that had previously been trained in apple juice flavor

attributes (Tree Top, Inc., Selah, WA). Each panelist received two samples that were color coded to indicate a control and a treatment sample. Panelists were not aware of the relationship of color coding to treatment and were instructed prior to the evaluation on the proper method for filling out the ballot describing their choices. Each evaluation session was limited to two groups of two samples (four juice samples in total).

The ballot consisted of three parts. Each panelist was asked to make a preference choice between the two samples after tasting the samples in the prescribed order. In addition, each panelist was asked to rate the intensity of the apple flavor for each sample using a nine-point scale where "one" indicated that the apple flavor was "too weak", "five" indicated that the apple flavor was "just right", and "nine" indicated that the flavor was "too strong". Finally, each panelist was asked to rate their overall "degree of liking" of each juice sample using a nine-point scale where "one" represented "dislike extremely", "five" represented "neither like nor dislike", and "nine" represented "like extremely".

Sensory analysis of whole apples: Consumers (N ≥ 100) were recruited at the North Carolina State University (NCSU) Sensory Service Center, Raleigh NC before each taste test. Consumers evaluated freshly cut and cored apple slices for overall liking, flavor and texture acceptability using a nine-point hedonic scale anchored on the left with "dislike extremely" and on the right with "like extremely" (Meilgaard et al., 1999). Apples were presented in a randomized, balanced order of presentation on paper plates with a random three-digit code designating each treatment on each plate. Prior to testing, each consumer panelist filled out an informed consent form for NCSU Human Subjects Approval and a brief questionnaire on apple consumption habits.

Consumer taste-panel demographics were similar for the various harvests each year. Consumers were fairly evenly split between genders (58% female, 42% male). Fifty-five percent were between 19 and 29 years old; 45% were between 30 and 55 years old. All consumer participants indicated a liking for apples, but frequency of apple consumption varied widely. Forty percent consumed apples seasonally or a least once per month, while 35% consumed apples a few times per week. Consumers were also asked to list the apple cultivars they consumed most often. 'Delicious' (62%), 'Fuji' (47%), 'Granny Smith' (58%), 'Golden Delicious' (51%), 'Gala' (42%) and 'McIntosh' (23%) were the most commonly cited cultivars. Eighty-five percent of consumer panelists indicated that flavor was a critical factor in apple choice and purchase; 72% indicated texture and 51% indicated that color was a critical factor in apple choice and purchase.

RESULTS AND DISCUSSION

AVG retarded flesh firmness and starch loss independent of harvest date but did not affect SSC, TA or SSC/TA ratio (Table 1). Delaying harvest by two weeks resulted in reduced flesh firmness and TA and increased starch hydrolysis. ETH treatments had no influence on fruit firmness but increased SSC/TA ratio (300 $mgXL^{-1}$ treatments only) and starch hydrolysis while having no effect on TA levels. A 2-week harvest delay again resulted in reduced firmness and increased starch hydrolysis but had no effect on SSC, TA or SSC/TA ratio. Fruit weight was not affected by bioregulator treatments but was greater on the second harvest date. The effects of delaying harvest produced similar responses in fruit quality parameters in both Trials 1 and 2.

AVG treatment resulted in reduced peel red color development that would not be visible to the average consumer due to the significant but small corresponding change in hue value (Table 2).

Table 1. Effects of aminoethoxyvinylglycine (AVG) or ethephon (ETH) applied preharvest on objective quality attributes of 'Delicious' (Scarletspur strain) apples at harvest (Trials 1 and 2, 2000).

Parameter	Firmness (N)	SSC (%)	TA (% malic)	SSC to TA ratio	Starch (1 to 6)
Bioregulator					
Control	72.2 b[z]	11.2 a	0.26 a	44.2 a	1.8 a
AVG 125 mg·L⁻¹ (4 WBH)[y]	76.5 a	11.2 a	0.25 a	45.6 a	1.7 b
Harvest					
20 Sept.	75.7 a	11.2 a	0.26 a	43.9 a	1.5 b
4 Oct.	73.0 b	11.2 a	0.25 a	45.9 a	2.0 a
Bioregulator × harvest	NS	NS	NS	NS	NS
Bioregulator					
Control	70.4 a	11.5 b	0.24 a	48.9 b	2.6 c
ETH 150 mg·L⁻¹ (2 WBH)[x]	68.9 a	12.3 a	0.24 a	52.0 ab	3.3 ab
ETH 300 mg·L⁻¹ (2 WBH)[x]	68.2 a	12.6 a	0.24 a	53.7 a	3.4 a
ETH 150 mg·L⁻¹ (1 WBH)[w]	69. 8 a	12.2 a	0.24 a	51.9 ab	3.1 b
ETH 300 mg·L⁻¹ (1 WBH)[w]	68.3 a	12.3 a	0.23 a	52.6 a	3.2 ab
Harvest					
18 Sept.	71.5 a	12.1 a	0.24 a	51.3 a	2.5 b
2 Oct.	66.7 b	12.2 a	0.23 a	52.3 a	3.8 a
Bioregulator × harvest	NS	NS	NS	NS	NS

