Plant Physiol. (1982) 69, 317–322
0032-0889/82/69/0317/06/$00.50/0

Enhancement of Wound-Induced Ethylene Synthesis by Ethylene in Preclimacteric Cantaloupe[1]

Received for publication May 26, 1981 and in revised form August 20, 1981

NEIL E. HOFFMAN AND SHANG FA YANG
Department of Vegetable Crops, University of California, Davis, California 95616

ABSTRACT

Although intact fruits of unripe cantaloupe (*Cucumis melo* L.) produce very little ethylene, a massive increase in ethylene production occurred in response to excision. The evidence indicates that this wound ethylene is produced from methionine via 1-aminocyclopropanecarboxylic acid (ACC) as in ripening fruits. Excision induced an increase in both ACC synthase and the enzyme converting ACC to ethylene. Ethylene further increased the activity of the enzyme system converting ACC to ethylene. The induction by ethylene required a minimum exposure of 1 hour; longer exposure had increasingly larger effect. The response was saturated at approximately 3 microliters per liter ethylene and was inhibited by Ag^+. Neither ethylene nor ACC had a promotive or inhibitory effect on ACC synthase beyond the effect attributable to wounding.

Since Adams and Yang (2) found the pathway of ethylene biosynthesis in ripening apples to be methionine → SAM[2] → ACC → ethylene, several workers have examined the biosynthesis of wound ethylene (5, 12, 28). They confirmed that ACC served as an intermediate of wound ethylene. Both Yu and Yang (28) and Boller and Kende (5, 12) found that stimulation of ACC synthase activity occurred as a result of wounding. They concluded that increases in production of stress ethylene were the result of an increase in the activity of ACC synthase, which catalyzes the conversion of SAM to ACC. The enzyme catalyzing the conversion of ACC to ethylene (EFE) has not been demonstrated in a cell-free system. The characteristics of this reaction in tissue suggest that this enzyme is membrane-associated, labile, and subject to disruption by various treatments (10, 13). In most of the vegetative systems examined, EFE appears to be constitutive (6, 9, 21, 27) since addition of ACC to various plant tissues causes a pronounced increase in ethylene production. Addition of ACC to preclimacteric fruit tissue, however, results in very minor stimulation (25). This has led to the conclusion that both ACC synthase and EFE are restricted in unripe preclimacteric fruit, and that the ripening process results in increased activity of both enzymes. Tissue slices cut from preclimacteric cantaloupe fruits showed dramatic increases in ethylene production and respiration in response to excision (17). It seems that the intact young preclimacteric fruits are potentially capable of synthesizing ethylene, but that the synthesis is suppressed by an unidentified mechanism; the ripening process, as well as wounding, is capable of relieving this restraint. Tissue slices of preclimacteric cantaloupe are excellent experimental tissue for studies of the regulation of ethylene biosynthesis because they undergo dramatic increases in ethylene production in response to cutting, are responsive to ethylene (17), and can be easily infiltrated with chemicals. In this study we investigated the roles of ACC and of ethylene in the regulation of ethylene synthesis, with respect to ACC synthase and the *in vivo* activity of EFE, by the excised tissue of unripe cantaloupe fruit.

MATERIALS AND METHODS

Plant Material. Cantaloupes (*cucumis melo* L., cv. Powdery Mildew Resistant No. 45, "Gusto" from Associated Seeds) were field harvested at the "mature" preclimacteric stage. These fruit weighed 0.6 to 1 kg and had complete netting, mature seeds, and fully pigmented flesh. Only those fruits producing less than 0.2 nl/g·h ethylene were used in experiments. The selected fruits were harvested from actively growing plants 1 d prior to use, and were then kept at 25°C.

Both the top and bottom portions of cantaloupes were removed by making two equatorial cuts leaving the central portion of the fruit approximately 4 cm in thickness. Using a cork borer and knife, plugs 1 cm (diameter) × 2 cm (length) were removed by longitudinal insertions, rinsed in 2% KCl, and briefly blotted dry. Discs 3 mm thick were then cut from the plugs. Chemicals were vacuum injected into plugs (8) or vacuum infiltrated into discs (23).

Chemicals. AVG·HCl was the generous gift of J. P. Scannel (Hoffman La Roche). AVG·HNO_3 was prepared by passing AVG·HCl solution through an ion exchange resin, Dowex-1 (NO_3^- form), and its concentration was determined by ninhydrin assay. ACA was kindly supplied by S. Sakamura, Hokkaido University, Sapporo, Japan. CHI, HEPPS, and DTT were purchased from Sigma, pyridoxal phosphate and ACC were purchased from Calbiochem, ethylene and 1-butene were purchased from Matheson Inc., SAM from Boehringer, and L-[3-^{14}C]methionine was from Research Products International. $Ag(S_2O_3)_2^{3-}$ was prepared from reagent grade $AgNO_3$ and $Na_2S_2O_3$ as described by Veen (22).

