Benzyladenine as A Chemical Thinner for ‘McIntosh’ Apples. I. Fruit Thinning Effects and Associated Relationships with Photosynthesis, Assimilate Translocation, and Nonstructural Carbohydrates

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ABSTRACT. BA was applied at 50 or 100 mg·L⁻¹ to ‘More-Spur McIntosh’/Malling 7 (M.7) apple trees [*Malus sylvestris* (L.) Mill var. *domestica* (Borkh.) Mansf.] at the 10 mm stage of fruit development. BA thinned fruit and increased fruit size. There were two distinguishable peaks of fruit abscission during ‘June drop’. BA accentuated the naturally occurring waves of fruit abscission, and enhanced translocation of ¹⁴C-sorbitol from leaves to fruit when applied directly to the fruit, but not when applied directly to the leaves. Net photosynthesis was decreased and dark respiration was increased when temperature following BA application was high (30 °C), whereas there was no effect when temperature was lower (20 °C). Total nonstructural carbohydrates, total soluble sugars, and starch in the leaves decreased dramatically over the 12- or 13-day observation period, regardless of BA treatment. These carbohydrate concentrations in the leaves were lowered further by BA application. Abscising fruit, based on specific reddening of the pedicel, had higher carbohydrate levels than persisting fruit, regardless of BA application. We conclude that BA thins fruit, at least in part, by increasing dark respiration and decreasing net photosynthesis. Chemical name used: N-(phenylmethyl)-1H-purine-6-amine [benzyladenine (BA)].

Fruit thinning is one of the most important management practices a commercial grower is required to do to produce high quality apples (*Malus sylvestris* var. *domestica*). Thinning improves fruit size, color, and quality at harvest, and increases return bloom the following year, thereby reducing alternate bearing (Childers et al., 1995). Chemical thinning is superior to hand thinning because it is less expensive and it can be done earlier (Childers et al., 1995).

Since the initial suggestion by McLaughlin and Greene (1984) that BA might be a good chemical thinner for apples, there have been a number of reports that have confirmed its effectiveness as a chemical thinner on many apple cultivars (Bound et al., 1991; Elfving and Cline, 1993; Greene et al., 1990; Wismer et al., 1995). Recently Accel (Abbott Laboratory, North Chicago, IL), an altered Promalin formulation that contains 90% BA and 10% gibberellins₄₊₇ (GA₄₊₇), was registered for use as a chemical thinner for apples. The mechanism whereby BA thins is unclear. Previous reports showed that BA at 50 to 100 mg·L⁻¹ is generally required to thin effectively (Bound et al., 1991; Greene and Autio, 1989; Greene et al., 1990), and the maximum thinning response occurs when BA is applied at the 10 to 14 mm stage of fruit development, which is frequently ≈14 to 21 d after full bloom (Bound et al., 1991; Greene and Autio, 1989). BA, similar to another chemical thinner naphthaleneacetic acid (NAA) (Schneider, 1978), thins apples more effectively when applied to the leaves than to the fruit (Greene et al., 1992). Lack of significant thinning can not be attributed to lack of BA absorption by the fruit because considerably more BA enters the fruit than enters through either surface of the leaves (Greene et al., 1992). BA treatments that thinned also increased ethylene production in both

leaves and fruit 24 h after application, but the magnitude of increase was quite small and not considered large enough to be the primary cause for thinning (Greene et al., 1992).

Carbohydrates have been implicated in fruit set and fruit development. It has been suggested that the mechanism of fruit thinning by NAA is due to reduced energy available to developing fruit either by interference with photosynthesis (Stopar et al., 1997) or by the reduced translocation of metabolites, including photosynthates, from leaves to the fruit (Schneider, 1975, 1978). Two days of artificial shade decreased photosynthesis, reduced the carbohydrates available to the fruitlets, and induced more apple abscission than NAA, ethephon (2-chloroethylphosphonic acid), or carbaryl (1-naphthalenyl methylcarbamate) + oil spray. Shading of whole trees for 3 d caused 98% fruit abscission (Byers et al., 1991). Fruit from artificially shaded trees or those from trees receiving the photosynthetic inhibitor terbacil (3-tert-butyl-5-chloro-6-methyluracil) had lower total sugars and reducing sugars than those not treated (Polomski et al., 1988). However, Abruzzese et al. (1995) reported that abscised apples had higher levels of soluble reducing sugars and sucrose than persisting fruit. Also, abscising naval orange fruit [*Citrus sinensis* (L.) Osbeck] had higher reducing sugars and total sugars than persisting fruit (Ruiz and Guardiola, 1994).

