

Characterizing the Interaction between NAA and BA on Apple Fruit Abscission and Development

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Abstract. NAA and BA are important compounds for regulating crop load in apples (*Malus domestica* Borkh.). When used for fruit thinning, both induce abscission, but at an equivalent crop load NAA tends to reduce and BA to increase fruit size. There is a strong interaction between NAA and BA when used together on ‘Delicious’ and ‘Fuji’, leading to excessive development of pygmy and small fruit (<65 mm diameter). The combination of BA (as Promalin, 1:1 BA + GA₄₊₇) applied at king bloom (KB) and NAAm (amide) at petal fall increased the percentage of small fruit by 3.3- or 5.1-fold compared with BA or NAAm alone. Similar results were obtained with BA (Promalin) at KB oversprayed with NAA at 10 to 12 mm king fruit diameter (KFD). When NAA was oversprayed with BA during fruitlet development, i.e., 5 to 6 mm, 10 to 12 mm, and ≈18 mm KFD, the greatest inhibition of fruit growth occurred at the 10- to 12-mm KFD stage, and there was no significant effect at 18 mm KFD. Inhibition by treatment at the 5- to 6-mm stage was intermediate and trees were overthinned. NAA + BA inhibition of fruit growth in ‘Delicious’ and ‘Fuji’ was not crop load-dependent. In all experiments, crop load (wt basis) of trees treated with NAA + BA was similar or less than of those treated with NAA or BA alone, but they produced 2.5- to 5-fold more small fruit. NAA + BA increased the number of fruit per cluster, many of which failed to fully develop. Increasing the ratio of BA to NAA from 25:15 to 125:15 mg·L⁻¹ increased small fruit formation. The presence of GA₄₊₇ in commercial formulations of BA (0:100, Maxcel; 10:100, Accel; 50:50, Promalin) did not significantly affect the NAA + BA response. Fruit growth was not inhibited by the NAA + BA combination in large-fruited ‘Golden Delicious’ and ‘Jonagold’ and was increased in small-fruited ‘Elstar’ and ‘Gala’ compared with the nontreated control.

The discovery of auxins provided the basis for current chemical fruit thinning of apples. The promotion of postbloom fruit abscission was first reported over 60 years ago (Davidson et al., 1945), and since then, synthetic auxins, NAA and its amide (NAAm), have become dominant apple fruit-thinning compounds (Dennis, 2000). Numerous studies have documented their effectiveness for fruit thinning, but their performance has been inconsistent and is subject to environmental conditions, particularly temperature and relative humidity during and for a short period after application (Luckwill and Lloyd-Jones, 1962; Wertheim, 2000; Westwood and Batjer, 1960; Williams, 1979).

Also, NAA has been reported to cause a transitory depression in the rate of fruit growth for a brief period after treatment and thus, the increase in size of the persisting fruit

may not be commensurate with the reduction in crop load (Greene and Autio, 1994; Luckwill, 1953; Marsh et al., 1960). In some cultivars, e.g., ‘Delicious’ and ‘Fuji’, the population of small fruit (<65 mm diameter) may be increased and small, seedless fruit (pygmy) may develop that persist until harvest. These effects are usually more pronounced in late postbloom applications and, more so, with NAAm than with NAA (Hoffman et al., 1955; Wise et al., 2007).

The discovery of the cytokinins and their pronounced effects on cell division and morphogenesis (Letham, 1963; Miller et al., 1995) provided an opportunity to increase fruit growth and perhaps overcome the negative effects of NAA on fruit development.

N⁶-Benzyladenine [BA, N-(phenylmethyl)-1H-purine-6-amine, bz⁶Ade], a synthetic cytokinin, and zeatin are the most widely used cytokinins in research and commercial application (Horgan, 1984). BA has increased fruit size in several apple cultivars (Elfving and Cline, 1993; Ferree, 1996; Greene, 1993; Greene et al., 1990; Stover

et al., 2001), promoted enlargement of the cortical tissue in the calyx lobes in ‘Delicious’, and increased cell division in apple and pear fruit (Flaishman et al., 2005; Greenhalgh et al., 1977; Letham, 1968; Williams and Stahly, 1960). Numerous thinning studies have confirmed that cytokinins induce fruit abscission and increase fruit size in apple and pear and that the fruit size is often greater than can be accounted for by the thinning effect (Bukovac et al., 2000; Flaishman et al., 2005; Greene et al., 1992; Wismer et al., 1995), thus suggesting a direct, positive effect on fruit growth.

In attempts to improve fruit thinning in hard-to-thin cultivars, combinations of chemicals have been used for aggressive thinning (Wertheim, 2000). An early, perhaps first, study using NAA and BA on ‘Delicious’ identified a strong interaction leading to excessive production of pygmy fruit (Miller, 1985). We observed a similar increase, not only in pygmy fruit, but also in the population of small fruit (<65 mm) in a grower study when NAA was used at ≈8 mm king fruit diameter (KFD) to thin ‘Delicious’ that were previously treated with Promalin at 80% king bloom (KB) to improve fruit typiness (E. Wittenbach, unpublished data). This response was similar, but of a greater magnitude, to that obtained with late or high concentrations of NAA (Hoffman, et al., 1955; Williams, 1979). This observation has been confirmed (Bound et al., 1991) and effects on pygmy and small fruit in ‘Delicious’ have been observed also when both chemicals were used for postbloom thinning on the same trees in the same season (Bukovac et al., 1995; Greene and Autio, 1994). In contrast, no significant negative cytokinin + NAA interaction has been observed on fruit growth in several other cultivars (Basak, 2006; Bukovac et al., 1995; Dennis, 2000; Stopar and Lokar, 2003).

Interactions between auxins and cytokinins on growth and differentiation are well established. Skoog and Miller (1957) demonstrated that the ratio (and concentration) of cytokinin to auxin in the culture media had a dramatic effect on organogenesis of cultured, undifferentiated tobacco tissue. In the presence of low auxin level (≈2 mg·L⁻¹), increasing the cytokinin concentration (≈0.02 to 10 mg·L⁻¹) induced root formation. On increasing the auxin:cytokinin ratio further, the tissue went through a rapid undifferentiated, proliferation stage and then developed buds. A high cytokinin:auxin ratio resulted in an undifferentiated callus and growth was inhibited. This interaction between auxins and cytokinins has been demonstrated for numerous plant tissues and, once the culture medium is optimized, provides the basis for regeneration and micropropagation of plants. Such findings have led to the suggestion that the ratio of these two hormones plays a defining role in controlling differentiation and growth in intact plants. Both auxins and cytokinins are present in apple fruitlets (Letham and Williams, 1969; Luckwill, 1953). However, the role of neither

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endogenous nor exogenous cytokinins in apple fruit development is understood.

