Effects of S-Absciscic Acid and (+)-8'-Acetylene Absciscic Acid on Fruit Set and Stomatal Conductance in Apple

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Abstract. Fruit set of apple can be reduced by cloudy weather, short-term shade treatments, or application of photosynthetic inhibitors when the young fruit are ≈ 8 to 15 mm in diameter, indicating that fruit are sensitive to a transient carbohydrate stress during this period. We investigated the potential for S-absciscic acid (ABA) and an ABA analog [(+)-8'-acetylene ABA] to chemically thin apple fruit by causing a stomatal limitation of photosynthesis. Stomatal conductance (g_s) of 'Imperial Gala'/M.7 was reduced by 60% 3 h after application of 250 mg·L⁻¹ ABA or 25 mg·L⁻¹ (+)-8'-acetylene ABA. Stomatal conductance began to recover 4 days after application but did not return to control levels until 19 days after treatment. Application of 250 mg·L⁻¹ ABA combined with 100 mg·L⁻¹ 6-benzyladenine (6-BA) when mean fruit diameter was ≈ 10 mm reduced fruit set of 'Gala'/M.7 but not 'Pink Lady'TM/M.7 or 'Morganspur Delicious'/MM.111. Fruit set of 'Pink Lady'TM/M.7 was reduced by application of 20 mg·L⁻¹ (+)-8'-acetylene ABA + 100 mg·L⁻¹ 6-BA at full bloom or 10 mg·L⁻¹ (+)-8'-acetylene ABA + 100 mg·L⁻¹ 6-BA at the 10-mm fruit diameter stage. Fruit set of 'Morganspur Delicious'/MM.111 was reduced by application of 25 mg·L⁻¹ (+)-8'-acetylene ABA, either alone or in combination with 75 mg·L⁻¹ 6-BA, at the 10-mm fruit diameter stage. ABA and (+)-8'-acetylene ABA triggered leaf abscission at rates above 250 mg·L⁻¹ and 25 mg·L⁻¹, respectively. Fruit set and g_s data from the present studies indicate the biological activity of (+)-8'-acetylene ABA is 10-fold higher than ABA. These results suggest that ABA and (+)-8'-acetylene ABA reduced fruit set by causing a stomatal limitation in photosynthesis that resulted in a transient carbohydrate stress. Thinning responses to ABA and (+)-8'-acetylene ABA at the concentrations used in these experiments were reduced compared with standard concentrations of currently available chemical thinning agents. However, increasing the concentration of ABA or (+)-8'-acetylene ABA to levels that would achieve comparable thinning are also likely to result in unacceptable leaf abscission.

Developing apple (*Malus × domestica*) fruit represent a relatively small carbohydrate sink between budbreak and bloom. Fruit are insensitive to an environmentally induced carbohydrate stress during this period and

the carbohydrates required for growth are derived from reserves (Lakso, 2011). However, as the supply of carbohydrates from reserves is depleted and the fertilized fruit begin to grow rapidly, they become increasingly dependent on current assimilates. Shoot growth has priority over fruit growth for carbohydrate partitioning when light levels during the first 40 d after bloom are limiting (Bepete and Lakso, 1998). The negative effect of post-bloom shade treatments (Byers et al., 1985, 1990, 1991; McArtney et al., 2004; Zibordi et al., 2009) or photosynthetic inhibitors (Basak, 2011; Byers et al., 1985, 1990; Clever, 2007; Deckers et al., 2010; Dorigoni and Lexxer, 2007; Lafer, 2010; McArtney and Obermiller, 2012a, 2012b) on fruit set provides evidence that a transient limitation in

carbohydrate supply can act as a strong trigger for abscission of apple fruit.