The overall goal of this investigation was to determine the mode of action of BA as a chemical thinner on apples. To accomplish this, the specific objectives of this study were to 1) evaluate the effect of BA on the translocation of assimilate from leaves to young growing fruit; 2) measure net photosynthetic rates and mitochondrial respiration rates of apple leaves at different temperatures following BA application; and 3) determine the influence of BA on the nonstructural carbohydrate status in the leaves and fruits of apples and its relation to apple abscission.

Materials and Methods

All orchard experiments were conducted at the University of Massachusetts, Horticultural Research Center, Belchertown, Mass.

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¹⁴C-ASSIMILATE MOVEMENT IN 1995 (EXPT. 1). Fruiting spurs of 'More-Spur McIntosh'/Malling 7 (M.7) apples were excised on 31 May 1995 when average fruit size was 10 mm. They were placed in water and transported to the laboratory. Thirty uniform spurs with three leaves and a 10 mm diameter size fruit were selected, and placed in individual flasks with distilled water at room temperature ($\approx 27^\circ\text{C}$). A randomized complete block design with 10 replications was used. Leaves on one group of 10 spurs and fruit on another group of 10 spurs were dipped into BA solution (ABG-3062, Abbott Laboratory, North Chicago, Ill.) at $100\text{ mg}\cdot\text{L}^{-1}$. The third group of 10 spurs were not treated and served as the control. After the BA solution dried, a circle 2 cm in diameter was outlined with dots of India ink on the abaxial surface of one leaf on each spur. Sixteen L^{14}C -sorbitol ($2.96 \times 10^4\text{ Bq}$) were applied within the marked area on the abaxial surface of the leaf using a microsyringe attached to a repeating dispenser (Model PB 600-1; Hamilton, Reno, Nev.). Twenty four hours after treatment, spurs were harvested and separated into fruit, stems, 2 cm marked portions of the treated leaves, the remaining treated leaves, and the other nontreated leaves. The treated leaf disks were each rinsed with 5 mL distilled water containing 0.1% X-77 surfactant and then 5 mL distilled water. The samples were sliced, dried at 70°C for 5 d, and oxidized for 5 min in a biological material oxidizer (model OX-400; Harvey Instrument Corp., Hillside, N.J.). Evolved $^{14}\text{CO}_2$ was collected in an external trap containing 15 mL Harvey ^{14}C cocktail. Radioactivity was determined by a liquid scintillation counter (Model 3801; Beckman, Fullerton, Calif.). Counts were adjusted to account for quenching and for biological oxidizer efficiency.

¹⁴C-ASSIMILATE MOVEMENT IN 1996 (EXPT. 2). The effect of BA on the movement of assimilate from leaves to young growing fruit was similarly evaluated in 1996. Fruiting spurs with average fruit size diameter of 10 mm were harvested on 30 May and treated as above. At 24 and 48 h after application of $16\text{ }\mu\text{L}^{14}\text{C}$ -sorbitol ($2.96 \times 10^4\text{ Bq}$) within the marked area on the abaxial surface of the leaf, spurs were harvested, separated into component parts, and analyzed for ^{14}C as described above.

