Physiological Changes Associated with Increased Lateral Branching of Apple Trees Treated with Aminoethoxyvinylglycine

Eric A. Curry

Department of Horticulture and Landscape Architecture, Washington State University, Pullman, WA 99164

Max W. Williams

ARS/USDA, Tree Fruit Research Laboratory, 1104 N. Western Avenue, Wenatchee, WA 98801

Abstract. In late September of 1981 and 1982, eight-year-old ‘Oregon-spur Delicious’ apple (Malus domestica) trees on M7 rootstock were sprayed immediately after harvest with 500 ppm AVG. The following spring of both years the number of spur leaves and lateral shoots on one-year-old wood was increased on treated trees. Total N was reduced and sucrose and fructose were increased in dormant one-year-old shoots of AVG-treated trees. Cold hardiness was not affected. Throughout the dormant period both apical and one-year-old lateral buds excised from treated trees and incubated in the dark at 24°C produced less ethylene over a period of 24 hr than buds from untreated trees. In situ ethylene production from apical buds of treated trees was also reduced as growth resumed in the spring. Chemical name used: N-(phenylmethyl)-6H-purin-6-amine (AVG).

AVG was first identified in 1973 (15). An analog of rhizobitoxine, it inhibits the biosynthesis of 1-amino-1-cyclopropane-1-carboxylic acid, (ACC) the immediate precursor of ethylene (22). Recently the involvement of ethylene in apical dominance and bud break was briefly reviewed (21).

This study was undertaken to determine if fall-applied AVG altered carbohydrate and N reserves in shoot tissue and to establish if these influenced lateral branching the following spring. We also extended our earlier studies that indicated increased carbohydrates might increase hardiness of treated trees. The persistence of AVG in the tissue was examined by measuring the inhibition of ethylene production in apical and lateral buds throughout the dormant period.

Materials and Methods

In September of 1981, twenty-four 8-year-old ‘Oregon-spur Delicious’ trees of similar size, vigor, and cropping history on Malling 7 (M7) rootstock were randomly assigned to one of 2 treatments. Immediately following harvest 12 trees were sprayed to the drip point with 500 ppm AVG plus 0.1% Regulaid, a commercial nonionic surfactant. Control trees were treated with water plus Regulaid. Six of the 12 trees in each treatment were used for growth measurements the following spring. These measurements were made by selecting ten 1-year-old shoots of similar length and caliper and removing them in early August the following year for measurements of new growth. The 6 trees were sampled periodically for tissue analysis during the dormant period. In September of 1982, the same experiment was repeated using different trees in the same experimental block.

Total N and carbohydrate analysis. At the time of treatment and at about 3-week intervals thereafter, the distal 30-cm section of eight 1-year-old shoots from each tree was removed. The shoots from each tree were pooled and dried for 4 days in a convection oven at 50°C. The tissue was ground in a Wiley mill to pass a 40-mesh screen and kept in a dessicator at −20°C until analysed. Total N was measured on a Technicon Autoanalyzer II (16). Fructose, glucose, sucrose, and sorbitol were analyzed using a Hewlett-Packard 5880A gas chromatograph equipped with a 1.85 m × 0.63 cm I.D. column of 3% SE-30, 80–100 mesh and an FID detector according to methods previously described (19, 20).

Hardiness examination. In January and February, the 30-cm distal section of eight 1-year-old shoots from each tree was removed for cold hardness evaluation using the regrowth and conductivity method of Ketchie et al. (12) modified as follows. The proximal 15-cm section of the shoot was used for conductivity measurements. These were cut into 1-cm pieces, subdivided into 4 groups of 4 g each, and subjected to the following temperatures: 0°C, −20°C, −30°C, and −40°C. Samples were frozen at a cooling rate of 2.5°C/hr, removed after one hr at the test temperature, and thawed for 2 hr at room temperature. Twenty ml of deionized water were added to each sample, and after 24 hr at 22°C, conductivity was determined with an electrical conductivity bridge (Leeds and Northrup Model 4959).

