Use of 1-Aminocyclopropane Carboxylic Acid and Metamitron for Delayed Thinning of Apple Fruit

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Abstract. The normal window for application of thinning chemicals in apple extends from bloom until 3 weeks after bloom, when the fruit reach a mean diameter of ≈ 16 mm. After this time fruit are generally insensitive to standard chemical thinning sprays. The potential for the photosystem II (PSII) inhibitor metamitron and the ethylene precursor 1-aminocyclopropane carboxylic acid (ACC) to thin apple fruit after the traditional thinning window was investigated in field experiments over three years. A standard rescue thinning spray of carbaryl plus ethephon plus naphthaleneacetic acid (NAA) reduced fruit set of Gale ‘Gala’ if applied when the mean fruit diameter was 18, 20, and 27 mm in 2010, 2011, and 2012, respectively. The thinning activity of 400 mg·L⁻¹ ACC was equivalent to the standard rescue thinning spray in 2010, whereas 350 mg·L⁻¹ metamitron reduced fruit set more effectively than either the standard or ACC in 2010. Application of 400 mg·L⁻¹ ACC plus 350 mg·L⁻¹ metamitron when the mean fruit diameter was 18 mm reduced fruit set to almost no crop in 2010. The combination of metamitron plus ACC exhibited thinning activity after application at 25 and 33 mm mean fruit diameter in 2011 and 2012, respectively. Increased ethylene evolution was found in detached ‘GoldRush’ fruit 24 h after applications of ACC from 11 mm to 27 mm mean fruit diameter, but not when ACC was applied at 31 mm mean fruit diameter. Ethylene evolution was much higher after application of ACC at the 11 mm or 17 mm mean fruit diameter stage compared with application when fruit diameter was 23 mm or 27 mm. The thinning activity of ACC was related to the period of maximum ethylene response. The effects of delayed applications of ACC and metamitron on fruit set tended to be greater when these two chemicals were combined, suggesting that the creation of a carbohydrate stress and the capacity to convert ACC to ethylene are both required to trigger abscission of apple fruit larger than 18 mm in diameter.

Chemical stimulation of young fruit abscission during the first 3 weeks after bloom is a key management consideration in modern apple (*Malus ×domestica* Borkh.) production systems. Sequential applications of chemical thinners with different modes of action are normally made during this period to reduce fruit set to commercially acceptable levels that eliminate the need for hand thinning, increase fruit size at harvest, and increase the probability of adequate return

bloom in the next year. Young fruit generally become insensitive to thinning chemicals after they reach a diameter of ≈ 16 mm, coincident with increasing carbohydrate reserves within the tree (Lakso et al., 1999). Studies describing the effects of shade treatments on fruit set in apple (Byers et al., 1985, 1990, 1991; McArtney et al., 2004; Zibordi et al., 2009), together with studies of altered gene expression after the imposition of abscission stimuli including shade (Zhou et al., 2008; Zhu et al., 2011), 6-benzyladenine (Botton et al., 2011), and naphthaleneacetic acid (Zhu et al., 2011), provide evidence in support of the hypothesis that a carbohydrate deficit in the fruit is one of the earliest responses to chemical or environmental stimuli that trigger abscission in apple fruit.

Commercial recommendations for delayed or rescue thinning (Cornell Pest Management Guidelines for Commercial Tree Fruit Production, 2012; Integrated Orchard Management Guide for Commercial Apples in the Southeast, 2012; Pennsylvania 2012–2013 Tree Fruit Production Guide, 2012) rely almost exclusively on 2-chloroethylphosphonic acid (ethephon) and 1-naphthyl methylcarbamate (carbaryl), which are both coming under

increasingly stringent regulatory pressures worldwide (Anon, 2006, 2009). Thinning responses after application of ethephon can be more erratic than other thinning materials and high ambient temperatures after application of ethephon at bloom may result in excessive thinning (Jones and Koen, 1985). In addition, ethephon may cause the fruit of some cultivars to flatten (Basak, 2006; Williams and Fallahi, 1999). The availability of an effective chemical strategy for delayed thinning, when fruit range from 18 to 30 mm in diameter, would provide apple growers with increased options for thinning in years when primary thinning sprays have not reduced fruit set to a commercially acceptable level.

Two new chemicals have been reported to have thinning activity in apple. The PSII inhibitor metamitron exhibited thinning activity when applied to apple fruitlets at the 10 to 12 mm diameter stage (Basak, 2011; Clever, 2007; Deckers et al., 2010; Dorigoni and Lexner, 2007; Lafer, 2010) and to ‘SunCrisp’ apple when mean fruit diameter was ≈ 20 mm (McArtney and Obermiller, 2012). Metamitron disrupts the photosynthetic apparatus for 7 to 10 d after application, reducing electron transport rates by up to 60% (McArtney and Obermiller, 2012). These data suggest that thinning activity of metamitron is a response to the creation of a transient carbohydrate stress within the fruit. If a carbohydrate surplus is responsible, at least in part, for lack of activity of conventional chemical thinning agents when applied later than 3 weeks after bloom, then application of a PSII inhibitor such as metamitron may increase sensitivity of fruit to abscission agents during periods of natural carbohydrate surplus at this time or even earlier.