NET PHOTOSYNTHESIS AND RESPIRATION IN 1996 (EXPT. 3). Twenty four 3-year-old young 'More-Spur McIntosh'/M.7 apple trees growing in 19-L containers outside the greenhouse at the University of Massachusetts were blocked based upon vigor into eight groups (replications) of three trees each. A randomized complete block design was used. One uniform fully expanded leaf (the fifth or sixth from the shoot apex) with good light exposure was tagged. One tree in each replication received a dilute spray of BA at 50 or $100\text{ mg}\cdot\text{L}^{-1}$. One tree in each replication was not sprayed and served as a control. Exchange rate of CO_2 was measured using a portable photosynthesis system (LI-6200; LI-COR, Lincoln, Nebr.) equipped with a 250 mL leaf chamber (total system gas volume = 390 mL). The rectangular area of the leaf enclosed in the closed leaf chamber was 13.6 cm^2 . Measurements were made at 0, 1, 6, and 8 d after BA application. Leaf net photosynthesis (P_n) at high temperature was measured between 9:30 AM and 12:30 PM on clear, sunny days. Environmental conditions during measurements were as follows: photosynthetic photon flux, 1800 to $2000\text{ }\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; air temperature $30 \pm 2^\circ\text{C}$; leaf temperature, $29 \pm 2^\circ\text{C}$; ambient CO_2 concentration, $330 \pm 10\text{ }\mu\text{mol}\cdot\text{mol}^{-1}$. The mitochondrial respiration of leaves at high (air temperature $30 \pm 2^\circ\text{C}$; leaf temperature, $29 \pm 2^\circ\text{C}$) and low temperature (air temperature, $20 \pm 0.5^\circ\text{C}$; leaf temperature, $19.5 \pm 0.5^\circ\text{C}$) were measured using black cloth to cover the leaf chamber and eliminate light. Complete blocks were measured to minimize environmental effects during any one measurement date.

FRUIT THINNING, ABSCISSION, AND NONSTRUCTURAL CARBOHY-

DRATE STATUS IN 1995 (EXPT. 4). Twenty-four mature 'More-Spur McIntosh'/M.7 apple trees were selected and grouped based upon vigor and blossom cluster density into eight blocks of three trees each. A randomized complete block design was used. Two limbs on each tree, 12 to 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) at either 50 or $100\text{ mg}\cdot\text{L}^{-1}$ on 1 June 1995, at the 10 mm stage of fruit development. One tree in each block was not sprayed and served as a 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.

Fifteen fruits and 20 spur leaves were collected from each tree at 0, 5, 8, and 12 d after BA application, and were placed immediately in a container with dry ice and transported to the laboratory. All the samples were dried in a forced-air oven at 70°C for 5 d, weighed, and ground in Wiley mill to pass a 40-mesh (0.635-mm) screen and used for analysis of total soluble sugars and starch.

The method of Belding and Young (1987) was used to extract plant material for total soluble sugars. Total soluble sugars were determined by the colorimetric method of Flood and Priestley (1973). Starch was extracted and hydrolyzed with amyloglucosidase (Sigma, St. Louis, Mo.) (Dekker and Richards, 1971). The glucose concentration produced from the enzymic hydrolysis of starch was assayed according to Flood and Priestley (1973).

FRUIT THINNING, ABSCISSION, AND NONSTRUCTURAL CARBOHY-DRATE STATUS IN 1996 (EXPT. 5). Effects of BA on fruit thinning, and nonstructural carbohydrate levels in leaves and fruits were examined similarly on a block of mature 'More-Spur McIntosh'/M.7 apple trees. Twenty-four trees were selected and grouped based upon vigor and blossom cluster density into eight blocks of three trees each. BA (ABG-3062, Abbott Laboratory, North Chicago, IL) was applied on 30 May 1996, at the 10-mm stage of fruit development. Fruit set and fruit quality were determined similarly to the manner described in Expt. 4. Fruits and leaves for analysis of total soluble sugars and starch, were collected at 0, 5, 9, and 13 d after BA application, and were determined as described previously.