[z]Means in a column within bioregulator or harvest not followed by a common letter are significantly different ($P \leq 0.05$).
[y]AVG applied 4 weeks before first harvest.
[x]ETH applied 2 weeks before first harvest.
[w]ETH applied 1 week before first harvest.
[NS]Nonsignificant.

Table 2. Effects of aminoethoxyvinylglycine (AVG) or ethephon (ETH) applied preharvest on objective color attributes of 'Delicious' (Scarletspur strain) apples at harvest and firmness values after regular atmosphere (RA) and controlled atmosphere storage (Trials 1 and 2, 2000).

Parameter	Peel color		Flesh color		Firmness (N)	
	L*	hue E	L*	hue E	RA (60 d)	CA (150 d)
Bioregulator (Trial 1)						
Control	27.6 a[z]	13.7 a	71.5 a	95.2 b	58.3 b	65.1 b
AVG 125 mg·L⁻¹ (4 WBH)[y]	27.3 a	13.1 b	71.3 a	97.3 a	65.1 a	69.1 a
Harvest						
20 Sept.	27.9 a	13.6 a	68.9 a	96.7 a	65.1 a	69.8 a
4 Oct.	27.0 b	13.2 a	72.1 a	96.0 a	58.2 b	64.3 b
Bioregulator × harvest	NS	NS	NS	NS	NS	NS
Bioregulator (Trial 2)						
Control	27.5 a	13.4 a	73.0 a	97.3 a	61.1 a	64.6 a
ETH 150 mg·L⁻¹ (2 WBH)[x]	26.8 a	13.5 a	71.5 b	94.8 b	57.3 bc	60.8 b
ETH 300 mg·L⁻¹ (2 WBH)[x]	27.0 a	13.8 a	71.3 b	94.5 b	54.7 c	55.3 c
ETH 150 mg·L⁻¹ (1 WBH)[w]	27.3 a	13.6 a	72.7 a	96.4 a	59.1 ab	60.4 b
ETH 300 mg·L⁻¹ (1 WBH)[w]	27.0 a	13.7 a	71.7 b	94.8 b	56.5 bc	56.3 c
Harvest						
18 Sept.	27.7 a	14.1 a	73.8 a	97.6 a	59.6 a	60.5 a
2 Oct.	26.6 b	13.1 b	70.3 b	93.5 b	56.1 b	58.5 b
Bioregulator × harvest	NS	NS	NS	NS	NS	NS

[z]Means in a column within bioregulator or harvest not followed by a common letter are significantly different ($P \leq 0.05$).
[y]AVG applied 4 weeks before first harvest.
[x]ETH applied 2 weeks before first harvest.
[w]ETH applied 1 week before first harvest.
[NS]Nonsignificant.

A change in hue value of one or more unit is required to visually identify color differences (Hunter and Harold, 1987). AVG treatment reduced color development in other less highly colored apple cultivars (Greene, 2002; Wang and Dilley, 2001). Delaying harvest by 2 weeks in this study resulted in small changes in "L" color values but no observable change in peel hue value. In contrast, AVG treatment produced apple flesh color that was more green (higher hue value) than non-treated apples. Greener flesh color could be related to slower maturity, indicated by the reduction in starch hydrolysis (Table 1). ETH did not influence the peel color of apples regardless of the amount of ETH applied or time of application. This result did not agree with the increased red color observations of Larrigaudiere et al. (1996) and Stover et al. (2003), but

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those studies were not conducted with the Scarletspur strain of 'Delicious', which is characterized by intense, early red color development in the peel. However, ETH produced a darker flesh color (lower "L" values), with less green and more yellow. The lower hue values associated with ETH application indicated a flesh color typical of more mature fruit.   These changes in flesh color were very evident regardless of the amount of ETH applied or time of application. The higher ETH concentration tended to have more influence on flesh color than the lower (150 mg•L^{-1}), particularly when applied at 1 WBH, which allowed less time for the ETH effect to be developed.

After both RA (60 days) and CA (150 days) storage, AVG-treated fruit were firmer than non-treated apples, regardless of harvest date (Table 2). After both 60 days RA and 150 days CA storage, all fruit met or exceeded the minimum Washington State grade standard for firmness of 54 N (Washington Agriculture Code, 1990). However, AVG-treated fruit were substantially firmer than untreated fruit, indicating that AVG treatment resulted in better tissue texture conservation under RA and CA storage conditions.

