Ethylene Production and Bruise Injury in Apple[1]

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Abstract. Ethylene production decreased linearly in 'Golden Delicious' apples given from 1 to 8 uniform bruises while CO_2 evolution was generally high in bruised apples. Primary regions of ethylene production and O_2 utilization were located under the skin and in the core.

In apple, a bruise can be considered to be cell injury and flesh browning from force application (8). Notwithstanding the economic significance of bruising there have been relatively few physiological studies on the subject. Experimental techniques to bruise fruit include dropping (9, 10) and pressure application by the thumb tip (8) or compression and impact testing apparatus (7). Studies involving various fruits have demonstrated increased CO_2 evolution after bruising (10, 12, 13, 14). The increase in O_2 utilization as a result of bruising was greatly exceeded by the increase in CO_2 output. The common field observation that "wormy" apples are ripened prematurely led us to speculate that bruise injury might be associated with increased level of ethylene production. The present study was initiated to explore the relationship between ethylene production and bruising, hopefully to develop an assay to evaluate bruising injury.

Materials and Methods

Bruise-free 'Golden Delicious' fruits were utilized out of 1°C storage 6 weeks after harvest. The fruit was bruised immediately after removal from storage using the apparatus illustrated in Fig. 1. A 190g stainless steel weight with a contact surface area of 7 cm^2 was dropped 30 cm from an electromagnet through a plastic tube and onto an apple which rested on a cork ring. The ring distributed the force and prevented bruising on the lower side of the fruit. The tube standardized the distance from the magnet to the apple, and insured that the weight fell to provide a continuously uniform contact surface on the fruit. A fairly constant fruit surface contour was insured by obtaining a snug fit between the tube rim and the fruit surface. The weight bounced only slightly after impact.

A series of studies were made using several methods of obtaining internal gas samples including insertion of hypodermic needles directly into the core region, underwater evacuation under slight vacuum, and simple diffusion in sealed jars. The last method was superior and is reported here because it caused no injury and, assuming ready gas diffusion throughout the fruit, gave a representative reading for the whole apple. The experimental unit was a 4 liter jar containing 4 apples. Jars were sealed and held at 21°C. Five 1 ml samples were taken for ethylene determinations every 24 hr through small septa in the jar covers. Ethylene levels were determined by gas chromatography using flame ionization and an activated alumina column, and identified by retention time, co-chromatography with commercial ethylene, and removal from gas samples by a saturated mercuric perchlorate solution. Immediately following each ethylene sampling, the jars were opened and the apples were removed to a flow-through type infra-red CO_2 analyzer where they were allowed to equilibriate for 30 min before the CO_2 evolution rate was determined. They were then resealed, and the process was repeated every 24 hr.

Localization of CO_2 and ethylene production in fruits was accomplished by halving uniform, small apples (6 cm diam), taking a 12 mm diam plug through the center of each half, and subdividing each plug into 11 disks each 2.5 mm thick from the peel to the central core region. Ten apples were included in each experiment, with the adjacent halves of each apple cored and subdivided for either ethylene or CO_2 analysis. Disks were incubated in manometric or Erlenmeyer flasks containing 2 ml 0.0005 M phosphate buffer with 0.25 M sucrose, 0.001 M

