Effects of the Bioregulators Aminoethoxyvinylglycine and Ethephon on Brix, Carbohydrate, Acid, and Mineral Concentrations in ‘Scarletspar Delicious’ Apple Juice

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Abstract. In a study conducted over three crop seasons, Ethrel (ETH) increased the Brix, sucrose, and sorbitol content of ‘Scarletspar Delicious’ apple juice while reducing the fructose content. Both longer preharvest exposure to, and higher concentrations of, ETH had a stronger influence than application closer to harvest and/or at lesser amounts. Time of ETH application tended to influence individual carbohydrates more so than amount of ETH applied. ETH reduced total acidity and also reduced apple juice individual acid (quinic and malic) contents with longer preharvest exposure or higher concentrations. Aminoethoxyvinylglycine [AVG (ReTain)] reduced both Brix and sucrose content of ‘Scarletspar Delicious’ apple juice, but had no influence on either total acidity or individual acid contents. Combinations of AVG with ETH tended to counteract the influence of either used alone on total Brix, carbohydrates, total acidity and individual acids. Mineral content of ‘Scarletspar Delicious’ apple juice was not strongly influenced by application of either ETH or AVG.

Bioregulators have been used for multiple purposes in tree fruit production and many physiological processes in tree fruit can be influenced by exogenous application of such products. Aminoethoxyvinylglycine [AVG (ReTain), Valant BioSciences, Walnut Creek, Calif.] and ethephon [ETH (Ethrel) Bayer CropScience, Research Triangle Park, N.C.] have been widely used alone or in combination to manipulate fruit tree physiology. Control of vegetative growth and regulation of flowering (Elfving and Cline, 1990; Elfving et al., 2003; Halder-Doll and Bangert, 1987), preharvest drop (Bangert, 1978; Greene et al., 2000; Greene, 2002), fruit maturity (Byers, 2002; Greene et al., 2000; Byers et al., 2000; Larrigaudiere, 1996; Wang and Dilley, 2001; Watkins and Rosenberger, 1999; Williams, 1980) have been manipulated with the use of bioregulators. There is little, if any, information on the compositional characterization of apple juice from apples treated with bioregulators, even though there has been extensive characterization of apple juice from non-treated cultivars (Drake and Eisele, 1999; Eisele and Drake, 2003; Eisele et al., 1996; Lee and Wrolstad, 1988). Therefore, the objective of this study was to determine the influence of the bioregulators AVG and ETH on the Brix, titratable acidity, carbohydrate, acid and mineral concentrations in apple juice using the ‘Scarletspar Delicious’ apple strain, widely grown in Washington today.

Materials and Methods

This study was conducted over three crop seasons using mature ‘Scarletspar Delicious’ apple trees (Malus domestica Borkh.) on MM.111 rootstock. Trees were located in a commercial orchard in East Wenatchee, Wash., and were planted in 1979 at a spacing of 3.3 × 6.6 m.

Trial 1 (2000). Applications of ETH were made to single-tree plots in six randomized complete blocks to run-off using a hydraulic hand-gun sprayer. Apple trees were sprayed at either 1 or 2 weeks before first (commercial) harvest (1 WBFH, 2 WBFH) with either 150 or 300 mg active ingredient (a.i.)/L ETH. All sprays were supplemented with 0.1% v/v Regulaid surfactant (Wilbur-Ellis, Fresno, Calif.) to runoff using a hydraulic hand-gun sprayer. The six double-tree plots in each block served as an unsprayed control. The other four double-tree plots previously treated with AVG were individually sprayed to runoff using a hydraulic handgun sprayer with the following combinations of ETH and timing: ETH 150 mg a.i./L at either 2 WBFH or 1 WBFH or ETH 300 mg a.i./L at either 2 WBFH or 1 WBFH. All ETH sprays were supplemented with 0.1% v/v Regulaid surfactant.