After 60 days of RA storage, all fruit, regardless of concentration, time of application, or harvest date, met the minimum Washington State grade standard for firmness, although the ETH 300 (2 WBH) treatment just barely met the standard (Table 2). After 150 days of CA storage, control fruit were firmer than any ETH treatment, and again all treatments met the minimum firmness standard, with the high ETH concentration fruit just exceeding that value. Fruit from the later harvest date were less firm after both 60 and 150 days of storage, but all fruit met the minimum firmness standard.

Applications of ETH alone to apples at 150 or 300 mg•L^{-1} at 1 or 2 WBH increased the preference, intensity and liking sensory scores of apple juice on both harvest dates over juice from untreated fruit (Table 3, Trial 2, 2000). Apple juice flavor intensity scores for the ETH treatments groups were closer to the 5.0 score of "just right" while the control group flavor intensity score was more toward "weak." At the second harvest the improvement in quality perception from preharvest ETH applications when compared to controls was not as conspicuous. Apple juice flavor intensity from the ETH 2 WBH group was near the 5.0 scale of "just right" while the juice from the ETH 1 WBH group and the control group were toward the "weak" intensity score. Liking scores followed a similar pattern in which the 2 WBH group apple juice exhibited higher scores than the 1 WBH group. Because of better flavor intensity and liking scores, apple juice from all the second harvest treatment groups except the 300 mg•L^{-1} ETH (1 WBH) were preferred over the juice from the control group. At the second harvest (II), control fruit had matured enough that only apples treated with 300 mg•L^{-1} ETH at 2 WBH produced juice with preference, intensity and liking scores superior to non-treated fruit.

Apple juice samples from apples treated with AVG alone were similar in preference, intensity and liking to juice from control fruit at the first harvest (Table 3, Trial 3, 2001). Juice from apples treated with ETH in addition to AVG was preferred to juice made from control fruit, except when 300 mg•L^{-1} ETH was applied 1 WBH; in this instance there was no difference in preference. Apple juice flavor intensity scores were similar among bioregulator treatments and control fruit. Apple juice from apples treated with a combination of AVG and ETH (150 or 300 mg•L^{-1}, 2 WBH) was scored higher for liking when compared to juice from control fruit. All other treatments received similar scores to control fruit juice for liking.

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Table 3. Effects of aminoethoxyvinylglycine (AVG) and ethephon (ETH) applied alone or sequentially, preharvest on sensory evaluation of 'Delicious' (Scarletspur strain) apple juice from fruit at harvest (Trial 2, 2000; Trial 3, 2001; Trial 4, 2002).

Treatment/year	N	Harvest I			Harvest II		
		Preference[z]	Intensity	Liking	Preference	Intensity	Liking
Trial 2, 2000							
Control	100		3.9 b[y]	6.0 b		4.4 b	7.3 ab
ETH 150 mg·L⁻¹ (2 WBH)[w]	25	T	5.2 a	7.3 a	T	5.2 a	7.8 a
ETH 300 mg·L⁻¹ (2 WBH)[w]	25	T	5.3 a	7.4 a	T	5.0 ab	8.0 a
ETH 150 mg·L⁻¹ (1 WBH)[v]	25	T	5.2 a	7.5 a	T	4.6 ab	6.8 b
ETH 300 mg·L⁻¹ (1 WBH)[v]	25	T	5.1 a	7.4 a	N	4.6 ab	6.9 b
Trial 3, 2001							
Control	125		4.5 a	6.3 b		4.8 abc	6.9 a
AVG 125 mg·L⁻¹ (4 WBH)[x]	25	N	4.7 a	6.5 b	N	4.5 bc	6.6 a
AVG + ETH 150 mg·L⁻¹ (2 WBH)[w]	25	T	4.9 a	7.1 ab	T	5.2 a	7.4 a
AVG + ETH 300 mg·L⁻¹ (2 WBH)[w]	25	T	5.1 a	7.4 a	T	5.2 a	7.4 a
AVG + ETH 150 mg·L⁻¹ (1 WBH)[v]	25	T	4.8 a	7.4 a	T	5.3 a	7.3 a
AVG + ETH 300 mg·L⁻¹ (1 WBH)[v]	25	N	4.9 a	7.0 b	N	4.4 c	6.5 a
Trial 4, 2002							
Control	250		4.0 b	6.4 a		4.8 ab	7.3 a
AVG 125 mg·L⁻¹ (4 WBH)[x]	50	T	4.6 a	6.9 a	N	5.0 a	7.3 a
ETH 75 mg·L⁻¹ (2 and 1 WBH)[u]	50	T	4.3 ab	6.8 a	N	4.7 ab	7.5 a
ETH 150 mg·L⁻¹ (2 WBH)[u]	50	N	3.9 b	6.4 a	C	3.9 d	6.3 b
AVG + ETH 75 mg·L⁻¹ (2 and 1 WBH)[u]	50	T	4.4 a	6.9 a	C	4.4 bc	6.9 ab
AVG +ETH 150 mg·L⁻¹ (2 WBH)[u]	50	T	4.5 a	6.8 a	C	4.4 cd	7.2 a

