Storage Responses of 'Empire' Apples to Benzyladenine and Other Chemical Thinners

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Additional index words. NAA, carbaryl, GA₄₊₇, Promalin, postharvest, fruit maturity, fruit quality, *Malus domestica*

Abstract. In three trials over 3 years, foliar BA applications for fruitlet thinning of 'Empire' apple (*Malus domestica* Borkh.) trees produced small and inconsistent effects on flesh firmness at harvest and after air storage. Soluble solids concentrations at harvest and after air storage were consistently increased by BA alone or together with GA₄₊₇ [Promalin (PR)], and were also increased by CB in one trial. Starch hydrolysis was slightly delayed by BA applications in 1990. Ethylene evolution at harvest was increased by NAA in 1988 and slightly increased by PR applied 29 days after full bloom (DAFB) in 1990, while poststorage ethylene evolution was stimulated by BA and PR treatments in 1990 except BA at 29 DAFB. Incidence of poststorage disorders was low and largely uninfluenced by thinning treatments. Chemical names used: *N*-(phenylmethyl)-1*H*-purine-6-amine [benzyladenine (BA)]; BA plus gibberellins A₄ and A₇ (GA₄₊₇) [Promalin (PR)]; 1-naphthaleneacetic acid (NAA); 1-naphthalenyl methylcarbamate [carbaryl (CB)].

Cytokinins such as BA, *N*-phenyl-*N*'-1,2,3-thiadiazol-5-ylurea (thidiazuron), and *N*-(2-chloro-4-pyridyl)-*N*'-phenylurea (CPPU) reduce fruit set in apple trees (Bound et al., 1991; Byers and Carbaugh, 1991; Elfving, 1989; Greene, 1989; Greene and Autio, 1989; Greene et al., 1990). Elfving and Cline (1993) reported on thinning responses to BA and PR on 'Empire' apple. The trials reported here evaluated harvest and storage responses of 'Empire' apples to thinning treatments of BA, PR, NAA, and CB in the same experiments.

Materials and Methods

Three field trials were conducted between 1988 and 1990 at the Horticultural Experiment Station, Simcoe, Ont., Canada (Elfving and Cline, 1993). All trials used single, whole-tree plots in randomized complete-block designs. All chemical treatments were applied to mature 'Empire'/M.26 trees as dilute sprays with a hydraulic handgun sprayer. Postharvest and poststorage characteristics of fruit harvested on two dates 1 week apart were evaluated at the Dept. of Horticultural Science (DHS), Univ. of Guelph. Fruit samples of 12 to 15 kg from each replication were counted, weighed, and sent to the DHS immediately after harvest. Subsamples from each replication were used for harvest evaluations. The remaining fruit were stored in air at 0.5°C and 90% to 95% RH for 19 to 21 weeks before poststorage evaluations.

BA vs. NAA (Expt. 1, 1988). Each of the following seven treatments was applied to five replicate, 8-year-old trees: 1) control (no treatment); 2) BA at 50, 100, or 150 mg a.i./liter; or 3) NAA at 5, 10, or 15 mg a.i./liter. Treatments were applied on 1 June (13 DAFB), when fruit diameter averaged 11.0 ± 1.1 mm. The trees had an average initial trunk cross-sectional area (TCSA) of 74.9

cm². They were spaced 3.7 × 5.5 m on a well-drained Bookton loamy sand consisting of 40 to 100 cm of sandy sediments over glaciolacustrine clays (Hohner and Presant, 1989). Apple samples were harvested from each tree on 20 and 27 Sept.