The ethylene precursor ACC was recently shown to have thinning activity in apple (McArtney, 2011; Schupp et al., 2012). Ethylene evolution from detached spurs was highest 1 d after application of ACC and gradually declined to control levels over the next 8 to 10 d. Lack of an autocatalytic ethylene response to exogenous application of ACC suggests that system 2 ethylene was not operating at this time. Ethylene evolution from fruiting spurs of ‘Cripps Pink’ was greatly reduced after application of ACC 31 d after bloom, when the mean fruit diameter was 20 mm compared with applications made at full bloom or 16 d after bloom (McArtney, 2011). The thinning activity of ACC is thought to be directly related to its rapid metabolism to ethylene during periods when the abscission zone cells are sensitive to this hormone. Loss of the capacity to convert exogenously applied ACC to ethylene might reflect a reduction in activity of *ACC oxidase* (ACO). Loss of ACO activity beginning 3 weeks after bloom might also be partly responsible for the decreased sensitivity of immature fruit to chemical thinners at this time.

Botton et al. (2011) proposed a hypothetical model for immature fruit abscission in apple in response to 6-benzylaminopurine, in which sugar starvation in the fruit cortex

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ultimately triggered ethylene signaling pathways, specifically upregulation of *ACC synthase* and *Ethylene Responsive Factor* genes. It was proposed that ethylene generated in the fruit cortex in response to a nutritional stress (synonym carbohydrate deficit) diffused to the seed, ultimately halting embryogenesis and polar auxin transport from the fruit. Zhu et al. (2011) also found that shading or NAA induced expression of genes involved in ethylene biosynthesis and perception and repressed the expression of genes involved in auxin transport in the fruit abscission zone. This decrease in polar auxin transport was associated with increases in ethylene production and expression of ethylene biosynthesis and signaling related genes (Zhu et al., 2011). Botton et al. (2011) proposed that generation of a carbohydrate deficit in the fruit cortex provides the primary stimulus for fruit abscission, and ethylene is not only an integral part of downstream signaling pathways, but increased ethylene levels provide the stimulus that ultimately activates the abscission zone in the fruit pedicel. According to this hypothesis, the PSII inhibitor metamitron and the ethylene precursor ACC may provide useful chemical tools with which to investigate the roles of carbohydrates and ethylene in abscission of apple fruit later than 3 weeks after bloom. The objectives of the present study were to evaluate the potential for delayed applications of the ethylene precursor ACC and the PSII inhibitor metamitron to thin apple fruit.

Materials and Methods

Delayed thinning of Gale 'Gala': Expts. 1 to 3

A planting of mature Gale 'Gala'/M.9 trees at the Mountain Horticultural Crops Research and Extension Center (MHCRC) in Mills River, NC, was used for thinning experiments in three consecutive years (2010–12). Trees that were uniform in size and cropload were selected each year. All trees in the planting were chemically thinned with 1200 mg·L⁻¹ carbaryl (Sevin XLR Plus; Bayer CropScience, Research Triangle Park, NC) when the fruit were 10 mm in diameter in a spray volume of 1496 L·ha⁻¹. This thinning spray was applied on 23 Apr., 12 Apr., and 19 Apr. in 2010, 2011, and 2012, respectively. Maximum/minimum air temperatures and average daily solar radiation on the day of application were 28/10 °C and 24.9 MJ in 2010, 21/7 °C and 10.3 MJ in 2011, and 19/ 10 °C and 8.6 MJ in 2012. There was still excessive cropload after this chemical thinning treatment each year.

Expt. 1 (2010). When the mean fruit diameter was 18 mm, as determined by measuring all the persisting fruit on a random sample of 100 spurs (14 May), the following eight treatments were applied to fully guarded single tree plots arranged in a randomized complete block (RCB) design experiment with six blocks: 1) an unthinned control; 2) a standard rescue thinning spray of 1200 mg·L⁻¹ carbaryl (Carbaryl 4L; Drexel

