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In situ, real-time monitoring of wound-induced ethylene in cherry tomatoes by two infrared laser-driven systems

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Abstract

Two laser-based detection setups, one involving photothermal deflection and the other photoacoustics, have been used to follow ethylene release when ripening cherry tomatoes (cv. Favorita) were mechanically wounded. Removing the calyx caused a double peak in ethylene release, the first peak over 1–2 h and the second over the next 4–7 h. Wounding the stem-scar yields similar results. However, wounding part of the fruit skin led to modest and variable releases of ethylene, much less than given on calyx-removal or stem-scar wounding. This higher emission does not originate from ethylene accumulated in the tomato already before wounding. Laser systems are shown to be useful in quantifying ethylene-releasing systems.

Keywords: Tomato; Wound; Ethylene; Photothermal deflection; Photoacoustics

1. Introduction

After the elucidation of the ethylene biosynthesis pathway by Yang and Hoffman (1984), the effect of wounding on the ethylene production could be clarified. Wounding is assumed to exert its effect at the step where S-adenosyl-methionine (SAM) is converted into ACC, the direct precursor of ethylene. Normally this step, regulated by the enzyme ACC synthase, is rate limiting in the cascade of events leading to ethylene production. By means of "stress" the rate can be increased.

Several types of stress that affect ethylene biosynthesis are known (Abeles et al., 1992). These include thermal stress such as freezing, chilling (Lurie and Klein, 1991) and high temperatures, drought, flooding, externally applied chemicals, gamma irradiation (Larrigaudière et al., 1991) and mechanical wounding, e.g. cutting (Meigh et al., 1960; Yang and Pratt, 1978; Boller and Kende, 1980; Kende and Boller, 1981;

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Parsons and Mattoo, 1991). One of the first examples of wound-ethylene production is quoted in the Bible (Amos 7:14; Galil, 1968). The development of figs was accelerated by gashing, already known by the early Egyptian civilizations dating back to 1100 B.C. Actually, the study of the effect of wounding on the ripening of fruit is important from an agricultural point of view, because fruits are often damaged during growth, harvest, storage and transport, which can lead to untimely ripening.

The stimulation of ethylene production by wounding typically occurs with a lag of 10–30 min and disappears after several hours (Yang and Hoffman, 1984). As demonstrated by Smith et al. (1986), wounding unripe tomato pericarp tissue by slicing it into 5-mm cubes resulted in a constantly increasing ethylene production during 3 h, after a lag of 20 min. This involves the expression of specific genes. However, the time lag depends on the maturity stage of the tomato according to Kende and Boller (1981); a lag of 15–30 min for green (pre-climacteric) and red (post-climacteric) tissue and instantaneous production for maximally respiring tomatoes is observed. The total amount of produced ethylene for wounded orange tomatoes is higher than for green and red fruit.

Stress mostly acts locally. An example is the increased ACC synthase activity in excised mesocarp of winter squash, which is localized in the outer 1 mm of cut tissue (Hyodo et al., 1989). Again, twice as much ethylene production has been found from the epidermis of an excised tomato fruit as compared to the underlying fleshy tissue (Ketsa, 1985).

Removal of the calyx at the fruit-pedicel junction, a late developing abscission zone (Osborne, 1989), is also a form of mechanical wounding as will be discussed below. The wounding effects of a small area of an otherwise intact tomato is investigated here. To estimate a small local increase in ethylene production due to wounding superimposed on an already high production level, sensitive laser-driven equipment is employed, monitoring total evolution of the hormone both in situ and in real-time.

In an attempt to understand the recovery process of a tomato after being mechanically damaged, attention is paid to (1) the time dependence of the ethylene evolution after local wounding of an otherwise intact tomato, (2) the absolute rates due to local wounding, and (3) the possible diffusion of accumulated ethylene through newly exposed cut surfaces.

2. Materials and methods

Fruit material

Experiments were carried out with cherry tomatoes (*Lycopersicon esculentum* cv. Favorita) at various stages of maturity. The tomatoes were obtained from commercial growers and had an individual weight of about 14 g and a diameter of approximately 30 mm. Three types of wounding were tested; (i) removal of the calyx, (ii) 10-fold puncturing into the tissue (depth 2 mm) by means of a sharp needle (diameter 0.5 mm) through the epidermis and/or the pericarp within an area of 5 mm diameter through the stem-scar area, and (iii) cutting the epidermis and pericarp layer over a length of 20 mm with a scalping-knife (depth 2 mm).

