

# Continuous Requirement of Ethylene for Apple Fruit Volatile Synthesis

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Pre- and postclimacteric Super Red Delicious apple (*Malus domestica* Borkh.) fruit were treated with ethephon, aminoethoxyvinylglycine (AVG), and diazocyclopentadiene (DACP) after harvest. Ethylene and volatile production were measured 1, 5, and 13 days after treatment. Production of some esters by preclimacteric fruit was enhanced after ethephon treatment, but the same treatment reduced production of various esters by postclimacteric fruit. Ethylene and volatile production were inhibited by AVG and DACP in preclimacteric fruit. Volatile acetate esters were not affected by any treatment, whereas AVG and DACP inhibited production of other volatile esters in postclimacteric fruits. The results suggest synthesis of some volatile esters requires continuous ethylene action and a high rate of ethylene production.

**Keywords:** Apple; ethylene; ethylene action; volatile biosynthesis

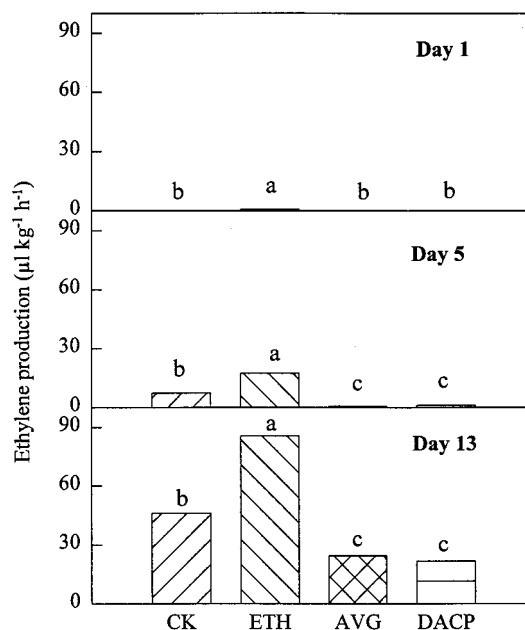
## INTRODUCTION

Climacteric fruit ripening is the sum of a series of events including increased ethylene synthesis, increased rates of respiration and softening, and changes in color and aroma (Abeles et al., 1992). A clear role of ethylene in controlling fruit respiration, softening, and color changes has been established (Theologis, 1992). The change in aroma compounds during ripening is also considered to be mediated by ethylene; however, no direct relationship has been reported. Oeller et al. (1991) demonstrated that tomato fruit produced by plants transformed with a 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase antisense gene failed to produce climacteric ethylene. The authors mentioned that the fruits did not develop an aroma characteristic of ripe fruit, although no data were presented. Compounds such as methyl jasmonate that increase ethylene production also enhance ester production in preclimacteric apple fruit (Fan et al., 1997), but it is unclear whether synthesis of ripening-related volatiles requires ethylene production and action.

Production rates of several classes of volatile compounds including esters, alcohols, aldehydes, and acids change during fruit ripening (Heath and Reineccius, 1986). The largest change during apple ripening is an increase in ester production (Brown et al., 1966). This increase in esters is responsible in part for development of the characteristic flavor and aroma of apple fruit during maturation and ripening.

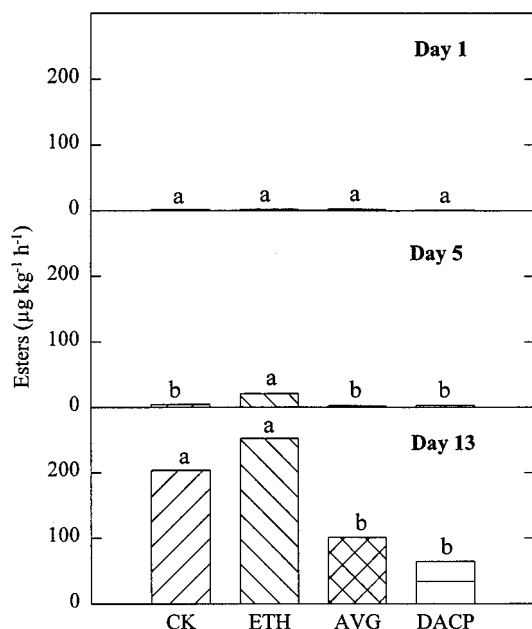
Inhibitors of ethylene synthesis and action are valuable tools for the study of control of ethylene-mediated responses. Aminoethoxyvinylglycine (AVG) is a potent inhibitor of ethylene production that inhibits the conversion of *S*-adenosylmethionine to ACC (Amrhein and Wenker, 1979). Preharvest application of AVG can retard production of ethylene and ripening-related volatiles in apple fruit during storage (Bangerth and Streif, 1987). The cyclic olefin compound 2,5-norbornadiene

