

## AMINOETHOXYVINYLGLYCINE AND FOLIAR ZINC TREATMENTS PLAY A KEY ROLE IN PRE-HARVEST DROPS AND FRUIT QUALITY ATTRIBUTES OF 'WILLIAM'S PRIDE' APPLE

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### ABSTRACT

Effects of pre-harvest aminoethoxyvinylglycine (AVG, 250 mg L<sup>-1</sup>) and zinc (0.3% ZnSO<sub>4</sub>) treatments on pre-harvest fruit drops, internal ethylene concentration (IEC), flesh firmness, color characteristics (L\*, chroma and hue angle), soluble solids content (SSC), acidity, total phenolics, antioxidant activity, flesh and leaf micronutrients and macronutrients of 'William's Pride' apples, were investigated in this study. AVG was sprayed 4 weeks before the anticipated harvest date and Zn was sprayed when the fruits reached the size of a walnut. As compared to the control, both the single AVG and zinc treatments significantly reduced the pre-harvest drops. However, combined AVG + Zinc treatments were found to be more effective in reducing the pre-harvest drops. At the last measurement date (15<sup>th</sup> of August), 60% fruit drop was measured in control, but 22% in AVG + Zinc treatment. AVG and AVG + Zinc treatments reduced ethylene synthesis. On the other hand, single zinc treatments stimulated ethylene synthesis. AVG treatments retarded red skin color development, but zinc treatments again stimulated red color development. As compared to the control and single zinc treatments, AVG-treated fruits had greater firmness and acidity values, but lower SSC values. While AVG treatments increased the total phenolics and antioxidant activity, zinc treatments decreased these attributes. As compared to the control, zinc treatments significantly increased flesh Cu, N, P and K contents and leaf Mn, N and P contents. Based on present findings, it was concluded that AVG could be used as an efficient tool to reduce the pre-harvest drops in apples.

**Key words:** color, DPPH, firmness, internal ethylene concentration, *Malus × domestica* Borkh., micronutrients, phenolics

### INTRODUCTION

Pre-harvest fruit drop is a common problem experienced by apple growers. Since the fruits drop before they reach the optimum size and color, is serious, economic losses are evident [Greene 2006]. Fruit

ripening and drops are closely related to internal hormone balance, especially the ethylene balance. Therefore, several researches have been conducted with different growth regulators to reduce or totally

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prevent the pre-harvest fruit drops [Byers 1997, Greene and Schupp 2004, Kang et al. 2007, Nawaz et al. 2008, Yildiz et al. 2012]. A synthetic hormone, NAA, has been used for this purpose for years. Increasing ethylene synthesis toward ripening is known to increase abscission [Yuan and Carbaugh 2007]. With this case in mind, researchers experimented with the effects of ethylene-inhibiting AVG treatments to prevent or reduce pre-harvest fruit drops and they mostly reported quite successful outcomes [Greene and Schupp 2004, Kang et al. 2007, Yildiz et al. 2012, Ozturk et al. 2015]. In several studies carried out with different apple cultivars, AVG was found to be more efficient than NAA both in controlling pre-harvest drops and in preservation of fruit flesh firmness [Greene et al. 1987, Yildiz et al. 2012].

Nutrient deficiency or competition for nutrients is also considered a significant factor for abscissions [Luckwill 1970, Boyton and Burrell 1994]. Despite insufficient number of studies carried out with apples, zinc-like micronutrients together with growth regulators were found to be quite efficient in various physiological process of several fruit species and also reported to play a significant role in delaying the fruit drops [Singh and Ram 1983, Babu et al. 1984, Khan et al. 1993]. Proper combinations of growth regulators and nutrients were also reported to be effective in controlling the pre-harvest fruit drops in citrus species [Rodriguez et al. 2005, Saleem et al. 2005]. Nutrient deficiency was also reported to destruct internal hormone balance controlling the abscission [Razi et al. 2011, Ashraf et al. 2012]. Zinc was reported to play a crucial role in auxin metabolism and Zn-deficiency was reported to reduce auxin synthesis significantly [Cakmak et al. 1989, Alloway 2004, Kramer and Clemens 2006].

Although there are several studies about the effects of AVG treatments on pre-harvest fruit drops in apples, there is limited number of studies available about the effects of zinc on such fruit drops. There is also limited information about the effects of AVG treatments on total phenolics and antioxidant activity of apples. Together with the comprehension of health impacts of antioxidant substances, consumers now are more interested in foodstuffs with high antioxidant activity. In this sense, researchers have recently

interested in and focused on the effects of different cultural practices on antioxidant capacity of the fruits [Ruiz-Garcia and Gomez-Plaza 2013]. Therefore, this study was designed to determine the effects of pre-harvest AVG and zinc treatments on pre-harvest fruit drops and some fruit quality attributes as well as on phenolics and antioxidant activity of 'William's Pride' apples.