The actions (i), (ii) and (iii) took place while the tomato was exposed to air and the flow was stopped momentarily. Measurements were performed under normal atmospheric conditions either with static air (PTD) or in a flow-through system (PAD).

Laser-driven ethylene monitors

We employed both local photothermal deflection (PTD) and sensitive photoacoustic detection (PAD). PTD is based upon a change in refractive index (Jackson et al., 1981). In our application two laser beams were crossed at about 1 mm above the wounded area of the cherry tomato, under normal atmospheric conditions. One of these lasers, a continuous CO_2 infrared laser, excites ethylene into a vibrational-rotational level. By non-radiative collision decay, the absorption region is heated so that the refractive index changes. A second laser beam, a HeNe probe laser, passes over this region 31 times, at a distance of 0.7 mm from the CO_2 laser waist, and is deflected by the mirage effect (De Vries et al., 1992). The deflection is proportional to the concentration of the absorbing gas and to the available laser power and is measured with a position-sensing detector (type Centronic QD 50-2). The detection limit for ethylene is 0.5 nl l^{-1}. The whole setup is computer controlled; experiments can be extended up to one week.

Next to the PTD technique a PAD setup was applied as developed in our laboratory (Harren et al., 1990). In this setup, cuvette measurements yield emission rates for ethylene and are performed in a continuous flow system. A KOH column is used to scrub CO_2 and water. In addition, a liquid nitrogen cooling trap at a temperature of $-150°C$ serves to remove other interfering gases, like ethanol. The cooling trap was filled with a wire mesh for optimal thermal contact at low temperatures. After these precautions the gas flow coming from the cuvette is admitted to the acoustical resonator, the central part of the PAD setup. For ethylene, a minimum concentration of 6 pl l^{-1} can be detected. Switching between cuvettes with a flow of 1 l h^{-1} causes a delay of 4 min, as it is necessary to refill the photoacoustic cell completely.

PAD is closely related to PTD; instead of making use of a change in refractive index, a pressure change in an acoustical resonator pipe is monitored. Sound is produced at the resonance frequency of this pipe at 1600 Hz; the modulation frequency of the pump beam is made equal to the resonator frequency. PAD has been employed for kinetic measurements of e.g. *Cymbidium* orchids (Woltering et al., 1988), *Rumex* species (Voesenek et al., 1993) and tomatoes (Bijnen et al., 1994).

3. Results

PTD measurements of ethylene concentrations above the stem-scar area are shown, starting immediately after removal of the calyx (consisting of pedicel and sepals), at $t = 0$ (Fig. 1). The increased ethylene production persisted over a period of nearly two hours.

By examining integral emission rates with PAD, the action of wounding consequently momentarily interrupted the flow. This has led — as an artifact — to a

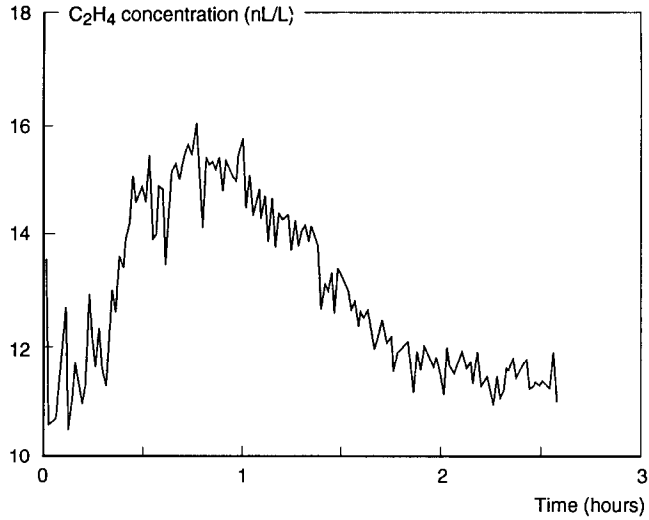

Fig. 1. Locally increased ethylene levels after removal of the calyx of a single mature orange cherry tomato at $t = 0$, using PTD.