Determination of Gas Concentrations and ACC. Ethylene, butene, and O_2 were determined by GC of 1-ml samples. 1-Butene produced from ACA was verified by co-chromatography with an authentic sample. Labeled ethylene was determined as described by Yu and Yang (27). ACC, extracted in $HClO_4$, was determined according to the method of Lizada and Yang (15) with slight modification as described by Hoffman and Yang (10). After $HClO_4$ extraction, labeled ACC was partially purified by adsorption onto ion exchange resin, Dowex 50 (H^+), followed by elution with 2 N NH_4OH. After concentration *in vacuo* at 50°C, the extract was chromatographed on Whatman 3 MM paper with 1-butanol: acetic acid:water (4:1:1, v/v/v) as developing solvent. Radioactiv-

[1] Supported by National Science Foundation Research Grant PCM 78-92078.

[2] Abbreviations: SAM, *S*-adenosylmethionine; ACC, 1-aminocyclopropane-1-carboxylic acid; EFE, ethylene forming enzyme which catalyzes the conversion of ACC to ethylene; AVG, aminoethoxyvinylglycine; ACA, (+)-allocoronamic acid [(1R,2S)-1-amino-2-ethylcyclopropane-1-carboxylic acid]; CHI, cycloheximide; HEPPS, 4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid.

ity was determined by radioscanner.

Gas Treatments. Gas treatments were conducted in both flow and static systems. For flow system experiments, 500 μl/l ethylene in N_2 was mixed with humidified air or N_2 to deliver 20 μl/l ethylene. Ethylene and ethylene-free N_2 or air were delivered, at a rate of 10 l/h, to 50-ml Erlenmeyer flasks containing 5-g tissue samples on moist filter paper. The high flow rate kept the ethylene concentrations negligible in the ethylene-free treatments. For the static system, 5-g tissue samples were placed in Erlenmeyer flasks containing moistened filter paper. The flasks were closed with rubber serum caps and the appropriate amount of ethylene was introduced by syringe. Gas concentrations were verified by gas chromatography. Ethylene-free treatments contained less than 5 nl/l ethylene and anaerobic treatments contained less than 500 μl/l O_2.

Assay of ACC Synthase. ACC synthase was extracted from the tissue and assayed according to Yu *et al.* (26). When AVG-treated tissues were used, the concentration of pyridoxal phosphate was increased to 4 μM in the dialysis buffer, and to 50 μM in the enzyme assay mixture.

Assay of EFE. Activity of EFE *in vivo* was determined by measuring conversion of ACA to butene or ACC to ethylene. All tissue samples were treated with AVG immediately after excision and incubated at 25°C. After incubation for various periods of time as indicated, ACA or ACC was vacuum infiltrated into the plugs or discs. The vacuum infiltration process was effective in removing all exogenously supplied ethylene. One h after infiltration, each 1 g tissue sample was enclosed in a 6-ml plastic syringe for 1 h; accumulated ethylene and butene was then determined by gas chromatography.

RESULTS

Biosynthesis of Wound Ethylene. It has been reported that ethylene production increases in preclimacteric cantaloupe in response to cutting (17). Recently, Yu and Yang (28) and Boller and Kende (5, 12) have studied changes in the levels of ACC in relation to increases in ethylene production in excised citrus peel and tomato fruit tissues. The response in cantaloupes is now shown to be very similar to those systems. Intact preclimacteric cantaloupes produce very little ethylene and contain little ACC. As illustrated in Figure 1A, cutting caused an increase in ethylene production from less than 0.1 $nl \cdot g^{-1} \cdot h^{-1}$ to over 60 $nl \cdot g^{-1}\ h^{-1}$ and an increase in ACC from less than 0.1 $nmol \cdot g^{-1}$ to over 350 $nmol \cdot g^{-1}$ over a period of 25 h. In tomato, the maximum response occurred within 9 h with ACC reaching about 10 $nmol \cdot g^{-1}$ and ethylene 15 $nl \cdot g^{-1} \cdot h^{-1}$ (28). To examine the ability to convert methionine to ethylene, cantaloupe plugs were infiltrated with L-[3-^{14}C]methionine at 0, 4, 9, or 22 h after cutting. In response to excision, there was an increase in the incorporation of label into ACC and into ethylene (Fig. 1B), indicating that the pathway of wound ethylene production in cantaloupe is similar to that of postclimacteric apple (2).

Increase in EFE Activity Due to Wounding. In tissue excised from preclimacteric cantaloupes, the capacity to produce ethylene increases as a response to the excision, and ethylene production eventually reaches a rate comparable to that of the ripe intact fruit. Cantaloupe plugs were vacuum-injected with ACC (100 nmol/g plug) at various times after cutting. Ethylene production was measured between 2 to 3 h after the addition of ACC. As compared to controls, a 3- to 5-fold increase in ethylene production resulted when ACC was added immediately after cutting, and a 1- to 3-fold increase resulted when ACC was added 4 h later (Fig. 2). These ACC-enhanced production rates, however, were only 5 and 40 $nl \cdot g^{-1} \cdot h^{-1}$, respectively, which are strikingly less than the ethylene production of control tissue at a later time after excision. The rate of ethylene production achieved by control tissue 25 h after excision was greater than 200 $nl \cdot g^{-1} \cdot h^{-1}$ (Fig. 2). It is