[z]Significantly preferred ($P \leq 0.05$): C = control, T = treatment, N = neither control or treatment preferred.
[y]Means in a column not followed by a common letter are significantly different ($P \leq 0.05$).
[x]AVG applied 4 weeks before first harvest.
[w]ETH applied 2 weeks before first harvest either with or without AVG applied 4 weeks before first harvest.
[v]ETH applied 1 week before first harvest either with or without AVG applied 4 weeks before first harvest.
[u]ETH applied at either 2 weeks or 2 weeks and 1 week before first harvest either with or without AVG applied 4 weeks before first harvest.

At the second harvest, juice from control fruit was preferred to juice made from apples treated with AVG alone (Table 3, Trial 3, 2001). When ETH was applied in conjunction with AVG, preference scores for juice were superior to juice from control fruit, except again in the instance where ETH was applied at 300 mg•L⁻¹ 1 WBH. By the second harvest, control fruit had matured enough that juice scores for apple flavor intensity were similar to all AVG+ETH treatments, although AVG alone had a lower intensity score than three of four AVG+ETH treatments. When AVG and ETH were used sequentially, juice liking scores from some of the treatments were preferred at the first harvest, but no differences in juice "liking" were detected at the second harvest.

In the final year of the study, apple juice from fruit treated with AVG was preferred when compared to juice from control fruit, but only at the first harvest (Table 3, Trial 4, 2002). At the second harvest no preference was indicated between juice from AVG treated or control apples. At the first harvest, juice from treated ETH fruit was preferred, except when ETH was applied at 150 mg•L⁻¹ (2 WBH). In this instance apple intensity was also reduced and no preference between juice from control fruit or juice from treated fruit was indicated. At all other rates and application times, apple flavor intensity was enhanced by the use of ETH. Panel scores indicated no difference in liking for juice from apples at the first harvest, regardless of the use of AVG or ETH. At the second harvest, no preference was evident between juice from treated (AVG alone or AVG + 75 mg•L⁻¹ 2+1 WBH) or control fruit. However, juice from control fruit was preferred over juice from apples treated with AVG + 150 mg•L⁻¹ ETH 2 WBH or 75 mg•L⁻¹ ETH 2+1 WBH and 150 mg•L⁻¹ ETH 1 WBH. Apple juice intensity scores were also reduced for juice from these treatments, particularly for juice from apples treated with AVG+150 mg•L⁻¹ ETH 2 WBH. Panelists indicated the juice to be "weak" in flavor intensity. At the second harvest, liking scores were similar between treatments except for the juice score from apples treated with AVG + 150 mg•L⁻¹ ETH 2WBH, which was reduced when compared to juice from all other treatments.

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In 2001, there was little difference in fruit firmness at harvest among treatments (Table 4). Application of ETH following AVG did not reduce firmness at harvest but did increase SSC and SSC/TA ratio. Bioregulator treatments did not affect TA at harvest. Delaying harvest by two weeks resulted in decreased flesh firmness and TA and increased SSC and SSC/TA ratio. Apples treated with AVG alone displayed a reduced overall sensory acceptance score when compared to control fruit or fruit treated with a combination of AVG and ETH (Table 4). Only apples with AVG and 300 mg•L^{-1} ETH applied 2 WBH were considered superior in overall acceptance to control fruit, while apples treated with other combinations of AVG and ETH were rated as similar to control fruit. Sensory flavor scores for apples treated with AVG alone were similar to flavor scores of control apples, but lower than flavor scores for fruit treated with a combination of AVG and ETH. The sensory flavor score for apples treated with AVG plus 300 mg•L^{-1} ETH 2 WBH was superior to control apples. As expected, fruit texture scores were similar between control apples and treated apples either with AVG alone or in combination with ETH. Previous research has indicated that AVG-treated apples maintain firmness better than non-treated fruit (Beaudry and Jayanty, 2003; Wang and Dilley, 2001). In the present study the whole-apple sensory panel findings reflected the virtual absence of differences in the objective firmness data recorded for these fruit. Firmness loss in apples due to ETH application has been reported in other trials (Drake et al., 2002). In this case AVG followed by ETH did not result in significant sensory texture deterioration at harvest. Delay of harvest permitted additional fruit maturation and resulted in an increase in overall sensory acceptance, flavor and texture scores.