The objective of our study was to provide a better understanding of this interaction by characterizing how the addition of BA modifies the effect of NAA on apple fruit abscission and development.

Materials and Methods

General. Studies were conducted at the Clarksville Horticultural Experiment Station on mature (10- to 14-year-old) trees of moderate vigor and receiving recommended horticultural and crop protection practices. Irrigation (trickle) was provided as needed. The designated chemicals were applied with a hand gun as a high-volume, foliar spray (2.4 MPa) to single trees, selected for uniformity of bloom density, replicated four to 10 times in random complete block designs. The effect on fruit abscission was indexed in all experiments by measuring yield per tree and fruit size ('Delicious' and 'Empire') by grading the fruit (per tree basis) into seven size classes (<51, 51 to 56, 57 to 64, 65 to 69, 70 to 75, 76 to 82, >82 mm diameter) using a Greefa Grader, Tricht-Geldermalen, Holland. In addition, the number of fruit per tree in Expt. 2 and Expt. 3 was estimated by dividing the total weight of fruit in each size class by the appropriate mean weight of a 100-fruit sample. For the other cultivars (Fuji, Elstar, Gala, Golden Delicious, Jonagold), all fruit was harvested on a per-tree basis and individual fruit were counted and weighed (Compac-Computerized Grading System, Auckland, New Zealand). The raw data were then sorted and assigned to designated size classes. Basic statistics was performed on all data. When appropriate, data were also subjected to analysis of variance or regression analysis using SAS (SAS Institute, Cary, NC) or Sigma Plot (SPSS, Chicago).

Chemicals and abbreviations. The following chemicals were used: BA formulated as Promalin (1.8% BA + 1.8% GA₄₊₇), BA formulated as Accel (1.8% BA + 0.18% GA₄₊₇), BA formulated as Maxcel (1.9% BA) from Abbott Laboratories, now Valent Biosciences Corp. (Libertyville, Ill), NAAm (Amid-Thin W, 8.4%, 1-naphthaleneacetamide), and NAA (Fruitone N, 3.5%, 1-naphthaleneacetic acid as the sodium salt) from AMVAC Chemical Corp. (Los Angeles, CA). No spray additives were used. The time of treatment was based on flower and fruit development, 80% KB and designated mean diameter of the king fruitlet (KF). A non-thinned control (NTC) and/or hand-thinned control (HT) was included for comparison. HT was performed during or immediately after June drop (≈25 mm fruit diameter).

We found no significant differences between the effects of the Maxcel and Accel formulations of BA in these studies and in a direct comparison on thinning (M.J. Bukovac, unpublished data). Therefore, we identify the formulations used for each experiment but refer to both as BA in the "Results" and "Discussion".

Table 1. Effects of Promalin and NAAm, applied alone and Promalin oversprayed with NAAm, on total yield and yield in fruit diameter size classes of less than 65 mm and greater than 70 mm in Redchief 'Delicious' apple.

Treatment ^y	Yield ^z		Fruit size class (kg) ^z			
	kg/tree	NTC ^x (%)	<65 mm	NTC (%)	>70 mm	NTC ^x (%)
NTC	125 a	100	23 ab	100	33 b	100
Promalin	106 ab	85	11 b	48	64 ab	194
NAAm	85 b	68	7 b	30	78 a	236
Promalin + NAAm	84 b	67	36 a	157	59 ab	179

^zMeans within a column followed by the same letters are not significantly different ($P = 0.05$) by the Tukey's honestly significant difference test.

^yPromalin (35 mg·L⁻¹) applied at 80% king bloom and NAAm (50 mg·L⁻¹) at 80% petal fall as high-volume sprays.

^xNTC = nontreated control.

Table 2. Effects of Promalin applied at king bloom (KB) and oversprayed with NAA at KB or at king fruit diameters (KF) of ≈10 mm and ≈18 mm on total yield and yield in fruit diameter size classes less than 65 mm and greater than 70 mm in Redchief 'Delicious' apple.

Treatment ^y	Yield ^z			Fruit size class (kg) ^z			
	kg/tree	Percent thinned	Calculated fruit (no./tree) ^x	<65 mm	NTC (%) ^w	>70 mm	NTC (%) ^w
NTC ^w	88 a	0	600	6.7 ab	100	80 bc	100
HT ^w	81 a	8	489	1.6 a	24	92 ab	115
Promalin _{KB} (P)	85 a	3	612	9.9 bc	148	73 cd	91
NAA _{KF 10 mm}	66 a	25	420	4.9 ab	73	88 abc	110
P + NAA _{KB}	27 b	69	145	1.8 a	27	95 a	119
P + NAA _{KF 10 mm}	58 ab	34	559	25.0 c	373	54 d	68
P + NAA _{KF 18 mm}	84 a	5	638	10.0 c	149	69 cd	86

^zMeans within a column followed by the same letters are not significantly different ($P = 0.05$) by Tukey's honestly significant difference test.

^yPromalin (35 mg·L⁻¹) applied at 80% KB and NAA (15 mg·L⁻¹) as high-volume sprays.

^xSee text for calculation.

^wNTC = nontreated control; HT = hand-thinned.

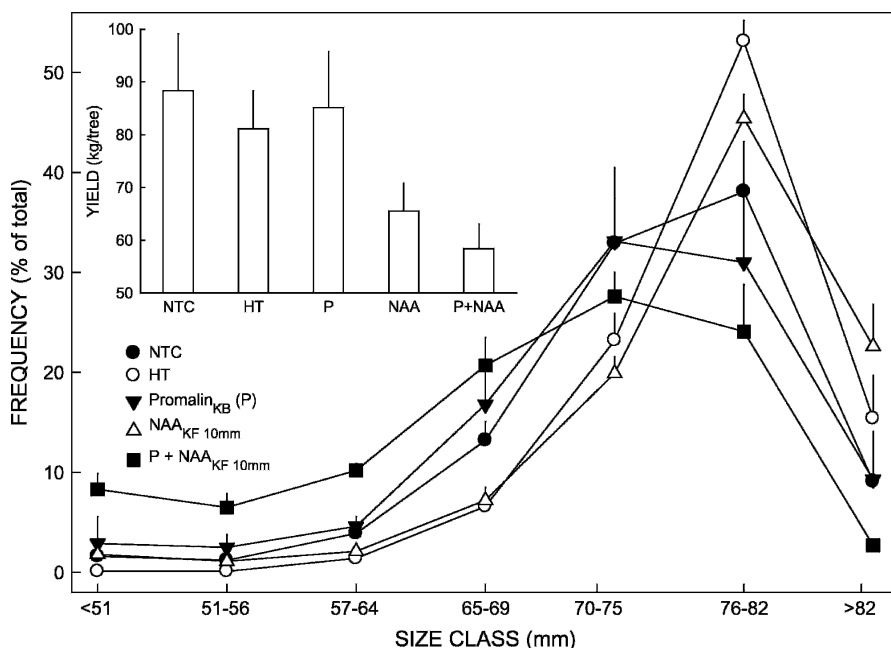


Fig. 1. Fruit size distribution profiles at harvest of Redchief 'Delicious' treated with Promalin (35 mg·L⁻¹) at 80% king bloom (KB), NAA (15 mg·L⁻¹) at 10 mm king fruit diameter (KFD), and Promalin (KB) oversprayed with NAA (10 mm KFD) compared with nontreated (NTC) and hand-thinned (HT) controls. Yield data in inset. Bars = SE.