Chemical Co., Memphis, TN) + 300 mg·L⁻¹ ethephon (Ethrel; Bayer CropScience) + 10 mg·L⁻¹ NAA (Fruitone L; AMVAC Chemical Corp., Los Angeles, CA); 3) a standard rescue thinning spray of 1200 mg·L⁻¹ carbaryl + 300 mg·L⁻¹ ethephon + 0.1% v/v Silwet L-77 (Helena Chemical Co., Collierville, TN); 4) 400 mg·L⁻¹ ACC (VBC-30160; Valent BioSciences, Libertyville, IL); 5) 350 mg·L⁻¹ metamitron (Goltix; Makhteshim Agan of North America, Inc., Raleigh, NC); 6) a tank mix of 400 mg·L⁻¹ ACC + 350 mg·L⁻¹ metamitron; 7) a tank mix of 350 mg·L⁻¹ metamitron + 300 mg·L⁻¹ ethephon; and 8) a tank mix of 350 mg·L⁻¹ metamitron + 400 mg·L⁻¹ ACC + 10 mg·L⁻¹ NAA. Thinning treatments were applied with an airblast sprayer calibrated to deliver 1496 L·ha⁻¹. The number of actively growing fruit was counted on three sample limbs on each tree on 13 May, just before applying the treatments. The number of persisting fruit was counted on each limb at the completion of fruit drop (30 June) and from these data, fruit set was calculated as the percent of initial fruit that remained. A random sample of 40 fruit per tree was removed at harvest for determination of treatment effects on mean fruit weight and fruit shape. The number of fruit within this sample that exhibited a dropped calyx or that were flattened was counted.

Expt. 2 (2011). The following three chemical thinning treatments were applied to fully guarded single-tree plots when mean fruit diameter was 20 mm (6 May), 25 mm (16 May), or 31 mm (24 May): 1) a standard rescue thinning spray of a tank mix of 1200 mg·L⁻¹ carbaryl (Carbaryl 4L; Drexel Chemical Company, Memphis, TN) + 300 mg·L⁻¹ ethephon + 10 mg·L⁻¹ NAA; 2) 175 mg·L⁻¹ metamitron (Metamitron 150 SG; Makhteshim Agan of North America, Inc.); and 3) a tank mix of 175 mg·L⁻¹ metamitron + 200 mg·L⁻¹ ACC (VBC-30160; Valent BioSciences). There was an unsprayed control in addition to the nine chemical thinning treatments. Thinning treatments were applied with an axial fan airblast sprayer calibrated to deliver 1496 L·ha⁻¹. Treatments were arranged in a RCB experiment with five blocks. Treatment effects on fruit set were measured as in the previous study. No measurements of fruit quality at harvest were made in this study.

Expt. 3 (2012). In addition to an unthinned control treatment, the following two thinning treatments were applied when mean fruit diameter was 20 mm (30 Apr.), 27 mm (11 May), or 33 mm (18 May): 1) a standard rescue thinning spray of 1200 mg·L⁻¹ carbaryl + 300 mg·L⁻¹ ethephon + 10 mg·L⁻¹ NAA; or 2) 350 mg·L⁻¹ metamitron (Metamitron 150 SG; Makhteshim Agan of North America, Inc.) + 400 mg·L⁻¹ ACC (VBC-30160; Valent BioSciences). Thinning treatments were applied with an axial fan airblast sprayer calibrated to deliver 1637 L·ha⁻¹. Treatments were arranged in a RCB experiment with six blocks. Treatment effects on fruit set were measured as in the previous studies. A random sample of 40 fruit per tree

was removed at harvest for determination of treatment effects on mean fruit weight and fruit shape (length/diameter ratio). The number of fruit within this sample that exhibited a dropped calyx or that were flattened was counted.

Metamitron ± 1-aminocyclopropane carboxylic acid timing study

The effect of metamitron and the time of application of 200 mg·L⁻¹ ACC (Sigma-Aldrich Corp., St. Louis, MO) on fruit set and ethylene evolution from detached fruiting spurs was investigated in 2011 on mature 'GoldRush'/M.7 apple trees growing at the MHCRC in Mills River, NC. A total of 166 fruiting spurs, each with four actively growing fruit, was selected on each of eight individual trees on 28 Apr. 2011. Metamitron (100 mg·L⁻¹) was applied to four trees on 28 Apr. with an airblast sprayer calibrated to deliver 935 L·ha⁻¹. ACC was applied to the foliage and fruit on 16 spurs per tree with a trigger sprayer on 28 Apr. (mean fruit diameter 11 mm), 5 May (mean fruit diameter 17 mm), 12 May (mean fruit diameter 23 mm), 19 May (mean fruit diameter 27 mm) or 26 May (mean fruit diameter 31 mm). Mean fruit diameter on each day of application was determined by measuring the diameter of each fruit within a random sample of 30 fruiting spurs removed from untreated trees within the same orchard. An organosilicone surfactant (Silwet® L-77; Helena Chemical Co.) was included in each treatment at a final concentration of 0.1% v/v. Thirty-six fruiting spurs were left untreated on each tree. Four treated spurs and four untreated spurs were removed 1 d after each ACC application for measurement of ethylene evolution, leaving 12 spurs of each treatment on each tree for measurement of final fruit set. Ethylene evolution was measured from the detached fruit only, including the pedicel. Fruit were sealed in 236-mL glass jars for 4 h at 20 °C before ethylene measurement. A 1.0-mL gas sample was withdrawn from the headspace and injected onto a gas chromatograph (Model GC-8A; Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and an activated alumina column (Supelco Division, Sigma-Aldrich, Bellefonte, PA). The rate of ethylene production was expressed as nL ethylene per gram fresh weight per hour (nL·g⁻¹·h⁻¹).