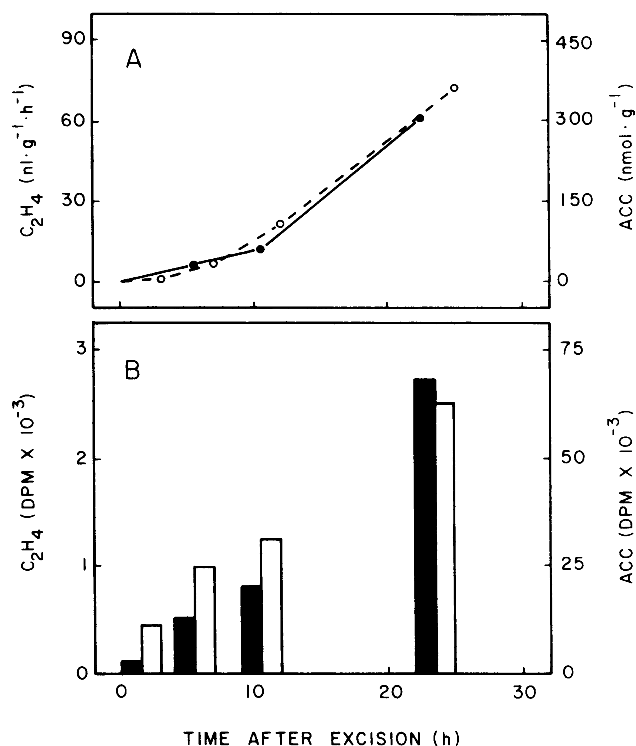

FIG. 1. Excision-induced increase in ACC content and ethylene production rate (A) and conversion of methionine to ethylene (B). Each preclimacteric cantaloupe plug was infiltrated with 20 μl 2% KCl solution containing 0.5 μCi and 10 nmol of methionine at 0, 4, 9, and 22 h after cutting and incubated for 3 h in 50-ml Erlenmeyer flasks. Total ethylene (●——●), radioactive ethylene (shaded bars), ACC (○---○), and radioactive ACC (open bars) were measured as described under "Materials and Methods."

apparent from these data that freshly excised tissue has a smaller capacity for converting ACC to ethylene than has similar tissue at a time later after excision. It is also indicated, however, that there was only a small percentage increase in ethylene production caused by ACC added 9 or 22 h after the tissue was cut, presumably because endogenous ACC had reached such a high level (Fig. 1) that added ACC was in excess of the requirement. AVG has been shown to block the conversion of SAM to ACC, but not the conversion of ACC to ethylene (2). When AVG was infiltrated into tissue immediately after the plugs were cut, it effectively inhibited ethylene production over the 25-h period. When ACC was added to AVG-treated plugs at 0 h, the stimulation of ethylene production, measured 2 to 3 h after the addition, was again very small. If ACC was added to AVG-treated plugs 9 or 22 h after wounding, however, it caused a 50-fold stimulation of ethylene production above that of tissue treated with AVG only (Fig. 3). This indicates that during a period of 9 h or more, the ability of the tissue to convert ACC to C_2H_4 was significantly increased. The stimulation of ethylene production caused by addition of ACC was approximately the same at 9 or 22 h, whereas the ethylene production of control plugs increased continuously over this time. The addition of ACC at 22 h to AVG-treated plugs failed to establish a rate of ethylene production equal to that of control tissue. This suggests the possibility that, in addition to inhibition of ACC synthase, AVG may exert a nonspecific inhibitory effect on EFE activity. No such effect was observed in postclimacteric apple tissue, however (2). Alternatively, the development of EFE activity might be regulated by the level of ACC or of ethylene, either of which may be too low in AVG-treated tissue to elicit maximal development of EFE. If this is the case, one may further

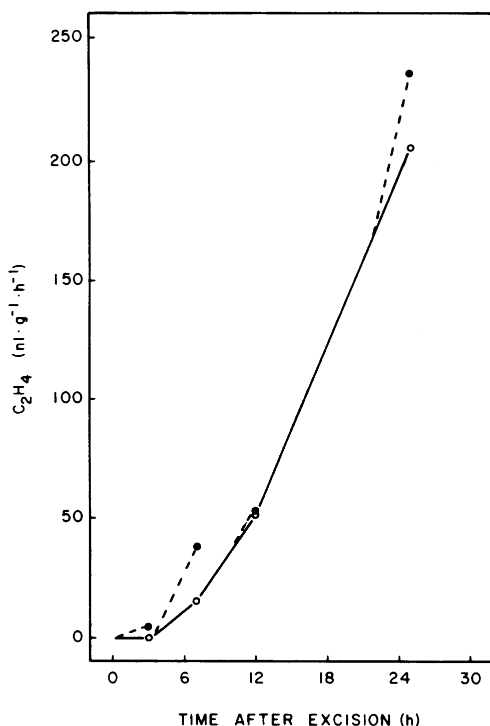

FIG. 2. Effect of exogenous ACC on ethylene production rate. Each preclimacteric cantaloupe plug weighing 1 g was injected with 100 nmol of ACC at 0, 4, 9, or 22 h after excision. Ethylene was collected from 2 to 3 h after adding the ACC. Control (○), +ACC (●).