Table 4. Effects of aminoethoxyvinylglycine (AVG) applied alone or sequentially with ethephon (ETH) on consumer sensory evaluation of 'Delicious' (Scarletspur strain) apples at harvest (Trial 3, 2001).

	Objective quality				Sensory evaluation (whole apples)[z]		
	Firmness (N)	SSC (%)	TA (% malic)	SSC to TA ratio	Overall	Flavor	Texture
Bioregulator							
Control	74.2 b[y]	11.3 b	0.28 a	41.9 c	6.4 b	6.2 bc	6.7 ab
AVG 125 mg·L^{-1} (4 WBH)[x]	73.7 b	11.3 b	0.28 a	42.1 c	6.1 c	5.8 c	6.5 b
AVG + ETH 150 mg·L^{-1} (2 WBH)[w]	76.5 ab	11.7 a	0.28 a	43.4 bc	6.6 ab	6.5 ab	6.9 ab
AVG + ETH 300 mg·L^{-1} (2 WBH)[w]	74.4 b	12.0 a	0.27 a	45.5 ab	6.7 a	6.7 a	7.0 a
AVG + ETH 150 mg·L^{-1} (1 WBH)[v]	77.0 ab	11.7 a	0.27 a	44.4 ab	6.5 ab	6.4 b	7.0a
AVG + ETH 300 mg·L^{-1} (1 WBH)[v]	78.2 a	11.8 a	0.26 b	46.4 a	6.5 ab	6.5 ab	6.9 ab
Harvest							
I	80.0 a	10.5 b	0.31 a	34.3 b	6.3 b	6.2 b	6.7 b
II	71.3 b	12.8 a	0.24 b	53.6 a	6.6 a	6.5 a	7.0 a
Bioregulator × harvest	NS	NS	NS	NS	NS	NS	NS

[z]N = 240.
[y]Means in a column within bioregulator or harvest not followed by a common letter are significantly different ($P \leq 0.05$).
[x]AVG applied 4 weeks before first harvest.
[w]AVG applied at 125 mg·L^{-1} 4 weeks before first harvest followed by ETH at 150 or 300 mg·L^{-1} at 2 weeks before first harvest.
[v]AVG applied at 125 mg·L^{-1} 4 weeks before first harvest followed by ETH at 150 or 300 mg·L^{-1} at 1 week before first harvest.
[NS]Nonsignificant.

In 2002, the double application of ETH (75 mg•L^{-1}) resulted in a reduction in flesh firmness at harvest; all other treatments had no effect on flesh firmness (Table 5). AVG alone reduced SSC at harvest while ETH increased SSC. ETH reversed the effect of previous AVG on SSC and SSC/TA ratio at harvest; SSC and SSC/TA ratio values were as high as or higher than the control. TA at harvest was unaffected by bioregulator treatments. Delaying harvest by 2 weeks resulted in reduced flesh firmness and higher SSC and SSC/TA ratio values, while having no detectable effect on TA.

Table 5. Effects of aminoethoxyvinylglycine (AVG) and ethephon (ETH) applied alone or sequentially on objective and consumer sensory evaluation of 'Delicious' (Scarletspur strain) apples at harvest (Trial 4, 2002).

	Objective quality				Sensory evaluation (whole apples)[z]		
	Firmness (N)	SSC (%)	TA (% malic)	SSC to TA ratio	Overall	Flavor	Texture
Bioregulator							
Control	79.8 a[y]	11.8 b	0.25 a	48.4 bc	6.2 bc	6.2 bc	6.5 bc
AVG 125 mg·L⁻¹ (4 WBH)[x]	80.2 a	11.4 c	0.24 a	47.0 c	6.0 c	5.9 c	6.4 bc
ETH 75 mg·L⁻¹ (2 and 1 WBH)[w]	77.3 b	12.1 a	0.24 a	51.3 ab	6.3 abc	6.3 ab	6.3 c
ETH 150 mg·L⁻¹ (2 WBH)[w]	78.4 ab	12.2 a	0.24 a	50.5 ab	6.5 a	6.5 a	6.5 bc
AVG + ETH 75 mg·L⁻¹ (2 and 1WBH)[v]	80.1 a	12.4 a	0.23 a	52.9 a	6.4 ab	6.3 ab	6.7 ab
AVG + ETH 150 mg·L⁻¹ (2 WBH)[v]	78.7 ab	12.0 ab	0.24 a	50.2 ab	6.5 a	6.4 ab	6.8 a
Harvest							
I	81.0 a	11.6 b	0.24 a	49.0 b	6.2 b	6.1 b	6.5 a
II	77.2 b	12.4 a	0.24 a	51.1 a	6.5 a	6.5 a	6.6 a
Bioregulator × harvest	NS	NS	NS	NS	NS	NS	NS