Delayed thinning of 'Cameo'

Twenty-one six-year-old 'Cameo'/M7 apple trees uniform in size and cropload were chosen from within a planting at the MHCRC in Mills River, NC, in 2012. The following seven treatments were applied in a RCB design experiment with three replications: 1) unthinned control; 2) standard rescue thinning spray of 1200 mg·L⁻¹ carbaryl + 300 mg·L⁻¹ ethephon + 10 mg·L⁻¹ NAA; 3) 350 mg·L⁻¹ metamitron; 4) 400 mg·L⁻¹ ACC; 5) 350 mg·L⁻¹ metamitron + 400 mg·L⁻¹ ACC; 6) 350 mg·L⁻¹ metamitron + 0.1% (v/v) organosilicone surfactant (Silwet L-77); and 7) 400 mg·L⁻¹ ACC followed by covering

the trees for 5 d with a neutral density 70% shade cloth. The spray treatments were applied on 30 Apr. (mean fruit diameter 19.1 mm) to fully guarded single-tree plots with an axial fan airblast sprayer calibrated to deliver 935 L·ha⁻¹. Fruit set was recorded on a random sample of 50 fruiting spurs on two-year-old wood on each tree. Ethylene evolution from detached fruiting spurs was measured 1 d and 3 d after all treatments except for Treatment 7 as previously described. Chlorophyll fluorescence (F_v/F_m) was measured on four recently expanded leaves per tree using a portable chlorophyll fluorometer (OS1p; Opti-Sciences, Hudson, NH) with a modulated light source of 0.2 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at 660 nm and a saturation pulse from a white light light-emitting diode with an intensity of 7700 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for a duration of 0.8 s. Leaves were dark-adapted for 30 min before measurement to ensure that all capable PSII reaction centers were fully oxidized.

Statistical analysis

The fruit set data were analyzed using SAS software (SAS Institute Inc., Cary, NC). Analysis of variance was conducted using the proc generalized linear model and mean separations by LSMEANS. Differences between treatment means were assessed by Duncan's multiple range test at $P \leq 0.05$.

Results and Discussion

Fruit set on Gale 'Gala' trees that were not re-thinned, expressed as a percent of the number of fruit at the time that the treatments were applied, was 44%, 54%, and 82% in 2010, 2011, and 2012, respectively (Figs. 1 to 3). Differences in fruit set in the control trees each year reflect differences in the extent of natural June drop or in the activity of the carbaryl thinning spray applied at 10 mm fruit diameter. The fruit drop that occurs from 15 to 40 d after full bloom can be triggered by short periods (2 d to 4 d) of cloudy weather (Byers et al., 1991) and may be stimulated by shading treatment lasting as few as 3 d (Byers et al., 1991; McCartney et al., 2004), indicating that the fruit remain sensitive to a transient carbohydrate deficit until ≈ 40 d after bloom. Application of a standard rescue thinning spray (carbaryl + ethephon + NAA) when mean fruit diameters ranged from 18 to 20 mm significantly reduced fruit set on Gale 'Gala' trees relative to the control in each year (Figs. 1 to 3) and reduced fruit set when applied at the 27-mm stage in 2012. The standard re-thinning spray did not result in thinning if it was applied when fruit were greater than 25 mm in 2011 or greater than 33 mm in 2012. Substituting a surfactant (Silwet L-77) for NAA in the standard rescue thinning spray resulted in similar thinning (Fig. 1), indicating that this strategy could be used to thin cultivars such as 'Spur Red Delicious' strains or Fuji where delayed applications of NAA would normally stunt fruit growth and increase the proportion of pygmy fruit at harvest (Greene and Autio, 1994; Jones et al., 1991; Rogers and Williams, 1977).