corresponding dip in the measured ethylene level, yielding an immediate decrease in ethylene levels, but was followed by a slowly increasing emission during the next 20 min. The increase in ethylene emission, which is observed in Fig. 2a at $t = 0$, was due to the interrupted flow. This figure shows a long-term experiment in which the calyx was removed at $t = 0$. The basal ethylene production revealed the first maximum after about 45 min and in particular a second maximum after 9 h (arrows). Thereafter a normal production was observed, while the fruit ripened as for non-wounded fruits. In another experiment, the effect of removal of the calyx (after 2.3 h) was clearly observed on top of the normal emission (Fig. 2b). The full width of this peak was also about two hours. It should be noted that the ethylene pattern has been corrected for the momentarily interrupted flow due to the action of wounding.

The local effect of wounding on the total ethylene emission at the stem-scar site and the stylar end in situ was demonstrated by PTD (Fig. 3). To this end the calyx was removed 10 min before starting the ethylene measurement; an increase in ethylene emission was observed at the stem-scar site (*A*). Ethylene levels at the stylar end were constant at 5 nl l^{-1} (*B*). Subsequently, measurements above the stem-scar (*C*) yielded an ethylene concentration slightly higher than that found at $t = 1.9$ h. Then, two cuts were made over 20 mm on the stylar through the epidermis and pericarp layer, one at $t = 3.2$ h and the other at $t = 4.6$ h. After cutting the tissue, the ethylene levels were followed above the cut-area at the stylar end (*D* and *F*), resulting in a negligible increase as compared to concentrations above an intact epidermis (*B*). The stem-scar site (*E* and *G*) gave similar readings to those found earlier in this experiment (*A* and *C*).

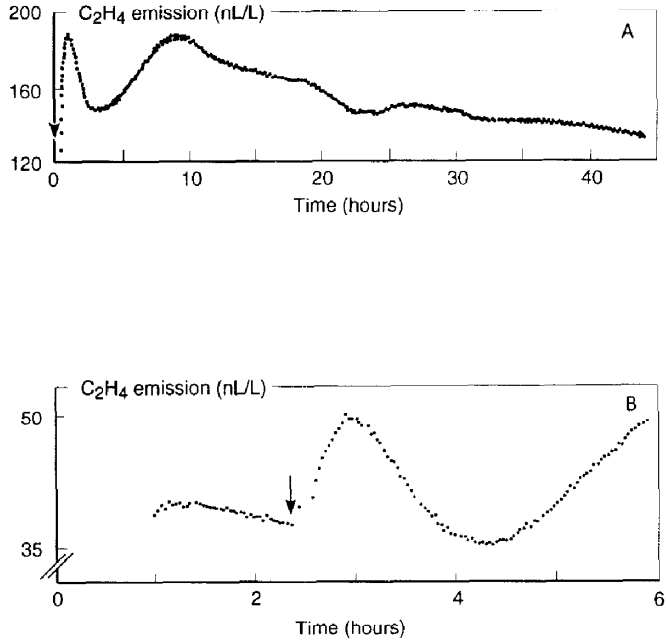

Fig. 2. (a) First, an increase in ethylene is visible immediately after removal of the calyx of a single mature orange cherry tomato at the start of the experiment, followed by a second peak and afterwards a normal climacteric ethylene pattern. (b) After 2.3 h, the effect of wounding is directly observable as a peak on the total ethylene emission rate of a single mature red cherry tomato, monitored by PAD.