Exogenously applied ABA during early fruit development stimulates fruit abscission in apples (Edgerton, 1971; Greene et al., 2011), European pears (*Pyrus communis*) (Greene, 2012), and cherries (*Prunus avium*) (Zucconi et al., 1969) at concentrations ranging from 150 to 1000 mg·L⁻¹. The exact mode of action of ABA in apple fruitlet abscission is unknown but is probably related to a combination of ethylene induction (Edgerton, 1971) or the creation of a transient carbohydrate stress resulting from stomatal limitation of photosynthesis. Lakso (1979) reported a close linear correlation between g_s and net photosynthesis in apple trees growing under a wide range of water stress conditions, indicating that photosynthesis is primarily limited by g_s under non-limiting light and temperature conditions. More recently, Massonnet et al. (2007) reported that the relationship between g_s and photosynthesis was different in 'Braeburn' and 'Fuji' apples, although net CO₂ assimilation rate in both cultivars was positively related to g_s .

ABA is rapidly catabolized in plants, principally through oxidation of the 8'-methyl group to 8'-hydroxy ABA, which is itself further cyclized to biologically inactive phasic acid (Balsevich et al., 1994). This reaction is catalyzed by ABA 8' hydroxylase, a cytochrome P450 monooxygenase (Krochko et al., 1998). It has been suggested that ABA levels in plants could be regulated by controlling ABA catabolizing enzymes, resulting in increased stress tolerance or plants with altered seed germination and composition traits (Rose et al., 1997). This possibility was recently demonstrated by Kondo et al. (2012) who reported prompt stomatal closure and increased dehydration tolerance in apple seedlings after treatment with a specific inhibitor of ABA 8' hydroxylase [Abscizazole-F1, an analog of uniconazole with no growth retardant activity (Todoroki et al., 2009)]. An analog of ABA, (+)-8'-acetylene ABA (Fig. 1), was shown to weakly inhibit ABA 8'-hydroxylase (Rose et al., 1997). (+)-8'-acetylene ABA is a poor substrate for ABA 8' hydroxylase, resulting in greater persistence and stronger hormonal activity than ABA itself (Cutler et al., 2000). The objectives of the present study were to confirm the thinning activity of ABA and to compare the effects of ABA and (+)-8'-acetylene ABA on fruit set and g_s in apple.

Materials and Methods

All trees used in this series of experiments were growing at the North Carolina State University Mountain Horticultural Crops Research and Extension Center in Mills River, NC, and exhibited uniform heavy bloom in the year of treatment. The trees were in mature orchards planted at 6.0 m between rows and 3.0 m between trees in the row (556 trees/ha), trained to a central leader, and managed according to accepted commercial practices as described in the Integrated Orchard Management Guide for Commercial

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Apples in the Southeast (Anon., 2013). An industry standard thinning spray of 75 to 100 mg·L⁻¹ 6-BA plus 600 mg·L⁻¹ carbaryl was included in all thinning experiments.

'Imperial Gala'/M.7—2010. Forty-two uniform trees were selected at bloom from a block of 15-year-old 'Imperial Gala'/'Malling 7' (M.7) trees. The following seven treatments were assigned to fully guarded single-tree plots in a randomized complete block design experiment with six replications: 1) an untreated control; 2) a standard chemical thinning treatment of 100 mg·L⁻¹ 6-BA (MaxCel; Valent BioSciences, Libertyville, IL) plus 600 mg·L⁻¹ carbaryl (Carbaryl 4L; Drexel Chemical Co., Memphis, TN); 3) 100 mg·L⁻¹ 6-BA plus 250 mg·L⁻¹ ABA (Valent BioSciences); 4) 100 mg·L⁻¹ 6-BA plus 500 mg·L⁻¹ ABA; 5) 100 mg·L⁻¹ 6-BA plus 750 mg·L⁻¹ ABA; 6) 100 mg·L⁻¹ 6-BA plus 7.5 mg·L⁻¹ naphthalene acetic acid (NAA, Fruitone L; AMVAC Chemical Corp., Los Angeles, CA); and 7) 500 mg·L⁻¹ ABA plus 7.5 mg·L⁻¹ NAA. Throughout this article, ABA refers to the S-enantiomer of abscisic acid. The treatments were applied with an airblast sprayer calibrated to deliver 1700 L·ha⁻¹ when the mean diameter of spur

