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# The Role of Epidermis in Wound Ethylene Production by Fruit Pericarp Tissue of *Rin* Mutant Tomato *(Lycopersicon esculentum* Mill.)

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## ABSTRACT

Wound-induced ethylene production by fruit pericarp tissue of *rin* mutant tomato was studied in relation to role of the epidermis. Disks with intact epidermis produced more wound ethylene than disks without epidermis. Disks with intact epidermis were stimulated to produce more ethylene than disks without epidermis in response to 1-aminocycopropane-1-carboxylic acid (ACC) application. This suggests that the compact cells close to the epidermis may at least be the location of enzyme(s) or other cofactor(s) required for the conversion of ACC to ethylene.

## INTRODUCTION

Ethylene is considered to be a plant hormone because it is a natural product of metabolism and acts in trace amounts (Burg and Burg 1964; Pratt and Goeschl 1969). Its production rates during the development of higher plants vary from organ to organ and time of development (Abeles, 1973). The site of ethylene synthesis in plant cells is not completely known. A number of investigators has suggested that ethylene biosynthesis may take place in a cytoplasmic membrane or the plasmalemma. (Lieberman, 1979). Recently, Guy and Kende (1984) compared the distribution of ACC between the vacuole of isolated pea *(Pisum sativum* L.) protoplasts and the remainer of the cell and found that over 85% of the ACC was localized in the vacuole. Isolated protoplasts and vacuoles evolved ethylene. Over 80% of the ethylene production by protoplasts could be accounted for as originating from the vacuole. The vacuole is probably one but perhaps not the sole organelle of the cell where ethylene is synthesized.

In order to exhibit wound ethylene production the wounding process must not destroy all the cells. Imaseki *et al.* (1968) reported that disease-induced ethylene production in sweet potato tissue was observed only by tissue plugs of sweet potato which consisted of both fungus-invaded and non-invaded parts. The fungus-invaded part alone did not release ethylene. Similarly, plugs which were removed from the fungus-invaded part did not produce an appreciable amount of ethylene. Elstner and Konze (1974) froze various areas of beet leaf disks and found that ethylene production increased with the increase in area of frozen tissue until 50% of the leaf area was damage. Further increase in damage reduced ethylene production. The cells adjacent to the injured or dead cells appeared to produce the surge in ethylene production. In this paper reports herein the role of the epidermis on wound ethylene production by disks from pericarp tissue of *rin* tomato.

## MATERIALS AND METHODS

**Plant material and incubation.** Plants of *rin* tomato (*Lycopersicon esculentum* Mill.) were grown and trained to a single stem. Unless noted otherwise, disks of pericarp tissue (diameter:

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1.5 cm; thickness: 0.25 cm), with intact epidermis from mature-green fruits (40 ± 1 days after anthesis) were prepared from equatorial part of tomato fruits with a cork borer, template and *razor*. Therefore, all disks were uniformly prepared in size and fresh weight. 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.

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

## RESULTS

Disks were cut into different thicknesses ranging 0.05 to 0.45 cm thickness resulting in cut surface areas ranging from 2.00 to 3.89 cm$^2$. The rate of wound ethylene production was directly proportional to the cut surface area of disks when expressed on a cut surface area basis (Figure 1). The rates of wound ethylene production by all disks reached a maximum 4 hours after cutting and decreased thereafter. The rate of wound ethylene production was increased when the epidermis of disks was closer to the major cut surface (Figure 2). The thinner the disk, the greater the wound ethylene production when expressed on a per unit weight basis. Disks with different thicknesses were the same as those in Figure 1. The rates of wound ethylene production by all disks reached a maximum 4 hours after cutting and then declined. The rates of wound ethylene production among disks with major cut surface 0.15, 0.25, 0.35 and 0.45 cm away from the epidermis were slightly different while disks with the cut surface only 0.05 cm away from the epidermis produced significantly greater amounts of wound ethylene throughout the incubation period.