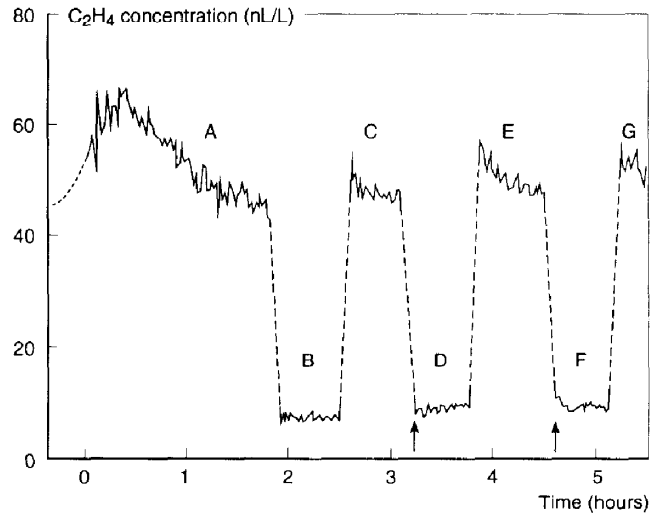

Fig. 3. The difference in removing the calyx (10 min before the experiment) and making a cut at the stylar end of the tomato (mature orange stage) at t = 3.2 h and at t = 4.6 h is visible. Alternatively the CO_2 laser in the PTD setup is scanned over the stem-scar (*A*, *C*, *E* and *G*) and stylar end (*B*, *D* and *F*).

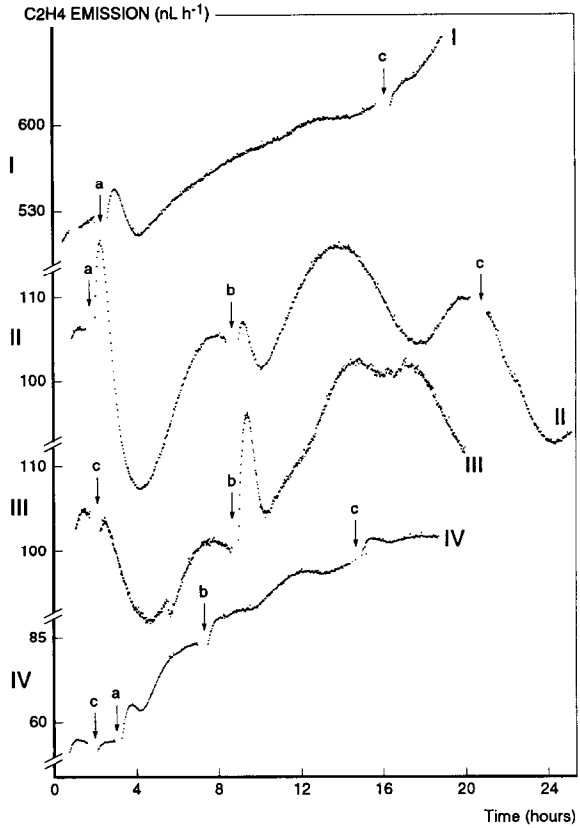

Fig. 4. Emission rates before and after locally wounded cherry tomatoes — all in mature orange stages — are determined with PAD. Arrows indicate the timing of wounding; *a* = removal of the calyx; *b* = 10-fold penetration (depth 2 mm) of the stem-scar; *c* = 10-fold penetration (depth 2 mm) of the pericarp layer. Four different curves are presented at different ethylene production scales indicated on the left.

To estimate absolute ethylene concentrations this local effect was followed by PAD, too (Fig. 4). The effects of removal of the calyx on the ethylene production (indicated by *a*), of puncturing the stem-scar tissue 10 times (indicated by *b*) and the pericarp layer (also 10 times; indicated by *c*) were studied. The sequence in first wounding the stem-scar area and then the pericarp layer, or vice versa, was varied for four experiments, resulting in four different curves (Fig. 4). The emission rates, dependent on the particular tomato involved, are indicated for each trace on the vertical axis.

After wounding the tissue at different sites, the ethylene evolution demonstrated quite different behaviour (Fig. 4). General features were: (i) in each curve it is demonstrated that wounding the pericarp layer (action *c*) yielded a much smaller effect as compared to wounding the stem-scar region (action *a* and *b*); (ii) removal of the calyx, or puncturing the stem-scar area always resulted in substantial ethylene