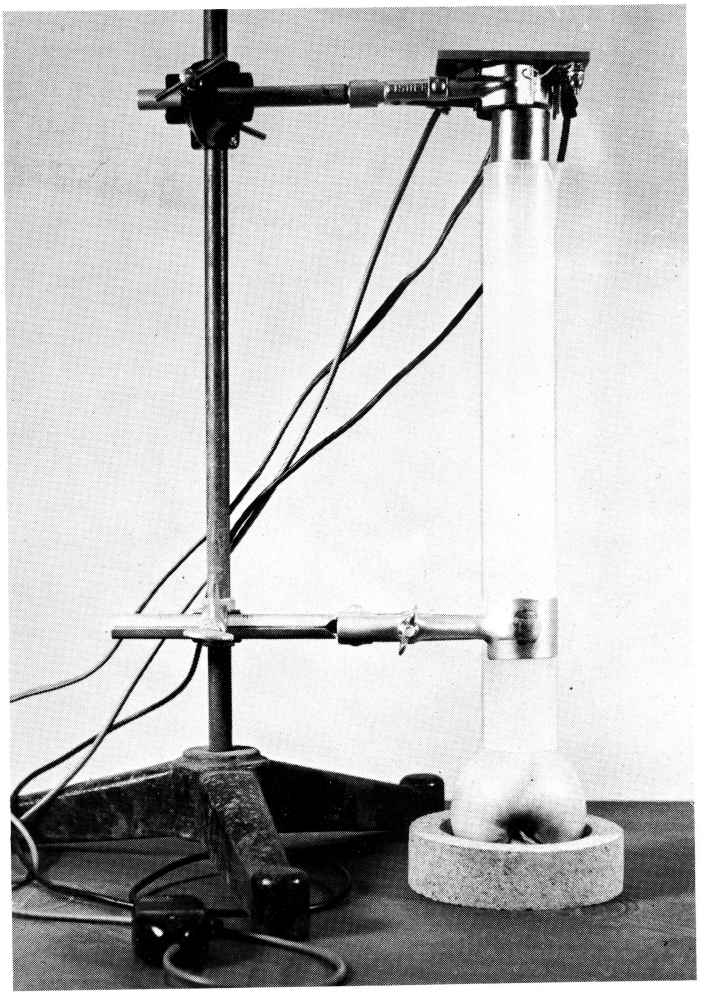

Fig. 1. The bruising apparatus consisted of an electromagnet, weight, and a plastic tube guide to insure uniform fruit surface contact area.

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$Ca(NO_3)_2$ and 0.0002 M $MgSO_4$ at pH 6.0. Approx 15 min elapsed from the initial cut to the placement into flasks in constant 30°C baths.

An indication of metabolic activity was obtained by measuring O_2 uptake with a Gilson respirometer. Flasks, incubated for 10 min at 30°C before the start of the run, contained 5N KOH in the center well and saturated mercuric perchlorate in the side arm to remove CO_2 and ethylene respectively. Maximum differences in O_2 uptake were noted after 30 min. Ethylene levels were determined as previously described after incubating sealed flasks for 90 min at 30°.

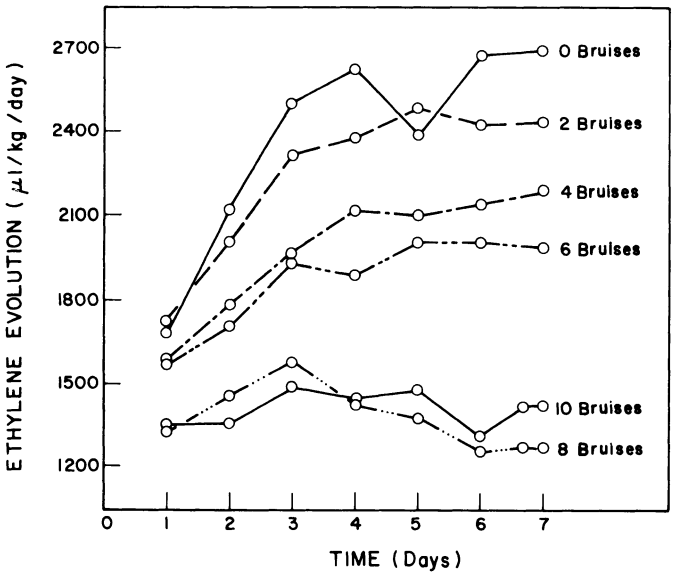

Fig. 2. Effect of a number of bruises on ethylene production from 'Golden Delicious' fruit. Ethylene diffused from the fruit into sealed jars, and was measured by gas chromatography.