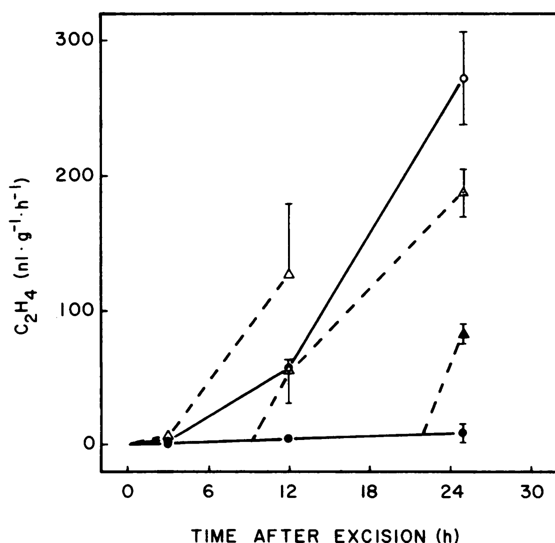

FIG. 3. Effect of exogenous ACC on the rate of ethylene production rate by cantaloupe plugs which were pretreated with AVG. Except for control plugs (○) which received no AVG, all other plugs were injected individually with 100 nmol AVG (●) immediately after excision. ACC, 100 nmol/plug was injected at 0 (△), 9 (△), or 22 h (▲) after excision. Ethylene was measured as in Figure 2. Data are averages ± SE from two separate experiments using two different cantaloupes.

predict that the ethylene production rate in AVG-treated plugs would increase as the time of exposure to ACC increased. The results of Figure 3 are consistent with this prediction. Plugs treated with AVG and ACC at 0 h were, by 12 h, producing ethylene at a rate as great or greater than that of the controls. Similarly, AVG-treated plugs fed ACC at 9 h were producing almost as much ethylene at 25 h as the control receiving no AVG.

When a similar experiment was performed substituting CHI for AVG, there was no increase in EFE (Fig. 4). ACC added at 0 h caused a slight increase in ethylene production; however, CHI-treated plugs, which were infiltrated with ACC at 9 h or later, produced as little ethylene as plugs infiltrated only with CHI. This implies that protein synthesis may be involved in the development of EFE. Yu and Yang (28) reported that extractable ACC synthase activity could not be demonstrated immediately after excision. During incubation, however, there was a steady increase in activity, accompanied by an increase in ACC level; these increases were inhibited by CHI. No ACC synthase activity was observed in cantaloupe tissue immediately after cutting, but as in tomato, a considerable level of activity was observed 12 h later. Although ethylene production was inhibited by CHI (100 nmol/g cantaloupe plug), levels of ACC and ACC synthase activity were comparable to the control, thus suggesting that the synthesis of EFE protein is more vulnerable to inhibition by CHI than is ACC synthase. Both ACC accumulation and increases in ACC synthase activity in

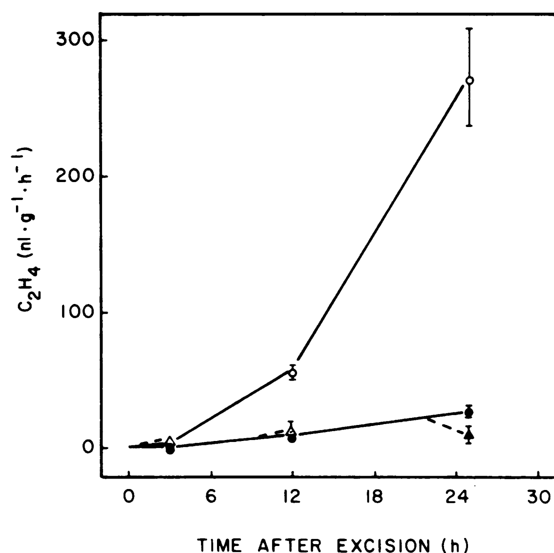

FIG. 4. Effect of exogenous ACC on the rate of ethylene production rate by cantaloupe plugs pretreated with CHI. Except for control plugs (○) which received no CHI, all other plugs were individually infiltrated with 100 nmol of CHI (●) immediately after excision. ACC (100 nmol/plug) was added at 0 (△), 9 (△), or 22 (▲) h after excision as described in Figure 3.

Table I. *Effect of Pretreatment with ACC or Ethylene on the Development of the Activity of the System Which Converts ACA to Butene*

Immediately after excision each plug received 100 nmol AVG, 100 nmol ACC, or both, and was then sealed in a 125-ml Erlenmeyer flask containing either air or ethylene (5 or 100 μl/l) in air. After incubation for 12 h, each plug was fed either 100 nmol ACC or 400 nmol ACA. One h later, the butene and ethylene produced during the following 1-h period were measured. Each value represents the mean ± SE of three plugs.