fruit was 9.7 mm. No additional surfactants were included with any of the treatments. Three uniform sample limbs were selected on each tree just before bloom and the total number of flower clusters on spurs was counted on each limb. The number of spur fruit remaining on each limb was counted after the completion of fruit drop. Fruit set was calculated as the number of remaining spur fruit per 100 spur clusters. Total fruit number per tree, total fruit weight, and fruit size distribution were measured at harvest.

'Pink LadyTM'/M.7—2012. A randomized complete block design experiment with five blocks and eight treatments was established in a planting of mature 'Pink LadyTM'/M.7 apple trees. The treatments included an untreated control, three chemical thinning treatments applied at full bloom, and four thinning treatments applied when the mean fruit diameter was 10 mm. The three chemical thinning treatments applied at full bloom were 1) 100 mg·L⁻¹ 6-BA plus 250 mg·L⁻¹ ABA; 2) 100 mg·L⁻¹ 6-BA plus 10 mg·L⁻¹ (+)-8'-acetylene ABA; and 3) 100 mg·L⁻¹ 6-BA plus 20 mg·L⁻¹ (+)-8'-acetylene ABA. The four thinning treatments applied at the 10-mm fruit diameter stage

were 1) a standard chemical thinning treatment of 100 mg·L⁻¹ 6-BA plus 600 mg·L⁻¹ carbaryl; 2) 100 mg·L⁻¹ 6-BA plus 250 mg·L⁻¹ ABA; 3) 100 mg·L⁻¹ 6-BA plus 10 mg·L⁻¹ (+)-8'-acetylene ABA; and 4) 100 mg·L⁻¹ 6-BA plus 20 mg·L⁻¹ (+)-8'-acetylene ABA. The spray treatments were applied to fully guarded single-tree plots with an airblast sprayer calibrated to deliver 1420 L·ha⁻¹. No additional surfactants were included with any of the treatments. Fruit set was measured as in the previous experiment. Trees in this study did not receive any additional hand-thinning. Total fruit number and weight per tree were recorded at harvest and mean fruit weight was calculated from the data.

'Morganspur Delicious'/MM.111—2013. Thirty-five uniform trees were selected at bloom from a block of 'Morganspur Delicious'/'Malling Merton 111' (MM.111) trees. The following seven treatments were assigned to fully guarded single tree plots in a randomized block design experiment with five replications: 1) an untreated control; 2) a standard chemical thinning treatment of 75 mg·L⁻¹ 6-BA plus 600 mg·L⁻¹ carbaryl; 3) 75 mg·L⁻¹ 6-BA; 4) 25 mg·L⁻¹ (+)-8'-acetylene ABA; 5) 25 mg·L⁻¹ (+)-8'-acetylene ABA plus 75 mg·L⁻¹ 6-BA; 6) 250 mg·L⁻¹ ABA; and 7) 250 mg·L⁻¹ ABA plus 75 mg·L⁻¹ 6-BA. The treatments were applied with an airblast sprayer calibrated to deliver 1324 L·ha⁻¹ to fully guarded single tree plots when the mean diameter of spur fruit was 11.1 mm. A non-ionic organosilicone surfactant was included with Treatments 4 and 6 at 0.05% (Break Thru; Plant Health Technologies, Lathrop, CA). No additional surfactants were included with the other thinning treatments because they included a commercial formulation of 6-BA that has been formulated with a surfactant. Fruit set was measured as in the previous experiments. Total number and weight of fruit per tree were recorded at harvest. *g_s* was measured on six leaves per tree at 1- to 2-d intervals on the untreated control (Treatment 1), 25 mg·L⁻¹ (+)-8'-acetylene ABA (Treatment 4), and 250 mg·L⁻¹ ABA (Treatment 6). *g_s* was measured with a steady-state diffusion porometer (Model SC-1; Decagon Devices, Pullman, WA). The youngest fully expanded leaves on vegetative shoots were chosen for conductance measurements. Total fruit number and weight per tree were recorded at harvest and mean fruit weight

Fig. 1. Structures of S-absciscic acid (ABA) and (+)-8'-acetylene absciscic acid.