[z]N = 210.
[y]Means in a column within bioregulator or harvest not followed by a common letter are significantly different ($P \leq 0.05$).
[x]AVG applied 4 weeks before first harvest.
[w]ETH applied at 75 mg·L⁻¹ 2 weeks and 1 week or 150 mg·L⁻¹ at 2 weeks before first harvest.
[v]AVG applied at 125 mg·L⁻¹ 4 weeks before first harvest followed by ETH at 75 mg·L⁻¹ 2 weeks and 1 week or 150 mg·L⁻¹ at 2 weeks before first harvest.
[NS]Nonsignificant.

In a second year of consumer panel evaluation of whole apples, fruit treated with AVG were similar to control apples in overall acceptance, flavor and texture scores. Overall sensory acceptance and flavor were only enhanced with the use of AVG + the highest ETH rate used (150 mg•L⁻¹ ETH 2 WBH). In most instances, including ETH after AVG enhanced overall sensory acceptance and flavor when compared to apples treated with AVG alone. Delay of harvest again resulted in increased overall sensory acceptance and flavor scores, but did not change sensory texture scores. In the demographics survey prior to sensory analysis of whole apples, 85% of consumers indicated that flavor was a critical factor in apple choice and purchase; 72% indicated texture and 5 % indicated that color was a critical factor in apple choice and purchase. This emphasis on apple flavor by consumers has also been reported in another study (Washington Apple Commission, 2002).

During the second year of this study, AVG alone or a combination of AVG and ETH along with harvest time had a strong influence on starch hydrolysis and firmness after RA and CA storage. At the first harvest, starch content was unaltered by AVG alone. Following AVG with ETH had no effect on starch hydrolysis by the first harvest date except for a slight increase when ETH was applied at the higher concentration the longest interval before harvest (300 mg•L⁻¹, 2 WBH). By the second harvest, fruit treated with AVG displayed less starch hydrolysis than control fruit. Adding ETH as a post-AVG treatment stimulated starch hydrolysis so that starch levels were similar to control fruit.

After 60 days of RA storage, fruit treated with AVG alone were firmer than fruit treated with AVG + ETH regardless of the amount of ETH or time of application; especially at the first harvest (Table 6). By the second harvest, AVG treated fruit were still firmer than fruit treated with AVG + 300 mg•L⁻¹ ETH, regardless of time of application, but equivalent in firmness to fruit treated with AVG plus ETH at the lower concentration. In all cases, treatment with AVG resulted in firmer fruit than controls, with or without ETH. In CA storage (120 days), fruit treated with AVG alone were only firmer than fruit treated with AVG + 300 mg•L⁻¹ ETH at (2 WBH). Again, nearly all AVG treatments maintained flesh firmness better than control fruit. Regardless of treatment, firmness of all fruit (60 RA, 120 CA) met or exceeded Washington

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State grade standards. After 180 days of CA storage, AVG alone and AVG + ETH treated apples were similar in firmness (66 to 68 N) and firmer (59.8 N) than control apples when harvested early. If harvest was delayed 2 weeks, only AVG and AVG + ETH at 150 mg•L^{-1} met or exceeded Washington State grade standards (53.4 N) for firmness after 180 days of CA storage. Late-harvested control fruit and any fruit treated with AVG + ETH at 300 mg•L^{-1} failed to meet grade standards.

Table 6. Effects of aminoethoxyvinylglycine (AVG) applied alone or sequentially with ethephon (ETH) and harvest date on starch level at harvest and flesh firmness of 'Delicious' (Scarletspur strain) apples after regular (RA) or controlled atmosphere (CA) storage (Trial 3, 2001).