Expt. 1: Promalin oversprayed with NAAm. We evaluated the effect of NAAm on fruit growth and abscission of Redchief 'Delicious'/MM.111 (13 years old) previously treated with Promalin at KB to enhance fruit form. Four single-tree treatments, replicated four times, were established: 1) NTC;

2) Promalin (35 mg·L⁻¹) at KB; 3) NAAm (50 mg·L⁻¹) at PF; and 4) Promalin (35 mg·L⁻¹) at KB followed by NAAm (50 mg·L⁻¹) at postbloom (PF). Other experimental conditions were as noted in general methods.

Expt. 2: Promalin oversprayed with NAA. A similar study was performed using Redchief

'Delicious'/M.106. (14 years old) the next season to evaluate the interaction of NAA with Promalin when NAA was oversprayed on Promalin (KB) at different stages of flower/fruitlet development (KB→KFD_{18mm}). This increased the time between applications of the two compounds and bracketed the most active phase of cell division in the cortex of the apple fruit. Promalin (35 mg·L⁻¹) was applied at KB. NAA (15 mg·L⁻¹) was applied alone (KFD_{10mm}) and to previously Promalin (35 mg·L⁻¹)-treated trees at KB, KFD of 10 mm and 18 mm. A NTC and a HT control were included. Five single-tree replications were used. Other experimental conditions were as described under general methods.

Expt. 3: Time relationship between NAA and BA application—'Delicious'. The effect of applying both NAA and BA at the same stage of fruitlet development, and NAA before or after BA at different fruitlet developmental stages, during or after the active cell division period, was determined using Redchief 'Delicious'/M.106. NAA (15 mg·L⁻¹) was oversprayed with BA (50 mg·L⁻¹; Accel), on the same day, at fruitlet stages of ≈6, 12, and 18 mm KFD. For the two treatments in which NAA and BA were applied at different stages (6 mm versus 12 mm KFD), the NAA and BA treatments were applied at 6 mm KFD, and the NAA-treated trees were oversprayed with BA and the BA-treated trees with NAA at 12 mm KFD. Each single tree treatment was replicated four times. Other experimental parameters were as described in the general methods.

Expt. 4: Time relationship between NAA and BA application—'Fuji'. A similar study on effect of time of NAA versus BA application on fruit growth and abscission was conducted on 6-year-old Myrared 'Fuji'/G.30. NAA (15 mg·L⁻¹) and BA (125 mg·L⁻¹; Maxcel) were applied both as independent treatments and together (NAA oversprayed with BA on the same day at 12 mm KFD). Two additional combination treatments were established: NAA (15 mg·L⁻¹, 6 mm KFD) was oversprayed with BA (125 mg·L⁻¹, 12 mm KFD) and BA (125 mg·L⁻¹, 6 mm KFD) was oversprayed with NAA (15 mg·L⁻¹, 12 mm KFD). A NTC was used for comparative purposes. Each single tree treatment was replicated 10 times. Treatment effects on abscission were indexed by yield and number of fruit per tree and on size by individual fruit weight/diameter at harvest. Other experimental parameters were as described in the general section.

Expt. 5: BA:NAA ratio. 'Delicious'/MM.111 and 'Empire'/M.106 were selected to establish the effect of an increasing ratio of BA to NAA on the NAA effect on fruit growth and abscission. 'Delicious' was selected because of the marked inhibition of NAA + BA on fruit growth and 'Empire' because, in an earlier study (Bukovac et al., 1995), this combination had no effect or increased fruit size. This evaluation was performed as two separate experiments in

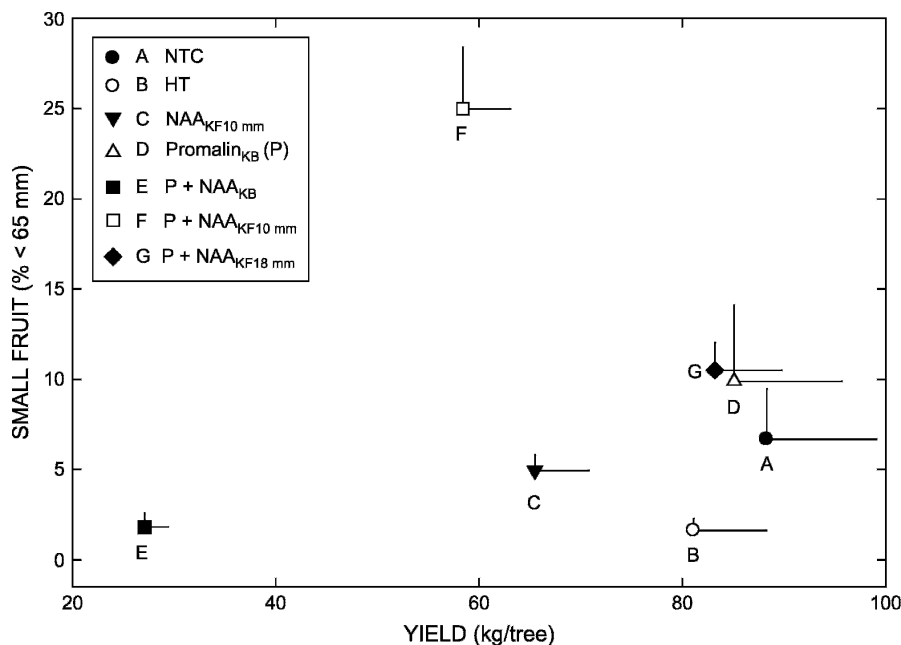


Fig. 2. Relationship between percentage of small fruit and yield in Redchief 'Delicious' treated with Promalin (P, 35 mg·L⁻¹) at 80% king bloom (KB) and oversprayed with NAA (15 mg·L⁻¹) at KB, 10 mm or 18 mm king fruit diameter (KFD) compared with Promalin (P) at KB alone, NAA (10 mm, KFD) alone and nontreated (NTC) and hand-thinned (HT) controls. Bars = SE.

the same orchard and in the same year. In the 'Delicious' study, treatments consisted of NAA at 0 and 15 mg·L⁻¹, and NAA (15 mg·L⁻¹) in combination with 0, 25, 50, and 100 mg·L⁻¹ BA (Accel). NAA was applied at 8 to 10 mm KFD and the trees were oversprayed with BA 2 to 4 h after the NAA spray application dried. Each single tree treatment was replicated four times. In the 'Empire' study, treatments consisted of NAA at 0 and 15 mg·L⁻¹ alone and in combination (oversprayed) with BA at 0, 25, and 50 mg·L⁻¹. Other experimental factors were as described for 'Delicious' previously. The amount of small fruit produced was used as an index for the effect on fruit growth.