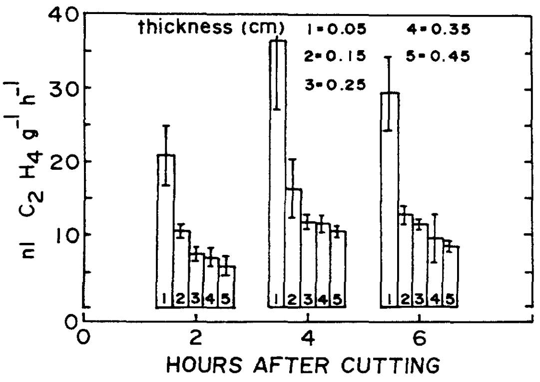

**Fig. 1.** **Effect of cut surface area of freshly cut disks on wound ethylene production. Disks with intact epidermis were cut into 0.05, 0.15, 0.25, 0.35 and 0.45 cm thickness resulting in cut surface area of 2.00, 2.47, 2.95, 3.42 and 3.89 cm$^2$, respectively.**

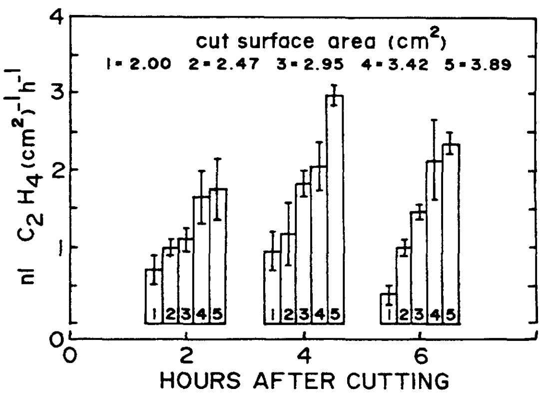

**Fig. 2.** **Effect ot the distance between the epidermis and the major cut surface on wound ethylene production of freshly cut disks. Disks with intact epidermis were cut into 0.05, 0.15, 0.25, 0.35 cm thickness.**

When disks with the same thickness were prepared from the same plug of pericarp tissue but different distances *from the* epidermis (see diagram in Figure 3), it was found that

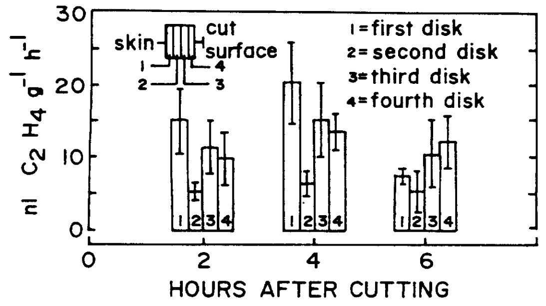

**Fig. 3.** Wound ethylene production by freshly cut disks from different parts of pericarp tissue. Plugs of pericarp tissue were cut into consecutive 0.1 cm thick disks beginning with the epidermis. Only the first disk had epidermis and the proximal cut surface of the second, third and fourth disk was covered with vaseline.

the first disk including the epidermis (intact epidermis) produced the most wound ethylene during the first 4 hours following the preparation of the disks. The second, third and fourth disks had one cut surface covered with vaseline. Six hours after preparing the disks, there was little difference in ethylene production between the disks from any of the four positions in the pericarp tissue.

Freshly cut disks with intact epidermis were stimulated to produce more ethylene than disks without epidermis in response to ACC application (Figure 4). However, disks with and without intact epidermis which had been incubated for 12 hours showed the role of intact epidermis more clearly than did freshly cut disks in response to ACC application but rates of ethylene production from the controlled disks with and without epidermis were almost the same throughout the incubation period (Figure 5).

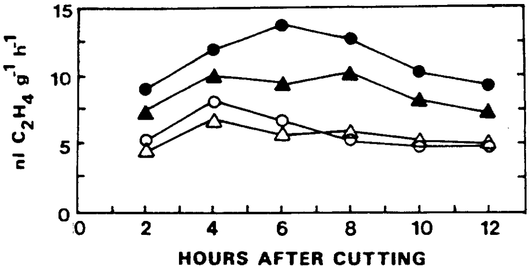

**Fig. 4.** Role of the epidermis of freshly cut disks on ACC-stimulated ethylene production. Disks without epidermis were prepared the same way as disks with intact epidermis, the cut surface of the proximal side was covered with vaseline. Twenty ul of $H_2O$ (distilled water) or 1 mM ACC were applied to the cut surface of disks (O—O : with epidermis + $H_2O$, △—△ : without epidermis + $H_2O$, ●—● : with epidermis + ACC, ▲—▲ : without epidermis + ACC).