Pretreatment	Substrate Fed	EFE Activity	
		Ethylene	Butene
		nl/g·h	
None		67.4 ± 12.5	0
AVG		0.4 ± 0.2	0
AVG	ACC	82.8 ± 7.4	0
AVG	ACA	0.6 ± 0.1	54.4 ± 17.5
AVG + ACC	ACA	16.3 ± 3.6	103.0 ± 18.2
AVG + ethylene, 5 μl/l	ACA	0.5 ± 0.1	99.7 ± 7.5
AVG + ethylene, 100 μl/l	ACA	0.3 ± 0.1	91.2 ± 7.0

response to wounding were inhibited in cataloupe discs vacuum infiltrated with 1 mM CHI (data not shown). This may have achieved more uniform distribution or higher concentration of inhibitor.

Inductive Effect of ACC and Ethylene. Since the enhancement of ethylene production in AVG-treated plugs was dependent upon the duration of exposure to ACC, it was assumed that either ACC or its product ethylene exerted the promotive effect on ethylene production. To examine this proposal we employed ACA, a 2-ethyl substituted analog of ACC. Although ACA is not normally present in plants, it is converted in the tissue to 1-butene, apparently by the same system as that which converts ACC to ethylene (11). Thus, by assaying the ability of the tissue to convert ACA to butene, it is possible to determine the activity of EFE independent of the level of endogenous ACC. This technique enables us to examine the effect of ACC on the development of EFE. Immediately after excision, each plug was infiltrated with 100 nmol AVG or with 100 nmol AVG + 100 nmol ACC. The plugs were then enclosed overnight in flasks containing either air or a known concentration of ethylene in air. After incubation for 12 h, each plug was infiltrated with 400 nmol of ACA. The ethylene and butene subsequently produced were measured. Table I shows that infiltration with ACC or exposure to 5 or 100 μl/l ethylene resulted in a 2-fold increase in the conversion of ACA to butene. This enhancement was even more pronounced in later experiments (Table II, Figs. 5 and 6), in which the endogenous ethylene was removed either by continuous flow system or by incubating tissue in larger flasks. Since ethylene was produced from the ACC-treated plugs and the level of ACC in the ethylene-treated plugs was very low (less than 0.1 nmol/g), it was considered that the increased conversion of ACA into butene by tissue infiltrated with ACC was not due to ACC itself, but was due to the ethylene derived from ACC.

Table II. *Inhibition of the Induction of Ethylene Production by Ag^+*

Immediately after excision, discs were vacuum-infiltrated with 1 mM AVG containing various concentrations of $Ag(S_2O_3)_2^{3-}$ as indicated. Discs were placed in 50-ml Erlenmeyer flasks through which ethylene-free air or air containing 20 μl/l ethylene was passed. ACC was added and ethylene measured as described in Table I. Each value represents means ± SE of three 1-g samples.

Ag^+ Treatment	Ethylene Production	
	Air	Ethylene (20 μl/l)
mM	*nl/g·h*	
0	13.9 ± 1.6	68.1 ± 3.9
1	11.5 ± 1.2	38.2 ± 4.1
5	16.1 ± 2.9	25.6 ± 4.1

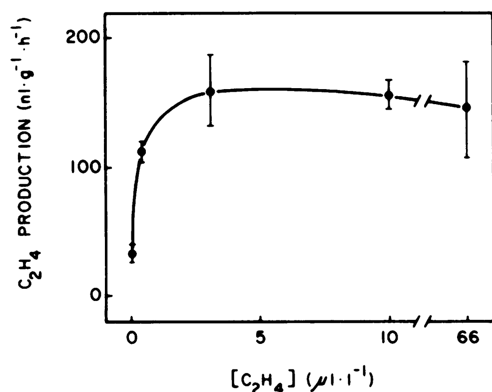

FIG. 5. The effect of ethylene concentration on the development of EFE activity. Cantaloupe plugs were infiltrated with 100 nmol AVG after excision. Five plugs were placed in each 2-liter Erlenmeyer flask and sealed. Ethylene was injected to give the desired concentration. Sixteen h after excision, 100 nmol ACC was injected into each plug. Ethylene was measured as described in Table I. Each point represents the mean ± SE of three plugs; high and low values were excluded.

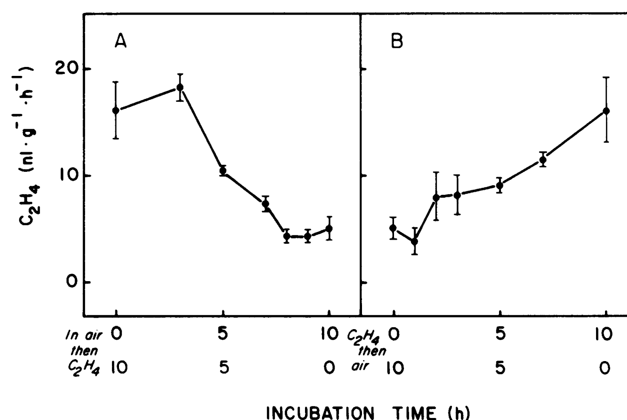

FIG. 6. Dependence of the development of EFE on duration of ethylene application. All discs were infiltrated in 1 mM AVG. Each 3-g sample of cantaloupe discs was placed in a 50-ml Erlenmeyer flask attached to a flow system which delivered (A) air for a specified period of time and then 20 μl/l ethylene, or (B) initially 20 μl/l ethylene for a specific period of time and air thereafter, for a total incubation period of 10 h. At the end of the incubation, the discs were infiltrated with 1 mM ACC and ethylene was measured, as described in Table I. All points represent means ± SE of three 1-g samples.