Table 1. Effect of various combinations of S-absciscic acid (ABA), benzyladenine (6-BA), carbaryl, and naphthalene acetic acid (NAA) thinning treatments on fruit set, yield and fruit number per tree, and mean fruit weight of 'Imperial Gala'/M.7 apple.²

Treatment (mg·L ⁻¹)	Fruit set (fruit/100 clusters)	Yield (kg/tree)	Fruit (no./tree)	Mean fruit wt (g)
Control	89 a	116 a ²	1087 a	106 d
6-BA (100) + carbaryl (600)	34 d	78 bc	592 d	133 a
6-BA (100) + ABA (250)	66 b	101 ab	908 ab	112 cd
6-BA (100) + ABA (500)	50 c	86 bc	722 bcd	118 bcd
6-BA (100) + ABA (750)	57 bc	75 bc	646 cd	117 bcd
6-BA (100) + NAA (7.5)	31 d	72 c	559 d	129 ab
ABA (500) + NAA (7.5)	62 bc	101 ab	812 bc	123 abc
Significance ^x	***	**	***	**

²Treatments were applied when the mean fruit diameter was 9.7 mm.

^xMeans within a column followed by different letters are significantly different at $P \leq 0.05$ as determined by Duncan's multiple range test ($n = 5$).

***, ** Significant at $P < 0.01$ or 0.001 , respectively.

Table 2. Effects of 6-benzyladenine (6-BA) combined with either S-absciscic acid (ABA) or (+)-8'-acetylene ABA on fruit set, yield, fruit number per tree, and mean fruit weight of 'Pink LadyTM'/M.7 apple trees.

Treatment (mg·L ⁻¹)	Time of application	Fruit set (fruit/100 clusters)	Yield (kg/tree)	Fruit (no./tree)	Mean fruit wt (g)
Control		111 b ²	112 a	788 a	142 de
ABA (250) + 6-BA (100)	Full bloom	130 a	106 abc	770 a	138 e
(+)-8'-acetylene ABA (10) + 6-BA (100)	Full bloom	99 bc	107 ab	720 a	150 cd
(+)-8'-acetylene ABA (20) + 6-BA (100)	Full bloom	87 c	95 abc	620 ab	155 bc
ABA (250) + 6-BA (100)	10 mm fruit diam.	99 bc	80 cd	522 bc	154 bc
(+)-8'-acetylene ABA (10) + 6-BA (100)	10 mm fruit diam.	64 d	84 bcd	512 bc	163 ab
(+)-8'-acetylene ABA (20) + 6-BA (100)	10 mm fruit diam.	58 de	81 cd	472 bc	172 a
6-BA (100) + carbaryl (600)	10 mm fruit diam.	46 e	68 d	396 c	172 a
Significance ^y		***	**	***	***

²Means within a column followed by different letters are significantly different at $P \leq 0.05$ as determined by Duncan's multiple range test ($n = 5$).