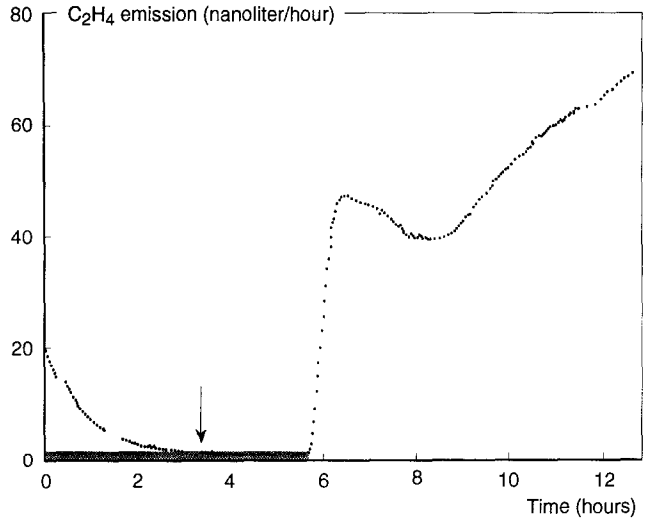

Fig. 5. Anoxic (at t = 0) and aerobic (at t = 5.6 h) conditions are interchanged for a single mature orange cherry tomato using the PAD setup. At t = 3.2 h the calyx is removed, yielding a continuous negligible emission rate.

production and double peak occurrence in time (see Fig. 2 and Fig. 4, actions *a* and *b* especially in curves II and III). Curve IV (Fig. 4) showed a less pronounced response, while curve I (Fig. 4) did not really display the second maximum; and (iii) the effect of wounding the pericarp layer twice yielded no pronounced first maximum before the ethylene production fell back to the broad minimum at about 3 h after wounding (Fig. 4, action *c* in curves II and III).

Finally, the calyx was removed under anaerobic conditions (Fig. 5). From the start of this experiment (t = 0) the tomato was exposed to anaerobic conditions. First (at t = 3.3 h), the calyx was removed resulting in an undisturbed ethylene emission. Then (at t = 5.75 h), aerobic conditions were restored, yielding an immediate increase, temporarily followed by a decrease and subsequent increase in ethylene emission.

4. Discussion

Concerning the temporal aspects of wounding tissue of an orange tomato, the data presented in Figs. 1 and 2 show good agreement with data from Kende and Boller (1981). In contrast to Yang and Hoffman (1984) and Smith et al. (1986), no time lag between wounding and enhanced ethylene production was found. Our observation of a second peak after wounding has not been reported before, probably due to the fact that experiments were performed on sliced tomato tissue which was then incubated. For tomato pericarp discs Riov et al. (1990) mentioned a slow second rise attributed to senescence; it could be greatly increased by applying abscisic acid (ABA).

In the present paper the described experiments were performed on locally wounded but otherwise intact tomatoes. The recovery after wounding could be followed over long periods for an entire tomato fruit. We suggest that the minimum in ethylene production between the first and second peak is due to suppressed ACC synthase activity as a consequence of the enhanced ethylene level (autoinhibition; Hyodo et al., 1985). At first there is an increase of ethylene emission originating from the direct effect of wounding, i.e. from the increased activity of ACC synthase (Yang and Hoffman, 1984). Then, with some delay the higher ethylene concentration diminishes the ACC synthase activity. This in turn leads to less ACC production and the ethylene evolution decreases without delay. Again, after some time the ACC synthase activity responds to the lowered ethylene level with an enhanced ACC and ethylene production yielding the second broader maximum in Fig. 2. Thereafter, the slow decay of ethylene production (typical for the post-climacteric phase of a tomato) dominates the picture. Note that the second peak was observed after removing the calyx. Puncturing the stem-scar area also revealed the second peak (Fig. 4, curves II, III, IV), but after wounding the pericarp layer the existence of a second peak is inconclusive, as will be discussed below. It cannot be excluded as an explanation that the rapid increase in ethylene production during the first rise comes from pre-existing ACC that is converted to ethylene by an increased activity of ACC oxidase in response to wounding (for apple, see Bufler, 1986). As for the second peak, an increased activity of ACC synthase induced by wounding will enhance ACC levels which may result in high rates of ethylene production.

The statement "stress acts locally" (Ketsa, 1985; Hyodo et al., 1989; Abeles et al., 1992) should be replaced by "stress varies locally" (Figs. 3 and 4); monitoring the local wound effect reveals a negligible increase in ethylene production at the pericarp site and a substantial effect on the stem-scar site after removal of the calyx.