Dependence of Development of EFE on Concentration of Ethylene and Duration of Ethylene Treatment. Figure 5 shows the effect of a range of ethylene concentrations on the development of the activity of EFE *in vivo*. The tissue responded significantly to 0.3 μl/l ethylene, the threshold level capable of inducing ripening in intact cantaloupe (16). The response reached a maximum at 3 μl/l or higher.

In many hormonal systems, continuous presence of the hormone is required for maximal effect. In all previous experiments, we exposed tissue to ethylene continuously for a minimum of 10 h. In the experiments described below, we added ethylene at different times for different durations during an incubation period totaling 10 h. Cantaloupe discs treated with AVG were first incubated in a flow system which delivered air only, and were subsequently transferred to flowing ethylene at various times, so that the combined time in the flow systems was 10 h (Fig. 6A). Exposure to ethylene for the last 7 h of the 10 h incubation period had as great an effect on ethylene production, and presumably on EFE, as did continuous exposure to ethylene. Ethylene treatments for the last 5 or 3 h of the 10 h period had smaller effects. Ethylene supplied during the last 2 h failed to cause any increase in ethylene production above that of discs incubated for the entire 10-h period in ethylene-free air. When the reverse experiment was done (Fig. 6B), in which discs from the same cantaloupe were first incubated in ethylene for a given period of time and then transferred to the ethylene-free system, a similar response was obtained. The data from this sequence indicate that continuous ethylene treatment was necessary to obtain the maximal response.

Effect of $Ag(S_2O_3)_2^{3-}$ on Development of EFE. Ag^+ is an inhibitor of ethylene action (3, 4); in banana and tomato fruit tissues, it inhibited the onset of ripening and thereby the autocatalytic synthesis of ethylene. It is of interest to examine the effect of Ag^+ on the inductive effect of ethylene on ethylene biosynthesis in the present system. Discs were infiltrated with 1 mM AVG·HNO_3 and 1 or 5 mM $Ag(S_2O_3)_2^{3-}$ in 2% KNO_3. Slight browning of the tissue occurred with 1 mM Ag^+; 5 mM caused

extensive browning. Ethylene production in the discs exposed to air was not affected, however, by 1 or 5 mM Ag^+. As observed previously, treatment of discs with 20 μl/l ethylene caused a 4-fold increase in ethylene production; 1 mM Ag^+ inhibited this induction by 55% and 5 mM Ag^+ inhibited it by 78% (Table II).

Effect of Ethylene and ACC on ACC Synthase. The autocatalytic effect of ethylene on ethylene production in ripening fruits is well documented (1), and ACC synthase is known to be the rate-limiting enzyme for ethylene synthesis in many plant systems (25). We have therefore examined the role of ethylene or ACC on the development of ACC synthase in this excision-induced ethylene production system. We assayed levels of ACC in plugs which were not pretreated with AVG but were incubated under either an ethylene-containing or an ethylene-free atmosphere for 12 h. The ACC levels in these two treatments were found to be identical (data not shown). Since AVG would inhibit ACC synthesis, we could not administer AVG to samples which were to be analyzed for ACC content. Failure to observe a difference between ethylene-free and ethylene-treated tissues in this experiment may have resulted from the fact that an amount of ethylene was produced from ACC sufficient to invalidate attempted ethylene-free conditions. Attempting to suppress endogenous ethylene production in the absence of AVG, we incubated the excised tissues under a nitrogen atmosphere (2). Freshly excised plugs, whether incubated 12 h under N_2 or under N_2 + ethylene, however, also contained identical levels of ACC (data not shown). In a further attempt to resolve whether ethylene may affect ACC synthase, we have undertaken another approach by administering AVG to cantaloupe plugs to inhibit endogenous ethylene production. The development of ACC synthase activity in plugs incubated under ethylene-free or under ethylene-containing atmospheres was then compared. As shown in Table III, no significant differences in extractable ACC synthase activity were observed between treatments. These data suggest that ethylene did not play a direct role in the development of ACC synthase.

DISCUSSION

It has been shown that regulation of ethylene synthesis either by IAA (27) or by excision (5, 12, 28) involves an increase in ACC synthase activity. Induction or activation of this enzyme is thus an important process. Regulation of ethylene synthesis via EFE, however, has received little attention. In the present study we have shown that excision of preclimacteric cantaloupe fruit tissue resulted in a spectacular increase in ethylene production which was accompanied by an increase in EFE as well as ACC synthase activity (Fig. 3). Ethylene treatment additionally enhanced EFE activity (Table I). This evidence supports the view that in fruit tissue both EFE and ACC synthase are involved in the regulation of ethylene biosynthesis. In vegetative tissues, in contrast, only ACC synthase appears to be rate-limiting (25). CHI inhibits the wound-induced formation of both enzymes, suggesting that both increase as a result of protein synthesis. Inhibition of the induction of ACC synthase formation requires higher concentrations of CHI than is required for EFE, however. This suggests that the synthesis of ACC synthase is less vulnerable to CHI inhibition than that of EFE. We know very little about how ethylene promotes EFE activity. It is clear, however, that this system is very sensitive to ethylene since as little as 0.3 μl/l ethylene induces half maximal EFE activity. Furthermore, the effect of ethylene requires 1 to 2 h to be manifested (Fig. 6), indicating that the response is mediated through a process requiring substantial metabolic activity. This process is dependent upon the continuous presence of ethylene, inasmuch as removal of ethylene results in reduced EFE activity (Fig. 6).