Trial 2 (2001). Five randomized complete blocks containing six double-tree plots each were selected from another part of the orchard that had not been previously treated with either AVG or ETH. Five double-tree plots per block (10 trees per block) were sprayed 4 WBFH with 125 mg a.i./L AVG plus 0.1% v/v Syldarg 309 organosilicon surfactant (Wilbur-Ellis, Fresno, Calif.) to runoff using a hydraulic hand-gun sprayer. The six double-tree plots in each block served as an unsprayed control. The other four double-tree plots previously treated with AVG were individually sprayed to runoff using a hydraulic handgun sprayer with the following combinations of ETH and timing: ETH 150 mg a.i./L at either 2 WBFH or 1 WBFH or ETH 300 mg a.i./L at either 2 WBFH or 1 WBFH. All ETH sprays were supplemented with 0.1% v/v Regulaid surfactant.

Trial 3 (2002). Seven randomized complete blocks containing four double-tree plots each were selected from another part of the orchard that had not been previously treated with either AVG or ETH. Three four-tree plots per block (12 trees per block) were sprayed 4 WBFH with 125 mg a.i./L AVG plus 0.1% v/v Syldarg 309 organosilicon surfactant to runoff using a hydraulic hand-gun sprayer. One four-tree plot in each block served as an unsprayed control. The other two four-tree plots previously treated with AVG were individually sprayed with either ETH 150 mg a.i./L at 2 WBFH or with ETH 75 mg a.i./L at 2 WBFH and again at 1 WBFH to run-off using a hydraulic hand-gun sprayer. The remaining two untreated four-tree plots were treated with ETH at either 75 or 150 mg a.i./L as described above. All ETH sprays were supplemented with 0.1% v/v Regulaid surfactant.

‘Scarletspar Delicious’ apples were harvested on two dates each year of the study corresponding to first commercial harvest and commercial harvest + 4 weeks (18 Sept. and 13 Oct., 2000; 10 Sept. and 8 Oct., 2001; 23 Sept. and 21 Oct., 2002). On each harvest date, thirty apples were harvested at random from each plot. After each harvest, the apples were held in refrigerated storage (1 week) before analysis.

Juice preparation. Each group of ten apples (treatment, harvest and replication) was washed, surface dried, sliced and juiced using a juicer (model 4000; Omega Products, Inc., Harrisburg, Pa.) at ambient temperature. Subsequent juice was centrifuged in an IEC (Centra-7; International Equ. Co., Needham Heights, Mass.) at 5000 rpm and then filtered.
through an odor free coffee filter to remove all insoluble solids. About 150 mL of juice from each group were placed in a plastic bottle and frozen until sufficient samples had been collected for analysis.

Previously frozen samples were thawed overnight at 2 °C. Titratable acidity (AOAC 942.15) and degrees Brix (AOAC 932.12) (AOAC, 1990) were measured on all samples prior to HPLC analysis. Carbohydrate and acid concentrations were determined as previously described (Drake and Eisele, 1999). Sodium, potassium, magnesium and calcium concentrations were determined by atomic absorption spectrophotometry using a modified AOAC 985.35 procedure. Ten ml aliquots of each juice sample were diluted to a final volume of 100 mL with 1 N HNO₃ containing 0.1% lanthium chloride. Further dilutions for each mineral as well as calibration curves, wavelengths and flame parameters were optimized for a GBC 932 AA in accordance with the instruments manufacturer’s recommendations (GBC Scientific Equipment, Inc., Arlington Heights, Ill.).

All data shown in the tables were normalized to 11.5° Brix to match the FDA definition for 100% apple juice. Total acidity data were expressed as percent of malic acid equivalents per 100 g of juice. Carbohydrate data were expressed as g/100 mL, acid data as mg/100 mL, and minerals as mg/100 mL. All juice dilutions, standards and reagents were prepared from 18 Mohm water. Analysis of variance was determined by MSTAT-C (1989) using bioregulators and harvest date as main effects. On the basis of significant F tests, bioregulator treatment means were separated by Tukey’s test (P ≤ 0.05). Harvest-date data were separated by single-df analysis of variance comparisons. Significant interactions between bioregulators and harvest dates were not found.