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**Figure 1.** Ethylene production by preclimacteric Super Red Delicious apples. Fruit were treated with ethephon, AVG, and DACP and then kept at 20 °C. Ethylene production was measured at 1, 5, and 13 days after treatment. CK, control; ETH, ethephon. Bars with the same letter are not significantly different ( $P < 0.05$ ).

(NBD), which reversibly blocks ethylene action, has been used to study ethylene action in stimulation of respiration (Gude et al., 1987), fruit softening (Blankenship and Sisler, 1989), and flower senescence (Sisler et al., 1986; Peiser, 1989). More recently, diazocyclopentadiene (DACP), a light-activated compound, has been shown to irreversibly inhibit ethylene action (Sisler and Blankenship, 1993a). DACP can inhibit softening, starch degradation, ethylene production (Blankenship and Sisler, 1993), and color changes (Sisler and Blankenship, 1993b; Sisler and Lallu, 1994) during fruit ripening. In the present study, ethylene production and action were manipulated using ethephon (an ethylene-



**Figure 2.** Total ester volatile production by preclimacteric Super Red Delicious apples. Fruit were treated with ethephon, AVG, and DACP and then kept at 20 °C. Ester volatile production was measured at 1, 5, and 13 days after treatment. CK, control; ETH, ethephon. Bars with the same letter are not significantly different ( $P < 0.05$ ).

releasing compound), AVG, and DACP to examine ethylene mediation of volatile compound synthesis in ripening apple fruit.

#### MATERIALS AND METHODS

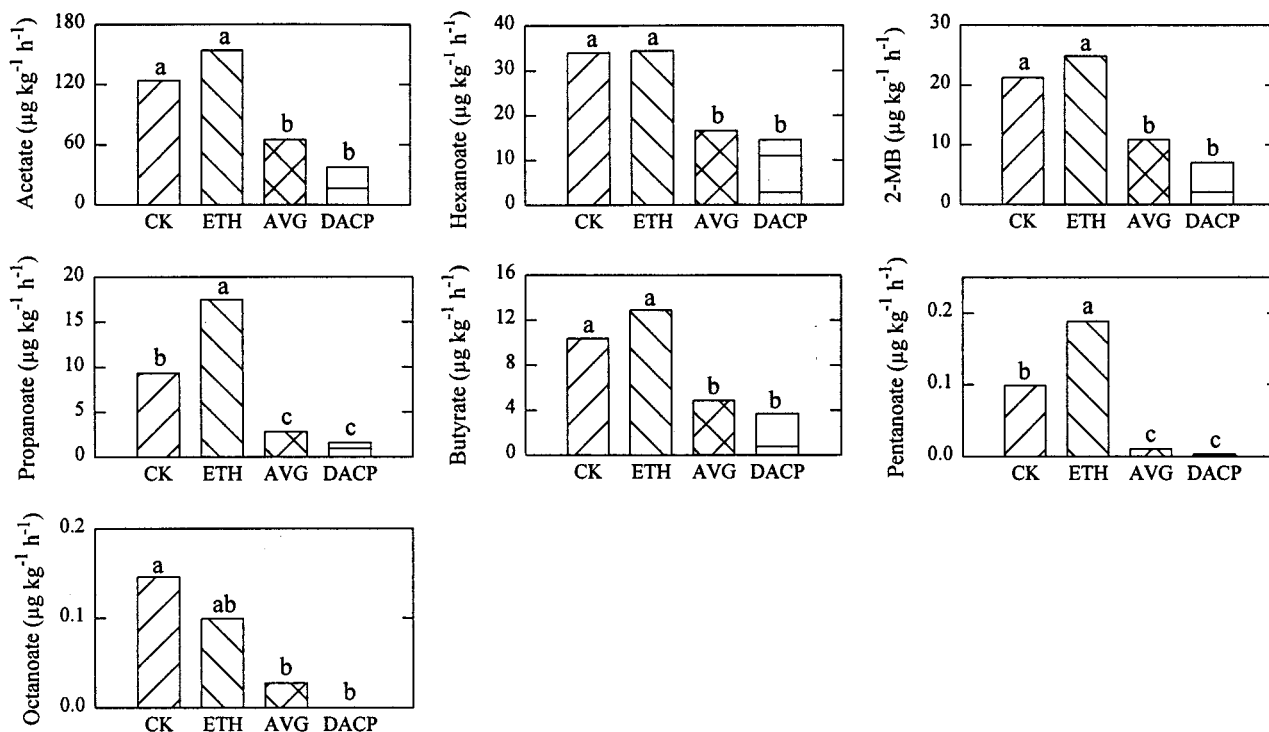
Super Red Delicious apples (*Malus domestica* Borkh.) were harvested from the Columbia View Experimental Plots near Wenatchee, WA. Internal ethylene concentration (IEC) was