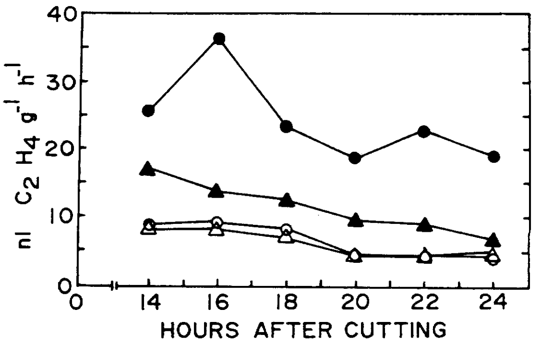

**Fig. 5.** Role of the epidermis of incubated disks on ACC-stimulated ethylene production. Disks without epidermis were prepared as previously described in Fig. 4. Disks were incubated on a layer of glass beads with water underneath in petri dishes for 12 hours. At the end of incubation period, 20 ul of $H_2O$ (distilled water) or 1 mM ACC were applied to the cut surface of disks (O—O : with epidermis + $H_2O$, △—△ : without epidermis + $H_2O$, ●—● : with epidermis + ACC, ▲—▲ : without epidermis + ACC).

## DISCUSSION

Increasing the cut surface area of freshly cut disks stimulated more wound ethylene production and this agrees with other reports with apple (Meigh *et al.*, 1960), citrus (Hyodo, 1977), sweet potato (Imaseki *et al.*, 1968), bean (Jackson and Osborne, 1970; Konze and Kwiathowski, 1981), rice (Imaseki *et al.*, 1971) and tomato (Craft, 1960; Herner and Sink, 1973; Kende and Boller, 1981; Yu and Yang, 1980). Perhaps the stimulation of wound ethylene production by increasing the cut surface area of disks is related to the amount of injured cells located on the wounded tissue (Imaseki *et al.,* 1968) which in turn more ACC synthase and more ethylene is produced (Kende and Boller, 1981; Yu and Yang, 1980).

The experiments with disks of different thicknesses and disks with and without intact epidermis from the same plug of pericarp tissue demonstrate that the epidermis and/or cells immediately below it play an important role in wound-induced ethylene production. Similarly, Wardale (1973) found that tomato plugs with skin produced more ethylene than plugs without skin during the onset of climacteric and post-climacteric. From chemical and morphological studies it has been shown that the epidermis of tomato fruits is composed primarily of lipid compounds and there are three to four layers of compact cells close to the epidermis (Batal *et al.,* 1972; Morse, 1971). Though the step from ACC to ethylene in the pathway of ethylene biosynthesis is totally dependent on oxygen (Adams and Yang, 1979), it is unlikely that when the cut surface was moved closer to the epidermis, as in the thinner disks, the compact cells were exposed to more oxygen.

In disks whose thickness was 0.45 cm, cells closest to the cut surface may produce more wound ethylene than those cells farther from the cut surface (Imaseki *et al.,* 1968) therefore, when the rate of wound ethylene production was expressed on a weight basis, the thicknest disks would appear to produce the least and the thinnest disks produce the most ethylene. When the rates of wound ethylene production of disks with equal size from the same plug of pericarp tissue were compared there were differences in the capacity to produce ethylene. The first disk containing the epidermis, initially produced the most ethylene, while disks farther from the epidermis produced less ethylene. This suggests that cells at different distances from the epidermis of tomato fruit pericarp tissue are unequally capable of producing ethylene.

The results from the experiment of freshly cut and incubated disks with and without epidermis in response to ACC application clearly showed that three to four layers of compact cell close to the epidermis are more active in the conversion of ACC to ethylene than those cells farther from the epidermis. This strongly supports the idea that the compact cells close to the epidermis play an important role in wound ethylene production by freshly cut disks from *rin* tomato fruits. Similarly, Sakai and Imaseki (1973) showed that mechanical destruction or removal of the epidermis from the hypocotyl segments of etiolated mungbean seedlings caused complete loss of ethylene production in response to auxin application. It is known that auxin stimulates ethylene production in plant tissues by inducing ACC synthase (Jones and Kende, 1979) which is the rate-limiting step in ethylene biosynthesis (Adams and Yang, 1979). This suggests that the epidermis may be the site of the enzyme(s) or other cofactor (s) required for the conversion of ACC to ethylene in hypocotyl segments of etiolated mungbean seedlings and probably in other plant tissues like tomato pericarp tissue. Peroxidase activity (van Fleet, 1959; Ku *et al.,* 1970) and phenolic compounds (Wardale, 1973) have been found to confine to the skin or the outermost and innermost layers of the pericarp of tomato fruits. Though Machackova and Zmrhal (1981) ruled out that peroxidase is not directly involved in the ethylene synthesis of wheat coleoptile segments but it can not be ruled out in tomato tissue. Phenol derivatives have been shown to stimulate ethylene production by citrus fruit peel (Fuch, 1970). The involvement of peroxidase and phenolic compounds in the compact cells close to the epidermis for *in vivo* ethylene production by tomato disks remains for further investigation.

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