FRUIT THINNING, ABSCISSION, AND NONSTRUCTURAL CARBOHY-DRATE STATUS IN 1997 (EXPT. 6). Effects of BA on the time course of fruit abscission, and the status of nonstructural carbohydrates in persisting and abscising fruit were evaluated in 1997. Eight mature 'More-Spur McIntosh'/M.7 apple trees, were selected and grouped based upon vigor and blossom cluster density into four blocks of two trees each. A randomized complete block design was used. Two limbs on each tree, 12 to 15 cm in diameter, were tagged and all blossom clusters were counted before bloom. One tree in each block was sprayed with BA at $100\text{ mg}\cdot\text{L}^{-1}$ on 6 June 1997, at the 10-mm

Table 1. Effects of BA application to leaves or fruit on the translocation of ^{14}C -sorbitol from leaves to the fruit of 'More-Spur McIntosh' apples (Expts. 1 and 2).^z

Treatments ^y	¹⁴ C (Bq·g ⁻¹ DW)		
	1995	1996	
	24 h ^x	24 h	48 h
Control	800 b ^w	3000 b	4233 b
BA to the fruit	1267 a	3833 a	5000 a
BA to the leaves	1000 ab	2683 b	4467 b

^zMean of 10 observations.

^yBA at $100\text{ mg}\cdot\text{L}^{-1}$ was applied at 10 mm stage of fruit development.

^xTranslocation period.

^wMean separation within columns by Duncan's multiple range test, $P < 0.05$.

Fig. 1. Effects of BA on (A) net photosynthetic rates, and mitochondrial respiration rates of 'More-Spur McIntosh' apple leaves at 30 °C (B) and 20 °C (C) (Expt. 3, 1996). Legend in C applies to all figures. Data are means $\pm$ SE (n = 8).

stage of fruit development. One tree in each block was not treated and served as a control. Fruit on tagged limbs were counted just before BA application and then fruit remaining on tagged limbs were counted every 3 or 4 d.

Two types of fruit were collected 12 d after BA application: persisting fruit which continued to develop to commercial harvest, and abscising fruit of the same age as persisting fruit. Abscising fruit were starting to show signs of abscission, as evidenced by a yellowing pedicel. A third type of fruit, abscising fruit with the same diameter as that of the persisting fruit sampled at 12 d after BA

application, were sampled 24 d after BA application. These fruit were also identified by a yellowing pedicel. Fifteen fruits of each type were collected from each tree, and weighed, and their equatorial diameter was measured. Total soluble sugars, starch, and total nonstructural carbohydrates were assayed as described in Expt. 4.

STATISTICAL ANALYSES. Statistical analyses included analysis of variance, Duncan's multiple range test, *t* test, and orthogonal polynomial comparison. When interactions of main effects were significant, sums of squares were partitioned into the sums of squares of one main effect nested within each level of the other main effect involved in the interaction. Statistical Analysis Systems Software for PC (SAS Inst., Cary, N.C.) was used to analyze these data.

Results

¹⁴C-ASSIMILATE MOVEMENT. Compared with nontreated spurs, more ¹⁴C-sorbitol was translocated from the leaves to the fruit when BA was applied directly to the fruit in a spur (Table 1). However, there was no difference in ¹⁴C-sorbitol movement from leaves to fruit between nontreated spurs and those on which only the leaves were treated. Overall, more ¹⁴C-sorbitol moved from the leaves to fruit in 48 h than in 24 h, regardless of treatments.

NET PHOTOSYNTHESIS AND RESPIRATION. BA at 50 or 100 mg·L⁻¹ equally decreased P_n of apple leaves at 1 and 6 d after treatment, but had no effect at 8 d after treatment (Fig. 1A). At 30 °C, BA treatments increased mitochondrial respiration rates of apple leaves 1 and 6 d after treatment (Fig. 1B). However, mitochondrial respiration rates of apple leaves treated with BA recovered to the control rate by 8 d after treatment. There was no significant difference in mitochondrial respiration rates of apple leaves between BA treatments. At 20 °C, BA had no significant influence on mitochondrial respiration rates of apple leaves (Fig. 1C). Mitochondrial respiration rates of apple leaves were considerably higher at 30 °C than at 20 °C regardless of BA treatment.

FRUIT THINNING, ABSCISSION, AND NONSTRUCTURAL CARBOHYDRATE STATUS. BA effectively thinned 'More-Spur McIntosh' apples in the three experiments reported herein (Table 2). The response to BA concentration was primarily linear. Fruit weight at harvest increased with increasing concentrations of BA (Table 2).