BA, NAA, and CB (Expt. 2, 1989). The following seven treatments were applied to five replicate, 9-year-old trees: 1) control (no treatment); 2) BA at 50 or 100 mg a.i./liter; 3) NAA at 5 or 10 mg a.i./liter; or 4) CB at 500 or 1000 mg a.i./liter. Treatments were applied on 6 June (13 DAFB), when fruit diameter averaged 9.8 ± 1.2 mm. The trees had an average initial TCSA of 62.9 cm². They were spaced 3.7 × 5.5 m on a well-drained Brant very fine sandy loam consisting of glaciolacustrine loams, silts, and fine sandy loam (Hohner and Presant, 1989). Apple samples were harvested from each tree on 26 Sept. and 3 Oct.

BA and PR vs. application timing (Expt. 3, 1990). In addition to a single control (untreated), each of the following four treatments was applied on each of three dates to six replicate, 9-year-old trees: 1) BA 75 or 150 mg a.i./liter or 2) PR 75 or 150 mg a.i./liter. BA and PR treatments were applied on 15 May (6 DAFB, mean fruit diameter 3.6 ± 0.9 mm), 29 May (20 DAFB, mean fruit diameter 10.2 ± 2.4 mm), or 7 June (29 DAFB, mean fruit diameter 17.1 ± 3.9 mm). The trees had an average initial TCSA of 61.1 cm², and were spaced 3.7 × 5.5 m on a well-drained Watford fine sandy loam consisting of mixed glaciolacustrine sands (Hohner and Presant, 1989). Apple samples were harvested from each tree on 20 and 27 Sept.

Fruit flesh firmness was measured on pared flesh on opposite sectors of each of 10 apples per replication with a penetrometer (Effegi, Alfonsine, Italy) using an 11.1-mm tip. Juice expressed from the apples during the firmness measurements was collected, and soluble solids concentration (SSC) was assessed on the composite juice sample with a hand refractometer (Fisher Scientific, Unionville, Ont.). Fruit maturity was assessed on the same 10 apples using the starch-iodine test, comparing starch patterns against an 'Empire' standard (Chu, 1988). After storage, 10 apples per replicate were assessed for flesh firmness and SSC. Disorders were rated visually on 25 apples per replication after storage plus 1 week at room temperature (≈20°C) on a scale from 0 (no symptoms) to 3 (severe symptoms).

At harvest and after storage, 10 apples per replication were

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Table 1. Effects of BA and NAA applications in 1988 on 'Empire' fruit quality characteristics at harvest, after 20 weeks in air at 0.5C, and after an additional week at 20C (Expt. 1).

Treatment ^z (mg·liter ⁻¹)	Harvest			Poststorage			
	Flesh firmness (N)	Soluble solids concn (%)	C ₂ H ₄ evolution (μl·kg ⁻¹ ·h ⁻¹)	After 1 day at ≈20C		After 1 week at ≈20C	
				Flesh firmness (N)	Soluble solids concn (%)	Scald ^y (%)	Internal breakdown ^y (%)
Control	65.8	11.1	0.3	43.1	11.3	0.8	0
BA 50	66.3	11.4	0.4	42.1	11.5	0.6	0
100	67.5	11.5	0.0	41.5	11.8	2.9	1
150	69.0	11.9	0.0	41.0	11.9	2.1	3
NAA 5	66.7	11.4	0.3	43.6	11.5	0.1	1
10	67.0	11.2	0.9	43.2	11.4	0.6	1
15	67.6	11.4	1.2	43.2	11.5	0.2	1
Significance							
Concentration (C)	**	****	*	****	*	*	*
GR ^x × C	*	NS	*	NS	NS	NS	NS
BA	L***	L***	NS	L**	L*	L*	L*
NAA	L*	NS	L*	NS	NS	NS	NS
Harvest date ^w							
20 Sept.	68.1 a	11.3 b	0.4 a	43.4 a	11.7 a	0.5 a	0 b
27 Sept.	66.1 b	11.5 a	0.5 a	41.7 b	11.4 b	1.1 a	2 a

^zEach mean contains 10 observations.

^yPercentage of 25-fruit samples showing moderate (rating of 2) to severe (rating of 3) symptoms.

^xGR = growth regulator.