Expt. 6: Cultivar comparison. The effects of NAA and BA alone and NAA oversprayed with BA (on the same day) were evaluated on two small-fruited ('Elstar'/M.9 and Imperial 'Gala'/Bud 9) and two large-fruited (Smoothie 'Golden Delicious'/G.30 and 'Jonagold'/G.30) cultivars. Concentration and stage of fruit development were as follow: 1) 'Elstar'—NAA (15 mg·L⁻¹), BA (50 mg·L⁻¹), and NAA + BA (same concentrations) at 7 mm KFD; 2) 'Gala'—NAA (15 mg·L⁻¹) BA (100 mg·L⁻¹), and NAA (10 mg·L⁻¹) + BA (100 mg·L⁻¹) at 9 mm KFD; 3) 'Golden Delicious'—NAA (15 mg·L⁻¹), BA (125 mg·L⁻¹), and NAA + BA (same concentrations) at 13 mm KFD; and 4) 'Jonagold'—NAA (10 mg·L⁻¹), BA (75 mg·L⁻¹), and NAA + BA (same concentrations) at 11 mm KFD. Each treatment was applied to 10 trees in a randomized complete block design. Treatment effects were assessed by total yield, size, and number (data not presented) of fruit per tree. Other experimental factors were as described earlier.

Results

Expt. 1: Promalin oversprayed with NAA. Promalin applied at KB had no significant effect on fruit abscission as indexed by yield per tree (Table 1). NAA reduced yield (≈32%), and the effect of NAA was not altered by the KB Promalin treatment. The yield of small fruit (<65 mm) was reduced by ≈52% and 70% by Promalin and NAA, respectively, compared with the NTC (Table 1). However, the combination of Promalin and NAA increased the percentage of small fruit by 3.3-fold and 5.1-fold compared with Promalin and NAA alone, respectively. The percentage of large (>70 mm) fruit was increased significantly by NAA, but not significantly greater than Promalin or NAA plus Promalin (Table 1).

Expt. 2: Promalin oversprayed with NAA. Fruit size profiles for NTC, HT, and NAA only treatments were similar, all having the greatest amount of fruit in the 76- to 82-mm size class (Fig. 1). Fruit from the Promalin-treated trees had a similar profile, but the greatest amount of fruit was shifted to the next smaller class, i.e., 70 to 75 mm. The fruit size distribution curve for the NAA (KFD 10 mm) treatment after Promalin (KB) was skewed to smaller size classes with consistently more fruit in the less than 51 mm to 70-mm classes and less in the two largest classes (76 to 82 mm, >82 mm) than in all other treatments and intermediate in the 70- to 75-mm class.

Promalin alone had no effect on yield (Table 2). Although NAA appeared to reduce yield by ≈25%, the effect was not significantly different from the NTC. NAA oversprayed on Promalin at KB, or KFD of 10 mm and 18 mm, thinned fruit, as indexed by yield,

by ≈69%, 34%, and 5%, respectively, but only the Promalin + NAA_{KB} treatment differed significantly from NTC (Table 2). The thinning response based on calculated number of fruit per tree closely followed the treatment effects indexed by weight.

Compared with the NTC, neither Promalin nor NAA alone had a significant effect on percentage of small (<65 mm) fruit produced. When Promalin-treated trees were oversprayed with NAA at KB, they were overthinned and the percentage of small fruit was reduced to 27%. However, overspraying Promalin with NAA at 10 mm KFD increased the percentage of small fruit by 3.7-fold, and application at KFD of 18 mm increased the percentage by 1.5-fold compared with the NTC; the latter equaled Promalin alone. Promalin plus NAA applied at KFD of 10 mm reduced crop load by 31% relative to Promalin plus NAA at 18 mm, but increased the percentage of small fruit 2.5-fold. Thus, the effect of the combination on inducing small fruit differed markedly with stage of flower/fruit development. Furthermore, Promalin + NAA at 10 mm KFD decreased crop load by 34% compared with the NTC but increased the percentage of small fruit by 3.7-fold. Thus, the Promalin + NAA effect on small fruit formation was not related to crop load (Table 2; Fig. 2).

Expt. 3: Time relationship between NAA and BA application—'Delicious'. The effects of BA and NAA on fruit abscission and growth were related not only to the stage of fruitlet development at time of treatment, but also to the sequence of their application (Table 3; Fig. 3). When BA was oversprayed on NAA on the same day (same developmental stage) during early fruitlet development (6 to 18 mm KFD), the thinning response, compared with NTC, was greatest at 6 mm KFD and then decreased with fruit development (Table 3). The response at 18 mm KFD was almost the same as for the NTC. Application of NAA (6 mm KFD) before BA (12 mm KFD) was more effective than applying BA (6 mm KFD) before NAA (12 mm KFD). Similar treatment effects on thinning were apparent based on calculated number of fruit per tree.

Fruit size profiles document distinctly different effects of NAA + BA on fruit growth depending on time and sequence of treatment (Fig. 3). When both compounds were applied to 'Delicious', the profiles for NAA + BA at 18 mm KFD and the NTC were identical (Fig. 3A). When applied at 6 mm KFD, the profile was skewed to larger fruit, but ≈23% of the total population was still less than 65 mm. The profile after treatment at 12 mm KFD was normally distributed; it peaked at 65- to 69-mm class and tailed with shallow slopes in both directions (Fig. 3A). Overspraying NAA and BA on the same trees at two different stages of fruitlet development revealed that NAA applied early (6 mm KFD) and BA late (12 mm KFD) resulted in more small fruit than when BA was applied early and NAA late (Fig. 3B).