### Results and Discussion

**Table 1.** ETH effects on Brix, total acidity, carbohydrate and acid concentrations in ‘Scarletspr Delicious’ apple juice (Trial 1).

| Parameter          | Carbohydrates (g/100 mL) | Acids (mg/100 mL) |
|--------------------|--------------------------|------------------|
|                    | Brix°  | Sucrose | Glucose | Fructose | Sorbitol | acidity (%) | Quinic | Malic | Citric |
| Bioregulator       |        |         |         |          |          |             |        |       |        |
| Control            | 10.9 b  | 2.27 c  | 2.53 a  | 6.00 a   | 0.29 c   | 0.27 a      | 88.6 a | 338.1 a | 3.5 a  |
| ETH @ 150 ppm      | 11.8 a  | 2.59 ab | 2.53 a  | 5.64 cd  | 0.36 ab  | 0.23 b      | 79.4 b | 306.0 ab | 3.7 a  |
| ETH @ 300 ppm      | 11.9 a  | 2.69 a  | 2.49 a  | 5.57 d   | 0.39 a   | 0.23 b      | 75.3 b | 303.6 ab | 3.5 a  |
| ETH @ 150 ppm      | 11.4 ab | 2.50 b  | 2.55 a  | 5.85 ab  | 0.30 bc  | 0.24 ab     | 82.7 ab | 319.9 ab | 3.8 a  |
| ETH @ 300 ppm      | 11.7 a  | 2.53 ab | 2.58 a  | 5.80 bc  | 0.33 bc  | 0.23 b      | 81.2 ab | 298.8 ab | 3.6 a  |
| Harvest            |         |         |         |          |          |             |        |       |        |
| 18 Sept            | 11.1 b  | 2.47 b  | 2.52 a  | 6.06 a   | 0.27 b   | 0.26 a      | 91.1 a  | 334.6 a | 3.8 a  |
| 13 Oct             | 12.0 a  | 2.56 a  | 2.55 a  | 5.48 b   | 0.39 a   | 0.22 b      | 71.8 b  | 262.0 b | 3.5 b  |

*Means in a column within bioregulators or harvest not followed by a common letter are significantly different (P ≤ 0.05).

**Table 2.** AVG and ETH effects on Brix, total acidity, carbohydrate and acid concentrations in ‘Scarletspr Delicious’ apple juice (Trial 2).

| Parameter          | Carbohydrates (g/100 mL) | Acids (mg/100 mL) |
|--------------------|--------------------------|------------------|
|                    | Brix°  | Sucrose | Glucose | Fructose | Sorbitol | acidity (%) | Quinic | Malic | Citric |
| Bioregulator       |        |         |         |          |          |             |        |       |        |
| Control            | 10.8 bc | 2.22 a  | 1.50 c  | 6.17 ab  | 0.26 a   | 0.31 ab     | 137.2 b | 427.2 a | 4.91 a |
| AVG @ 125 ppm      | 10.5 c  | 2.04 b  | 1.53 c  | 6.27 a   | 0.26 a   | 0.32 a      | 150.8 a | 415.8 a | 4.26 b |
| AVG + ETH @ 150 ppm| 11.3 ab | 2.31 a  | 1.68 a  | 5.98 bc  | 0.30 a   | 0.30 ab     | 135.2 bc | 383.6 ab | 3.58 c |
| AVG + ETH @ 300 ppm| 11.9 a  | 2.28 a  | 1.85 a  | 5.87 c   | 0.31 a   | 0.27 b      | 120.2 c  | 366.4 ab | 3.19 d |
| AVG + ETH @ 150 ppm (1 WBFH) | 11.3 ab | 2.31 a  | 1.65 bc | 6.02 bc  | 0.28 a   | 0.28 bc     | 126.5 c  | 381.4 ab | 3.47 cd |
| AVG + ETH @ 300 ppm (1 WBFH) | 11.8 a  | 2.30 a  | 1.79 ab | 5.84 c   | 0.31 a   | 0.25 c      | 121.3 c  | 369.1 ab | 3.54 cd |
| Harvest            |         |         |         |          |          |             |        |       |        |
| 10 Sept            | 11.0 c  | 2.17 c  | 1.66 b  | 6.25 a   | 0.26 a   | 0.33 a      | 165.9 a | 446.4 a | 4.38 a |
| 8 Oct              | 11.9 a  | 2.42 a  | 1.75 a  | 5.80 a   | 0.31 a   | 0.25 b      | 98.3 b  | 334.7 b | 3.27 b |

*Means in a column within bioregulators or harvest not followed by a common letter are significantly different (P ≤ 0.05).

