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Effect of 1-Aminocyclopropane-1-Carboxylic Acid (ACC) Application Time on Wound-Induced Ethylene Production from *Rin* Tomato Disks

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ABSTRACT

ACC stimulated wound ethylene production by fruit pericarp tissue or *rin* mutant tomato (*Lycopersicon esculentum* Mill.). The later ACC was applied after cutting, the greater the stimulation of wound ethylene production, Severely damaged disks resulting from freezing lost their ability to convert ACC to ethylene.

INTRODUCTION

Most vegetative tissues are capable of converting applied ACC to ethylene (Cameron *et al.*, 1979). This indicates that the enzyme converting ACC to ethylene or ethylene forming enzyme (EFE) is constitutive and usually not rate limiting (Yang and Hoffman, 1984). Addition of ACC to preclimacteric fruit tissues results in very minor stimulation of ethylene production (Liu *et al.*, 1985 ; Bufler, 1986). This has let to the conclusion that both ACC synthase and EFE are low in activity in unripe preclimacteric fruit (McKeon and Yang, 1987). The increases in ethylene production from ripening fruits and wounded tissue are the results of an increase in the activity of ACC synthase and EFE (Yang, 1985). EFE has been demonstrated in a cell-free system (Kende *et al.*, 1986). In the present study we report the effect of application time of ACC on wound-induced ethylene production from *rin* tomato disks.

MATERIALS AND METHODS

Plant Material and Incubation

Plants of *rin* and wild-type tomatoes (*Lycopersicon esculentum* Mill.) were grown in the greenhouse and trained to a single stem. Flowers were tagged at anthesis; only one flower per cluster being pollinated. All others in a cluster were excised. Mature-green fruits were harvested at 40 $\pm$ 1 days after anthesis. Disks of pericarp tissue (diameter: 1.5 cm; thickness: 0.25 cm) were prepared from the equatorial part of tomato fruits using a cork borer and template. The rate of ACC-stimulated ethylene production was studied using a static system. Single disks of pericarp tissue were placed with epidermis on a layer of glass beads in 20 ml scintillation vials. Vials were flushed with ethylene-free air and sealed with a serum cap then incubated in darkness at 20°C.

Chemical Treatments

Twenty μl of 1 mM ACC solution or distilled water (control) were applied to the cut surface of disks opposite the epidermis.

Ethylene Determination

At the end of every 2 h period, wound ethylene production by disks was withdrawn with a 1-ml gas tight syringe through the serum cap was assayed on a gas chromatograph (Varian Aerograph Series 1700) which equipped with a flame ionization detector. The vials were then flushed with ethylene-free air, sealed and returned to the incubation conditions. Each experiment consisted of five to six disks and was repeated three times. Data represent mean values.

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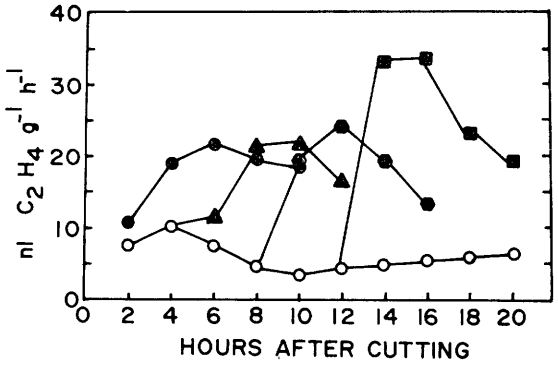

Figure 1. Effect of ACC application time on the stimulation of wound ethylene production by disks from mature-green fruits of *in* tomato. Twenty μl of H_2O at O h (○) and 1 mM ACC were applied to the cut surface of disks at O (●), 4(△), 8(▲) or 12(■) after cutting.

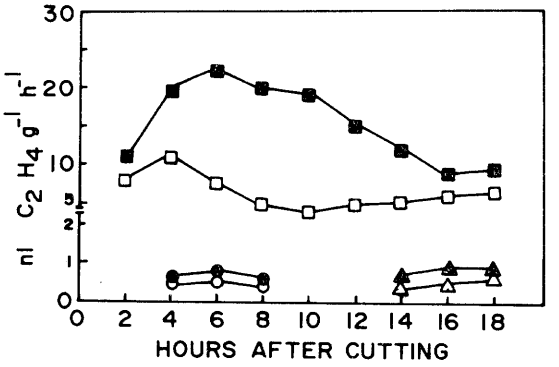

Figure 2. Effect of ACC application time on wound ethylene production by severely demaged disks from mature-green fruits of *rin* tomato. Disks were frozen for 1 h at 0 and 12 h after cutting and returned to 20°C. After return to 20°C for 1 h disks were blotted with paper towels and 20μl of H_2O or 1 mM ACC were applied to the cut surface (□ : non-frozen disk + H_2; ■ : non-frozen disk + ACC; ○ : frozen disk at 0 h + H_2O; ● : frozen disk at 0 h + ACC; △ : frozen disk at 12 h + H_2O; ▲ : frozen disk at 12 h + ACC).