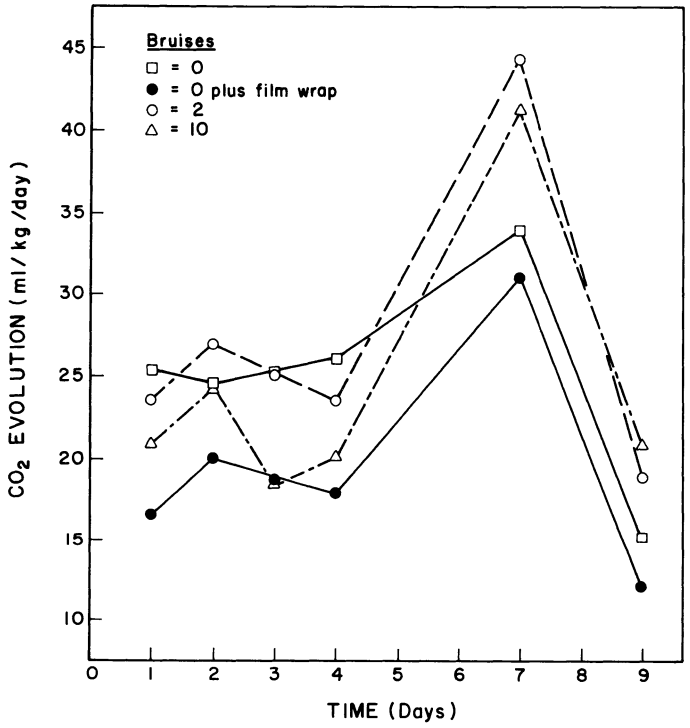

Fig. 3. Influence of bruising on fruit CO_2 production rates, as measured in a flow-through type infra-red CO_2 analyzer.

Results and Discussion

Fruit ethylene evolution decreased with bruising. The decrease was directly proportional to the number of bruises up to 8 or approx ½ of the surface area of the apple (Fig. 2). We considered that bruising might block lenticels on the fruit, thereby decreasing diffusion of the gas to the outside. Sealing off half of the non-bruised fruit surface area with a film (Saran wrap), however, failed to affect ethylene levels. There was no significant effect of bruising on CO_2 production rates (Fig. 3), also suggesting that the decrease in ethylene evolution by bruising was not a diffusion effect.

Consecutive disks, taken from the peel to the center of the fruit, were used to localize areas of highest metabolic and ethylene production activity. The outermost disk, including the peel, and the innermost disk from the central core region, showed the highest rates for both O_2 uptake and ethylene evolution (Fig. 4). This suggested that areas of highest metabolic activity in the fruit are responsible for most of the ethylene production. Browning reactions appeared to occur at equal rates in disks from peel to core suggesting that differences in rates of O_2 uptake could be due to differences in respiration rates and not oxidative browning reactions. The high rate of physiological activity in the outermost disk correlates well with anatomical structure. The outermost disk includes the epidermis and hypodermis, regions comprising approx 10 layers of small,