## MATERIALS AND METHODS

### Experimental area

Experiments were carried out in an apple orchard located in Horticultural Research Center of Gaziosmanpaşa University (40°20'02.19"N latitude, 36°28'30.11"E longitude and 623 m altitude). Soil texture is clay loam with 22% sand, 50% clay and 28% silt and 0.7% organic matter. The soil pH is 8.16.

### Plant material and experimental design

Twenty-four 6 year-old 'William's Pride' apple trees (*Malus × domestica* Borkh.) grafted on M9 rootstocks were used in this study. Trees were grouped (randomized block design) into three blocks of 12 trees based on proximity in orchard and fruit load. The trees were spaced at 1.5 × 3.0 m and trained to Slender Spindle System. Standard cultural practices (pruning, fertilization, irrigation and etc.) were regularly performed. Irrigations were carried out through drip irrigation and micronutrients and macronutrients were supplied in four aliquots on April 1, May 1, June 1 and July 1. A total of 15 g N (nitrogen), 25 g K<sub>2</sub>O (60%, potassium oxide), 5 g NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> (monoammonium phosphate) and 25 g K<sub>2</sub>SO<sub>4</sub> (potassium sulfate) were supplied to trees. Additionally, 5 g calcium nitrate [Ca (NO<sub>3</sub>)<sub>2</sub>] was supplied once on August 1.

### Treatments

In each block, 3 trees were selected for control, 3 trees for Zn treatments (0.3% ZnSO<sub>4</sub>), 3 trees for 250 mg L<sup>-1</sup> AVG treatments [containing 150 mg aminoethoxyvinylglycine g<sup>-1</sup> (Valent BioScience Corp. Libertyville, USA)] and 3 trees for Zn + AVG treatments (250 mg L<sup>-1</sup> AVG + 0.3% ZnSO<sub>4</sub>). AVG was sprayed 4 weeks (July 18, 2012) before the anticipated harvest date (August 15, 2012). Zn was

sprayed on trees when the fruits reached a walnut size [fruit development stage (June 15, 2012)].

The experimental trees were uniformly sprayed with an aqueous solution containing AVG, Zn and Sylgard 309 [(0.05%, v/v), Dow Corning, Istanbul, Turkey] as a surfactant until run-off with a low-pressure hand sprayer. Only water (pH 6.48) + surfactant was used in control. Sprays were completed in a non-windy day during favorable weather conditions when rainfall was not forecasted for the following 24 h. Amount of solution to be applied was calculated using the equation developed by researchers [Block-specific Sprayer Calibration Worksheet 2012] and 1000 mL solution was sprayed to each tree.

One tree was designated to be the sample tree, from which fruits were collected for ethylene and quality analysis at certain dates. No fruits were harvested from two trees until normal harvest time and they served to follow the progression of fruit drop. Twenty fruits from the sample tree of each block were harvested randomly from the whole canopy to determine the quality attributes. Apple fruits with uniform shape, color and size and free from visual symptoms of any disease or blemishes, were selected. The fruits were immediately transported to laboratory to determine the quality attributes and bioactive compounds.

**Cumulative drop ratio (%).** To determine the pre-harvest fruit drop ratio, starting 30 days before the anticipated harvest date, fruits fallen under tree were counted twice a week until the harvest. Then, fruits remaining on the trees were harvested and cumulative drop ratio was calculated.

**Internal ethylene concentration and flesh firmness.** To evaluate the internal ethylene concentration, 1-mL air sample from core cavity of each fruit was injected into a gas chromatograph equipped with an active alumina column and Flame Ionization Detector (Perkin Elmer-Clarus 500, USA), using the method of Bramlage et al. [1980]. The resultant peaks were compared to that of 100  $\mu\text{L L}^{-1}$  ethylene standard and the internal ethylene concentration was calculated. Flesh firmness was measured on three sides of equatorial line of each fruit using a press-mounted Effegi penetrometer (FT 327; McCormick Fruit Tech. Torino, Italy) with 11.1 mm tip.

**Color characteristics.** Color characteristics ( $L^*$ , chroma and hue angle) was measured at opposite sides of each fruit with a colorimeter (Minolta, model CR-400, Japan). Chromatic analyses were conducted in accordance with the CIE (Commission Internationale de l'Eclairage) system of 1976. Values of  $L^*$ ,  $a^*$  and  $b^*$  were used to define a three-dimensional color space. The chroma value was calculated with the formula  $C^* = (a^{*2} + b^{*2})^{1/2}$ , and the hue angle with  $h^\circ = \tan^{-1} b^*/a^*$ .