RESULTS AND DISCUSSION

Freshly cut disks from mature-green fruits of *rin* tomato evolved wound ethylene during incubation at 20°C in the dark, reaching a maximum about 4 h after cutting (Figure 1). The rate of wound ethylene production then declined to a minimum 10 h after cutting and slightly increased again thereafter. ACC applied to the cut surface of disks at difference times after cutting caused different ethylene production. The later ACC was applied to disks after cutting the more ethylene production was stimulated (Figure 1.). The effect of differences in absorption of ACC can be ruled out because 2 h after its application, all ACC solution had completely disappeared from the cut surface of all disks. As the disks passed through the time of maximum wound ethylene production, it would be expected that endogenous levels of free ACC decreased (Yu and Yang, 1980). This would lower the ACC pool of disks and when ACC was applied it would be converted more effectively to ethylene then it was applied earlier. One may argue that since the rates of wound ethylene production by disks without applied ACC at eighth and twelfth hour after cutting were the same, the difference of ACC levels at eighth and twelfth hour should not make a great difference in response to applied ACC. This had led to the question as to whether or not wounding can increase the activity of enzyme required for the conversion of ACC to ethylene. In fact, Hoffman and Yang (1982) and Bufler (1986) have shown that excision can induce the enzyme activity converting ACC to ethylene in preclimiacteric cantaloupe and apple respectively. If wounding indeed increases the enzyme activity required for the conversion of ACC to ethylene, then applying ACC later to disks would be expected to stimulate more ethylene production, This suggests that wounding can induce an increase in both ACC synthase (Kende and Boller, 1981) and the enzyme converting ACC to ethylene or EFE (Hoffman and Yang, 1982 ; Bufler, 1986). Although ACC-dependent ethylene production can be readily demonstrated in intact tissues, no enzyme had yet been isolated that displays all the properties of the *in vivo* activity (Kende *et al.*, 1986).

ACC applied to disks which had been frozen and thawed at different times after cutting had little response to ACC and there was no difference in ethylene production because of ACC application time (Figure 2). This could be due to inactivation of enzyme responsible for the conversion of ACC to ethylene or freezing may

have caused the breakdown of compartment which would possibly bring inhibitors in contact with EFE. Konze and Kende (1979) reported that the homogenates of tomato fruits failed to show the ability to convert ACC to ethylene. Many reports suggest that EFE is membrane associated, labile and subject to disruption by various treatments (Lieberman, 1979 ; Hoffman and Yang, 1980). Therefore, the freezing condition may deactivate the EFE activity resulting in no difference in response to earlier and later ACC application.

LITERATURE CITED

Bufler, G. 1986. Ethylene-promoted conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene in peel of apple at various stages of fruit development. Plant Physiol. 80 : 539-543.

Cameron, A.C., C.A.L. Fenton. Y.B. Yu, D.O. Adams and S.F. Yang. 1979. Increased production of ethylene by plant tissues treated with 1-aminoclopropane-1-carboxylic acid. HortScience 14 : 178.

Hoffman, N.E. and S.F. Yang. 1982. Enhancement of wound-induced ethylene synthesis by ethylene in preclimacteric cantaloupe. Plant Physiol. 69 : 317-322.

Kende, H., A.B. Bleecker, W.H. Kenyon and R.G. Mayne. 1985. Enzymes of ethylene biosynthesis, pp. 120-128. *In* M. Bopp (ed.). Proc. 12th Int. Conf. Plant Growth Substances. Springler-Verlag, Berlin.

Kende, H. and T. Boller. 1981. Wound ethylene and 1-aminocyclopropane-1-carboxylate synthase in ripening tomato fruits. Planta 151 : 476-488.

Lieberman, M. 1979. Biosynthesis and action of ethylene. Ann. Rev. Plant Physiol. 30 : 533-391.

Liu, Y., N.E. Hoffman and S.F. Yang. 1985. Promotion by ethylene of the capability to convert 1-aminocyclopropane-1-carboxylic acid to ethylene in preclimacteric tomato and cantaloupe fruits. Plant Physiol. 77 : 407-411.

McKeon, T.A. and S.F. Yang. 1987. Biosynthesis and metabolism of ethylene, 94-112. *In* P.J. Davies (ed.). Plant Hormones and Their Role in Plant Growth and Development. Martinus Nijhoff Publishers, Dordrecht.

Yang, S.F., 1985 Biosynthesis and action of ethylene. HortScience 20 : 41-45.

Yang, S.F. and N.E. Hoffman. 1984. Ethylene biosynthesis and its regulation in higher plants. Ann. Rev. Plant Physiol. 35 : 155-189.

Yu, Y.B. and S.F. Yang. 1980. Biosynthesis of wound ethylene. Plant Physiol. 66 : 281-285.