Expt. 4: Time relationship between NAA and BA applications—'Fuji'. In 'Fuji', all

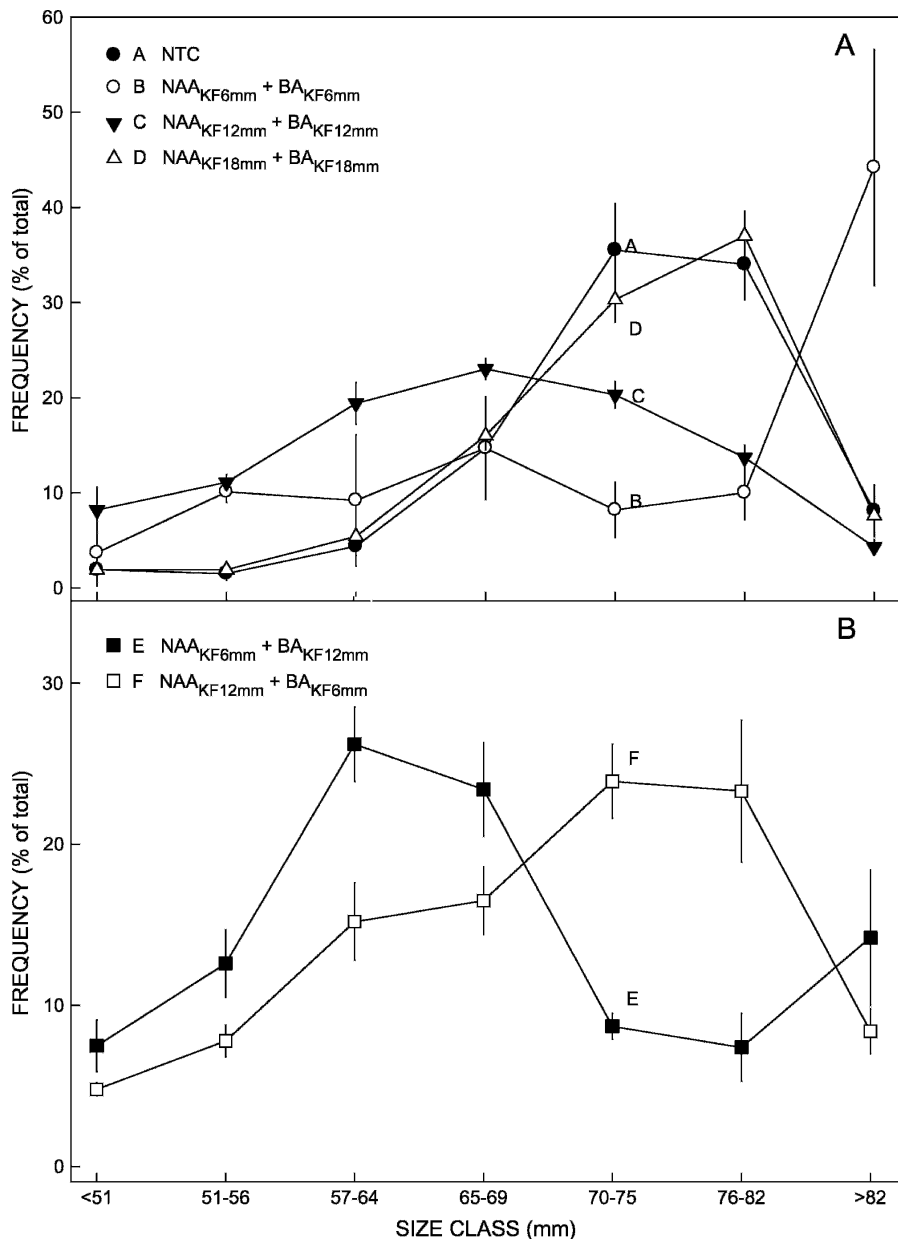


Fig. 3. Fruit size distribution profiles at harvest of Redchief 'Delicious' treated with NAA (15 mg·L⁻¹) and oversprayed with BA (50 mg·L⁻¹) at king fruit diameters (KFD) of 6 mm, 12 mm, and 18 mm compared with nontreated control (A) and when NAA was applied at 6 mm KFD followed by BA at 12 mm KFD and the reciprocal sequence (B). Bars = SE.

Table 3. Effects of NAA and BA applied at the same or different stages of early fruitlet development on yield of Redchief 'Delicious' apple^z.

NAA/BA treatment ^x	Yield ^y		
	kg/tree	NTC (%)	Fruit no./tree ^w
None (NTC)	85 a	100	596
Both at the same KFD of			
≈6 mm	5 d	6	41
≈12 mm	67 bc	79	715
≈18 mm	81 ab	95	584
NAA before or after BA			
NAA _{6 mm} /BA _{12 mm}	20 d	24	218
BA _{6 mm} /NAA _{12 mm}	50 c	59	443

^zFoliar sprays (high volume) of BA (50 mg·L⁻¹; Accel) were applied 4 to 6 h after the NAA (15 mg·L⁻¹) spray dried when both compounds were applied at the same stage of fruit development.

^yMeans followed by the same letters are not significantly different (*P* = 0.05) by the Tukey's honestly significant difference test.

^xHigh-volume sprays were made at the noted diameters of the king fruit (KFD).

^wCalculated number; see text for calculation.

BA treatments reduced yield significantly (Table 4). NAA alone reduced yield, but not significantly. Number of fruit per tree was closely related to yield except for NAA + BA applied at 12 mm KFD, in which a large number (404/tree) of small fruit (average 105 g, $\approx 30\%$ less than the NTC) were produced. Yield and number of fruit per tree were similar for NAA + BA treatments applied at two different stages (KF_{6mm} versus KF_{12mm}), but mean fruit size was significantly less when BA was applied before, rather than after, NAA.

NAA and BA applied on the same day at 12 mm KFD dramatically increased the percentage of small fruit (<75 mm) compared with application of either alone (Fig. 4A). Similar fruit size distribution profiles were obtained whether NAA was applied before or after BA. The percentage of small fruit, based on number of fruit per tree, was ≈ 1.4 -fold greater than when expressed on a fresh weight basis (Fig. 4A versus 4B). Significantly more small fruit were present when NAA was used alone compared with BA alone. There were no significant differences between the fruit size profiles relative to the sequence of NAA/BA applications.

Expt. 5: BA:NAA ratio. The ratio of the concentration of BA to NAA had a significant effect on formation of small fruit in 'Delicious', but not in 'Empire' (Fig. 5). Increasing BA concentration (0 to 150 mg·L⁻¹) alone resulted in a slight linear ($Y = 0.06x + 1.65$, $r^2 = 0.94$) increase in percentage of small fruit in 'Delicious'. In contrast, in the presence of NAA (15 mg·L⁻¹), the percentage of small fruit increased linearly ($y = 0.38x + 11.7$, $r^2 = 0.99$) between 0 and 50 mg·L⁻¹ BA and then plateaued, giving $Y = 10.90 + 0.57x - 0.004x^2$ for the 0 to 100 mg·L⁻¹ range. The effect of NAA plus BA on 'Delicious' small fruit formation was independent of crop load (Fig. 5, inset). Neither NAA nor BA alone or in combination significantly altered the percentage of small fruit formed in 'Empire'.