**Soluble solids content (SSC) and titratable acidity.** A sample of juice was taken from one piece of each of ten fruits per tree, and 3 different measurements were obtained from each replication. SSC was determined with a digital refractometer (PAL-1, McCormick Fruit Tech., Yakima, Wash). For titratable acidity (TA), 10 ml of extract was taken from each sample, 10 ml distilled water was added and the value corresponding to consumed sodium hydroxide (NaOH) during the titration with 0.1 N NaOH to increase the pH of samples to 8.1 was expressed in malic acid ( $\text{g malic acid } 100 \text{ mL}^{-1}$ ).

**Total phenolics and antioxidant activity.** At the anticipated harvest date, flesh and skin samples of one piece of five fruits were homogenized and placed into 4 different tubes and measurements were made from each tube in each replication. The fruit samples were kept in 50 mL tubes at  $-20^\circ\text{C}$  for bioactive analysis. Samples were thawed at room temperature ( $\approx 21^\circ\text{C}$ ) and homogenized in a food grade blender. The resultant slurry was centrifuged ( $12000 \times g$ ) for 30 min at  $4^\circ\text{C}$  to separate the juice from the pulp. Freshly obtained juice material was diluted with distilled water, divided into multiple sample aliquots, and refrozen at  $-20^\circ\text{C}$  until used in phenolics, antioxidant and anthocyanin assay procedures.

**Total phenolics:** A portion of 300  $\mu\text{L}$  from each sample was diluted with 4.3 mL distilled water and 100  $\mu\text{L}$  Folin-Ciocalteu reagents were added. After an interval of 3 min, 20%  $\text{Na}_2\text{CO}_3$  was added to 300  $\mu\text{L}$  portions and the mixture was vortexed and incubated at room temperature for 30 min. Absorbance was then read on a UV-Vis (Perkin Elmer, Lambda-1050 spectrophotometer, CA, USA) spectrophotometer at 760 nm. Gallic acid was used as the standard. The results were expressed as grams (g) of gallic acid equivalents (GAE) per kilogram of fresh weight (f.w.) ( $\text{g GAE kg}^{-1} \text{ f.w.}$ ) [Beyhan et al., 2010].

**ABTS<sup>+</sup> radical scavenging activity:** 2 mM of ABTS<sup>+</sup> [2,2'-azino-bis (3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt] and 2.45 mM of K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> solutions were prepared by 0.1 M of PO<sub>4</sub><sup>-3</sup> buffer solution (pH 7.4). The ABTS and K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> solutions were mixed in (1 : 2) ABTS-K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> and incubated for 6 h in dark. The absorbance of the mixture was read at 734 nm and it was diluted with PO<sub>4</sub><sup>-3</sup> buffer if the value was greater than 0.75. Finally, 20 µL samples were taken out of the mixture into tubes, 1 mL of ABTS-K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> solution was added to each tube and buffer solution was added to make the total sample volume 4 mL. Following vortexing, they were incubated for 30 min and absorbance was read at 734 nm. The results were expressed as mmol Trolox equivalents (TE) per kg of fw (mmol TE kg<sup>-1</sup> f.w.) [Pellegrini et al. 1999].

**Ferric ions (Fe<sup>+3</sup>) reducing antioxidant power assay (FRAP):** Portions of 120 µL were taken from the samples, 0.2 M of phosphate buffer (PO<sub>4</sub><sup>-3</sup>) (pH 6.6) was added to obtain a volume of 1.25 mL and then 1.25 mL of 1% potassium ferricyanide (K<sub>3</sub>Fe(CN)<sub>6</sub>) solution was added. After vortexing, they were incubated at 50°C. Afterwards, 1.25 mL of 10% TCA (trichloroacetic acid) and 0.25 mL of 0.1% FeCl<sub>3</sub> were added to the samples. The absorbance of the resultant solution was read on an UV-Vis spectrometer at 700 nm. The results were expressed as mmol Trolox equivalents (TE) per kilogram of f.w. (mmol TE kg<sup>-1</sup> f.w.) [Benzie and Strain 1996].