^wMean separation within columns by F test ($P \leq 0.05$). Each mean contains 35 observations.

NS,*,**,***,**** Nonsignificant or significant at $P \leq 0.05, 0.01, 0.001$, or 0.0001 , respectively. L = linear effect by radiating regression analysis. Curvilinear effects nonsignificant ($P \leq 0.05$).

Table 2. Effects of BA, CB, or NAA applications in 1989 on 'Empire' fruit quality characteristics at harvest, after 19 weeks in air at 0.5C, and after an additional week at ≈20C (Expt. 2).

			Poststorage					
Harvest			After 1 week at ≈20C					
Treatment	Flesh firmness ^z	Soluble solids concn ^z	Flesh firmness (N)		Soluble solids concn ^{z,y}	Brown core ^{z,y}	Internal breakdown (%)	
(mg·liter ⁻¹)	(N)	(%)	1 day ^z	1 week ^z	(%)	(%)	26 Sept. ^{y,x}	3 Oct. ^{y,x}
Control	71.1	11.6	48.9	44.8	11.8	0.0	0.0	0.0
BA 50	69.8	11.8	46.6	43.4	12.5	0.2	0.0	0.6
100	68.9	12.1	45.4	42.5	12.4	1.0	0.0	2.8
CB 500	69.5	11.8	47.0	44.0	12.5	0.0	0.0	0.0
1000	70.0	12.0	46.3	43.7	12.7	0.6	0.0	0.2
NAA 5	70.0	11.8	46.7	44.1	12.4	0.0	0.0	0.0
10	70.2	11.9	47.1	44.4	12.3	0.1	0.2	0.2
Significance								
Concentration (C)	NS	*	NS	NS	*	NS	NS	NS
GR ^w × C	*	*	*	*	*	*	NS	**
BA	L*	L*	L**	L**	L**,Q*	L*	NS	L***
CB	NS	L*	L*	NS	L**	NS	NS	NS
NAA	NS	NS	NS	NS	L**,Q*	NS	NS	NS
Harvest date ^v								
26 Sept.	70.3 a	11.5 b	49.1 a	45.1 a	12.3 a	0.2 a	0.0 b	—
3 Oct.	69.5 b	12.2 a	44.7 b	42.6 b	12.2 a	0.2 a	—	0.2 a

^zEach mean contains 10 observations.

^yPercentage of 25-fruit samples showing moderate (rating of 2) to severe (rating of 3) symptoms.

^xSignificant treatment × harvest date interaction ($P \leq 0.05$). Each mean contains five observations.

^wGR = growth regulator.

^vMean separation within columns or variable by F test ($P \leq 0.05$). Each mean contains 35 observations.

NS,*,** Nonsignificant or significant at $P \leq 0.05$ or 0.01 , respectively. L = linear effect, Q = quadratic effect by radiating regression analysis.

placed in a flow-through system to measure ethylene production at 20°C (Lougheed and Franklin, 1968). The effluent air was automatically sampled and 3-ml samples were injected into a gas chromatograph (model GC-5; Beckman Instruments, Fullerton, Calif.) with dual-flame ionization and thermal-conductivity detectors (Lougheed et al., 1969). Ethylene production was calculated from the concentration in the effluent airstream.

Data were analyzed using a radiating regression model described by Cochran and Cox (1957) and Elfving and Allen (1987). All percentage values were transformed using the arcsin of the square root before analysis. All analyses were conducted using the General Linear Models (GLM) procedure of the Statistical Analysis System (SAS) program package (SAS Institute, Cary, N.C.). Quadratic and cubic regression models were analyzed based on available degrees of freedom in the treatment arrangement. Nonsignificant higher-order effects were incorporated into the error term of less complex models, which were then re-analyzed. Where interactions among main effects were not significant, interactive effects were incorporated into the error term of main-effects models.