Table III. *Effect of ACC and Ethylene Treatments on Extractable ACC Synthase Activity*

Immediately after excision, discs were infiltrated with 2% KCl containing 1 mM AVG or 1 mM AVG + 1 mM ACC as specified and incubated for 12 h under a flow of air or air containing 20 μl/l ethylene. Extractable ACC synthase activity in the discs at the end of the incubation period were assayed as described under "Materials and Methods." Values represent means ± SE of two 5-g samples.

Treatment	ACC Synthase Activity
	nmol ACC/mg protein·3 h
Control (−AVG)	1.9 ± 0.1
AVG	1.7 ± 0.6
AVG + ACC	1.5 ± 0.2
AVG + ethylene	2.0 ± 0.1

The present cantaloupe system has allowed us to investigate the role of ethylene in ethylene synthesis. If ethylene action is required to induce ethylene biosynthesis, then we would expect to see an inhibition of enzyme induction by Ag^+, which is known to antagonize ethylene action (3, 4). Indeed, Ag^+ inhibited the ethylene-promoted development of EFE, although it had no effect on ethylene synthesis in ethylene-free air (Table II). As in the present excised preclimacteric cantaloupe, EFE is also induced in intact climacteric fruits during ripening. If ethylene plays a corresponding inductive role in the development of EFE in ripening fruits, we would expect Ag^+ to inhibit ethylene synthesis. Indeed, Ag^+ has been shown to inhibit ethylene synthesis in ripening banana and tomato fruits (20). Ag^+ was also reported to inhibit ethylene production in postclimacteric apple (20) which already contains EFE (25). This may be explained on the basis that EFE undergoes turn over and that ethylene plays a role in maintaining a high level of EFE and consequent maximal rates of ethylene production as in excised cantaloupe tissue shown in Figure 6. Veen (22) found that Ag^+ inhibited ethylene production in cut carnations and also proposed that the effect of Ag^+ was to inhibit ethylene action on ethylene production. Our assay for EFE activity is based on the assumption that ACC and ACA are substrates for the same enzyme; supporting evidence is described elsewhere (11).

The autocatalytic effect of ethylene on ethylene production in ripening fruits and other senescing tissues is well documented (1). Since ACC synthase is a rate-limiting enzyme, it might be expected that ethylene would induce ACC synthase in these developmental processes. In the present excision-induced ethylene production system, ACC synthase activity was induced by excision but not by ethylene.

Several reports indicate that wound-induced ethylene production is greater in mature preclimacteric than in immature preclimacteric fruit. This behavior has been observed for apple, tomato, and avocado (14), papaya (18), and cantaloupe (N. E. Hoffman, unpublished). In intact fruits of cantaloupe (19), avocado (7), and banana (24), ethylene is known to be less effective in initiating ethylene production in immature fruits than in mature fruits. Ethylene failed to induce ethylene production in intact 9-day-old cantaloupe (19). Plugs excised from immature 9-day-old cantaloupes produced very little ethylene (about 20 nl/g·h) but after they were treated with ethylene for 12 h following excision and then supplied ACC, their rate of ethylene production rate increased to 200 nl/g·h (N. E. Hoffman, unpublished). This rate is comparable to that of tissue excised from mature preclimacteric fruits, in which exogenous ACC caused no further stimulation (Fig. 2). This suggests that ethylene production, in tissue excised from immature fruit, is limited by an inadequate supply of endogenous ACC. Although ethylene was not capable of increasing ACC levels in excised 9-day-old tissue, it did induce EFE activity. Similarly, the development of wound-induced ACC synthase activity increased in excised tissue of tomato fruit as ripeness of the fruits advanced (12; T. McKeon, unpublished). In excised tissue of immature fruits of both cantaloupe and tomato, it appears that

induction of ACC synthase in response to excision is restricted, and that this restriction is relaxed as the fruit undergoes maturation and ripening. By contrast, EFE development is induced by excision and ethylene treatment to the same degree in both immature and mature preclimacteric fruits. In intact preclimacteric fruit, the synthesis of both ACC synthase and EFE are suppressed. We speculate that during the maturation process a repressor which restricts the development of ACC synthase is destroyed, resulting in the development of ACC synthase; excision is, however, ineffective in destroying this repressor. The role of ethylene in autocatalytic ethylene production may be to accelerate the destruction of this repressor resulting in synthesis of ACC synthase in addition to promoting the development of EFE activity. In this study we have shown that ethylene promotes the development in EFE activity in excised tissue of preclimacteric cantaloupe. It is yet to be shown, however, whether ethylene also induces the development of EFE in intact preclimacteric fruit.