#### Micronutrients and macronutrients

A total of five fruits were randomly harvested from each tree in each block for each treatment at the anticipated harvest date. Hundred apple leaves were collected from the middle parts of annual shoots, from different directions to represent each tree at the anticipated harvest date. To determine the micronutrients (Fe, Zn, Cu and Mn) and macronutrients (N, P and K), fruits and leaves were washed with 0.01% HCl solution and distilled water, dried in an oven at 70°C for two days and ground with a blender (Waring, Torrington, USA). Ground samples were then dry-combusted in an ash oven [Kacar and Inal 2008]. P, K, Fe, Zn, Mn and Cu readings were performed on resultant extracts with ICP-OES (Perkin Elmer – 100DV Optima, USA) device. Nitrogen was determined using Kjeldahl distilla-

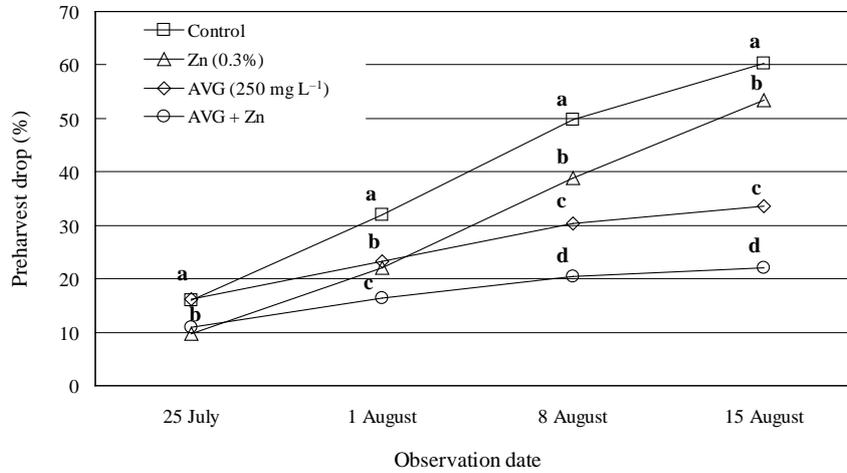
tion method [Bremner 1965]. Results were expressed as mg kg<sup>-1</sup> d.w. (dry weight) for micronutrients or g kg<sup>-1</sup> d.w. for macronutrients.

#### Statistical analysis

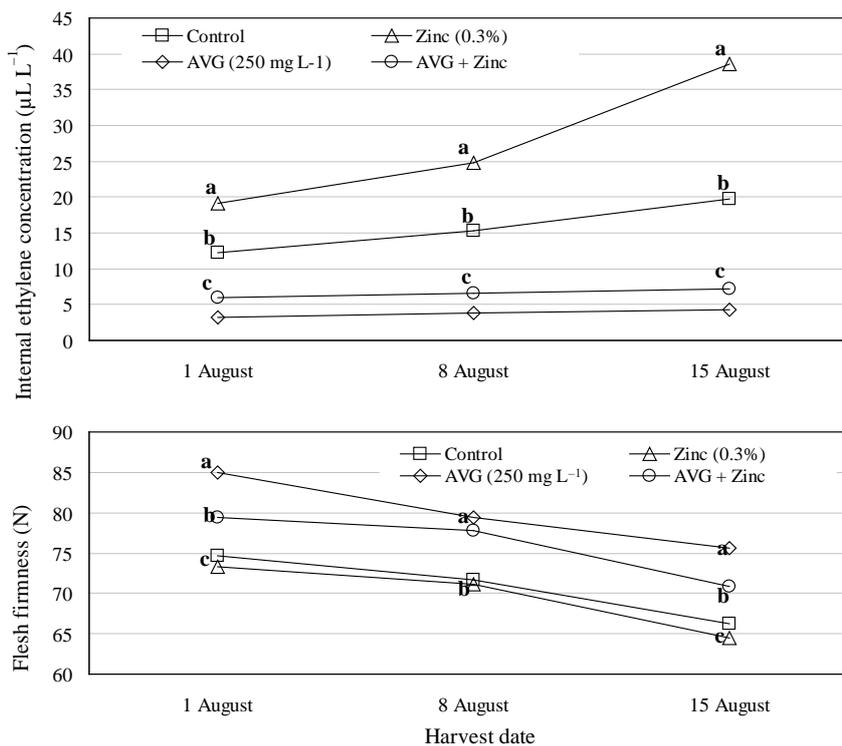
Data normality was confirmed by the Kolmogorov-Smirnov test and the homogeneity of variances by the Levene's test. The data sets were analyzed with one-way ANOVA using SAS Version 9.1 (SAS Institute Inc., Cary, NC, USA) software. When the F test was significant, means were separated by Duncan's Multiple Range Test ( $P \leq 0.05$ ).