The extent of the reduction in ethylene concentration immediately after the first peak due to wounding, is quite different for curves I–IV (Fig. 4) from the various actions such as removal of the calyx (*a*), 10-fold penetration of the stem-scar (*b*), and 10-fold penetration of the pericarp layer (*c*). In several cases the minimum level is even lower as compared to the ethylene concentration just before the action of wounding [curve I (action *a*) and curve II (actions *a* and *b*)]. This means that the autocatalytic effect of increased ethylene levels is dependent on the particular tomato. In Fig. 4 the action of wounding the pericarp layer (action *c*; curves II and III) twice resulted in decreasing ethylene emission rates, while in Fig. 4, curves I and IV, action *c* caused a small increase, due to a so far unknown reason.

Comparing our results with those of Hoffman and Yang (1982) and Smith et al. (1986), a strong increase in ethylene production is not observed in our experiments after wounding the pericarp layer, probably due to the more subtle way of wounding the tissue; slicing and incubating is a much more drastical measure than making a small cut or a few fine holes locally through the skin. Wounding the stem-scar region either by removal the calyx or, after its removal by making small holes with a needle, yields higher ethylene evolution than does wounding an area of the epidermis and pericarp as big as the stem-scar. Our suggestion is that the stem-scar area is a highly sensitive tissue as far as ethylene production is concerned, in contrast to the pericarp

layer. The stem-scar also serves as main avenue for ethylene and CO_2 exchange (Burg and Burg, 1965; Cameron and Yang, 1982; De Vries et al., 1995). More than 90% of the total ethylene is released through the stem-scar area. This indicates that the surrounding tissue to the stem-scar is well capable of synthesizing ethylene, possibly due to its open structure allowing oxygen to be available for the conversion of ACC to ethylene.

We have tested the theory that the elevated ethylene levels are really due to wounding, i.e. damage leads to increased production and is not due to accumulated ethylene which diffuses out of the tissue through surface cuts (Fig. 5). With the help of the continuous flow system and the technique of exchanging aerobic for anoxic conditions, we were able to block the conversion of ACC into ethylene. Thus elevated levels, detected under anoxic conditions, could only be caused by accumulated ethylene concentrations already present in the fruit before wounding. These levels were not observed (Fig. 5). The ethylene peaks in Figs. 1 to 4 are unequivocally due to wound effects, and that returning the tissue to aerobic conditions directly results in renewed ethylene production (De Vries et al., 1995). A similar conclusion was indirectly drawn by Saltveit and Dilley (1978) after wounding etiolated pea stems. It was observed that removal of endogenous ethylene by repeated evacuation and flushing of tissue sections with ethylene-free air did not significantly affect the timing or rate of wound-ethylene synthesis. Confirmation of the absence of accumulated ethylene was supported by the observed time lag of 26 min before wound-ethylene becomes detectable. The burst of ethylene production — observed after transferring the wounded tissue from anaerobic to aerobic conditions — indicate that ACC formed and accumulated under anaerobiosis is rapidly converted to ethylene by ACC oxidase; oxygen is required for this conversion (De Vries et al., 1995).

The laser-based PTD and PAD systems are able to detect low ethylene concentrations for the investigation of small and temporary effects of mechanical damage. The first system (PTD) permitted us to measure concentrations down to 0.5 nl l^{-1}, locally, in a normal atmosphere. Its time response amounts to 0.1 sec. The second apparatus coupled to a flow-through system yields an even higher sensitivity of 6 pl l^{-1} with a time response of 4 min (flow at 1 l h^{-1}). These systems allow us to increase our knowledge about the physiological response in recovery from wounding (subsequent increase-decrease-increase in ethylene production) for specific parts of fruit or intact organs.

5. Summary

The laser-driven PTD and photoacoustic setups permit us to observe in detail the effect of wounding on cherry tomatoes. Three main results are:

(1) a double peak structure in ethylene production, after wounding the stem-scar area;

(2) a higher ethylene production at the stem-scar site as compared to the pericarp site, for comparable wounding conditions;

(3) no observable ethylene accumulation in the tissue which is mechanically wounded.

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