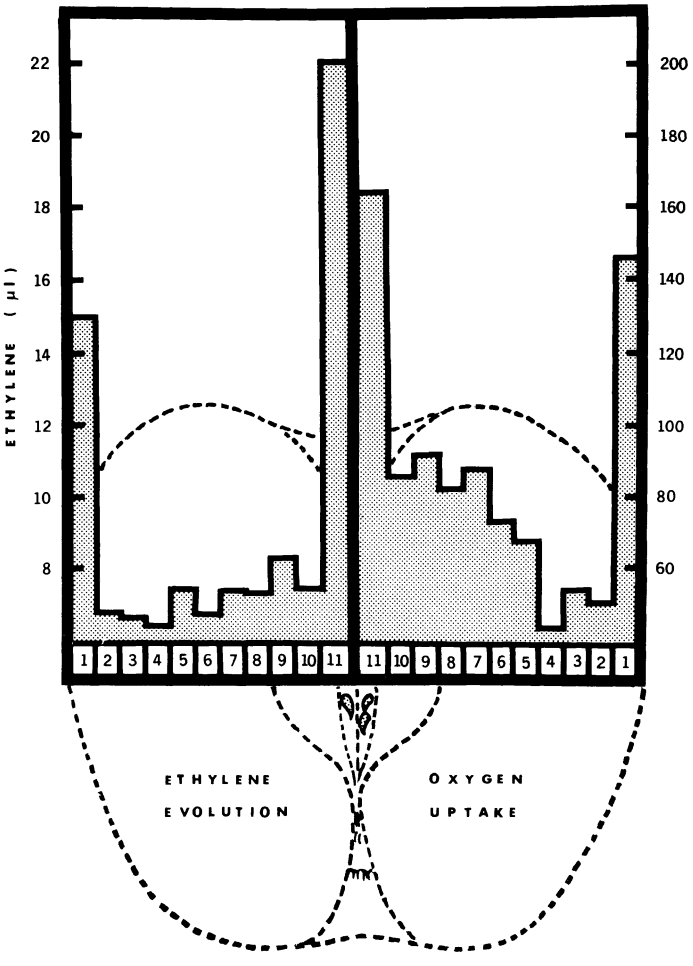

Fig. 4. Ethylene evaluation and O_2 uptake from successive disks of 'Golden Delicious' taken from the edge to the core of the fruit.

regular cells which differ from the large, irregular cells of the cortex.

Bruising may decrease ethylene evolution in apple fruits by crushing the cells included in the region responsible for a large percentage of the ethylene production. Masceration drastically reduced ethylene production by apple fruits (5). Crushing cells may also increase the activity of oxidative enzymes which might lower the O_2 concn beneath the unbroken skin enough to depress ethylene synthesis (18). Since interior apple tissues produce considerable ethylene, it was expected that bruising could only partially reduce ethylene evolution. However, 8 evenly spaced bruises covering about half of the fruit surface area have maximum reduction in ethylene evolution that could be obtained by bruising. It is possible that bruising affected areas peripheral to those actually contacted. The effect of bruising may not be necessarily localized. In the tomato a partial or complete loss of phosphorylative capacity upon bruising was found to be dependent on the severity of bruising and on the time elapsed after bruising, and complete loss of phosphorylative capacity occurred in a few hours, even though the bruised area was restricted to 1 part of the fruit (16).

If a high respiratory rate was responsible for the high rate of O_2 uptake in the outermost layer, then a decrease in fruit CO_2 production should occur after that layer is destroyed by bruising. That CO_2 production was unaffected or actually increased after 7 days (Fig. 3) suggests that CO_2 production may not be a good indication of normal respiration rate after bruising. The CO_2 from other sources might compensate for decreased respiration. In cherries, much of the increase in CO_2 output after bruising resulted from the decarboxylation of malate (15).

Our results were contrary to expectations that wounding increases ethylene production. It appears that different types of wound, i.e. slicing vs. masceration may give rise to different physiological responses. Fruit slices react differently than intact fruit. Slicing often increases respiration rate[3,4], but the response of apple tissue is variable and influenced by size, morphology, cultivar, and maturity of the fruit, and by the permeability of the peel to gases (1, 2, 3). The effect of slicing on ethylene production also differs with species. Slicing causes increased production of ethylene and other volatiles from fruit tissue of pear (4) and tomato (5), and ethylene production in apple slices or plugs was reported to remain unchanged (5) or decrease in proportion to the size of the cut surface (6). Masceration, however, caused a considerable decline in ethylene evolution in both apple and tomato fruit (5).

We conclude that bruising decreases ethylene production in apple by destroying the production region under the skin.

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