There were two distinguishable fruit abscission peaks during 'June drop' in 'More-Spur McIntosh' apples regardless of BA treatment (Fig. 2). The large and small abscission peaks occurred 14 and 24 d after treatment, respectively. About 54% of then existing fruit on BA treated trees abscised from 10 to 14 d after BA treatment, compared with 25% fruit abscission on control trees.

Table 2. Effects of BA on fruit set and mean fruit weight at harvest of 'More-Spur McIntosh' apples in 3 years (Expts. 4, 5, and 6).^z

BA ^y (mg·L ⁻¹)	1995		1996		1997	
	Fruit/ cm ² LCSA ^x	Fruit wt (g)	Fruit/ cm ² LCSA	Fruit wt (g)	Fruit/ cm ² LCSA	Fruit wt (g)
0	13.2	125	6.3	156	11.3	111
50	9.8	152	3.9	163	---	---
100	7.8	161	3.0	181	5.5	171
Significance						
Linear	**	***	**	***	*** ^w	*** ^w
Quadratic	NS	**	NS	NS		

^zMean of eight observations.

^yBA was applied at 10 mm stage of fruit development.

^xLCSA = limb cross-sectional area.

^wMean separation within columns by *t* test.

NS, **, *** Nonsignificant or significant at *P* < 0.01 or 0.001, respectively.

Generally, the concentration of total nonstructural carbohydrates, total soluble sugars, and starch in the apple leaves decreased dramatically with time regardless of BA treatment in the two (Figs. 3 and 4) experiments reported herein (Figs. 3 and 4). In 1995, BA reduced the concentration of total nonstructural carbohydrates, total soluble sugars, and starch in apple leaves 5 and 8 d after treatment, but this difference disappeared 12 d after treatment (Fig. 3). In 1996, total nonstructural carbohydrates and total soluble sugars were considerably lower in the leaves of BA-treated trees than those of the control trees at 5 and 9 d after treatment (Fig. 4). BA had no significant influence on starch levels in the leaves. The trend of carbohydrate content in the leaves and its response to BA application were similar to that of the concentration of carbohydrates in the leaves (unpublished data).

BA did not significantly affect concentrations of total nonstructural carbohydrates, total soluble sugars, and starch in the fruit in either year, except in 1996, where BA at 100 mg·L⁻¹ reduced starch concentration in fruit 9 d after treatment (Figs. 5 and 6).

On 18 June 1997, 12 d after BA application, average fruit size of abscising fruit was 32% smaller than that of persisting fruit, whereas the fruit size of the fruit abscising 12 d later (30 June) was similar to persisting fruit (18 June) (Table 3). Abscising fruit the same age had lower fresh weight (FW) and dry weight (DW) than persisting fruit. Persisting fruit had higher water content than abscising fruit either 12 or 24 d after BA application.

Concentrations of total soluble sugars and total nonstructural carbohydrates were greater in abscising fruit than in persisting fruit (Table 4). The concentration of starch was highest in abscising fruit the same size as persisting fruit, whereas starch concentration was similar in persisting fruit and abscising fruit at the same harvest date.

Discussion

Photosynthates required for young fruit to grow and develop are produced in the leaves, and must be translocated from the leaves to

Fig. 2. Effects of BA on the patterns of fruit abscission of 'More-Spur McIntosh' apples during 'June drop' (Expt. 6, 1997). Data are means $\pm$ SE (n = 8).

the fruit. Schneider (1978) suggested that the mechanism of fruit thinning by NAA was interference with photosynthate transport from leaves to fruit since NAA decreased both the concentration and content of reducing sugars in the flesh of young fruit (Schneider and Lasheen, 1973) and reduced translocation of sugars from leaves to the developing apples (Schneider, 1975). In our investigation, BA enhanced rather than reduced translocation of ¹⁴C-sorbitol from spur leaves to fruit when applied directly to the 'More-Spur McIntosh' fruit, but not when applied directly to the leaves (Table 1). Similarly, BA promoted sugar movement from the foliage to the developing fruit in citrus (Mauk et al., 1986). Sorbitol and sucrose are the principal carbohydrates translocated in apples (Webb and Burley,

Fig. 3. Effects of BA on nonstructural carbohydrate levels of 'More-Spur McIntosh' apple leaves (Expt. 4, 1995). (A) Total soluble sugars, (B) starch, (C) total nonstructural carbohydrates. Legend in A applies to all figures. Data are means $\pm$ SE (n = 5).