Expt. 6: Cultivar comparison. The combination of NAA + BA reduced yield by 8% to 49% compared with the NTC (Table 5). Mean fruit weight was significantly increased in all four cultivars. The percentage of total yield of small fruit (<60 mm) was significantly reduced in the small-fruited cultivars, Elstar and Gala, but not in the large-fruited 'Golden Delicious' and 'Jonagold' (Table 4).

Discussion

Fruit thinning 'Delicious' trees with NAAm or NAA, previously treated with Promalin to increase tyness and size of the fruit, often increased pygmy fruit formation. Further studies established an interaction between NAA and BA that resulted in inhibition of fruit growth in 'Delicious' (Bound et al., 1991; Bukovac et al., 1995). Our data confirmed that NAA + BA increased pygmy fruit formation, but also dramatically increased the percentage of small (<65 mm) fruit. Furthermore, the effect on apple fruit growth was dependent on stage of fruitlet

Table 4. Effects of over-spraying NAA with BA at the same or different stages (6 mm versus 12 mm) of king fruitlet diameter (KFD) on yield and mean fruit weight of 'Fuji' apple.

Treatment and stage of fruitlet development ^z	Yield		Mean fruit wt	
	kg/tree ^y	No./tree	g/fruit ^y	NTC (%) ^x
NTC ^x	65 a	437 a	149 c	100
NAA _{12 mm}	60 a	374 a	156 c	105
BA _{12 mm}	36 b	154 b	227 a	152
NAA _{12 mm} + BA _{12 mm}	44 b	404 a	105 d	71
NAA _{6 mm} + BA _{12 mm}	42 b	196 b	215 a	144
BA _{6 mm} + NAA _{12 mm}	46 b	239 b	189 b	127

^zFor the overspray treatments at 12 mm KFD, NAA (15 mg·L⁻¹) and BA (125 mg·L⁻¹), the chemicals were applied as separate high-volume sprays on the same day at 4- to 6-h intervals.

^yMeans within a column followed by the same letter are not significantly different at $P = 0.05$ by the Tukey's honestly significant difference test.

^xNTC = nontreated control.

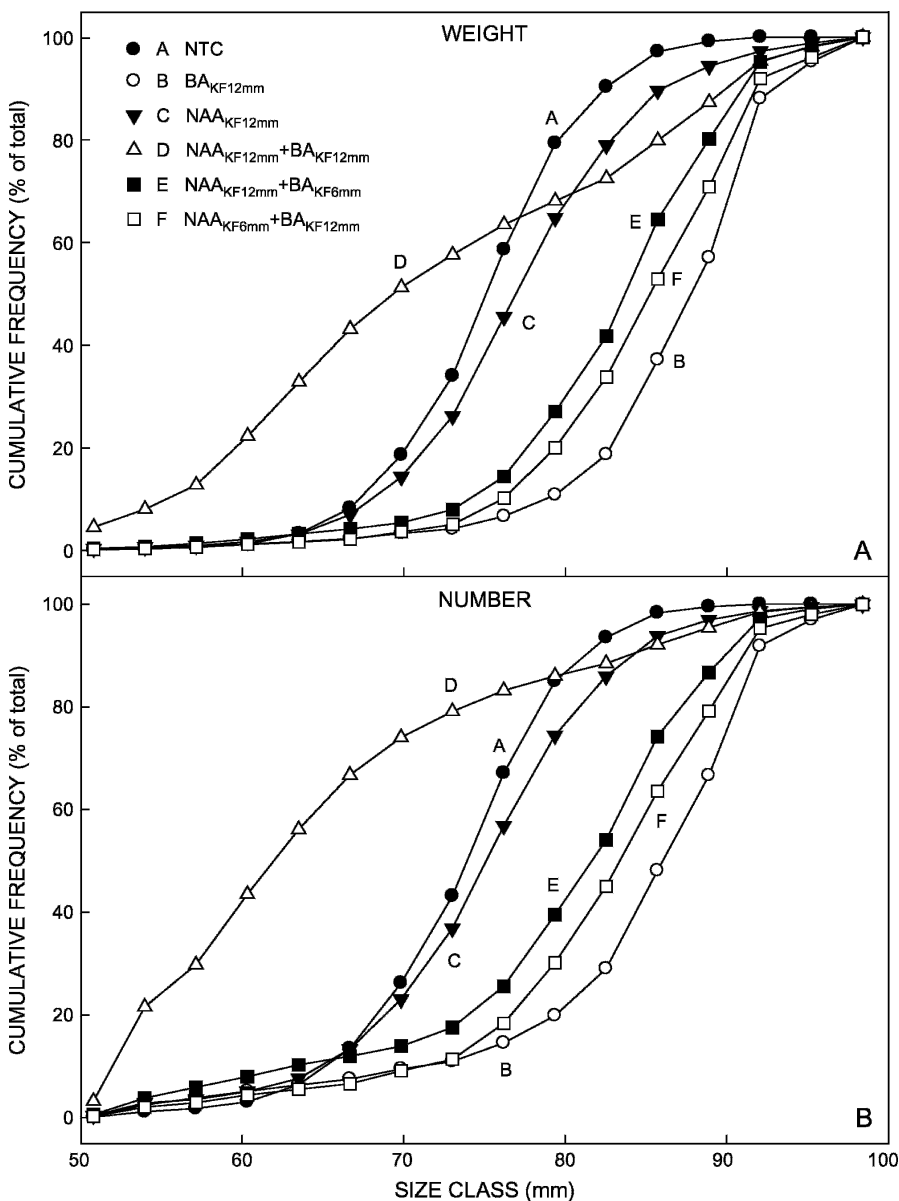


Fig. 4. Fruit size distribution profiles (cumulative frequency) at harvest of 'Fuji' from trees treated with NAA (15 mg·L⁻¹) and BA (125 mg·L⁻¹) alone and NAA oversprayed with BA at 12 mm king fruit diameter (KFD) compared with when NAA was applied at 6 mm KF and oversprayed with BA at 12 mm KFD and the reciprocal sequence and the nontreated control. Data presented as fruit weight (A) and number per tree (B).

development, the BA:NAA ratio, and cultivar and was independent of crop load and the presence of GA₄₊₇ in the BA formulations (Promalin, Accel).