Results

Experiment 1. At harvest, flesh was firmer the higher the concentrations of both BA and NAA (Table 1). SSC was higher with increasing BA concentration but was not affected by NAA. Thinning treatments did not affect fruit maturation as determined by starch-index values (data not shown), which averaged 5.8 and 7.4 for harvests on 20 and 27 Sept., respectively. Ethylene evolution was very low, but was higher at higher concentrations of NAA. There were no significant interactions between treatments and harvest dates for at-harvest attributes. Differences between harvest dates reflected the onset of ripening, although ethylene evolution was unaffected by sample harvest date.

After 20 weeks of air storage, BA-treated fruit were slightly softer the higher the BA concentration, while NAA had no effect (Table 1). As at harvest, SSC was higher in direct proportion to BA concentration. Ethylene evolution was much higher than at harvest (76 and 73 $\mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ for harvests on 20 and 27 Sept., respectively), but was not influenced by either thinner (data not shown). The overall incidence of moderate to severe storage disorders was minimal. Both storage scald and internal breakdown were slightly more common at the higher BA concentrations. Moderate to severe brown core had no relationship to thinner treatment (data not shown), averaging 0.2% and 1.8% for the two harvest dates. There were no significant interactions between treatments and harvest dates for poststorage quality attributes. Where harvest date effects were significant, they primarily reflected fruit maturity at harvest.

Experiment 2. At harvest, BA-treated fruit had a slightly but significantly reduced flesh firmness with increasing concentration, while NAA and CB had no effect (Table 2). SSC increased with higher BA and CB concentrations. Fruit maturation, as determined by starch index (means 4.2 and 5.8 for harvests on 26 Sept. and 3 Oct., respectively) and ethylene evolution (means 0.1 and 0.3 $\mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ for harvests on 26 Sept. and 3 Oct., respectively) were unaffected by any treatments (data not shown). There were no significant interactions between treatments and harvest dates for harvest parameters. Significant harvest-date effects reflected the onset of ripening over the harvest period.

After 19 weeks of air storage, both BA and CB applications slightly but significantly reduced flesh firmness when measured after 1 day at room temperature, and the influence of BA was still

detectable after 1 week out of storage (Table 2). After 1 week at room temperature, SSC was higher than the control for all thinners, but BA and NAA produced a curvilinear response. Although the incidence of all poststorage fruit disorders was very low, BA slightly increased the incidence of moderate to severe brown core. Among poststorage characteristics, only internal breakdown had a significant treatment $\times$ harvest-date interaction. Internal breakdown was slightly more common for higher BA concentrations for the second harvest date only. There was no moderate to severe storage scald (data not shown). Fruit from the later harvest date were softer and had a small amount of internal breakdown.

Experiment 3. At harvest, flesh was firmer for higher BA concentrations at all application timings and for higher PR concentrations at 6 DAFB (Table 3). The apparent increase in flesh firmness observed in fruit from the later harvest date likely resulted from an unavoidable change in laboratory personnel making manual firmness determinations between the first and second harvest dates. SSC was increased by higher concentrations of BA and PR, independent of application timing (Table 4). Fruit maturation as indicated by the starch index was slightly delayed by BA. Ethylene evolution at harvest was very low (means 0 and 0.4 $\mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ for harvests on 20 or 27 Sept., respectively) and increased slightly only with PR at 29 DAFB (data not shown). There were no significant interactions between treatments and harvest dates for at-harvest characteristics. Harvest date affected all at-harvest attributes except ethylene evolution. Other significant effects of harvest date on fruit attributes seem to reflect fruit ripening during the interval between harvests.