1962). Since BA must come in contact with the leaves to thin (Greene et al., 1992), it is unlikely that BA thinned apples directly by restricting the translocation of photosynthates from leaves to fruit.

Foliar application of BA reduced P_n of 'More-Spur McIntosh' apple leaves at $30 \pm 2^\circ\text{C}$ by 10% to 15% (Fig. 3A). This suppression persisted for ≈ 6 d. Following petal fall, fruit growth, leaf area, and shoot growth increase rapidly. There is a high demand for energy produced by photosynthesis of leaves at these multiple centers of metabolic activity (Hansen, 1971; Quinlan and Preston, 1971). Shading or application of photosynthetic inhibitors during this critical time, which decreases photosynthesis and thus reduces the carbohydrates available to the fruit, has been suggested as a primary

factor responsible for increasing early fruit abscission of apple (Byers et al., 1985; 1990; 1991; Schneider, 1978), peach [*Prunus persica* (L.) Batsch] (Byers et al., 1985), sweet orange [*Citrus sinensis* (L.) Osbeck] (Moss, 1976), and litchi [*Litchi chinensis* Sonn.] (Yuan and Huang, 1988). Even shading with cloth to reduce light interception of 'Delicious' apple trees by 25% for 2 d caused significant fruit abscission (Schneider, 1978). Carbon balance models indicate a potential limitation of carbon availability during the first 5 weeks after bloom, a critical period for fruit set and fruit cell division (Lakso and Corelli Grappadelli, 1992). Thus, it is reasonable to assume that a 10% to 15% reduction in P_n of apple leaves following BA application, sustained for several days, may be an important factor contributing to the early fruit abscission.

P_n is determined by three factors (Pasian and Lieth, 1989). First,

Fig. 4. Effects of BA on nonstructural carbohydrate levels of 'More-Spur McIntosh' apple leaves (Expt. 5, 1996). (A) Total soluble sugars, (B) starch, (C) total nonstructural carbohydrates. Legend in A applies to all figures. Data are means $\pm$ SE ($n = 5$).

Fig. 5. Effects of BA on nonstructural carbohydrate levels of 'More-Spur McIntosh' apples (Expt. 4, 1995). (A) Total soluble sugars, (B) starch, (C) total nonstructural carbohydrates. Legend in A applies to all figures. Data are means $\pm$ SE ($n = 5$).

Fig. 6. Effects of BA on nonstructural carbohydrate levels of 'More-Spur McIntosh' apples (Expt. 5, 1996). (A) Total soluble sugars, (B) starch, (C) total nonstructural carbohydrates. Legend in A applies to all figures. Data are means $\pm$ SE ($n = 5$).

gross leaf photosynthesis (P_g), is the total amount of carbon fixed by the plant. The second and third, which consume some of the photosynthates, are photorespiration (R_p) and mitochondrial respiration (R_m) of the leaves. Stated differently $P_n = P_g - (R_m + R_p)$, or $P_n + R_m = P_g - R_p$, and $P_n + R_m = P$, where $P = P_g - R_p$. In our investigation, there was no significant difference in the sum of P_n and mitochondrial respiration among treatments and control (data not presented) which meant that P did not change in value. Therefore, the reduced P_n following BA application resulted mainly from increased mitochondrial respiration caused by BA application. However, the response of leaf mitochondrial respiration to BA application was temperature dependent. Leaf respiration was increased when temperature following BA application was high ($\approx 30^\circ\text{C}$) (Fig. 1B), whereas there was no effect when temperature was lower (20°C) (Fig. 1C). The temperature-dependence of mitochondrial respira-

tion may explain the erratic thinning observed following BA application. Stopar et al. (1997) reported that BA at $50\text{ mg}\cdot\text{L}^{-1}$ reduced P_n by 10% in 'Redchief Delicious' but this was not statistically different from the control. However, they did not report the temperature when P_n was measured. Furthermore, the P_n that they reported ranged from 12 to $15\text{ }\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, which is relatively low compared with $\approx 20\text{ }\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ reported by Schechter et al. (1994) and 18 to $19\text{ }\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in our investigation.