## RESULTS AND DISCUSSION

**Cumulative drop ratio.** As compared to the control, while single AVG treatments did not result in significant variations in fruit drops in assessments made a week after AVG spray to trees (25 July), treatments resulted in distinctive decreases in fruit drops in subsequent observation dates (Fig. 1). Such a case supported the findings of Greene [2005] indicating that at least 10–14 days should pass for AVG to be effective. As it was in several previous studies indicating AVG as an efficient chemical in controlling fruit drops in apples [Greene and Schupp 2004, Wargo et al. 2004, Kang et al. 2007, Yildiz et al. 2012], AVG was also considered as an efficient chemical in present study. At the last harvest date, about 50% less fruit drops were achieved with AVG treatments as compared to the control. Single zinc treatments yielded distinctive decreases in fruit drops at all measurement dates. On the other hand, combined AVG + Zinc treatments were found to be quite more effective than single treatments and the lowest drop rates were obtained from the combined treatments at all measurement dates, except for the first measurement date. Such a fruit drop-reducing effect of zinc may be related to the role of zinc in auxin metabolism. Previous researchers also indicated significant role of zinc in auxin metabolism [Cakmak et al. 1989, Oguchi et al. 2004]. Supporting the present findings, delayed fruit drops were also reported by combined growth regulator and zinc treatments in different citrus species [Ashraf et al. 2013, Razzaq et al. 2013].



**Fig. 1.** Effects of aminoethoxyvinylglycine (AVG) and Zinc (Zn) on pre-harvest fruit drops in 'William's Pride' apples; means in columns with the same letter do not differ significantly according to Duncan's Multiple Range test,  $P < 0.05$



**Fig. 2.** Effects of AVG and Zinc treatments on internal ethylene concentration and flesh firmness of 'William's Pride' apples picked at different harvest dates;  $n = 9$  for the internal ethylene concentration (3 replications  $\times$  3 different measurements for each replication);  $n = 90$  for the flesh firmness (3 replications  $\times$  10 fruits  $\times$  3 different measurements for each fruit); the differences among the treatments indicated with the same letter vertically were not significant according to Duncan's Multiple Range test at  $P < 0.05$

**Internal ethylene concentration and flesh firmness.** Several studies indicated delayed pre-harvest drops in apples with AVG treatments through inhibition of ethylene synthesis [Byers 1997, Greene and Schupp 2004, Yildiz et al. 2012]. Similar findings were also clearly seen in the present study. In all three measurement dates, AVG treatments yielded lower ethylene levels as compared to the control. On the other hand, zinc treatments also yielded decreasing drop rates, while remarkable increases in ethylene synthesis (Fig. 2). Such a case then brought into the mind that zinc might have influenced the fruit drops independently on ethylene. Another remarkable outcome was observed in fruits with combined AVG + Zinc treatments. Although the lowest fruit drop rates were observed in this treatment, ethylene synthesis level of the combined treatment was quite close to the control. Further research is needed to better elucidate the more efficient nature of combined AVG + Zinc treatments in controlling the pre-harvest fruit drops.

At all three measurement dates, entire flesh firmness values were greater in AVG-treated fruits than the control (Fig. 2). AVG-induced retarded flesh softening is mainly related to inhibition of ethylene synthesis by AVG [Greene 2006, Yildiz et al. 2012].

**Color characteristics.** Several previous studies reported retarded red skin color development with AVG treatments [Byers 1997, Greene and Schupp 2004, Yildiz et al. 2012]. Similar findings were also obtained in the present study. Hue angles of AVG-treated fruits were greater than the control fruits at all three measurement dates. As compared to the control, while the AVG treatments did not yield significant variations in chroma values, treatments yielded significant increases in  $L^*$  values (Fig. 3). Lower hue angle and  $L^*$  values indicate less ripened nature of the fruits. Such a case supports the idea that AVG retarded fruit ripening [Greene and Schupp 2004, Greene 2006, Kang et al. 2007].