***, ** Significant at $P < 0.01$ or 0.001 , respectively.

Table 3. Effects of 6-benzyladenine (6-BA) combined with either S-abscisic acid (ABA) or (+)-8'-acetylene ABA on fruit set, yield, fruit number per tree, and mean fruit weight of 'Morganspur Delicious'/MM.111 apple trees.^z

Treatment (mg·L ⁻¹)	Fruit set (fruit/100 clusters)	Yield (kg/tree)	Fruit (no./tree)	Mean fruit wt (g)
Control	78 a ^y	61 a	306 a	198 bc
6-BA (75)	78 a	61 a	321 a	191 c
ABA (250)	70 abc	62 a	305 a	204 bc
(+)-8'-acetylene ABA (25)	59 bc	58 a	251 a	238 abc
ABA (250) + 6-BA (75)	72 ab	64 a	268 a	243 ab
(+)-8'-acetylene ABA (25) + 6-BA (75)	57 c	55 a	255 a	219 abc
6-BA (75) + carbaryl (600)	19 d	19 b	81 b	260 a
Significance ^x	***	***	***	*

^zTreatments were applied when the mean fruit diameter was 11.1 mm.

^yMeans within a column followed by different letters are significantly different at $P < 0.05$ as determined by Duncan's multiple range test ($n = 5$).

^x*, *** Significant at $P < 0.05$ or 0.001, respectively.

Fig. 2. Effect of 250 mg·L⁻¹ S-abscisic acid (ABA) or 25 mg·L⁻¹ (+)-8'-acetylene ABA on stomatal conductance (g_s) of 'Morganspur Delicious'/MM.111 apple trees. Initial measurements were taken 1 d before treatment and measurements on Day 0 were taken 3 h after treatments were applied. Absolute values of g_s are presented in A; g_s relative to the control value on each day is presented in B. Data points in A represent means $\pm$ SE ($n = 5$).

calculated from these data. In addition, the number of fully developed seeds and seed traces was counted in a random sample of 10 fruit per tree. The proportion of parthenocarpic fruit was calculated from this data. Fruit shape (length:diameter ratio) was measured on this sample.

'Imperial Gala'/M.7—2013. Ten uniform vegetative shoots were selected on each of five replicate 'Imperial Gala'/M.7 trees. One shoot

on each tree was sprayed with (+)-8'-acetylene ABA at 0, 2.5, 10, 25, or 50 mg·L⁻¹ or with ABA at 0, 25, 100, 250, or 500 mg·L⁻¹. A non-ionic organosilicone surfactant (Break Thru) was included with all of the treatments at 0.05%. Stomatal conductance was measured 1 d after treatment on five fully expanded leaves per shoot as previously described. The number of leaves that abscised on each shoot was counted weekly until 21 d after treatment.

Statistical analysis. The data were analyzed using analysis of variance procedures in SAS software (Version 9.2; SAS Institute Inc., Cary, NC). Treatment effects on fruit set, total yield, fruit number, and mean fruit weight were analyzed using the generalized linear model procedure and means separation by LSMEANS. Differences between treatment means were assessed by Duncan's multiple range test at the 0.05 P level.

Results

All of the treatments reduced fruit set compared with the control in the 2010 'Imperial Gala'/M.7 study (Table 1). The most aggressive thinning treatments were the commercial standard (6-BA plus carbaryl) and the combination of 6-BA plus NAA. Combinations of ABA and 6-BA reduced fruit set; however, there was no clear effect of ABA concentration. NAA plus 500 mg·L⁻¹ ABA reduced fruit set compared with the control. The thinning activity of this combination was significantly less than the combination of NAA and 6-BA. Taken together, these results indicate that although combinations of ABA with either 6-BA or NAA exhibited thinning activity, the level of thinning was significantly reduced compared with combinations of current commercially available thinning chemicals: 6-BA and carbaryl or 6-BA and NAA. Differences in yield and fruit number per tree and mean fruit weight of 'Imperial Gala'/M.7 at harvest appeared related to treatment effects on fruit set and cropload.