measured according to the method of Williams and Patterson (1962) on the day of harvest. Preclimacteric (IEC  $< 1 \mu\text{L L}^{-1}$ ) apples were harvested 142 days after full bloom (DAFB) and held at 20 °C for 24 h prior to treatment. Postclimacteric fruit were harvested 154 DAFB and then incubated at 20 °C for 7 days prior to treatment. On the day fruit were treated, IEC ranged from 74 to 300  $\mu\text{L L}^{-1}$ .

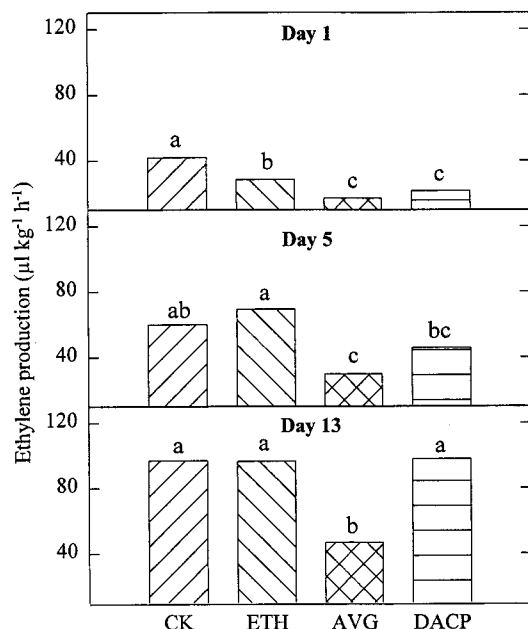
There were four treatments: ethephon, AVG, DACP, or water control. Control, ethephon- and AVG-treated fruits were dipped into water, 500 ppm of ethephon, or 500 ppm of AVG, respectively, for 5 min and then air-dried. Water temperature was 20 °C for all aqueous treatments. DACP was synthesized according to the method of Regitz and Liedhegner (1967), and the concentration was measured according to the method of Ramirez and Levy (1958). All chemicals used for synthesis and concentration measurements of DACP were purchased from Aldrich (Milwaukee, WI). Fruit were treated according to the procedure of Sisler and Blankenship (1993) with 2000  $\mu\text{L L}^{-1}$  (gas/gas) DACP in 12 L chambers for 12 h at 20 °C under laboratory light. Hydrated CaO (0.5 kg) was enclosed in each chamber to absorb  $\text{CO}_2$ . There were three replicates with three apples for each replicate. After treatment, fruit remained in the opened chambers at 20 °C.