The distal 15-cm portion of the one-year-old shoots used for the regrowth test was subjected to the same temperatures as samples used to measure conductivity. Their bases were recut after freezing and placed in distilled water under diffuse light at 26°C for 4 weeks. After that time, bud and leaf growth and discoloration of the basal end were rated.

Ethylene production from excised buds. In late November, December, January, and February, 30 lateral vegetative buds
were excised from each tree used for tissue analysis. Ten buds were placed in each of 3 separate 1.2 × 7.6 cm test tubes, sealed with a serum cap and placed for 24 hr in an incubator maintained at 24°C. Every 3 hr a 1-ml gas sample was removed from each test tube. After sampling, the tubes were opened, flushed with a slow stream of compressed air, and resealed. Ethylene was analyzed with a Hewlett-Packard 5880A gas chromatograph equipped with an FID and a 2-m Porpak Q 100/200-mesh column (2 mm I.D.) maintained at 90°C. Blank sample test tubes were analyzed for ethylene, and any background was subtracted from sample peaks.

Ethylene production from apical buds in situ. Beginning in late February, 10 shoot tips were selected on each of the trees used for growth measurements. A rubber sleeve stopper (13 × 20 mm I.D. × O.D.) with a hole 2 mm in diameter in the center was pushed over the terminal bud. A 20-ml test tube which had been cut to a volume of 6 ml was fitted onto the stopper and covered with a aluminum foil. This unit enclosing the 2-cm portion of the apical shoot tip remained in place for 4 hr. At that time a 1-ml gas sample was withdrawn through the rubber sleeve with a syringe and analyzed for ethylene as previously described. Measurements were made weekly at about the same time of day through the green tip stage.

Results and Discussion

Results from fall applications of AVG in 1981 and 1982 were similar. Therefore only the results from 1982 will be presented except when data from the 2 year differ significantly.

Growth measurements. Fall-applied AVG increased terminal growth, total lateral growth on 1-year wood, the number of 1-year branches longer than 1 cm in length, and the number of leaves per spur (Table 1). The increase in the number of leaves per spur appeared to be the result of new shoots being initiated but not elongating. Generally, the treated trees had a much bushier appearance due to the increase in foliage. Leaves on treated trees had a lighter color initially, as previously noted by Williams (18); however, this effect had disappeared by mid-June. Leaf size was also slightly larger shortly after bloom; however, this difference did not persist (data not shown).

Greene (8, 9) treated young spur-type 'Delicious' trees with 500 ppm AVG at 4 days before harvest and the following spring at full bloom, and at 2 weeks after full bloom. Spring applications increased the number of lateral branches but decreased the total lateral branch growth. The major effect of fall applications was to increase the number of leaves as well as the leaf size. We also found an increase in leaf weight in 1981, but not in 1982.

Total N and carbohydrate analysis. The main source of remobilized N in leaves is protein that may decrease up to 50% during leaf senescence (17). Harley et al. (10) reported a positive correlation between the level of storage N and the extent to which shoots extended the following spring. Trees treated with 500 ppm AVG in September often retained their leaves up to 4 weeks longer than untreated trees. We examined the possibility that leaf retention might prolong N remobilization, thereby increasing N available in the bud for the following year. Beginning 3 weeks after treatment, total N in one-year-old wood of treated trees was consistently lower than in untreated trees and remained so until silver-tip stage at which time it increased to levels similar to controls (Fig. 1). Thus, the inhibition of leaf abscission did not increase movement of N from the leaves to the above ground woody tissue. At higher concentrations, AVG has been reported to inhibit protein synthesis (13) and therefore may have prevented the synthesis or degradation of enzymes involved in this process (11). Even though the leaves often remained on the trees several weeks longer than untreated trees, they retained much of their pale-green color during that period, suggesting an inhibition of senescence.

Treated trees contained more sucrose and fructose in one-year-old wood tissue during the dormant period (Fig. 1), although there was no effect on the level of either glucose or sorbitol (data not shown).

Evaluation of cold hardiness. Several studies have related carbohydrate content of woody tissue of cold hardiness (3, 4, 12, 19). Sorbital (D-glucitol) is a major source of carbohydrate translocated through the phloem (1). Trees treated with AVG in the fall of 1982 were slightly hardier than controls at -20°C (ns), and no hardier at lower temperatures (data not shown).