Generally, there is more than one peak in fruit abscission during 'June drop' (Abruzzese et al., 1995). In our investigation, there were two waves of fruit abscission during the 'June drop' period on 'More-Spur McIntosh' apples, regardless of BA application (Fig. 2). These peaks appear to be innate but the peak value may be affected by the weather. Application of BA significantly increased the first peak, which occurred between 10 and 14 d after BA treatment. At this time, approximately half of then existing fruit abscised from treated trees in 4 d, whereas only 25% abscised from the control trees. Therefore, BA appears to accentuate naturally occurring waves of fruit abscission. It also indicated the importance of the time of BA application, since application made too far in advance or following a fruit abscission peak may result in insufficient thinning (Bound et al., 1991; Greene and Autio, 1989). Similarly, Byers et al. (1991) found that artificially shading for 2 to 3 d almost defruited trees when shaded 14, 21, or 28 d after full bloom, but it did not reduce fruit set when shaded 8, 35, or 42 d after full bloom.

Concentration of carbohydrates in 'More-Spur McIntosh' apple leaves decreased dramatically over the 12 or 13 d observation period (Figs. 3 and 4), suggesting that carbohydrates available to developing fruit and growing bourse shoots were potentially limiting during

Table 3. The effect of BA on fruit diameter, fruit fresh weight, fruit dry weight, and fruit water content of persisting and abscising 'More-Spur McIntosh' apples (Expt. 6, 1997).^z

BA ($\text{mg}\cdot\text{L}^{-1}$) ^y	Fruit		
	Persisting (18 June) ^x	Abscising (18 June) (same age) ^x	Abscising (30 June) (same size) ^w
	Fruit diam (mm)		
0	25.4	17.6	25.2
100	25.8 ^{NS}	17.2 ^{NS}	25.6 ^{NS}
Mean	25.6 a ^v	17.4 b	25.4 a
	Fresh wt/fruit (g)		
0	8.2	2.8	7.4
100	8.3 ^{NS}	2.9 ^{NS}	7.8 ^{NS}
Mean	8.2 a	2.9 c	7.6 b
	Dry wt/fruit (g)		
0	0.9	0.4	0.9
100	0.9 ^{NS}	0.4 ^{NS}	1.0 ^{NS}
Mean	0.9 b	0.4 c	1.0 a
	Water/fruit (g)		
0	7.3	2.5	6.5
100	7.4 ^{NS}	2.5 ^{NS}	6.8 ^{NS}
Mean	7.3 a	2.5 c	6.7 b

^zMean of four observations.

^yBA was applied on 6 June 1997, at 10-mm stage of fruit development.

^xTwelve days after BA application.

^wTwenty-four days after BA application.

^vMean separation within rows by Duncan's multiple range test, $P < 0.05$.

^{NS}Difference in mean between BA at $0\text{ mg}\cdot\text{L}^{-1}$ and $100\text{ mg}\cdot\text{L}^{-1}$ was nonsignificant according to t test, $P < 0.05$.