Application of ACC (400 mg·L⁻¹) or metamitron (350 mg·L⁻¹) when mean fruit diameter was 18 mm reduced fruit set of Gale 'Gala' in 2010, with metatiron having greater thinning activity than either ACC or the standard rescue thinning spray (Fig. 1). Rescue thinning treatments that included metatiron aggressively thinned Gale 'Gala' in 2010, reducing cropload to levels that were less than commercially acceptable. A combination of metatiron plus ACC thinned Gale 'Gala' more aggressively than either material alone in 2010 (Fig. 1), suggesting an additive effect of a carbohydrate stress and ethylene on fruit abscission. None of the treatments affected fruit shape (length/diameter ratio) or the percent of flattened fruit or fruit with a dropped calyx in 2010 or 2012 (data not shown). Application of standard rescue thinning treatment (carbaryl + ethephon + NAA) when mean fruit diameter was 20 mm exhibited thinning activity on Gale 'Gala' in 2011 but did not reduce fruit set

if applied when mean fruit diameter was greater than 25 mm (Fig. 2). In contrast, the combination of metatiron (175 mg·L⁻¹) plus ACC (200 mg·L⁻¹) exhibited thinning activity when applied at the 20 mm or the 25 mm fruit diameter stage, but not at the 31 mm diameter stage (Fig. 2). In 2012 the standard rescue thinning treatment had thinning activity if applied to Gale 'Gala' when the mean fruit diameter was 20 mm or 27 mm, but not when the mean fruit diameter was 33 mm (Fig. 3). Application of metatiron (350 mg·L⁻¹) plus ACC (400 mg·L⁻¹) at the 20-mm stage resulted in aggressive thinning of Gale 'Gala' in 2012, and some leaf yellowing and abscission was observed after treatment at this time. The combination of metatiron plus ACC also thinned Gale 'Gala' at the 27 mm and 33 mm fruit diameter stages in 2012, although the thinning activity was reduced compared with the earlier application timing (Fig. 3). Thus, these data indicate that delayed application

Fig. 1. Effects of delayed application of different combinations of 1-aminocyclopropane carboxylic acid (ACC) (400 mg·L⁻¹), metatiron (350 mg·L⁻¹), carbaryl (1200 mg·L⁻¹), ethephon (300 mg·L⁻¹), naphthaleneacetic acid (NAA) (10 mg·L⁻¹), and Silwet L-77 (0.05% v/v) on fruit set of Gale 'Gala' apples in 2010. Treatments were applied when the mean fruit diameter was 18 mm (14 May). Bars represent means $\pm$ SE (n = 6). Bars followed by the same letter are not significantly different at $P \leq 0.05$ using Duncan's multiple range test.

Fig. 2. Effects of delayed application of 1-aminocyclopropane carboxylic acid (ACC) (200 mg·L⁻¹), metatiron (175 mg·L⁻¹), carbaryl (1200 mg·L⁻¹), ethephon (300 mg·L⁻¹), and naphthaleneacetic acid (NAA) (10 mg·L⁻¹) on fruit set of Gale 'Gala' apples in 2011. Treatments were applied when the mean fruit diameter was 20 mm (6 May), 25 mm (16 May), or 31 mm (24 May). Bars represent means $\pm$ SE (n = 5). Bars followed by the same letter are not significantly different at $P \leq 0.05$ using Duncan's multiple range test.

Fig. 3. Effects of delayed application of 1-aminocyclopropane carboxylic acid (ACC) (400 mg·L⁻¹), metamitron (350 mg·L⁻¹), carbaryl (1200 mg·L⁻¹), ethephon (300 mg·L⁻¹), and naphthaleneacetic acid (NAA) (10 mg·L⁻¹) on fruit set of Gale 'Gala' apples in 2012. Treatments were applied when the mean fruit diameter was 20 mm (30 Apr.), 27 mm (11 May), or 33 mm (18 May). Bars represent means ± SE (n = 5). Bars followed by the same letter are not significantly different at $P \leq 0.05$ using Duncan's multiple range test.

of metamitron or ACC alone can effectively thin apple fruit up to 20 mm in diameter, and the combination of these two chemicals can exhibit thinning activity in some years even if application is delayed until the mean fruit diameter was 33 mm. We speculate that the thinning activity of metamitron will be dependent on the combined effects of the concentration applied and the ambient light and temperature conditions immediately after application. Previous research has shown an increasingly negative effect of metamitron concentration on the maximum potential quantum efficiency of PSII in apple leaves and a linear relationship between metamitron concentration and thinning activity (McArtney and Obermiller, 2012). We speculate that application of metamitron immediately before a period of carbohydrate deficit in the tree will have greater thinning activity compared with application before a period of carbohydrate surplus.