Ethylene and volatile production were measured 1, 5, and 13 days after treatment. To measure ethylene production, apples (three) were placed into 4 L glass jars. The jars were sealed using Teflon lids with two gas ports. Purified compressed air flowed into the jars at 100 mL  $\text{min}^{-1}$  for 4 h at 20 °C prior to sampling. A 1 mL gas sample was collected from the outlet of each jar, and ethylene was analyzed using a gas chromatograph equipped with a glass column (610 mm  $\times$  3.2 mm i.d.) packed with Porapak Q, 90–100 mesh. Oven, injector, and FID detector temperatures were 50, 50, and 200 °C, respectively. Gas flows for  $\text{N}_2$  carrier,  $\text{H}_2$ , and air were 30, 30, and 300 mL  $\text{min}^{-1}$ , respectively. Analysis for other volatiles was as previously described (Mattheis et al., 1991).

Statistical analyses were conducted using SAS (SAS Institute, Cary, NC). The SAS ANOVA procedure was used to examine treatment effects, and treatment mean separation was determined by Fischer's least significant difference ( $P < 0.05$ ).



**Figure 3.** Ester volatile production by preclimacteric Super Red Delicious apples. Fruit were treated with ethephon, AVG, and DACP and then kept at 20 °C. Ester volatile production was measured 13 days after treatment. Groups of the apple ester volatiles were acetate, hexanoate, 2-methylbutyrate (2-MB), propanoate, butyrate, octanoate, and pentanoate esters. CK, control; ETH, ethephon. Bars with the same letter are not significantly different ( $P < 0.05$ ).



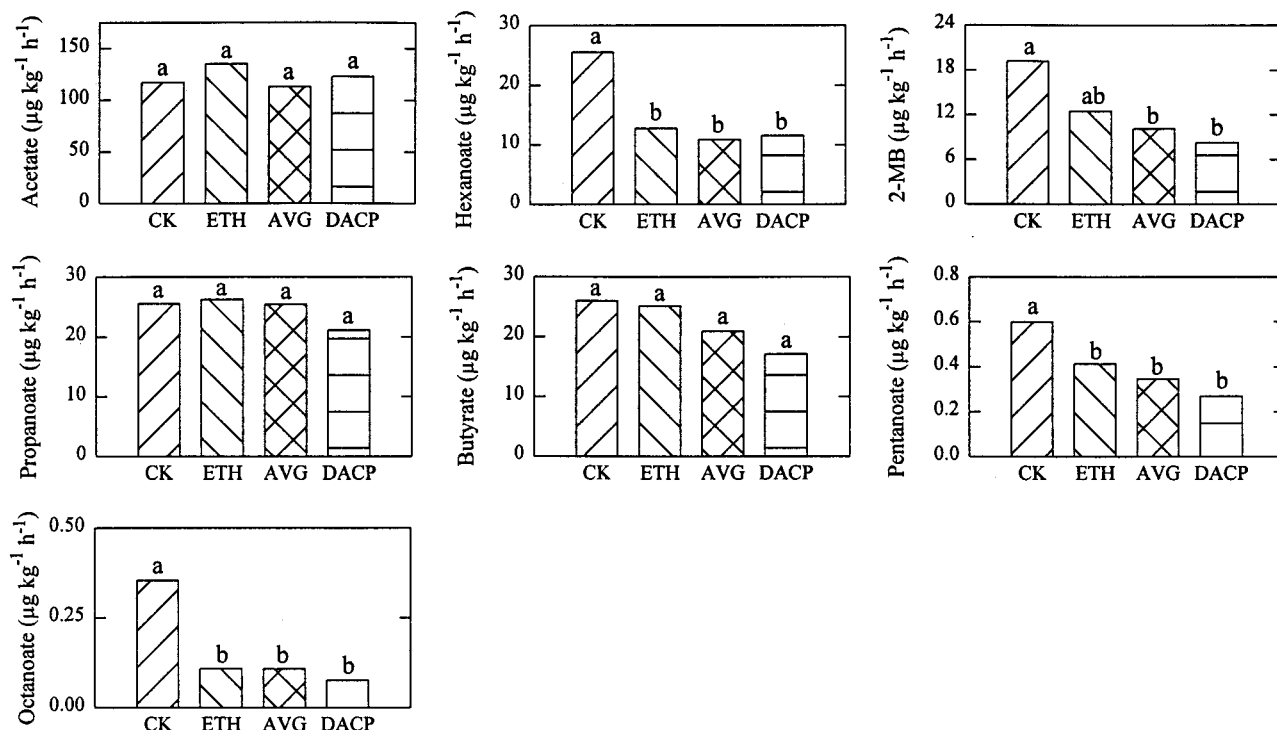
**Figure 4.** Ethylene production by postclimacteric Super Red Delicious apples. Fruit were treated with ethephon, AVG, and DACP and then kept at 20 °C. Ethylene production was measured at 1, 5, and 13 days after treatment. CK, control; ETH, ethephon. Bars with the same letter are not significantly different ( $P < 0.05$ ).