Bioregulator	Harvest	Starch (1–6) at Harvest	Firmness (N) RA (60 d)	CA (120 d)	CA (180 d)
Control	I	1.5 d^z	63.2 d	64.1 cd	59.8 b
	II	2.7 a	54.5 h	53.9 g	48.4 de
AVG 125 mg·L^{-1} (4 WBH)^y	I	1.4 d	68.5 a	69.0 a	68.1 a
	II	2.4b	59.6 e	61.8 de	57.5 bc
AVG + ETH 150 mg·L^{-1} (2 WBH)^x	I	1.6 cd	66.4 c	67.0 ab	66.8 a
	II	2.6 ab	58.7 ef	59.6 ef	53.3 cd
AVG + ETH 300 mg·L^{-1} (2 WBH)^x	I	1.8 c	66.6c	66.1bc	65.8a
	II	2.6 ab	56.8 g	58.3 f	47.5 e
AVG + ETH 150 mg·L^{-1} (1 WBH)^w	I	1.5 d	68.1ab	67.2 ab	67.3 a
	II	2.6 ab	59.4 ef	59.8ef	54.8 bc
AVG + ETH 300 mg·L^{-1} (1 WBH)^w	I	1.6 cd	67.0 bc	67.5 ab	66.6 a
	II	2.8 a	58.0 fg	58.0 f	52.2 cde
Bioregulator × harvest	*	*	*	*	*

^{z}Means in a column not followed by a common letter are significantly different ($P \leq 0.05$).
^{y}AVG applied 4 weeks before first harvest.
^{x}AVG applied at 125 mg·L^{-1} 4 weeks before first harvest followed by ETH at 150 or 300 mg·L^{-1} 2 weeks before first harvest.
^{w}AVG applied at 125 mg·L^{-1} 4 weeks before first harvest followed by ETH at 150 or 300 mg·L^{-1} 1 week before first harvest.
*Significant at $P \leq 0.05$.

AVG application did not influence the peel color of 'Delicious' (Scarletspur strain) apples after 60 days of RA storage or 120 days of CA storage, regardless of harvest time (Table 7). After 180 days of CA storage, AVG treated fruit displayed redder (lower hue) peel color than control fruit from both harvest, but only at this storage duration. The use of AVG + ETH (150 or 300 mg•L^{-1}) produced darker (lower "L" values), redder (lower hue value) apples than controls at all storage times, but only when apples were harvested early. By the second harvest, no bioregulator treatment affected peel color after 180 days CA storage.

Table 7. Effects of aminoethoxyvinylglycine (AVG) applied alone or sequentially with ethephon (ETH) and harvest date on Hunter peel color parameters of 'Delicious' (Scarletspur strain) apples at harvest and after regular (RA) or controlled atmosphere (CA) storage (Trial 3, 2001).

Bioregulator	Harvest	RA (60 d) L*	hue (E)	CA (120 d) L*	hue (E)	CA (180 d) L*	hue (E)
Control	I	32.4 a	18.9 a	31.8 a	18.7 a	31.6 a	20.2 a
	II	26.7 de	15.6 cd	26.4 de	15.9 cde	25.7 c	16.1 cdef
AVG 125 mg·L^{-1} (4 WBH)^y	I	30.9 b	18.3 ab	31.0 ab	18.5 ab	30.7 ab	18.2 b
	II	27.1 d	15.2 d	27.1 d	15.1 e	26.4 c	15.6 efg
AVG + ETH 150 mg·L^{-1} (2 WBH)^x	I	29.3 c	16.9 bc	29.8 c	17.0 bcd	29.9 b	17.5 bcde
	II	25.8 e	14.6 d	26.2 de	15.3 e	25.1 c	15.5 fg
AVG + ETH 300 mg·L^{-1} (2 WBH)^x	I	30.0 bc	17.1 b	29.6 c	17.3 abc	30.1 b	17.7 bc
	II	26.2 de	15.6 cd	25.8 e	15.7 de	22.2 d	13.8 g
AVG + ETH 150 mg·L^{-1} (1 WBH)^w	I	29.9 bc	17.1 b	30.1 bc	17.8 ab	30.2 b	17.6 bcd
	II	26.2 de	15.0 d	26.2 de	15.3 e	25.4 c	15.6 efg
AVG + ETH 300 mg·L^{-1} (1 WBH)^w	I	30.7 b	17.7 ab	30.1 bc	17.9 ab	30.6 ab	17.9 bc
	II	26.0 de	15.1 d	25.5 e	15.0 c	25.0 c	15.7 defg
Bioregulator × harvest		*	*	*	*	*	*

^{z}Means in a column not followed by a common letter are significantly different ($P \leq 0.05$).
^{y}AVG applied 4 weeks before first harvest.
^{x}AVG applied at 125 mg·L^{-1} 4 weeks before first harvest followed by ETH at 150 or 300 mg·L^{-1} 2 weeks before first harvest.
^{w}AVG applied at 125 mg·L^{-1} 4 weeks before first harvest followed by ETH at 150 or 300 mg·L^{-1} on week before first harvest.
*Significant at $P \leq 0.05$.