Because there were no significant interactions between metamitron and ACC in the 'GoldRush' study, only the main effects are presented. Application of metamitron (100 mg·L⁻¹) when the mean fruit diameter was 11 mm resulted in a significant reduction in fruit set ($P < 0.01$) from 1.5 fruit per cluster to 1.3 fruit per cluster (data not shown). The relatively mild thinning activity of metamitron at this time may be related to the low concentration (100 mg·L⁻¹), which was previously shown to have a minimal effect on the maximum potential quantum efficiency of PSII in 'Suncrisp' apples (McArtney and Obermiller, 2012). In previous work we reported a rapid increase in ethylene evolution after ACC application that peaked 1 d after application but persisted for up to 8 d after application (McArtney, 2011). This pattern of ethylene release was observed after application of ACC to 'Pink Lady' at full bloom or when mean fruit diameter was 10 mm (16 d after full bloom), but the ethylene response was greatly suppressed when ACC was applied at the 20-mm stage (31 d after full bloom). In the present study a significant increase in ethylene evolution from detached fruit was measured 1 d after

application of ACC (200 mg·L⁻¹) to 'GoldRush' when the mean fruit diameter was 11, 17, 23, and 27 mm but not 31 mm (Fig. 4A). Ethylene evolution was higher after application of ACC at the 11 mm or 17 mm fruit diameter stages compared with the 23-mm or 27-mm stages (Fig. 4A). ACC (200 mg·L⁻¹) reduced fruit set when it was applied at the 11 mm or 17 mm fruit diameter stages but did not reduce fruit set if applied when the mean fruit diameter was 23 mm or larger (Fig. 4B). These data suggest a positive relationship between the amount of ethylene released immediately after application of ACC and the final thinning response. In addition, the ethylene evolution data from this study provide additional support for the hypothesis that the activity of ACC oxidase is greatly reduced once the fruit reach ~18 to 20 mm in diameter. A decline in the ability of tissues to metabolize ACC into ethylene may be responsible, at least in part, for the loss of sensitivity to chemical thinning agents as fruits grow.

Application of metamitron (350 mg·L⁻¹) to 'Cameo' when the mean fruit diameter was 19 mm had no effect on ethylene evolution but reduced the maximum potential quantum efficiency of PSII (F_v/F_m) for at least 8 d (Table 1) and was without effect on fruit set. Inclusion of an organosilicone surfactant resulted in a greater reduction in F_v/F_m 8 d after treatment compared with metamitron alone and a significant reduction in fruit set compared with the control (Table 1). These fluorescence data support the findings of a previous study, which showed that the addition of an organosilicone surfactant further enhanced the negative effects of metamitron on F_v/F_m , the quantum photosynthetic yield of PSII (Φ_{PSII}), and the estimated relative electron transport rate (ETR) (McArtney and Obermiller, 2012). This was the only instance in the present study when delayed application of metamitron did not exhibit thinning activity. The lack of a thinning response in this study might be explained by the possibility that application of metamitron at this time did not create a severe enough carbohydrate

Fig. 4. Effects of fruit diameter at time of 1-aminocyclopropane carboxylic acid (ACC) (200 mg·L⁻¹) application on ethylene evolution from detached fruit (A) and final fruit set (B) of 'GoldRush' apples in 2011. Ethylene evolution was measured 1 d after each time of ACC application. Error bars represent SE (n = 4). NS, **, ***Nonsignificant or significant at $P \leq 0.001$ or $P \leq 0.01$, respectively. Bars followed by the same letter are not significantly different at $P \leq 0.05$ using Duncan's multiple range test.

deficit in the fruit to trigger abscission. In support of this argument, daily average solar radiation levels were relatively high during the 10 d period after metamitron application in this study (mean of 13.2 MJ per day; SD of 4.5 MJ per day). We have found that application of metamitron at similar rates to that used in this study reduced Φ_{PSII} and ETR across a range of apple cultivars by 40% to 60% (unpublished data). If such a reduction in photosynthetic performance is coincident with periods of low ambient light, then the likelihood of creating a carbohydrate deficit that is severe enough to trigger fruit abscission is higher compared with a similar reduction in photosynthetic performance during periods of adequate or high ambient light levels.

Application of ACC (400 mg·L⁻¹) to 'Cameo' when the fruit were 19 mm in diameter increased ethylene evolution from detached spurs and significantly reduced fruit set compared with the control but was without effect on F_v/F_m (Table 1). The fruit abscission response to ACC, in the absence of an effect on photosynthetic efficiency, suggests that a burst in ethylene alone can provide the trigger for fruit abscission during periods of positive carbohydrate balance in the tree. In previous studies we have observed that ACC can trigger fruit abscission

Table 1. Effects of 1-aminocyclopropane carboxylic acid (ACC) (400 mg·L⁻¹) and metamitron (350 mg·L⁻¹) application to 'Cameo' apple trees on 30 Apr. 2012 (mean fruit diameter 19.1 mm) on ethylene evolution from fruit, dark-adapted leaf chlorophyll fluorescence (F_v/F_m), and fruit set compared with a standard delayed thinning spray of carbaryl (1200 mg·L⁻¹) + ethephon (300 mg·L⁻¹) + naphthaleneacetic acid (NAA) (10 mg·L⁻¹).