**Table 3.** AVG and ETH effects on Brix, total acidity, carbohydrate and acid concentrations in ‘Scarletspr Delicious’ apple juice (Trial 3).

| Parameter          | Carbohydrates (g/100 mL) | Acids (mg/100 mL) |
|--------------------|--------------------------|------------------|
|                    | Brix°  | Sucrose | Glucose | Fructose | Sorbitol | acidity (%) | Quinic | Malic | Citric |
| Bioregulator       |        |         |         |          |          |             |        |       |        |
| Control            | 12.0 a  | 2.27 a  | 1.97 a  | 5.87 ab  | 0.37 a   | 0.24 ab     | 97.6 a  | 350.7 ab | 4.58 a |
| AVG @ 125 ppm      | 11.4 b  | 2.12 b  | 2.00 a  | 5.98 a   | 0.30 b   | 0.28 ab     | 93.6 a  | 365.3 a | 4.46 a |
| ETH @ 75 ppm (2 and 1 WBFH) | 12.2 a  | 2.06 ab | 2.04 a  | 5.80 b   | 0.41 a   | 0.22 b      | 93.6 b  | 365.2 a | 4.98 a |
| AVG + ETH @ 75 ppm | 12.2 a  | 2.17 a  | 2.12 a  | 5.73 b   | 0.36 a   | 0.23 b      | 94.4 a  | 347.7 ab | 4.36 a |
| AVG + ETH @ 150 ppm (2 WBFH) | 12.1 a  | 2.20 a  | 2.04 a  | 5.75 b   | 0.37 a   | 0.24 ab     | 90.5 ab | 343.4 ab | 4.37 a |
| Harvest            |         |         |         |          |          |             |        |       |        |
| 23 Sept            | 11.3 b  | 1.94 b  | 2.12 a  | 5.94 a   | 0.27 b   | 0.26 a      | 109.2 a | 386.0 a | 5.23 a |
| 21 Oct             | 12.8 a  | 2.30 a  | 1.95 b  | 5.68 b   | 0.45 a   | 0.21 b      | 75.3 b  | 313.7 b | 3.55 b |

*Means in a column within bioregulators or harvest not followed by a common letter are significantly different (P ≤ 0.05).

**AVG** applied 4 weeks before anticipated harvest.

**ETH** applied 2 weeks before anticipated harvest.
was reduced only by treatment with 300 ppm ETH, regardless of time before harvest. Delay of harvest resulted in apple juice with reduced total acidity and individual acid concentrations. The reduction in total acid concentration with delayed harvest has been previously reported (Drake et al., 2002b). The changes in individual acids with delayed harvest reported here have not been well documented.

Trial 2 (2001). AVG alone reduced sucrose concentration of apple juice at harvest but had no effect on glucose, fructose, sorbitol or total Brix (Table 2). ETH at 300 ppm applied either two or three weeks after AVG increased juice Brix and glucose levels above the levels in untreated fruit and decreased fructose concentration. Juice glucose was also increased by ETH at 150 ppm if applied 2 weeks after AVG (2 WBFH) but not if applied 3 weeks after AVG (i.e., 1 WBFH). Delaying harvest by 4 weeks resulted in increased juice Brix, sucrose, glucose and sorbitol concentrations and decreased fructose concentration, which is in agreement with observations the first year of the study.

AVG alone resulted in juice with increased quinic acid and reduced citric acid concentrations but had no influence on malic acid level or total acidity compared to control apple juice (Table 2). ETH treatment of previously AVG-treated apples resulted in reduced concentrations of quinic, malic and citric acids, with the exception of ETH 150 ppm at 2 WBFH. Despite these reductions in individual acid levels, only ETH at 300 ppm applied 1 WBFH reduced total acidity. Delaying harvest by 4 weeks resulted in reductions in total acidity as well as concentrations of all three of the individual acids measured.

