‘McIntosh’ apple fruit thinning by benzyladenine in relation to seed number and endogenous cytokinin levels in fruit and leaves

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Accepted 8 February 2000

Abstract

The relationship of 6-benzylaminopurine (BA)-induced fruitlet abscission to seed number and endogenous cytokinin levels in the fruit and leaves was examined in ‘McIntosh’ apples (Malus domestica Borkh.). BA applied at the 10 mm stage of fruit development effectively thinned apple fruit and increased fruit size. The seed number of abscising fruit increased as BA concentrations increased. In persisting fruit, BA reduced the number of normal seeds, increased the number of aborted seeds, but had no influence on the number of total seeds. The total number of seeds was significantly higher in persisting fruit than in abscising fruit. BA increased zeatin riboside levels in the fruit 2 days after application, but it had no effects on zeatin levels in the fruit. The levels of zeatin and zeatin ribosides in the leaves were not affected by BA application. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Malus domestica; Cytokinin; BA; Fruit thinning; Fruit set; Seed number

1. Introduction

Early fruitlet thinning removes excessive fruitlets from apple trees and is one of the most effective measures to increase fruit size, color, and quality in the
year of application, and to promote flowering the following year (Childers et al., 1995). Several reports have confirmed that benzyladenine (BA) is an effective chemical thinner on many cultivars (Greene et al., 1990; Bound et al., 1991; Elfving and Cline, 1993; Greene, 1993; Wismer et al., 1995). BA effectively thins fruit when 50–100 mg l\(^{-1}\) are applied at the 10 mm stage of fruit development. It thins more effectively when applied to the leaves than to the fruit (Greene and Autio, 1989; Bound et al., 1991; Greene et al., 1992). Accel, an altered Promalin formation that contains 90% BA and 10% GA\(_{4+7}\), was registered recently for commercial use as a chemical thinner for apples. It is unclear whether the thinning effect of BA on apples is due to BA itself, or to an increase in the natural cytokinin levels. BA is one of the most active cytokinins (Bayley et al., 1989; Matsubara, 1990), and recently, BA has been identified as a natural cytokinin in a number of plants (Van Staden and Crouch, 1996). Nevertheless, physiological responses to BA application may be associated with increased endogenous cytokinin concentrations (Kuiper et al., 1989; Van Staden and Crouch, 1996).

In general, there is a positive relationship among seed number, fruit size, and fruit set in apples, since seeds are rich sources of hormones (Luckwill, 1953; Crane, 1969). Fruit which contain the fewest or weakest seeds are usually the first to drop (Heinicke, 1917; MacDaniels, 1928; Murneek, 1954; Childers et al., 1995). Fruit size usually increases with increasing seed number (Heinicke, 1917; Denne, 1963; Dennis, 1986). While optimum seed number may vary with cultivar, a minimum of eight seeds was required to achieve maximum size of ‘Delicious’ apple fruit (Williams, 1977).

The objective of this experiment was to examine the effect of BA application on apple fruit thinning, seed number of abscising and persisting fruit, and endogenous cytokinin levels in leaves and fruit.

2. Materials and methods

Twenty-four mature ‘Morespur McIntosh’/M7 apple trees growing at University of Massachusetts Horticultural Research Center, Belchertown, MA, USA, were selected and grouped into eight blocks of three trees each based on vigor and blossom cluster number. A randomized complete block design was used. Two limbs on each tree, 12–15 cm in diameter, were tagged and all blossom clusters were counted before bloom. One tree in each block received a spray of BA (ABG-3062, Abbott Laboratory, North Chicago, IL) at either 50 or 100 mg l\(^{-1}\) on 30 May 1996, at the 10 mm stage of fruit development. One tree in each block was not sprayed and served as control. All fruit remaining on tagged limbs were counted at the end of the ‘June drop’ period in July. Fruit weight was evaluated at commercial harvest.
The apple fruit abscission peak often occurs 10–17 days after BA treatment applied at the 10 mm stage of fruit development (Yuan and Greene, 2000a,b). Therefore, 30 persisting fruits and 30 fruits which were starting to show signs of abscission as evidenced by a yellowing pedicel, were collected from each tree 13 days after BA application. The number of healthy and aborting seeds in each fruit were counted.