AVG treatment reduced IEC compared to control fruit at harvest and after all storage times, regardless of fruit harvest date (Table 8). Application of ETH following AVG treatment did not overcome the AVG-based control over fruit ethylene production, even after 180 days in CA. IEC was higher for fruit harvested on the second harvest date, but the relationship of fruit IEC to treatments remained the same. At both harvests, the amount of watercore present was similar regardless of the amount of ETH applied. After 60 days of RA storage, apples treated with AVG displayed significantly less watercore than apples treated with AVG + ETH, regardless of the amount of ETH applied. AVG + ETH treated apples displayed watercore at a similar level as control apples. These differences in watercore were also evident after 120 days of CA storage. After 180 days of CA storage, apples treated with AVG + ETH at 150 mg•L^{-1} (1 WBH) showed more watercore than all other treatments, which were the same.

Table 8. Effects of aminoethoxyvinylglycine (AVG) applied alone or sequentially with ethephon (ETH) on fruit internal ethylene concentration (IEC) and watercore incidence in 'Delicious' (Scarletspur strain) apples at harvest and after regular (RA) or controlled atmosphere (CA) storage (Trial 3, 2001).

	At harvest		RA (60 d)		CA (120 d)		CA (180 d)
	IEC (mg·L^{-1})	Watercore (%)	IEC (mg·L^{-1})	Watercore (%)	IEC (mg·L^{-1})	Watercore (%)	IEC (mg·L^{-1})
Bioregulator							
Control	2.9 a^z	8.0 a	39.0 a	12.0 ab	15.2 a	9.0 ab	20.2 a
AVG 125 mg·L^{-1} (4 WBH)^y	0.2 b	3.0 a	2.5 b	4.0 b	3.3 b	0.0 b	4.6 b
AVG + ETH 150 mg·L^{-1} (2 WBH)^x	0.3 b	13.0 a	5.0 b	28.0 a	2.7 b	25.0 a	4.4 b
AVG + ETH 300 mg·L^{-1} (2 WBH)^x	0.7 b	10.0 a	4.1 b	23.0 a	2.7 b	14.0 ab	2.5 b
AVG + ETH 150 mg·L^{-1} (1 WBH)^w	0.3 b	7.0 a	4.8 b	19.0 a	1.9 b	20.0 a	2.3 b
AVG + ETH 300 mg·L^{-1} (1 WBH)^w	0.3 b	12.5 a	5.7 b	18.5 a	3.9 b	18.0 a	3.6 b
Harvest							
I	0.2 b	0.0 b	5.6 b	0.0 b	2.4 b	0.0 b	5.1 b
II	1.4 a	8.3 a	14.7 a	17.5 a	7.5 a	14.3 a	7.5 a
Bioregulator × harvest	NS	NS	NS	NS	NS	NS	NS

^{z}Means in a column not followed by a common letter are significantly different ($P \leq 0.05$).
^{y}AVG applied 4 weeks before first harvest.
^{x}AVG applied at 125 mg·L^{-1} 4 weeks before first harvest followed by ETH at 150 or 300 mg·L^{-1} 2 weeks before first harvest.
^{w}AVG applied at 125 mg·L^{-1} 4 weeks before first harvest followed by ETH at 150 or 300 mg·L^{-1} 1 week before first harvest.
^{NS}Nonsignificant.

CONCLUSIONS

AVG and ETH are both tools used to manipulate fruit maturity and quality; AVG delays the changes associated with maturity and ripening while ETH accelerates those changes. In this study AVG delayed fruit maturity and flesh firmness loss, but reduced flavor. ETH reduced flesh firmness but aided flavor development. Because following AVG-treated fruit with ETH prior to harvest did not stimulate uncontrolled fruit ethylene evolution, combination treatments of AVG and ETH produced fruit with improved flavor and texture retention even after short or long-term storage. Using AVG and ETH overcame the disadvantages of either product when used alone. Fruit quality was best at harvest and after storage with a combination of both AVG and ETH.

Fruit continue to mature and ripen until harvested. In this study, quality attributes (objective and subjective) were superior for all treatments at harvest I (commercial) versus harvest II (2-week delay). Earlier-harvested fruit showed less water core, better firmness and better flavor, liking and preference scores. This harvest-date effect on fruit quality continued through both RA and CA storage. AVG treatment alone produced apples with optimum objectively measured characteristics but not the best flavor quality in all three years. ETH applied at 300 mg•L^{-1} reduced subjective perception quality scores, particularly for texture; this concentration was probably too high for this cultivar. The results of this study suggest that preharvest AVG

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followed by ETH at 2 WBH applied to fruit harvested at the commercial harvest date may be the best approach for optimizing all quality attributes, both objective and subjective, while maintaining long-term storability.

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