Treatment	Ethylene (nL·g ⁻¹ ·h ⁻¹)		F _v /F _m			Fruit set (fruit/spur)	Defruited spurs (%)
	+1 d	+3 d	+3 d	+5 d	+8 d		
Control	0.55 a ^z	0.29 a	0.79 a	0.80 a	0.77 a	2.1 a	12.7 a
Carbaryl + ethrel + NAA	1.62 a	1.30 bc	0.78 a	0.78 a	0.76 a	1.6 ab	26.7 abc
Metamitron	0.82 a	0.21 a	0.64 b	0.67 b	0.65 b	1.6 ab	21.3 ab
ACC	4.87 b	1.62 c	0.79 a	0.79 a	0.76 a	1.1 b	44.0 c
Metamitron + ACC	3.99 b	1.07 abc	0.68 b	0.69 b	0.64 b	0.9 b	44.7 c
Metamitron + Silwet L-77	1.03 a	0.33 ab	0.65 b	0.65 b	0.56 c	1.3 b	38.0 bc
ACC + shade ^y	—	—	—	0.77 a	0.73 a	0.0 c	98.7 d
Significance Pr > F	<0.0001	0.028	<0.0001	<0.0001	<0.0001	<0.001	<0.0001

^zMeans within a column followed by different letters are significantly different $P < 0.05$ by Duncan's multiple range test ($n = 3$).

^yTrees were covered with a neutral density black polythene shadecloth (80%) for 5 d after ACC application.

extremely rapidly; in this particular experiment, we noted significant fruit abscission occurring within 3 d of ACC application. This response is much more rapid than the existing thinning chemicals. The combination of ACC and metamitron (350 mg·L⁻¹) resulted in both increased ethylene evolution and a reduction in photosynthetic efficiency compared with the control but did not stimulate any additional thinning compared with ACC alone. In contrast, the combination of ACC followed by 5 d of artificial shade defruited almost all of the spurs (Table 1). Because there was not a shade-only treatment in this experiment, we cannot eliminate the possibility that the excessive thinning we measured in response to the ACC + shade treatment was not the result of shade alone. Nevertheless, previous studies with similar levels of shade have resulted in aggressive thinning (McArtney et al., 2004) but not in defruiting of almost all of the spurs like in the present study. Because metamitron alone did not reduce fruit set of 'Cameo' in 2012 nor did its application in combination with ACC stimulate any additional thinning compared with ACC alone, whereas the combination of ACC and 5 d of shade practically eliminated fruit set, we suggest that metamitron did not reduce the level of carbohydrates in the fruit to a critical level that would trigger the fruit abscission process.

Our results indicate that the PSII inhibitor metamitron and the ethylene precursor ACC can trigger fruit abscission in apple during periods when the fruit are generally insensitive to currently available thinning chemicals. The combined application of metamitron and ACC generally exhibited greater thinning activity than either chemical alone, even having thinning activity if applied when the mean fruit diameter was 33 mm in one study. Metamitron and ACC may be important thinning compounds in the future, particularly in light of the increasingly stringent regulatory pressures faced by some currently available thinning chemicals (Anon, 2006, 2009). Furthermore, metamitron and ACC

may provide useful biochemical probes to further investigate the relationship between carbohydrate supply and ethylene formation in the fruit abscission process in apple and how this relationship might change as fruit develop.

Literature Cited

- Anon. 2006. Review report for the active substance carbaryl. 26 Oct. 2011. <http://ec.europa.eu/food/plant/protection/evaluation/existactive/list-carbaryl_en.pdf>.
- Anon. 2009. Review of the existing maximum residue levels (MRLs) for ethephon. European Food Safety J. 7:1347.
- Basak, A. 2006. The effect of fruitlet thinning on fruit quality parameters in the apple cultivar 'Gala'. J. Fruit and Ornamental Plant Research. 14:143–150.
- Basak, A. 2011. Efficiency of fruitlet thinning in apple 'Gala Must' by use of metamitron and artificial shading. J. Fruit and Ornamental Plant Research. 19:51–62.
- Botton, A., G. Eccher, C. Forcato, A. Ferrarini, M. Begheldo, M. Zermiani, S. Moscatello, A. Battistelli, R. Velasco, B. Ruperti, and A. Ramina. 2011. Signaling pathways mediating the induction of apple fruitlet abscission. Plant Physiol. 155:185–208.
- Byers, R.E., J.A. Barden, R.F. Polomski, R.W. Young, and D.H. Carbaugh. 1990. Apple fruit abscission by photosynthetic inhibition. J. Amer. Soc. Hort. Sci. 115:14–19.
- Byers, R.E., D.H. Carbaugh, C.N. Presley, and T.K. Wolf. 1991. The influence of low light on apple fruit abscission. J. Hort. Sci. 66: 7–17.
- Byers, R.E., C.G. Lyons, and K.S. Yoder. 1985. Peach and apple thinning by shading and photosynthetic inhibition. J. Hort. Sci. 60: 465–472.
- Clever, M. 2007. A comparison of different thinning products applied to the apple variety 'Elstar Elshof' in the lower Elbe region. Erwerbs-Obstbau 49:107–109.
- Cornell Pest Management Guidelines for Commercial Tree Fruit Production. 2012. Cornell Univ. Coop. Ext. 25 June 2012. <<http://ipmguidelines.org/TreeFruits/Chapters/CH11/default-6.aspx>>.
- Deckers, T., H. Schoofs, and W. Verjans. 2010. Looking for solutions for chemical fruit thinning on apple. Acta Hort. 884:237–244.