Table 4. Effects of BA on total soluble sugars, starch, and total nonstructural carbohydrates (TNC) of persisting and abscising 'More-Spur McIntosh' apples (Expt. 6, 1997)^z

BA (mg·L ⁻¹) ^y	Fruit		
	Persisting (18 June) ^x	Abscising (18 June) (same age) ^x	Abscising (30 June) (same size) ^w
Total soluble sugars (mg·g ⁻¹ dry wt)			
0	198.4	228.2	312.7
100	194.6 ^{NS}	238.3 ^{NS}	320.0 ^{NS}
Mean	196.2 c ^v	233.2 b	316.3 a
Starch (mg·g ⁻¹ dry wt)			
0	18.8	15.65	27.5
100	15.0 ^{NS}	13.2 ^{NS}	26.3 ^{NS}
Mean	16.9 b	14.4 b	26.9 a
TNC (mg·g ⁻¹ dry wt)			
0	214.9	243.9	340.2
100	210.1 ^{NS}	251.5 ^{NS}	346.3 ^{NS}
Mean	212.2 c	247.7 b	343.3 a

^zMean of four observations.

^yBA was applied on 6 June 1997, at 10-mm stage of fruit development.

^xTwelve days after BA application.

^wTwenty-four days after BA application.

^vMean separation within rows by Duncan's multiple range test, $P < 0.05$.

^{NS}Difference in mean between BA at 0 mg·L⁻¹ and 100 mg·L⁻¹ was nonsignificant according to t test, $P < 0.05$.

the time of early fruit development. The carbohydrate concentration in apple leaves were lowered further by BA application, indicating that the available carbohydrate supply for developing fruit and growing bourse shoot was aggravated further by reduced P_n resulting from BA application, thus causing further carbon stress and additional fruit abscission. Similarly, reduced soluble carbohydrate level in the leaves and a limited carbohydrate supply caused by increased respiration at high temperature or low light was suggested as a primary factor responsible for serious premature abscission of fruit in apple (Kondo and Takahashi, 1987), cyathia in poinsettia (*Euphorbia pulcherrima* Willd.) (Miller and Heins, 1986), and young bolls of cotton (*Gossypium hirsutum* L.) (Guinn, 1976).

Our results show that abscising fruit, regardless of BA treatment, contained higher carbohydrate concentrations than persisting fruit (Table 4). Similar results were also observed in apples (Abruzzese et al., 1995) and navel oranges (Ruiz and Guardiola, 1994). Abscised or abscising apples had higher calcium content, and lower DW, nitrogen, potassium, amino acids, and proteins than persisting fruit (Abruzzese et al., 1995; Howlett, 1923). The reason for high soluble sugars in abscising or abscised fruit was attributed to a reduction in their use (Abruzzese et al., 1995), since fruit growth reduced or slowed down 1 to 2 weeks before abscission (Abruzzese et al., 1995; Ruiz and Guardiola, 1994), and fruit also had greatly lower respiration rates before abscission (Stopar, 1998). Byers et al. (1991) reported that fruit destined to abscise stopped growth from 1 to 6 d after a 3-d period of shading. Using metabolic heat rates of plant tissues as the indicator of metabolic activities of the tissues, Nzima et al. (1998) also found that shading of pistachio (*Pistacia vera* L.) trees reduced photosynthesis and resulted in reduced metabolic activities of inflorescence buds, thereby promoting inflorescence bud abscission. Therefore, reduced fruit respiration or metabolic activities results in limited energy production which in turn reduces the synthesis of amino acids and proteins, and lowers amino acid and protein concentrations in abscising or abscised fruit.

At the same time, reduced respiration causes accumulation of soluble sugars. In our investigation, there was no significant difference in carbohydrate concentrations of fruit of BA-treated trees and nontreated trees (Figs. 5 and 6). This may be attributed to the sample containing some abscising fruit which usually have considerably higher carbohydrates, since $\approx 83\%$ of then existing fruit of BA-treated trees abscised during 'June drop' (Fig. 2), and abscising fruit were not distinguished visually from persisting fruit until shortly before abscission.

In conclusion, it seems that BA thinned apples not directly by affecting the movement of carbohydrates from leaves to fruit, but by increasing mitochondrial respiration and decreasing net photosynthesis. Decreased P_n led to limited carbohydrate supply, thereby accentuating fruit abscission. This suggestion is also supported by the fact that BA thinned apples only when one leaf per fruit was on the girdled small fruiting spur, but not when leaf number was greater than two (Yuan and Greene, 2000).

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