Twenty fruits and 50 spur leaves were collected from each tree at 0, 2 and 5 days after BA application, and were washed with distilled water. All samples were lyophilized and ground to pass a 40-mesh screen. The method of Wagner and Beck (1993) was modified to extract and purify plant materials for cytokinins. Briefly, 2 g of ground leaf or fruit material were placed in an Erlenmeyer flask. To each Erlenmeyer flask, 40 ml of 80% methanol containing 40 mg l\(^{-1}\) butylated hydroxytoluene (BHT) as an antioxidant was added, and the tissue was then extracted overnight at 4°C. The crude extract was filtered through Whatman No. 2 paper and the residue was re-extracted twice in 80% methanol containing 40 mg l\(^{-1}\) BHT overnight at 4°C. The filtrates were combined, and evaporated to the aqueous phase in vacuo at 35°C. The aqueous phase was adjusted to pH 3.1 with 1 N acetic acid and passed through a column (10 cm×1.5 cm) of insoluble PVP, and the column was washed with 20 ml acidified water (pH 3.1, with dilute acetic acid). The combined elutes then passed through a DEAE-cellulose column (Whatman, DE-52, 10 cm×1.5 cm) that was first preconditioned with acidified water (pH 3.1, with dilute acetic acid). The column was washed with an equal column of acidified water (pH 3.1) and subsequently the cytokinins were eluted with 40 ml of 2 N NH\(_3\)OH. The elutes were further purified by reversed phase chromatography using a Sep-Pak C\(_{18}\) Cartridge (Waters, Millipore, 0.5 cm×1.5 cm, preconditioned with 1 column length of pure methanol and 1 column length of 80% methanol), which was rinsed with 10 ml of water before cytokinins were eluted with 5 ml of 90% methanol. The eluates were dried in vacuo at 35°C and dissolved in 1 ml of 100% HPLC grade methanol.

The samples were further purified by HPLC according to the method of MacDonald et al. (1981) with some modification. In brief, 200 µl of samples were applied to an analytical Octadecylsilica column (250 mm×4.6 mm, 5 µm particle size, Beckman Ultrasphere) at a flow rate of 1.0 ml min\(^{-1}\) with a 15–80% (v/v) methanol gradient over a period of 30 min in a buffer of 0.1 M sodium acetate (pH 3.5), followed by an increase to 100% methanol over 3 min. Fractions corresponding to zeatin and zeatin ribosides were collected, freeze-dried and redissolved in phosphate buffered saline (PBS) before assaying by radio-immunoassay (RIA). The detailed RIA procedures described by Weiler (1980) were used to quantify cytokinins. One hundred µl of \(^{3}\)H-zeatin riboside (approximately 5000 DPM) was added as internal standard to the plant samples to determine the recovery rate. The recovery rate for the zeatin riboside was about 49.3%.
3. Results

BA effectively thinned ‘McIntosh’ apples (Table 1). The response to BA concentration was primarily linear. Fruit weight at harvest increased with increasing concentrations of BA.

The seed number of abscising fruit during ‘June drop’ increased linearly with concentrations of BA applied (Table 2). In persisting fruit, BA linearly decreased

### Table 1
Effects of BA on fruit set and fruit weight at harvest of ‘McIntosh’ apple in 1996

<table>
<thead>
<tr>
<th>BA (mg l⁻¹)b</th>
<th>Blossom cluster/cm² limb X-sect. area</th>
<th>Fruit/cm² limb X-sect. area</th>
<th>Fruit wt. (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.1</td>
<td>6.3</td>
<td>156</td>
</tr>
<tr>
<td>50</td>
<td>7.0</td>
<td>3.9</td>
<td>163</td>
</tr>
<tr>
<td>100</td>
<td>6.8</td>
<td>3.0</td>
<td>181</td>
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</tbody>
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**Significance**

<table>
<thead>
<tr>
<th>L</th>
<th>NSd</th>
<th>**</th>
<th>***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

a Mean of eight observations.
b BA was applied on May 30, 1996, at 10 mm stage of fruit development.
c Fruit set on July 17, 1996.
d Nonsignificant.

** Significant at $P=0.01$.

*** Significant at $P=0.001$.

### Table 2
Effects of BA on seed count of abscising and persisting fruit of apples during ‘June drop’ (1996)

<table>
<thead>
<tr>
<th>BA (mg l⁻¹)b</th>
<th>Total seeds abscising (no.)</th>
<th>Persisting</th>
<th>Normal seeds (no.)</th>
<th>Aborted seeds (no.)</th>
<th>Total seeds (no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.1</td>
<td>9.5</td>
<td>0.4</td>
<td>9.9</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>7.7</td>
<td>8.6</td>
<td>1.1</td>
<td>9.6</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>8.0</td>
<td>8.2</td>
<td>1.6</td>
<td>9.7</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>7.6 e**</td>
<td>8.8 b</td>
<td></td>
<td>9.7 a</td>
<td></td>
</tr>
</tbody>
</table>

**Significance**

<table>
<thead>
<tr>
<th>L</th>
<th>**</th>
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<tr>
<td>Q</td>
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<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

a Mean of seven observations.
b BA was applied on May 30, 1996, at 10 mm stage of fruit development.
c Mean followed by different letters within the row differ significantly at $P=0.05$ level according to Duncan’s multiple range test.
d Nonsignificant.