Trial 3 (2002). At harvest, AVG-treated fruit showed reduced juice Brix, sucrose and

Table 4. ETH effects on selected mineral concentrations in apple juice from ‘Scarletspur Delicious’ apples (Trial 1).

| Parameter          | Na     | K     | Ca   | Mg   |
|--------------------|--------|-------|------|------|
| Control            | 0.72 a | 124.3 a | 2.12 a | 3.65 a |
| ETH @ 150 ppm (2 WBFH) | 0.67 a  | 117.8 ab | 1.98 a | 3.56 a |
| ETH @ 300 ppm (2 WBFH) | 0.64 a  | 122.3 ab | 2.14 a | 3.38 a |
| ETH @ 150 ppm (1 WBFH) | 0.62 a  | 110.7 b  | 2.17 a | 3.29 a |
| ETH @ 300 ppm (1 WBFH) | 0.57 a  | 115.5 ab | 2.09 a | 3.32 a |
| Harvest            |        |       |      |      |
| 18 Sept            | 0.62 a  | 118.8 a | 2.27 a | 3.54 a |
| 13 Oct             | 0.67 a  | 117.3a  | 1.93 b | 3.26 b |

*Means in a column within bioregulators or harvest not followed by a common letter are significantly different (P ≤ 0.05).
ETH applied 2 weeks before anticipated harvest
*ETH applied 1 week before anticipated harvest.

Table 5. AVG and ETH effects on selected mineral concentrations in apple juice from ‘Scarletspur Delicious’ apples (Trial 2).

| Parameter          | Na     | K     | Ca   | Mg   |
|--------------------|--------|-------|------|------|
| Control            | 0.64 ab | 138.6a | 1.12 ab | 1.98 a |
| AVG* @ 125 ppm     | 0.66 a  | 124.8 ab | 1.18 a | 1.90 a |
| AVG + ETH @ 150 ppm (2 and 1 WBFH) | 0.59 ab | 131.2 ab | 1.15 ab | 1.76 a |
| AVG + ETH @ 300 ppm (2 WBFH) | 0.48 b  | 114.9 b  | 0.85 bc | 1.72 a |
| AVG + ETH @ 150 ppm (2 and 1 WBFH) | 0.51 ab | 120.0 b  | 0.70 c  | 1.75 a |
| AVG + ETH @ 300 ppm (1 WBFH) | 0.49 b  | 131.1 ab | 0.85 bc | 1.91 a |
| Harvest            |        |       |      |      |
| 10 Sept            | 0.64 a  | 138.6 a | 1.14 a | 2.20 a |
| 8 Oct              | 0.49 b  | 115.0b  | 0.81 b  | 1.47 b |

*Means in a column within bioregulators or harvest not followed by a common letter are significantly different (P ≤ 0.05).
*AVG applied 4 weeks before anticipated harvest.
*ETH applied 2 weeks before anticipated harvest
*ETH applied 1 week before anticipated harvest.

Table 6. AVG and ETH effects on selected mineral concentrations in apple juice from ‘Scarletspur Delicious’ apples (Trial 3).

| Parameter          | Na     | K     | Ca   | Mg   |
|--------------------|--------|-------|------|------|
| Control            | 0.29 b | 100.8 b | 1.19 b | 1.45 b |
| AVG* @ 125 ppm     | 0.47 a  | 100.5 b | 1.15 b | 1.47 b |
| ETH @ 75 ppm (2 and 1 WBFH) | 0.45 a  | 105.2 ab | 1.30 ab | 1.64 ab |
| ETH @ 150 ppm (2 WBFH) | 0.21 b  | 111.3 ab | 1.44 a | 1.66 ab |
| AVG + ETH @ 75 ppm (2 and 1 WBFH) | 0.25 b  | 108.7 ab | 1.27 ab | 1.56 b |
| AVG + ETH @ 150 ppm (2 WBFH) | 0.21 b  | 113.1a  | 1.37 ab | 1.78 a |
| Harvest            |        |       |      |      |
| 23 Sept            | 0.35 a  | 113.1 a | 1.42 a | 1.81 a |
| 21 Oct             | 0.28 b  | 100.1 b | 1.15 b | 1.38 b |

*Means in a column within bioregulators or harvest not followed by a common letter are significantly different (P ≤ 0.05).
*AVG applied 4 weeks before anticipated harvest.
*ETH applied 2 weeks and again 1 week before anticipated harvest.
*ETH applied 1 week before anticipated harvest.
sorbitol concentrations compared to control juice, but glucose and fructose levels were unchanged (Table 3). ETH either alone or applied after AVG had no effect on Brix or any of the sugars measured compared to control juice samples. However, either ETH treatment following application of AVG returned total juice Brix and sucrose concentrations to control levels. As in the previous two trials, delaying harvest 4 weeks increased Brix, sucrose and sorbitol concentrations and reduced the amount of fructose present in apple juice. In this trial, juice glucose content was reduced at the later harvest date. This observation is in contrast to juice glucose content was reduced at the later harvest date. This observation is in contrast to juice glucose content was reduced at the later harvest date.