The combination of 20 mg·L⁻¹ (+)-8'-acetylene ABA plus 6-BA was the only bloom-thinning treatment to reduce fruit set of 'Pink Lady'TM/M.7 compared with the untreated control. All thinning treatments applied at the 10-mm fruit diameter stage exhibited some degree of thinning compared with the control (Table 2). The combination of 20 mg·L⁻¹ (+)-8'-acetylene ABA plus 6-BA reduced fruit more effectively than 250 mg·L⁻¹ ABA plus 6-BA, indicating the thinning activity of (+)-8'-acetylene ABA was $\approx$ 10-fold higher than ABA. Like in the previous study, differences in yield and fruit number per tree, and mean fruit weight of 'Pink Lady'TM/M.7 at harvest, appeared related to treatment effects on fruit set and cropload.

Thinning treatments that included 25 mg·L⁻¹ (+)-8'-acetylene ABA reduced fruit set of 'Morganspur Delicious'/MM.111 in

Fig. 3. Comparison of effect of S-abcisic acid (ABA) and (+)-8'-acetylene ABA concentration on stomatal conductance of 'Imperial Gala'/M.7 apple trees. Conductance values are plotted using a common scale for ABA and (+)-8'-acetylene ABA in **A**; the same data are presented in **B** except conductance values for (+)-8'-acetylene ABA concentration are multiplied by a factor of 10. Stomatal conductance was measured 1 d after treatment. Data points represent means $\pm$ SE ($n = 5$).

2013, whereas treatments that included 250 mg·L⁻¹ ABA were without effect on fruit set (Table 3). The standard thinning treatment of 75 mg·L⁻¹ 6-BA plus 600 mg·L⁻¹ carbaryl overthinned 'Morganspur Delicious'/MM.111, reducing fruit set to 19 fruit per 100 clusters compared with 78 fruit per 100 clusters for the control. This overthinning was also reflected by a similar reduction in yield and fruit number per tree and increased mean fruit weight at harvest. Unlike the previous experiments, trees in the 2013 'Morganspur Delicious'/MM.111 study were hand-thinned to a commercial cropload at the completion of natural fruit drop. For this reason, there were no effects of (+)-8'-acetylene ABA on tree yield, fruit number, and mean fruit weight at harvest.

Stomatal conductance of 'Morganspur Delicious'/MM.111 was reduced by 60% compared with the control within 3 h of application of either 250 mg·L⁻¹ ABA or 25 mg·L⁻¹ (+)-8'-acetylene ABA (Fig. 2) and conductance did not begin to recover until 4 d after application. Recovery of g_s was gradual with some inhibition still evident 19 d after application. Leaf g_s had completely recovered by 21 d after application of either ABA or (+)-8'-acetylene ABA. These data indicate that although (+)-8'-acetylene ABA exhibited higher biological activity than ABA, the persistence of these two chemicals was similar. (+)-8'-acetylene ABA was $\approx$ 10-fold more active than ABA with respect to inhibition of g_s of 'Imperial Gala'/M.7 (Fig. 3). A 50%

reduction in g_s 1 d after treatment resulted from application of ABA and (+)-8'-acetylene ABA at 25 mg·L⁻¹ and 2.5 mg·L⁻¹, respectively. Some leaf abscission was observed after application of 500 mg·L⁻¹ ABA and 50 mg·L⁻¹ (+)-8'-acetylene ABA (Table 4). In the case of ABA, little additional leaf abscission was observed 7 d after treatment, whereas leaves continued to abscise for 21 d after application of (+)-8'-acetylene ABA. Concentrations of ABA and (+)-8'-acetylene ABA that exhibited thinning activity (250 mg·L⁻¹ and 25 mg·L⁻¹, respectively) did not result in increased leaf abscission compared with the control. Furthermore, no leaf abscission was observed in any of the thinning studies reported here.