When NAAm was applied at PF after Promalin at KB, the small fruit (<65 mm) population was increased ≈ 5 -fold compared with NAAm alone and ≈ 3 -fold greater than

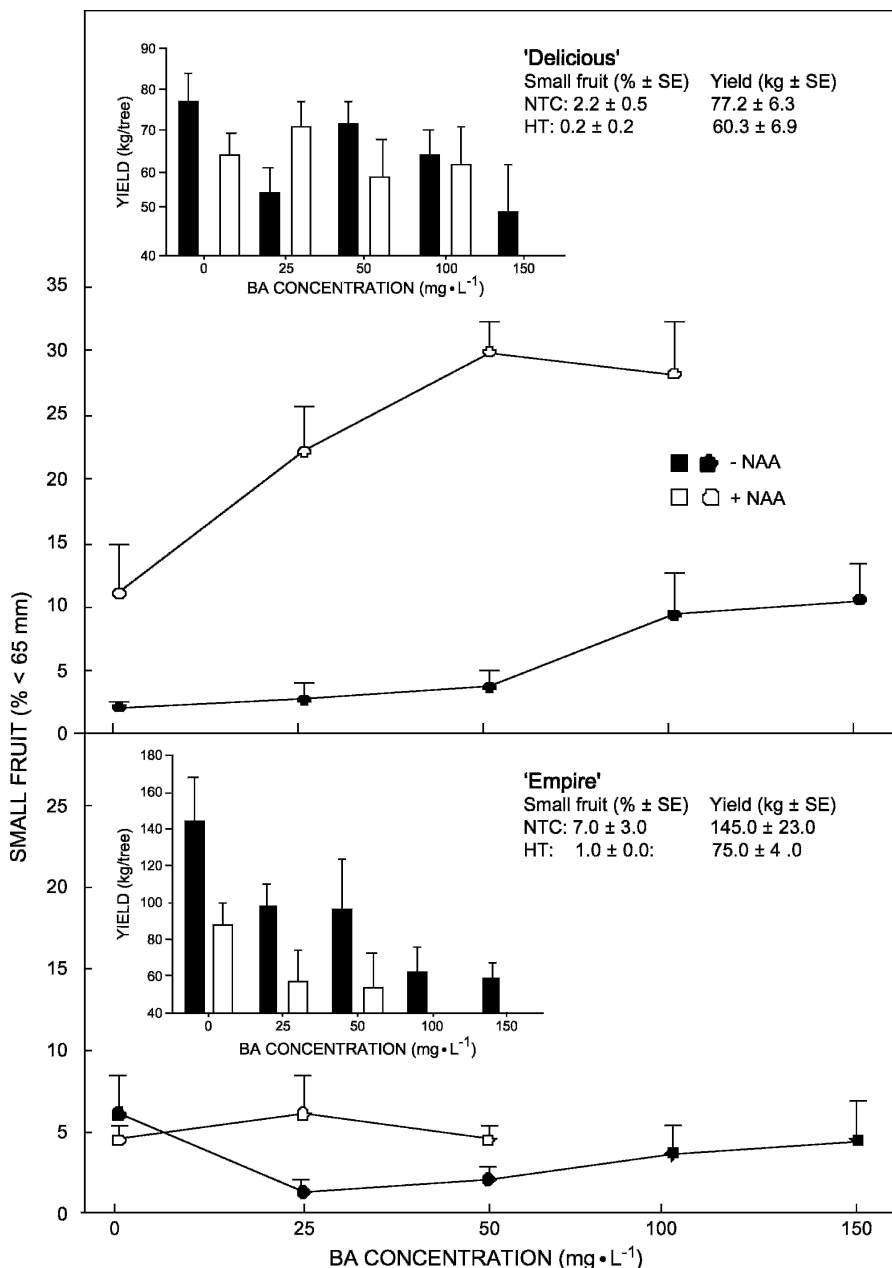


Fig. 5. Effects of increasing ratios of BA to NAA on small fruit formation and yield (inset) of 'Delicious' and 'Empire'. NAA (15 mg·L⁻¹) was oversprayed with BA (25 to 100 mg·L⁻¹) at 8 to 10 mm king fruit diameter. Bar graph (inset) indicates the effects of treatments on total yield per tree.

Promalin alone (Table 1). Similarly, NAA applied at 10 mm KFD to previously Promalin-treated (KB) 'Delicious' increased the small fruit population by 5-fold compared with NAA alone and 2.5-fold over Promalin (Table 2). In contrast, there was a reduction in percentage of small fruit when NAA followed Promalin at KB, and there was no inhibition of fruit growth when NAA at 18 mm KFD followed Promalin (KB) compared with Promalin alone (Table 2).

Similar effects on fruit growth of 'Delicious' were observed when NAA was oversprayed with BA at 6 to 18 mm KFD. The greatest inhibition of fruit growth occurred when both compounds were applied at 12 mm; no effect was apparent at 18 mm and an intermediate response occurred at 6 mm KFD

(Fig. 3). Interestingly, greater inhibition of fruit growth was obtained (46% versus 28% small fruit) in 'Delicious' when NAA was applied earlier than BA (6 mm versus 12 mm KFD) during fruitlet development (Fig. 3). The effect of applying NAA and BA at 12 mm KFD on 'Fuji' was almost identical to 'Delicious' (Fig. 4). However, there were no significant differences in the fruit size distribution curves when NAA preceded or followed BA (Fig. 4). The basis for the differences in fruitlet growth responses, relative to time and sequence, of NAA and BA applications during fruitlet development is not readily apparent. Often NAA decreases the growth rate of fruitlets compared with nontreated fruitlets, and this inhibition is usually less as fruitlets develop, e.g., ~17 to

Table 5. Effects of overspraying NAA with BA at king fruit diameters of 10 to 12 mm on yield, mean fruit weight, and percentage of small fruit of two small-fruited ('Elstar', 'Gala') and two large-fruited ('Golden Delicious', 'Jonagold') cultivars.

Treatment ^y	Measurement ^z		
	Yield (kg/tree)	Mean fruit wt (g/fruit)	Small fruit (% <60 mm)
Elstar ^x			
NTC ^w	45 b	113 b	11.1 a
NAA + BA	38 a	147 a	2.6 b
NTC (%)	84	130	23
Gala			
NTC ^w	37 a	111 b	2.9 a
NAA + BA	30 a	141 a	0.2 b
NTC (%)	81	127	7
Golden Delicious			
NTC ^w	41 a	171 b	1.2 a
NAA + BA	21 b	207 a	1.2 a
NTC (%)	51	121	100
Jonagold			
NTC ^w	37 a	217 b	0.4 a
NAA + BA	34 a	251 a	0.4 a
NTC (%)	92	116	100

^wMeans within a column, within each cultivar, followed by the same letter are not significantly different at $P = 0.05$ by the Tukey's honestly significant difference test.

^yHigh-volume foliar sprays of BA applied 4 to 6 h after NAA spray dried. See text for rates.

^xData for 'Elstar' adapted from Bukovac et al. (2000).

^wNTC = nontreated control.

21 mm KFD (Black et al., 1995; Southwick et al., 1962). We are not aware of comparable detailed data on the effect of BA on growth rate of apple fruitlets, but BA generally results in larger fruits at harvest than NAA at similar crop loads (Elfving and Cline, 1993; Greene, 1993). Nevertheless, the NAA + BA effect in 'Delicious' appears to be closely coupled to the active cell division phase of fruit growth (Blampied and Wilde, 1968). To understand the basis of this response will require new focused research.