After 21 weeks of air storage, flesh was firmer than that of the control after 1 day and 1 week at room temperature for fruit treated with BA at 6 DAFB and was softer for fruit treated with PR applied at 20 or 29 DAFB (Table 3). Changes in ethylene evolution were small, but significantly related to concentrations of all chemical applications except BA at 29 DAFB. The incidence of moderate to severe brown core was slightly increased relative to the control for BA and PR applied 6 DAFB only. Among poststorage attributes, only internal breakdown showed a significant treatment by harvest-date interaction. Internal breakdown was not affected by thinner treatments for fruit harvested on the first sampling date, but PR applied 29 DAFB resulted in a concentration-dependent increase in internal breakdown of fruit from the second harvest. There was no incidence of moderate to severe storage scald (data not shown). SSC after 1 week at room temperature was increased by BA and PR applications, independent of application timing (Table 4). Harvest-date effects seem to be related to fruit ripening over the harvest period.

Discussion

BA effects on flesh firmness in these experiments were small despite large increases in fruit size (Elfving and Cline, 1993). In addition, BA-induced firmness responses were not consistent from year to year and differed between harvest and poststorage evaluations in one of three trials. Increases or no effects on flesh firmness have been associated with BA applications for fruit thinning in other cultivars (Greene and Autio, 1989; Greene et al., 1982; 1990), as well as from applications of other cytokinins (Greene, 1989), although PR applications resulted in softer flesh in 'McIntosh' apple (Greene et al., 1982). Larger apples typically have larger intercellular spaces (Smith, 1940), normally associated with softer flesh. Because BA may influence apple fruit size, in part, by stimulating cell division (Greene et al., 1990; Letham and Williams, 1969), the small changes in flesh firmness at harvest and after storage may reflect a different tissue architecture than that associated with larger

Table 3. Effects of BA or PR applications in 1990 on 'Empire' fruit quality characteristics at harvest, after 21 weeks in air at 0.5C, and after an additional week at ≈20C (Expt. 3).

Treatment ^z (mg liter ⁻¹)	DAFB ^y	Harvest Flesh firmness ^z (N)	Poststorage					
			Flesh firmness (N)		After 1 week at ≈20C		Internal breakdown ^{w,v} (%)	
			1 day	1 week	C ₂ H ₄ evolution (μl·kg ⁻¹ ·h ⁻¹) ^x	Brown core ^w (%)		
							20 Sept.	27 Sept.
Control	—	74.3	47.0	44.7	54	0.4	0.1	0.0
BA 75	6	77.5	48.6	45.9	65	3.0	0.0	0.0
150	6	78.5	48.0	45.6	62	3.3	0.0	0.0
BA 75	20	75.9	46.6	43.7	64	0.3	0.1	0.1
150	20	78.6	47.3	44.2	64	0.4	0.0	0.2
BA 75	29	75.4	46.9	44.7	56	0.6	0.1	1.1
150	29	76.3	47.3	44.9	61	0.6	0.1	0.4
PR 75	6	76.4	47.1	43.7	61	3.0	0.0	0.2
150	6	76.6	46.6	44.2	57	3.0	0.4	0.0
PR 75	20	73.8	45.1	42.3	65	0.0	0.0	0.0
150	20	75.3	44.8	42.3	67	0.4	0.0	0.4
PR 75	29	73.7	45.1	42.2	64	0.4	0.1	0.4
150	29	74.1	44.9	42.6	58	0.3	0.2	7.3
Significance								
Concentration (C)		**	NS	NS	**	NS	NS	NS
GR ^u × C		***	****	****	NS	NS	NS	*
GR × Timing		***	***	****	**	***	NS	***
BA	6	L****	L*	L**	L**,Q*	L*	NS	NS
	20	L****	NS	NS	L*	NS	NS	NS
	29	L*	NS	NS	NS	NS	NS	NS
PR	6	L**	NS	NS	L*,Q*	L**	NS	NS
	20	NS	L***	L**	L**	NS	NS	NS
	29	NS	L***	L**	L**,Q*	NS	NS	L****
Harvest date ⁱ								
20 Sept.		73.8 b	47.8 a	45.1 a	59 b	1.7 a	0.0 b	—
27 Sept.		78.0 a	45.2 b	42.7 b	64 a	0.3 b	—	0.3 a

^zEach mean contains 12 observations.