There were no effects of ABA or (+)-8'-acetylene ABA on fruit shape of 'Morganspur Delicious'/MM.111 as measured by the length:diameter ratio; however, some treatments did reduce seed number per fruit and the proportion of parthenocarpic fruit at harvest (Table 5). When either 6-BA or (+)-8'-acetylene ABA was applied individually, they did not influence seed development; however, when they were applied in combination, the number of fully developed seeds per fruit was decreased and the number of parthenocarpic fruit at harvest was increased to 24% compared with only 2% in the control (Table 5). In comparison, application of ABA alone or in combination with 6-BA was without effect on seed development. Application of the commercial chemical thinning standard of 6-BA + carbaryl reduced the number of fully developed seeds per fruit and the proportion of parthenocarpic fruit at harvest more severely than the combination of 6-BA and (+)-8'-acetylene ABA (Table 5).

Discussion

Results from these experiments support previous findings that ABA can trigger abscission in young apple fruit (Edgerton, 1971; Greene et al., 2011). At the concentrations used in the present studies, ABA would be considered a mild thinner compared with commercially available chemical thinning agents. The mode of action of ABA as a thinning agent is probably related to the creation of a transient reduction in the supply of carbohydrates to developing fruit resulting from a stomatal limitation of photosynthesis, although other mechanisms cannot be ruled out. ABA reduced g_s by 60% within 3 h of application, and conductance did not begin to recover until 4 d after application. Full recovery of g_s was gradual, taking 21 d or more in the case of 'Morganspur Delicious'/MM.111. Given the direct positive relationship between g_s and net assimilation in apple (Lakso, 1979; Massonnet et al., 2007), this suppression is likely to have significantly reduced the supply of carbohydrates to developing fruits.

The efficacy of ABA as a chemical thinning agent in apple will be dependent on whole-tree net carbon assimilation as determined by the combined effects of environmental conditions

Table 4. Comparison of effects of S-abscisic acid (ABA) or (+)-8'-acetylene ABA concentration on leaf abscission (%) on vegetative shoots of 'Imperial Gala'/M.7 apple trees after treatment.

Treatment	Concn (mg·L ⁻¹)	No. of abscised leaves at different intervals after treatment (%)		
		7 d	14 d	21 d
ABA	0	0.0 a ^z	0.0 a	0.0 a
	25	0.0 a	0.0 a	0.0 a
	100	0.7 a	0.7 a	0.7 a
	250	5.7 a	5.7 a	5.7 a
	500	15.4 b	16.1 b	16.8 b
(+)8'-acetylene ABA	0	0.0 a	0.0 a	0.0 a
	2.5	0.0 a	0.7 a	0.7 a
	10	0.0 a	0.0 a	0.6 a
	25	6.5 a	7.3 a	10.5 ab
	50	23.3 c	27.1 c	32.3 c
Significance ^y		***	***	***

^zMeans within a column followed by different letters are significantly different at $P < 0.05$ as determined by Duncan's multiple range test ($n = 5$).

^y*** Significant at $P < 0.001$.

Table 5. Effects of 6-benzyladenine (6-BA) combined with either S-abscisic acid (ABA) or (+)-8'-acetylene ABA on seed development, parthenocarp, and fruit length:diameter (L:D) ratio of 'Morganspur Delicious'/M.111 apple trees.^z

Treatment (mg·L ⁻¹)	Seeds/fruit (no.)			Parthenocarpic fruit (%)	Fruit L:D ratio
	Full seeds	Trace seeds	Total seeds		
Control	7.8 a ^y	0.8 ab	8.7 ab	2 a	0.94
6-BA (75)	8.3 a	0.9 ab	9.2 a	4 a	0.96
ABA (250)	7.4 a	0.7 ab	8.1 bc	2 a	0.95
(+)8'-acetylene ABA (25)	8.7 a	0.4 a	9.0 a	0 a	0.92
ABA (250) + 6-BA (75)	7.4 a	1.1 ab	8.5 ab	4 a	0.96
(+)8'-acetylene ABA (25) + 6-BA (75)	5.8 b	1.7 b	7.4 c	24 b	0.96
6-BA (75) + carbaryl (600)	3.6 c	2.9 c	6.5 d	40 c	0.91
Significance ^x	***	***	***	***	NS

^zTreatments were applied when the mean fruit diameter was 11.1 mm.