**RESULTS AND DISCUSSION**

Ethephon treatment increased ethylene production of preclimacteric apples 1, 5, and 13 days after treatment (Figure 1). Ethylene production by apples treated with AVG and DACP was lower than that by controls 5 and 13 days after treatment. The inhibition was the strongest 5 days after treatment. Despite the inhibitory

effects by AVG and DACP, ethylene production increased during the 13 day ripening period. DACP competes with ethylene and “permanently” attaches to ethylene receptors under illumination with low levels of fluorescent light (Sisler and Blankenship, 1993a). Ethylene production by mature green tomato fruits treated with DACP was low initially after treatment, but after 10 days there was a rise in ethylene production which exceeded that of the controls (Sisler and Blankenship, 1993b). Our results with apples showed DACP-treated fruit had lower ethylene production than controls throughout the 13 day ripening period after treatment.

Ethephon treatment increased total ester production by preclimacteric apples, but only at 5 days after treatment (Figure 2). AVG and DACP inhibited total ester production 13 days after treatment. Volatile esters were grouped by acid moiety: acetate, hexanoate, 2-methylbutyrate, butyrate, propanoate, octanoate, and pentanoate esters (Figure 3). Acetate esters included ethyl acetate, propyl acetate, 2-methylpropyl acetate, butyl acetate, 2-methylbutyl acetate, pentyl acetate, and hexyl acetate; hexanoate esters included ethyl hexanoate, propyl hexanoate, butyl hexanoate, and hexyl hexanoate; 2-methylbutyrate esters included methyl 2-methylbutyrate, ethyl 2-methylbutyrate, butyl 2-methylbutyrate, 2-methylbutyl 2-methylbutyrate, and hexyl 2-methylbutyrate; butyrate esters included methyl butyrate, ethyl butyrate, butyl butyrate, pentyl butyrate, and hexyl butyrate; propanoate esters included ethyl propanoate, propyl propanoate, butyl propanoate, and hexyl propanoate; ethyl octanoate and ethyl pentanoate were the only octanoate and pentanoate esters, respectively. At 13 days after treatment, ethephon promoted production of only propanoate and pentanoate



**Figure 5.** Ester volatile production by postclimacteric Super Red Delicious apples 13 days after treatment. Fruit were treated with ethephon, AVG, and DACP and then kept at 20 °C. Groups of apple ester volatiles were acetate, hexanoate, 2-methylbutyrate (2-MB), propanoate, butyrate, octanoate, and pentanoate esters. CK, control; ETH, ethephon. Bars with the same letter are not significantly different ( $P < 0.05$ ).

esters, whereas AVG and DACP inhibited synthesis of all esters in preclimacteric fruit (Figure 3).