Ethylene production in excised lateral buds and apical buds in situ. Lateral buds excised in late November from 2-year-old wood of trees treated with AVG and held for 24 hr at 24°C showed reduced levels of ethylene production (Fig. 3). Suppression of ethylene production persisted throughout the dormant period until shortly after green tip (data not shown). It

### Table 1. Effect of 500 ppm AVG applied to 8-year-old 'Oregon-spur Delicious' immediately after harvest in 1981 or 1982 on shoot growth and leaf number measured in July the year following treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Terminal shoot length (cm)</th>
<th>No. lateral buds</th>
<th>Wt. shoot leaf (g)</th>
<th>Branches &gt;1 cm prior yr</th>
<th>Total lateral growth (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>43.4</td>
<td>7.2</td>
<td>2.3</td>
<td>1.3</td>
<td>8.6</td>
</tr>
<tr>
<td>AVG</td>
<td>56.1***</td>
<td>8.5</td>
<td>2.8</td>
<td>4.2**</td>
<td>29.8**</td>
</tr>
<tr>
<td>Control</td>
<td>36.6</td>
<td>6.2</td>
<td>3.4</td>
<td>1.1</td>
<td>9.4</td>
</tr>
<tr>
<td>AVG</td>
<td>45.0</td>
<td>11.4**</td>
<td>3.9</td>
<td>4.7**</td>
<td>43.8**</td>
</tr>
</tbody>
</table>

*Significantly different from control at the 5% (*) or 1% (**) level by Student's t test.

Fig. 1. Effect of 500 ppm AVG applied 24 Sept. 1982 to 'Oregon-Spur Delicious' apples on the percentage of total N throughout the dormant period in one-year-old shoot tissue. Bars represent + or − SE.
was necessary to monitor the ethylene production for at least 6 hr since differences were often not apparent after the first 2-hr reading. Temperatures above 35°C and below 20°C inhibited ethylene production in bud tissue (data not shown). This response to temperature is similar to that reported for apple fruit tissue (23) and avocado (5). Our data supports the results of others who have also shown that fall applications of 500 ppm AVG inhibited ethylene production in apple flowers the following spring (8, 10, 18).

Ethylene evolution from detached apical buds followed the same pattern as in lateral buds; however, too few buds were available for reliable data. Excised internodal tissue from one- or 2-year-old wood produced very little ethylene 9 hr after excision. This low ethylene production might have been due to the difference in volume and activity of the meristematic tissue in the bud vs. the internodal tissue. No carry-over effect of treatment with AVG in the fall of 1981 was detected in the winter of 1982–83.

Ethylene production from intact shoot tips of trees treated with AVG in the fall was indistinguishable from untreated trees until early bud break the following spring; at that time they continued to produce less than apices from control trees through the prepink stage (Fig. 4).

AVG applied at the rate of 500 ppm to mature spur-type ‘Delicious’ trees immediately after harvest reduces ethylene production in apical and lateral buds through the winter. Possibly, leaves metabolize or dilute the compound to the point where inhibition can no longer be detected. (The presence of AVG in the tissue was not chemically determined; however, the consistency of results over several years suggests the inhibition of ethylene might serve as a good indicator of its presence in the


239
tissue.) By early spring, one-year-old woody tissue contains an increased concentration of both sucrose and fructose. Even though total N is reduced during dormancy in one-year-old wood of AVG-treated trees, the level exceeds that in untreated trees shortly after bud break. The tree appears to be in a state of suppressed metabolism. The suppression of C2H4 production may also aid in relieving any ethylene induced restriction on growth (7).

AVG may inhibit protein synthesis (13). Inhibition of "auxin-like" compounds by AVG has been demonstrated in the apices of ‘Bartlett’ pear seedlings (2). This inhibition may interfere with apical dominance long enough to allow lateral buds to escape from control by the apical bud.

The type of lateral growth seen on ‘Delicious’ trees treated with 500 ppm AVG in the fall could be advantageous in the “feathering” of young trees or to promote lateral branching in trees afflicted with disorders such as “dead spur” (14).

**Literature Cited**