Acknowledgments—We thank Professor S. Sakamura of Hokkaido University, Japan, for the generous gift of (+)-allocoronamic acid, Dr. Mas Yamaguchi, Department of Vegetable Crops, University of California, Davis, for cantaloupes, and to Gordon Von Abrams, for critically reading the manuscript.

LITERATURE CITED

1. Abeles FB1973 Ethylene in Plant Biology, Chap 7. Academic Press, New York, pp 161–178
2. Adams DO, SF Yang 1979 Ethylene biosynthesis: identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. Proc Natl Acad Sci USA 76: 170–174
3. Beyer EM 1976 Silver ion: a potent inhibitor of ethylene action in plants. Plant Physiol 58: 268–271
4. Beyer EM 1979 Effect of silver ion, carbon dioxide, and O_2 on ethylene action and metabolism. Plant Physiol 63: 169–173
5. Boller T, H Kende 1980 Regulation of wound ethylene synthesis in plants. Nature 286: 259–260
6. Cameron AC, CAL Fenton, Y Yu, DO Adams, SF Yang 1979 Increased production of ethylene by plant tissues treated with 1-aminocyclopropane-1-carboxylic acid. HortScience 14: 178–180
7. Eaks IL 1980 Respiratory rate, ethylene production, and ripening response of avocado fruit to ethylene or propylene following harvest at different maturities. J Am Soc Hort Sci 105: 744–747
8. Frenkel C, I Klein, DR Dilley 1968 Protein synthesis in relation to ripening of pome fruits. Plant Physiol 43: 1146–1153
9. Gepstein S, KV Thimann 1980 The effect of light on the production of ethylene from 1-aminocyclopropane-1-carboxylic acid by leaves. Planta 149: 196–199
10. Hoffman NE, SF Yang 1980 Changes of 1-aminocyclopropane-1-carboxylic acid content in ripening fruits in relation to their ethylene production rates. J Am Soc Hort Sci 105: 492–495
11. Hoffman NE, SF Yang 1981 Conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene by plant tissues: conversion of stereoisomers of 1-amino-2-ethylcyclopropane-1-carboxylic acid to 1-butene. Plant Physiol 67: S-51
12. Kende H, T Boller 1981 Wound ethylene and 1-aminocyclopropane-1-carboxylate synthase in ripening tomato fruit. Planta 151: 476–481
13. Lieberman M 1979 Biosynthesis and action of ethylene. Annu Rev Plant Physiol 30: 533–591
14. Lieberman M, JE Baker, M Sloger 1977 Influence of plant hormones on ethylene production in apple, tomato, and avocado slices during maturation and senescence. Plant Physiol 60: 214–217
15. Lizada MCC, SF Yang 1979 A simple and sensitive assay for 1-aminocyclopropane-1-carboxylic acid. Anal Biochem 100: 140–145
16. Lyons JM, WB McGlasson, HK Pratt 1972 Ethylene production, respiration and internal gas concentrations in cantaloupe fruits at various stages of maturity. Plant Physiol 37: 31–36
17. McGlasson WB, HK Pratt 1964 Effects of wounding on respiration and ethylene production by cantaloupe fruit tissues. Plant Physiol 39: 128–132
18. Patil SS, CS Tang 1974 Inhibition of ethylene evolution in papaya pulp tissue by benzyl isothiocyanate. Plant Physiol 53: 585–588
19. Rowen KS, WB McGlasson, HK Pratt 1969 Changes in adenosine pyrophosphates in cantaloupe fruit ripening normally and after treatment with ethylene. J Exp Bot 20: 145–155
20. Saltveit ME, KJ Bradford, DR Dilley 1978 Silver ion inhibits ethylene synthesis and action in ripening fruits. J Am Soc Hort Sci 103: 472–475
21. Suttle JC, H Kende 1980 Methionine metabolism and ethylene biosynthesis in senescing petals of *Tradescantia*. Phytochemistry 19: 1075–1080
22. Veen H 1979 Effects of silver on ethylene synthesis and action in cut carnations. Planta 145: 467–470
23. Vendrell M 1969 Reversion of senescence: effects of 2,4-dichlorophenoxyacetic acid and indole acetic acid on respiration, ethylene production and ripening in banana fruit slices. Aust J Biol Sci 22: 601–610
24. Vendrell M, WB McGlasson 1971 Inhibition of ethylene production in banana fruit tissue by ethylene treatment. Aust J Biol Sci 24: 885–895
25. Yang SF, DO Adams, MCC Lizada, YB Yu, KJ Bradford, AC Cameron, NE Hoffman 1980. Mechanism and regulation of ethylene biosynthesis. *In* F Skoog, ed, Plant Growth Substances. Springer-Verlag, Berlin, p 219–229
26. Yu YB, DO Adams, SF Yang 1979 1-Aminocyclopropanecarboxylate synthase, a key enzyme in ethylene biosynthesis. Arch Biochem Biophys 198: 280–286
27. Yu YB, SF Yang 1979 Auxin induced ethylene production and its inhibition by aminoethoxyvinylglycine and cobalt ion. Plant Physiol 64: 1074–1077
28. Yu YB, SF Yang 1980 Biosynthesis of wound ethylene. Plant Physiol 66: 281–285