^yMeans within a column followed by different letters are significantly different at $P \leq 0.05$ as determined by Duncan's multiple range test ($n = 5$).

^xNS, *** Nonsignificant or significant at $P < 0.001$, respectively.

immediately after treatment and the extent and duration of a stomatal limitation of photosynthesis. For example, the thinning response is predicted to be much greater if cloudy days (reduced photosynthesis) and warm nights (high respiration) follow ABA application compared with sunny days (high photosynthesis) and cool nights (low respiration). In this regard, the efficacy of ABA as a chemical thinning agent may eventually be predicted using a carbon balance modeling approach (Lakso, 2011; Robinson and Lakso, 2011). However, such a strategy will require more complete description of the effects of ABA on whole-tree carbon acquisition rather than indirect (g_s) or direct (photosynthesis) measurements at an individual leaf level. Furthermore, if rates of ABA catabolism differ between apple cultivars, then the response to ABA may be cultivar-specific, making accurate prediction of the thinning response more difficult in a practical sense.

The ABA analog (+)-8'-acetylene ABA exhibited thinning activity at rates that were one-tenth that of ABA. Direct comparison of the g_s response to varying rates of ABA and (+)-8'-acetylene ABA provides clear evidence for the 10-fold increase in biological activity of (+)-8'-acetylene ABA compared with ABA. (+)-8'-acetylene ABA is perceived as ABA-like by the receptor complex (Abrams et al., 2011) and is more persistent than ABA

itself as a result of lower rates of catabolism (Cutler et al., 2000). (+)-8'-acetylene ABA was also shown to hyperinduce many ABA-regulated genes, including the transcription factor *MYBRI* suggested to be a negative regulator of ABA signal transduction (Huang et al., 2007). The incidence of parthenocarpic fruit at harvest was unaffected by ABA but was significantly increased by the combination of (+)-8'-acetylene ABA and 6-BA. Interestingly, the incidence of parthenocarpic fruit was even higher in fruit treated with a standard chemical thinning spray of 6-BA + carbaryl. The reason why (+)-8'-acetylene ABA + 6-BA stimulated parthenocarpic fruit development is unknown at this time. Because a relationship among seed number, fruit calcium content, and storage disorders is occasionally reported in apple (Bramlage et al., 1990), parthenocarpic fruit resulting from (+)-8'-acetylene ABA + 6-BA treatment may have a higher risk of postharvest disorder development.

The thinning activity of ABA or (+)-8'-acetylene ABA in the present studies was significantly lower than currently available thinning agents. Commercially acceptable levels of thinning after ABA or (+)-8'-acetylene ABA application would require concentrations that could trigger unacceptable levels of leaf yellowing and/or abscission, although it has been suggested that inclusion of 6-BA

can reduce ABA-related plant leaf yellowing (Greene et al., 2011; Liu et al., 2010). The leaf abscission we observed in the current study will probably limit the commercial potential for ABA or (+)-8'-acetylene ABA alone as a chemical thinning agent in apple. The inhibitory effects of ABA or (+)-8'-acetylene ABA on g_s at concentrations lower than 250 mg·L⁻¹ and 25 mg·L⁻¹, respectively, are likely to reduce the supply of carbohydrates to developing fruit. Carbon deficits within the tree at this time are believed to increase the sensitivity of developing fruit to chemical thinners. Thus, although ABA or (+)-8'-acetylene ABA might not exhibit direct chemical thinning activity, their application could potentially increase the thinning activity of commercially available thinning agents. Such a strategy might be useful in situations where the fruit are otherwise insensitive to chemical thinners; for example, when environmental conditions favor a positive carbon balance within the tree. Alternatively, creation of a transient carbohydrate stress by ABA or (+)-8'-acetylene ABA may permit equivalent thinning with reduced rates of currently available chemical thinning agents.

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