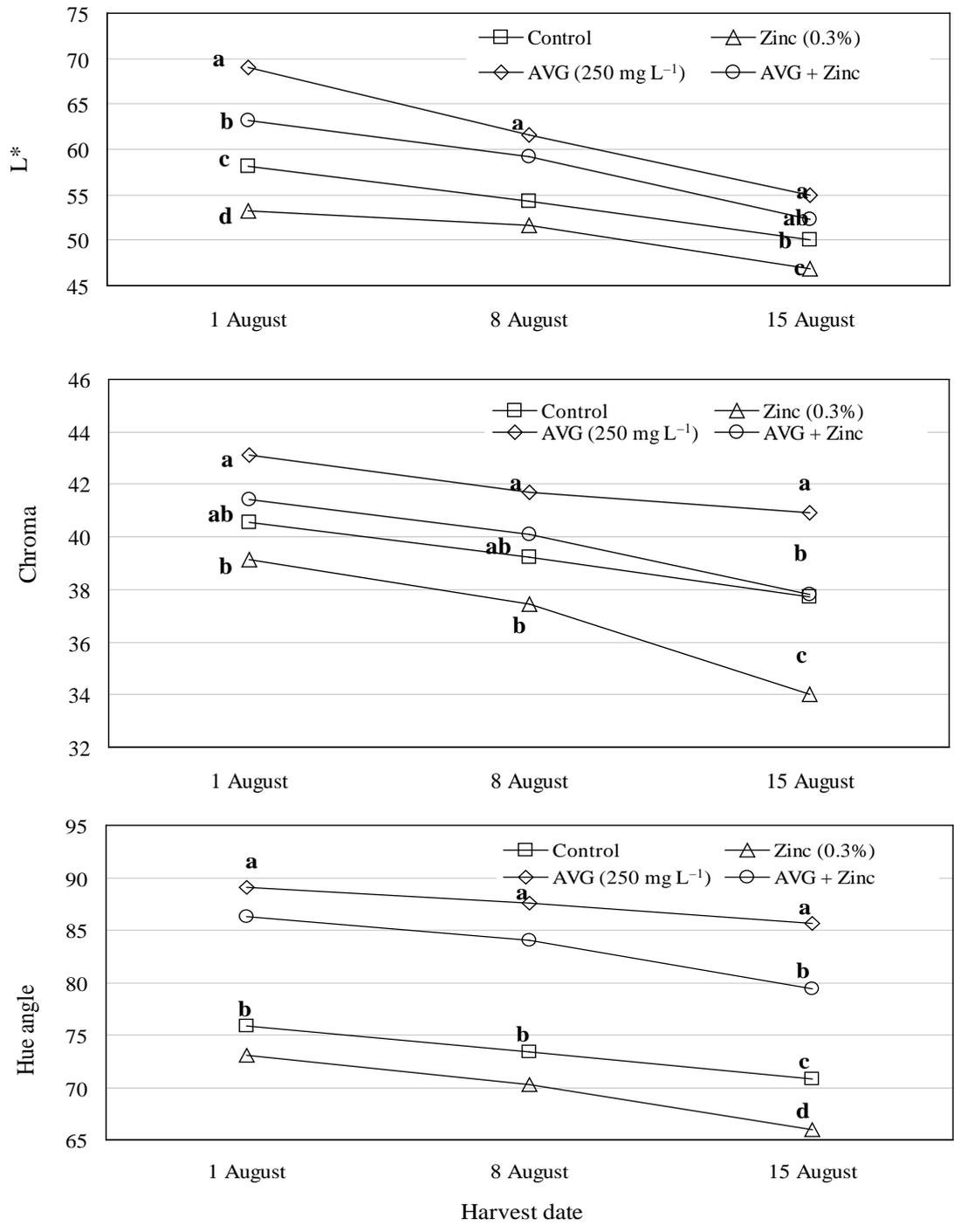
Although single zinc treatments did not change  $L^*$  values on the 8<sup>th</sup> of August, the treatments reduced  $L^*$  values on the 1<sup>st</sup> and 15<sup>th</sup> of August. While there were not significant differences in hue angle and chroma values of the control and single zinc treatments at the first two measurement dates, lower hue angle and chroma values were obtained from

single zinc treatments than the control at the last measurement date (Fig. 3). Zhang et al. [2013] worked with Gala and Fuji apples and reported decreasing hue angles with foliar zinc treatments. Considering the color parameters ( $L^*$ , hue angle and chroma) at the last harvest date, it can be stated that zinc treatments stimulated the red color development in 'William's Pride' apples. Similarly, Yogaratham and Johnson [1982] also reported increasing red color development in apples with zinc treatments. On the other hand, in combined AVG + Zinc treatments, effects of AVG were dominant. While AVG + Zinc treatments increased  $L^*$  values of the fruits at the first two measurement dates, combined treatments did not have significant effects on  $L^*$  values at the last measurement date. With regard to chroma values, there were not significant differences between the control and AVG + Zinc treatments at all three measurement dates. As compared to the control, AVG + Zinc treatments yielded higher hue angles at all three measurement dates.