Data from a related study with Redchief 'Delicious' provide insight on the NAA × BA interaction on fruit growth (Black et al., 2000). We found that fruit size on a given spur was not correlated with the presence of fruit on other spurs (interspur competition) on the same branch, but was highly correlated with fruit on the same spur (intraspur competition). Analysis of the effect of fruit position and number of fruit per spur revealed that the KF or a dominate lateral fruit (LF) in the absence of a KF was consistently the largest fruit with minimal variation (~4%) in the presence of one or two LF. However, the size (wt basis) of a single LF in the presence of a KF was ~20% less than the KF. If two LF were present, the secondary fruit was ~10% and tertiary ~28% smaller than the KF. Typical effect of BA + NAA on relative size of 'Delicious' fruit at maturity is illustrated in Figure 6.

The application of NAA and BA alone or together (oversprayed) affected the frequency of multifruited spurs (Black et al., 2000). NAA + BA increased the frequency by 31% compared with the NTC and ~73% and ~56% compared with NAA and BA, respectively.

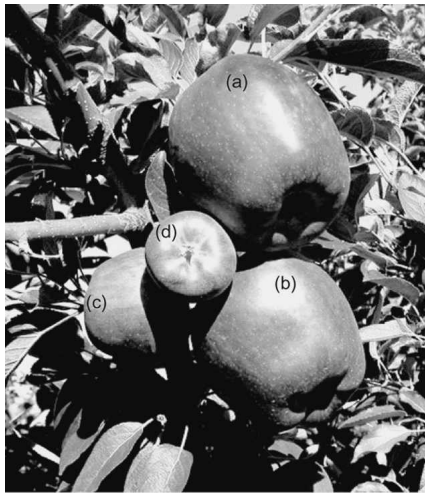


Fig. 6. Photograph of Redchief 'Delicious' fruit illustrating the difference in size of king and lateral fruit at harvest in response to a foliar spray of NAA ($15 \text{ mg}\cdot\text{L}^{-1}$) oversprayed with BA ($100 \text{ mg}\cdot\text{L}^{-1}$) at $\approx 10 \text{ mm}$ king fruit diameter. King fruit only per spur (A), king fruit (B) with a secondary (C) and tertiary (D) lateral fruit per spur.

Furthermore, the growth of a KF in the presence of a single LF was not significantly inhibited by NAA + BA, but the growth of the LF was depressed by 16%. Growth of a single LF per spur was not inhibited, but the size of a LF was reduced by 27%, in the presence of a KF, and by 14% if a second LF was present. NAA + BA also reduced seed number per fruit. Seed number in the KF averaged 3.4 in the absence of a LF and 2.8 in the presence of a LF. A more dramatic reduction was apparent in seed content of LF, from 4.4 (NTC) to 2.6 in the absence of the KF compared with 4.7 to 0.14 in the presence of a KF and from 5.5 (NTC) to 0.6 in the absence of the KF and in the presence of a second LF. Thus, the NAA + BA treatment at 10 to 12 mm KFD increased the number of multifruited spurs, thereby increasing the population of LF with low seed content. Such fruit are generally considered noncompetitive (Heinicke, 1920; Westwood et al., 1968) and develop into small fruit or abscise during June drop; however, we show that NAA + BA treatment improves their retention but not their capacity to fully develop.

The NAA + BA effect on fruit retention may be a direct auxin effect and that on fruit growth indirect, because developing seeds are critical for fruit growth (Dražeta et al., 2004). The low seed complement would be expected to reduce the sink strength of the fruit and also the supply of hormones necessary for fruit retention and growth. The high incidence of small fruit induced by the Promalin (at KB) + NAAm (at PF) treatment (Table 1) was in contrast to the response from NAA, but this may be related to overthinning and, thus, a reduction in the sensitive, lateral fruit population (Table 2). Similarly, few small fruit were produced when NAA was oversprayed with BA at the 6 mm KFD stage where overthinning also occurred (Table 3;

Fig. 3). Our data support the conclusion that NAA + BA did not inhibit cell enlargement in 'Delicious', because it had no effect on fruit growth when applied after cell division was completed (18 mm KFD; Fig. 3) in 'Delicious' (Blampied and Wilde, 1968). Our data also conclusively established that NAA + BA inhibition of fruit growth in 'Delicious' and 'Fuji' was not related to crop load. Crop load after treatments with NAA + BA was similar to or less than that after application of NAA or BA alone, but the NAA + BA treatment induced 2.5- to 5- fold more small fruit (Tables 1, 2, and 4; Figs. 1, 2, and 4).

The incidence of small fruit was increased by a broad range of BA to NAA ratios (25:15 to 125:15 $\text{mg}\cdot\text{L}^{-1}$) in 'Delicious' and 'Fuji', but not in 'Elstar', 'Gala', 'Golden Delicious', and 'Jonagold' (Fig. 5; Table 5). The amount of small fruit formation in the small-fruited cultivars Elstar and Gala was significantly reduced and mean fruit weight was increased in all cultivars (Table 5). These data are consistent with reports on NAA + BA increasing fruit size in several additional cultivars (Basak, 2006; Bukovac et al., 2007; Stopar and Lokar, 2003). The sensitivity of 'Delicious' and 'Fuji' to NAA + BA could have a genetic basis because 'Fuji' has 'Delicious' ('Delicious' \times 'Rawls Jenet') as one of its parents. Interestingly, NAA + BA does not inhibit fruit growth in 'Empire', which was derived from 'McIntosh' growing next to 'Delicious'. Resolving the mode of action of NAA and BA on fruit growth would be significant for understanding and regulating cropping in apple.

The possibility that the NAA + BA inhibition of fruit growth may be related to an effect on photosynthesis merits comment. NAA has been reported to depress CO_2 assimilation (Stopar et al., 1997; Schumacher et al., 1993) and phloem transport of photoassimilates from apple leaves (Schneider, 1978). CO_2 assimilation was significantly depressed by NAA in apple leaves of 'Delicious' and 'Empire'; BA was without effect. However, when the combination of NAA + BA was applied, BA overcame the NAA-induced inhibition of CO_2 fixation in 'Empire', but only partially, and in a second year failed to reduce the NAA effect in 'Delicious' (Stopar et al., 1997). In contrast, BA enhanced leaf CO_2 fixation and transport to developing fruit in citrus (Mauk et al., 1986). Direct evidence is not available suggesting enhanced partitioning of photoassimilates to apple fruit. Although these data provide some evidence for involvement of NAA and BA in carbon fixation in apple leaves, conclusive data are needed to couple these observations to fruit growth.

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