^yDAFB = days after full bloom.

^xAfter 1 day at ≈20C.

^wPercentage of 25-fruit sample showing moderate (rating of 2) or severe (rating of 3) symptoms.

^vSignificant growth regulator by harvest date interaction. Each mean contains six observations.

^uGR = growth regulator.

ⁱMean separation within columns or variable by F test ($P \leq 0.05$). Each mean contains 78 observations.

NS, *, **, ***, **** Nonsignificant or significant at $P \leq 0.05$, 0.01, 0.001, or 0.0001, respectively. L = linear effect, Q = quadratic effect by radiating regression analysis.

fruit size resulting primarily from cell enlargement. Flesh firmness at harvest or after storage was also increased by NAA applications in 1988, decreased by CB in 1989, and increased or reduced by PR in 1990, although these treatments did not affect fruit size to the same extent as BA alone (Elfving and Cline, 1993). Changes in fruit load associated with thinning also may have contributed to the observed differences in flesh firmness in these trials (Johnson, 1992).

The higher SSC observed with 'Empire' apples treated with BA in these trials is similar to levels reported with other cultivars (Greene and Autio, 1989; Greene et al., 1990). SSC increases were observed in all treatments producing a strong thinning response, a result suggesting reduced fruit load as a major factor contributing to differences in SSC.

Fruit maturation, as measured by starch index and ethylene evolution, showed little effect of BA, with only a slight delay in starch hydrolysis in one trial. Inconsistent effects on starch hy-

drolysis have been reported for 'McIntosh' after BA applications for fruit thinning (Greene and Autio, 1989).

The incidence of poststorage disorders was low in all three trials and was influenced to only a minor extent by the chemical treatments. PR had a much smaller effect on poststorage disorders in 'Empire' apple in these trials than was reported for 'McIntosh' (Greene et al., 1982). However, fewer seeds and an inhibitory effect on return bloom in response to PR treatments (Elfving and Cline, 1993) suggest that commercial formulations of BA that include equivalent concentrations of gibberellins may not be satisfactory chemical thinning agents for 'Empire' apple.

BA is an effective chemical thinner for 'Empire' apple (Elfving and Cline, 1993). The limited effects of BA on harvest and poststorage behavior were similar to those observed for the other chemical thinners included in these trials. The results presented here indicate little risk of adverse effects on fruit quality at harvest or after air storage as a result of thinning 'Empire' apples with BA.

Table 4. Effects of BA or PR applications in 1990 on 'Empire' fruit quality characteristics at harvest, after 21 weeks in air at 0.5C, and after an additional week at ≈20C (Expt. 3).

Treatment ^z (mg·liter ⁻¹)	Harvest		Poststorage
			After 1 week at ≈20C
	Soluble solids concn (%)	Starch index ^y	Soluble solids concn (%)
Control	11.0	4.4	11.8
BA 75	11.3	4.2	12.0
150	11.6	4.0	12.4
PR 75	11.4	4.5	11.9
150	11.5	4.4	12.0
Significance			
Concentration (C)	****	NS	****
GR ^x × C	NS	***	***
GR × Timing	NS	NS	NS
BA	L**	L**	L****
PR	L**	NS	L**
Harvest date ^w			
20 Sept.	11.1 b	3.8 b	11.9 b
27 Sept.	11.7 a	4.7 a	12.2 a

^zControl means contain 12 observations; growth regulator (GR) means contain 36 observations.

^yMean treatment rating on a scale from 1 to 9 (immature to overmature).

^xGR = growth regulator.

^wMean separation within columns by F test ($P \leq 0.05$). Each mean contains 78 observations.

NS, **, ***, **** Nonsignificant or significant at $P \leq 0.01$, 0.001, or 0.0001, respectively. L = linear effect by radiating-regression analysis. Curvilinear effects nonsignificant at $P \leq 0.05$.

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