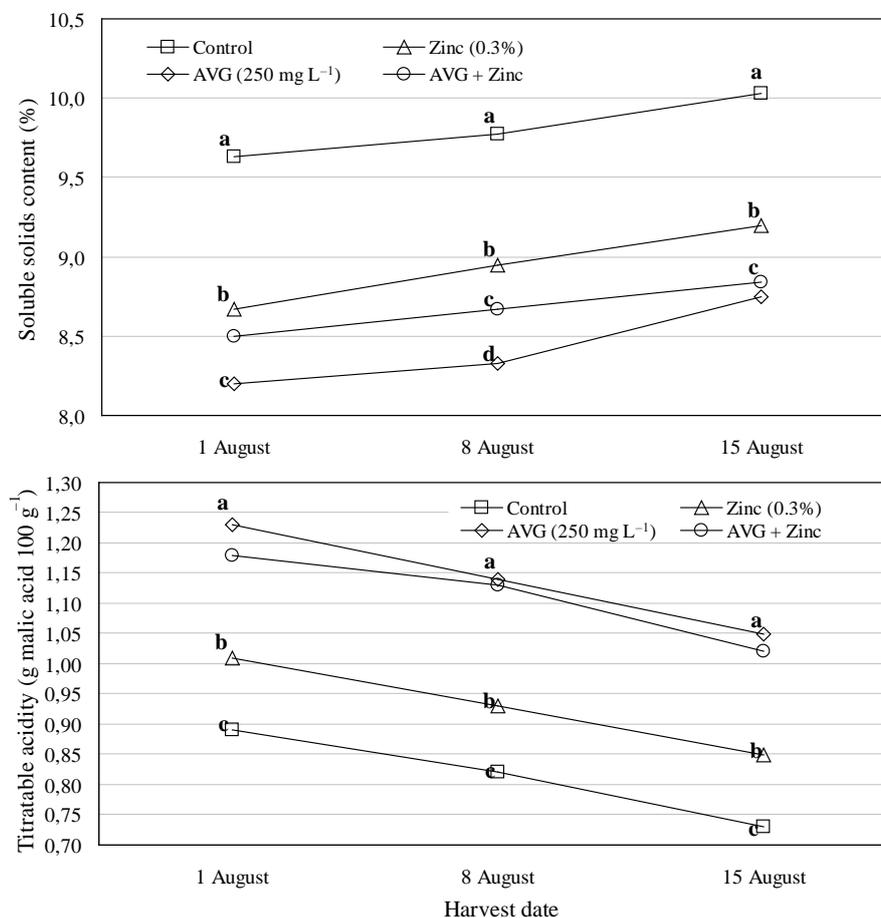
**Soluble solids content (SSC) and titratable acidity.** AVG-induced changes in titratable acidity and SSC values clearly indicated that AVG retarded fruit ripening. While AVG treatments increased acidity, they reduced SSC values of the fruits (Fig. 4). Present findings on acidity and SSC values comply with the findings of earlier studies carried out with different apple cultivars [Greene and Schupp 2004, WookJae et al. 2006, Yildiz et al. 2012].

As compared to the control, zinc both alone and combined with AVG, reduced the SSC values, but slightly increased acidity values (Fig. 4). Zhang et al. [2013] indicated that the effects of zinc on SSC and acidity of Gala and Fuji apples varied with the time of application.

**Total phenolics and antioxidant activity.** AVG and zinc treatments yielded significant changes in total phenolics and antioxidant activity of the fruits. While the greatest total phenolics was obtained from AVG treatments, the lowest value was observed in zinc treatments. On the other hand, total phenolics of AVG+Zinc-treated fruits were greater than single zinc-treated fruits, but lower than single AVG-treated fruits. In general, similar changes were also observed in antioxidant activity. In other words, antioxidant



**Fig. 3.** Effects of aminoethoxyvinylglycine (AVG) and Zinc (Zn) on L\*, chroma and hue angle of 'William's Pride' apples picked at different harvest dates; n = 30 for the L\*, chroma and hue angle (3 replications × 10 fruits × 2 different measurements for each fruit); means in columns with the same letter do not differ significantly according to Duncan's Multiple Range test, P < 0.05



**Fig. 4.** Effects of aminoethoxyvinylglycine (AVG) and Zinc (Zn) on SSC and titratable acidity of ‘William’s Pride’ apples picked at different harvest dates;  $n = 9$  for the SSC and titratable acidity (3 replications  $\times$  3 different measurements for each replication);  $n = 30$  for the fruit weight (3 replications  $\times$  10 fruits for each replication); means in columns with the same letter do not differ significantly according to Duncan’s Multiple Range test,  $P < 0.05$

**Table 1.** Effects of aminoethoxyvinylglycine (AVG) and Zinc (Zn) on total phenolics and antioxidant activity of ‘William’s Pride’ apples at anticipated harvest time

Treatments	(g GAE kg <sup>-1</sup> f.w.)	ABTS	FRAP
Control	0.38 c	17.9 bc	0.35 c
Zn (0.3 %)	0.27 d	16.2 c	0.20 d
AVG (250 mg L <sup>-1</sup> )	0.64 a	24.0 a	0.55 a
AVG+Zn	0.47 b	19.8 b	0.43 b

Antioxidant activity was expressed as mmol TE kg<sup>-1</sup>;  $n = 12$  for the total phenolics and antioxidant activity (3 replications  $\times$  4 different measurements for each replication); means in columns with the same letter do not differ significantly according to Duncan’s Multiple Range test,  $P < 0.05$