Ethephon treatment inhibited ethylene production 1 day after treatment of postclimacteric fruit, but no effect was observed 5 or 13 days after treatment (Figure 4). AVG inhibited ethylene production 1, 5, and 13 days after treatment. DACP inhibited ethylene production 1 day after treatment; however, no effect was observed 5 or 13 days after treatment. Total ester production was not inhibited by DACP or AVG in postclimacteric fruit, and ethephon did not promote total ester production during the 13 day ripening period after treatment (data not shown). There was no significant difference in ester production between the treatments 1 or 5 days after treatment (data not shown). DACP and AVG inhibited production of hexanoate, 2-methylbutyrate, octanoate, and pentanoate esters 13 days after treatment, but no effect on acetate, propanoate, or butyrate esters was observed (Figure 5). Ethephon also reduced production of hexanoate, octanoate, and pentanoate esters 13 days after treatment of postclimacteric fruits.

Although DACP and AVG inhibited both ethylene and ester production, the inhibition of ester production was delayed compared to the inhibition of ethylene production. For example, in preclimacteric fruit, DACP inhibition of ethylene production was observed 1 day after treatment, whereas inhibition of ester production was not observed until 5 days after treatment (compare Figures 1 and 2). The delay between DACP inhibition of ethylene and ester production indicates synthesis of some esters can continue for only a relatively short period in the absence of ethylene action. DACP inhibited production of all esters in preclimacteric fruit, indicating the initiation of ester production associated with apple fruit ripening requires ethylene action. The response of postclimacteric apples to DACP shows continuous ethylene action is required for production of some but not all esters once ripening-related ester production has been initiated. DACP inhibition of ethylene evolution was observed 1 day after treatment in postclimacteric fruit; however, the inhibition was transient. No significant inhibition was observed 5 or 13 days after treatment. Similar results have been reported for tomatoes (Sisler and Blankenship, 1993b). The recovery of ethylene production 5 or 13 days after DACP treatment (Figure 5) implies higher turnover of ethylene receptors in postclimacteric compared to preclimacteric apples. A higher dose or repeated DACP application may be necessary to block ethylene action in postclimacteric apples. DACP inhibited color change and respiration in tomato fruit at any developmental stage (Sisler and Lallu, 1994), indicating that these ripening processes require continuous ethylene action rather than being triggered by ethylene. Inhibition of ethylene biosynthesis by AVG also inhibits ester production in both pre- and postclimacteric apple fruit, suggesting a high rate of ethylene production is required to maintain a high production rate for some esters. Interestingly, exogenous ethylene (ethephon) application also inhibited production of some esters in postclimacteric fruit (Figure 5), indicating that volatile synthesis is closely regulated by fruit ethylene production during apple fruit ripening. Autoinhibition of ethylene production occurs rapidly after ethylene treatment (Vendrell and McGlasson, 1971) and influences ester production even though the fruit are still exposed to ethylene. In postclimacteric apples, DACP and AVG did

not inhibit production of acetate esters, the largest quantitative group of apple volatile compounds, but production of some other esters was reduced, indicating there is variation in ethylene regulation of ester synthesis.

Metabolic changes during fruit ripening result in increased catabolic activity, providing substrates for volatile production. It has been proposed that the formation of ripening-related volatiles in fruits such as apple is initiated by the climacteric rise in respiration (Heath and Reineccius, 1986). Ester production of Golden Delicious apples increases during maturation as respiration and ethylene production increase (Song and Bangerth, 1996). DACP inhibits respiration in tomato fruit (Sisler and Lallu, 1994). The inhibition of ester production by DACP in apples may be due to a low respiration rate as a result of inhibition of ethylene action by DACP.

In summary, production of ester volatiles by ripening apples is regulated by ethylene. DACP and AVG inhibited production of some volatile esters in both pre- and postclimacteric apple fruit, suggesting that biosynthesis of these ester volatiles requires not only continuous ethylene action but also continuous ethylene biosynthesis. Acetate ester production was not inhibited by DACP and AVG in postclimacteric fruit, indicating that sufficient acetate and the enzyme(s) required for ester synthesis were present after the initiation of apple ripening regardless of the status of ethylene production or action.

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