AVG alone had no influence on juice total acidity and the individual acids (quinic, malic and citric) compared to juice from control apples (Table 3). AVG effects on acid levels parallel the results reported in Table 2 except for citric acid, which was reduced by AVG. Changes in juice total acidity and the levels of quinic, malic and citric acids were not as clear when ETH was applied at 75 and 150 ppm (Table 3) than when applied at 150 and 300 ppm (Table 2). The results from this trial suggest that ETH must be applied at concentrations at or above 150 ppm to have a significant effect on acid levels. As with earlier results, delay of harvest reduced total acidity and the amounts of the three individual acids evaluated in apple juice.

**Trial 1 (2000).** Application of ETH had little or no influence on the mineral (Na, K, Ca, Mg) concentrations of apple juice compared to juice from control apples (Table 4). There was a decrease in juice K concentration when ETH was applied at 150 ppm 1 WBFH, but the reduction was very slight and would not be considered of interest. Delayed harvest reduced the Ca and Mg concentrations of juice but had no influence on the concentrations of Na or K. It is doubtful that any of the mineral changes due to ETH application or delay in harvest would have a significant impact on apple juice quality.

**Trial 2 (2001).** Application of AVG alone had no influence on the juice concentrations of Na, K, Ca, or Mg in treated apples (Table 5). Application of ETH after AVG had no effect on the Na, or Mg concentrations in juice. A few of the combination treatments of AVG and ETH produced small but statistically significant reductions in either or both K and Ca concentrations of apple juice. The amount of Ca present in apples has been directly related to multiple apple disorders (Fallahi et al., 1988; Fallahi and Simons, 1996). Delay of harvest resulted in a reduction in juice concentration of all minerals measured.

**Trial 3 (2002).** AVG applied alone the final year of the study increased the Na concentration of apple juice, but had no additional influence on other selected minerals (Table 6). The influence of AVG on Na concentration in 2002 may be related to the fact that juice Na concentration was much lower in 2002 as compared to the previous year (2001, Table 5). The juice concentrations of K, Ca, and Mg were similar among years. There were few significant differences in juice mineral concentrations associated with treatments, and it is unlikely that any of these differences would have materially affected either storability or perceived fruit quality. Delay of harvest decreased all mineral concentrations.

**Conclusions**

Preharvest ETH treatment of ‘Scarletspur Delicious’ apples enhanced the Brix and sucrose concentrations and reduced fructose concentration in juice. Effects on glucose and sorbitol levels were not consistent among years in this study. Exposure to either higher ETH concentration or to a longer preharvest ETH-to-harvest interval appeared to have a stronger influence than application closer to first harvest and/or at lower concentrations. Glucose content of apple juice was not consistently affected, regardless of the bioregulator used or the time of harvest. ETH reduced juice total acidity and, when applied early, and particularly at higher concentrations, reduced individual juice acid (quinic and malic) concentrations. Use of AVG reduced both juice Brix value and sucrose concentration in juice but had no influence on either juice total acidity or individual acid concentrations. Applying ETH following AVG tended to reverse the negative effects of AVG on solids and individual sugar levels, while pre-treatment with AVG appeared to partially mitigate ETH-induced reductions in acidity in juice. Increased juice Brix values coupled with reduced total acidity would improve the overall solids/acid ratio, thereby possibly having a strong positive influence on the sensory quality of ‘Delicious’ apples. Use of ETH, AVG, or a combination of AVG and ETH had little or no consistent influence on the juice mineral content of ‘Delicious’ apples and thus would not be expected to affect mineral-nutrient-related fruit quality characteristics.

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