**Table 2.** Effects of aminoethoxyvinylglycine (AVG) and Zinc (Zn) on fruit flesh and leaf micronutrients and macronutrients of 'William's Pride' apples at anticipated harvest time

Treatments	Micronutrients (mg kg <sup>-1</sup> )				Macronutrients (g kg <sup>-1</sup> )		
	Cu	Mn	Fe	Zn	N	P	K
	Fruit flesh						
Control	4.79 c	1.67 a	3.73 a	1.92 c	2.40 b	0.72 c	11.19 c
Zn (0.3 %)	5.81 a	1.31 ab	4.90 a	9.68 a	2.74 a	1.55 a	16.33 a
AVG (250 mg L <sup>-1</sup> )	5.28 b	1.24 b	5.50 a	1.51 c	2.79 a	0.89 b	11.25 c
AVG+Zn	4.99 bc	1.15 b	4.61 a	4.43 b	2.81 a	0.96 b	12.42 b
	Leaf						
Control	14.74 a	53.05 d	102.46 b	25.75 c	1.70 c	1.86 b	13.78 a
Zn (0.3 %)	13.41 b	62.32 c	95.66 c	154.39 a	2.40 b	2.12 a	11.56 b
AVG (250 mg L <sup>-1</sup> )	14.31 a	77.67 a	106.78 a	12.97 d	2.20 b	2.06 a	10.89 c
AVG+Zn	14.08 ab	73.36 b	93.58 d	106.26 b	3.03 a	2.29 a	13.28 a

n = 6 for the micronutrients and macronutrients (3 replications × 2 measurements for each replication); means in columns with the same letter do not differ significantly according to Duncan's Multiple Range test, *P* < 0.05

activity was greater in treatments with higher total phenolics and antioxidant activity was lower in treatments with lower total phenolics (Tab. 1). Such findings comply with the results of earlier studies indicating a positive correlation between total phenolics and antioxidant activity [Khanizadeh et al. 2008, Carbone et al. 2011]. Contrary to the present findings, decreased total phenolics were reported with AVG treatments in plums [Ozturk et al. 2012] and sweet cherries [Kucuker and Ozturk 2015]. On the other hand, insignificant effects of AVG treatments were reported on phenolic substances (quercetin glycosides, catechin, epicatechin, chlorogenic acid and phloridzin) of Cripp's Pink apples [Whale et al. 2008] and Jonagold apples [Awad and Jager 2002]. Considering earlier findings indicating AVG-induced increases in ethylene and phenolics substances [Cvikrova et al. 1991, Liang et al. 2013], increasing total phenolics with ethylene-inhibiting AVG were considered as quite reasonable. However, further research is needed about the ethylene-related or ethylene-independent effects of AVG on phenolic substances and the enzymes playing a role in synthesis of these substances. Despite the previous knowledge on potential impacts of zinc on several physiological

process of different fruit and vegetable species and on fruit quality attributes [Pandey et al. 2013, Saadati et al. 2013, Zhang et al. 2014], there is a limited information about the effects of zinc treatments on total phenolics and antioxidant activity of the fruits. It was observed in present study that foliar zinc treatments reduced total phenolics and antioxidant capacity of 'William's Pride' apples.

**Micronutrients and macronutrients.** Zinc and AVG treatments also yielded significant changes in flesh and leaf nutrient contents (Tab. 2). As it was expected, the most remarkable changes were observed in zinc contents. Zinc alone and combined with AVG yielded distinctive increases in flesh and leaf zinc contents. While single AVG treatments did not result in significant changes in flesh Zn contents, treatments resulted in significant decreases in leaf Zn contents. A remarkable change was also observed in leaf manganese contents, both the single zinc and AVG treatments and combined treatments yielded distinctive increases in leaf manganese contents. While there were not significant differences in flesh Fe contents of the treatments, significant differences were observed in flesh Fe contents of the treatments. Zn treatments increased flesh N, P and K contents,

whereas AVG treatments increased flesh N and P contents, but did not result in significant changes in K contents. Significant zinc and AVG-induced changes were also observed in leaf N, P and K contents. Butar et al. [2015] also reported significant changes in nutrient contents of Jersey Mac apples at different AVG doses.

Based on present findings, it was concluded that as it was in various other apple cultivars, AVG treatments were effective in controlling the pre-harvest fruit drops in 'William's Pride' apples and such an effect increased even further when combined with zinc treatments. Further research is recommended to better elucidate such a synergic impact mechanism and the mechanisms effective in pre-harvest fruit drops. It was also disclosed in this study that besides decelerated pre-harvest fruit drops and retarded ripening, AVG treatments had also positive impacts on total phenolics and resultant antioxidant capacity of apples.

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#### CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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