** Significant at $P=0.01$.

* Significant at $P=0.05$. 
the number of normal seeds, increased the number of aborted seeds, but had no effect on the number of total seeds. Regardless of BA application, the number of total seeds was higher in persisting fruit than in abscising fruit.

BA did not influence zeatin levels in ‘McIntosh’ apple fruit in the 5 days following application, but it increased zeatin riboside levels by approximately 80% 2 days after BA application (Fig. 1). The zeatin and zeatin riboside levels in both BA-treated and control apple leaves decreased with time, and they were not affected by BA application (Fig. 1).

4. Discussion

Seed number is positively related to fruit set, retention (Heinicke, 1917; MacDaniels, 1928; Luckwill, 1948; Murneek, 1954; Dennis, 1986) and growth (Denne, 1963) primarily because they are a rich source of hormones (Luckwill, 1948; Dennis, 1986). In our investigation, total seed number was lower in abscising fruit than in persisting fruit, irrespective of BA treatment (Table 2). Further, BA treatment increased the seed number in abscising fruit, indicating
that it induced abscission of multiseeded fruit that would normally have persisted. Seeds or seed-derived hormones presumably affect fruit retention indirectly (Dennis, 1986). Cell division occurs rapidly in fruit when BA is applied as a chemical thinner (Denne, 1963; Wismer et al., 1995), and fruitlets at this stage function as utilization sinks (Gillaspy et al., 1993; Mehouachi et al., 1995). High levels of hormones in the seed are thought to cause diversion of metabolites to the fruit and enable them to compete more efficiently with other growing organs of the plant (Crane, 1969). Therefore, fruit with more normal seeds should have higher levels of hormones and thus higher metabolic activity or higher sink strength, leading to their successful survival in the competition with other fruit, which have relatively lower seed number, and bourse shoot tips. The weak sinks, such as fruit with relatively lower seed number, will compete less effectively with strong sinks, and thus finally abscise (Addicott, 1982).

Fruit growth after bloom is dependent in large part on photosynthates supplied by spur leaves (Lakso, 1994). Shoot leaves do not become net exporters of carbohydrates until 10–12 leaves develop, usually when shoot length is 25–30 cm (Johnson and Lakso, 1986). When BA is applied to apple trees, the net photosynthesis of leaves is reduced and the carbohydrates available to developing fruits are further reduced (Yuan and Greene, 2000a,b). This leads to intensified competition among fruit, and between fruit and vegetative growth, thereby causing more fruits with a relatively high seed number to abscise. After the completion of ‘June drop’, fruit growth is attributed mainly to the cell enlargement (Wismier et al., 1995), and fruit at this stage act as storage sinks accumulating high levels of carbohydrates (Gillaspy et al., 1993; Mehouachi et al., 1995). Carbohydrate stress at this time will affect fruit growth but will not cause fruit abscission (Beruter and Droz, 1991; Mehouachi et al., 1995).

BA thins apple fruit more effectively when applied to the leaves than to the fruit (Greene et al., 1992). We have presented evidence that BA thins apple fruit by increasing dark respiration and decreasing net photosynthesis of apple leaves, thus leading to a limited carbohydrate supply to developing fruitlets (Yuan and Greene, 2000a,b). Our results that BA had no influence on the cytokinin levels in the leaves (Fig. 1) indicate that BA thins apple fruit not directly through affecting endogenous cytokinin levels in the leaves.

BA increases the rate of cell division in the fruit cortex (Wismier et al., 1995), and thus increases fruit size independent of its effects on reducing crop load, but only when applied directly to the fruit (Greene et al., 1992). The dramatic increase in zeatin riboside levels in fruit after BA application (Fig. 1) suggests that BA promotes cell division in apple tissue possibly by increasing zeatin riboside levels in the fruit. This suggestion is further supported by the observation that BA increases fruit size maximally at the 8–12 mm stage of fruit development (Greene, 1993), a time that coincides with maximum cell division within the fruit (Wismier et al., 1995).
References