- Dorigoni, A. and P. Lexxer. 2007. Chemical thinning of apple with new compounds. Erwerbs-Obstbau 49:93–96.
- Greene, D.W. and W.R. Autio. 1994. Combination sprays with benzyladenine to chemically thin spur-type 'Delicious' apples. HortScience 29: 887–890.
- Integrated Orchard Management Guide for Commercial Apples in the Southeast. 2012. North Carolina State Univ. North Carolina Coop. Ext. Service AG-572. 25 June 2012. <<http://www.ces.ncsu.edu/fletcher/programs/apple/2012orchard-management.pdf>>.
- Jones, K.M. and T.B. Koen. 1985. Temperature effects on ethephon thinning of apples. J. Hort. Sci. 60:13–19.
- Jones, K.M., T.B. Koen, S.A. Bound, and M.J. Oakford. 1991. Some reservations in thinning 'Fuji' apples with naphthalene acetic acid (NAA) and ethephon. N. Z. J. Crop Hort. Sci. 19:225–228.
- Lafer, G. 2010. Effects of chemical thinning with metamitron on fruit set, yield and fruit quality of 'Elstar'. Acta Hort. 884:531–536.
- Lakso, A.N., J.N. Wunsche, J.W. Palmer, and L. Corelli-Grappadelli. 1999. Measurement and modeling of carbon balance of the apple tree. HortScience 34:1040–1047.
- McArtney, S. 2011. Effects of 1-aminocyclopropane carboxylic acid on the rate of ethylene release from detached fruiting spurs and on fruit abscission in apple. J. Hort. Sci. Biotechnol. 86:640–644.
- McArtney, S., M. White, I. Latter, and J. Campbell. 2004. Individual and combined effects of shading and thinning chemicals on abscission and dry-matter accumulation of 'Royal Gala' apple fruit. J. Hort. Sci. Biotechnol. 79:441–448.
- McArtney, S.J. and J.D. Obermiller. 2012. Comparison of the effects of metamitron on chlorophyll fluorescence and fruit set in apple and peach. HortScience 47:509–514.
- Pennsylvania 2012–2013 Tree Fruit Production Guide. 2012. Penn. State Univ. AGRS-045. 25 June 2012. <<http://pubs.cas.psu.edu/FreePubs/PDFs/agrs045.pdf>>.
- Rogers, B.L. and G.R. Williams. 1977. Chemical thinning of spur-type 'Delicious' apple trees. Va. Fruit 65:23–28.
- Schupp, J.R., T.M. Kon and H. E. Winzeler. 2012. 1-aminocyclopropane carboxylic acid shows promise as a chemical thinner for apple. HortScience 47:1308–1311.
- Williams, K.M. and E. Fallahi. 1999. The effects of exogenous bioregulators and environment on regular cropping of apple. HortTechnology 9: 323–327.
- Zhou, C., A.N. Lakso, T.L. Robinson, and S. Gan. 2008. Isolation and characterization of genes associated with shade-induced apple abscission. Mol. Genet. Genomics 280:83–92.
- Zhu, H., C.D. Dardick, E.P. Beers, A.M. Callanhan, R. Xia, and R. Yuan. 2011. Transcriptomics of shading-induced and NAA-induced abscission in apple (*Malus domestica*) reveals a shared pathway involving reduced photosynthesis, alterations in carbohydrate transport and signaling and hormone crosstalk. BMC Plant Biology 11:138. <<http://www.biomedcentral.com/1471-2229/11/138>>.
- Zibordi, M., S. Domingos, and L. Corelli Grappadelli. 2009. Thinning apples via shading: An appraisal under field conditions. J. Hort. Sci. Biotechnol. ISAFRUIT Special Issue:138–144.