Effects of Wounding on Respiration and Ethylene Production by Cantaloupe Fruit Tissue [1, 2]

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Despite the difficulty of evaluating the effects of handling and wounding, tissue slices offer advantages for physiological or biochemical tests where small quantities of uniform tissues are required. This paper provides information on behavior of tissue slices from cantaloupe fruits, including the changes induced by wounding. Respiration, ethylene production, and the effect of applied ethylene on respiration were measured, and the responses of tissue slices from cantaloupe fruits of various chronological ages were compared with the responses of matched intact fruits.

It is well-known that wounding of plant tissue increases the rate of respiration (1, 5, 16), and may induce chemical changes and meristematic activity in the region of the wound (2, 3). Even shaking or dropping can cause an increase in respiration in avocado and citrus fruits (1, 9). Much information has been accumulated on changes in tissue cut from tubers and tuberous roots (12, 13, 15), but information on cut or wounded tissue from fruits is limited. The response of fruit tissues to wounding is variable and is influenced by variety and maturity (1, 10, 19, 20, 24). Other sources of variability are fruit size, morphology, and the permeability of the peel to gases. According to various authors (1, 10, 22), increased CO_2 evolution soon after cutting may indicate improved ventilation of the organ, rather than a direct effect of the wound on the cells involved. At least part of the burst of CO_2, produced by apples immediately after cutting, may be eliminated by pre-evacuation of the tissue (5). Studies by Burg and Thimann (7) and Burg (5) emphasized the importance of the time elapsed after cutting in interpreting the effects of wounding. Cutting or wounding generally causes increased production of ethylene and other volatiles by fruit tissue (11, 18). However, although Burg and Thimann (7) found an increased respiratory rate in tissues cut from postclimacteric apples, the rate of ethylene production was the same in plugs of tissue as in whole fruits, and the production by slices was appreciably lower. Release of existing ethylene from tissues may account for most of any increased production apparent just after cutting (5).

When apple tissue was soaked in water or hypotonic solutions, Burg and Thimann (7) found that ethylene production was depressed, but the respiration rate was not affected; hypertonic solutions prevented the inhibition of ethylene production. Even during short periods of immersion, apple tissue loses appreciable amounts of fresh and dry weight (7, 10).

Only avocado, banana, and tomato fruit tissues have been reported as stored for more than 2 days (1, 4, 5,). Avocado and banana tissue portions showed normal ripening changes when stored in moist air, but such were not reported for tomato tissue. Infection by microorganisms may be a factor in the increased respiration and ethylene production rates shown by cut tissue. While *Penicillium digitatum* is the only microorganism known to produce relatively large amounts of ethylene (26), Williamson (25) has found that the growth of disease organisms may stimulate ethylene production by several plant materials; this response, which he attributed to injury, occurred as long as the infected tissue was alive.

The following factors must be considered in the planning and interpretation of studies on ethylene production and respiration rate when fruit tissue slices are used: possible detrimental effects from washing or from immersion of the tissue in water, the length of time between cutting the tissue and the measurement of respiration and ethylene production rates, potential contamination from microorganisms, and the state of fruit maturity or ripeness.

Materials and Methods

The source and handling of the cantaloupe fruits (*Cucumis melo* L., var. reticulatis Naud., cv. Powdery Mildew Resistant No. 45) have been described elsewhere (16, 17). Respiration jars and equipment required for the handling and preparation of tissue slices were sterilized before each experiment, either by autoclaving or by rinsing with 70% ethanol. All operations associated with the preparation and handling of the tissue slices were conducted in a sterile transfer room to minimize contamination. Before cutting, the fruits were surface-sterilized by a 2-minute dip in 70% ethanol, followed by brief rinses in sterile distilled water and in a 10% hypochlorite solution (Clorox). These procedures were effective in

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preventing fungal contamination of the slices from the flesh but were not always effective for slices from the rind.

Since no dividing line is discernible between the true pericarp and the extra-carpellary tissues which make up the fruit wall in melons, the wall was divided arbitrarily into 2 regions on the basis of gross morphology. The region which includes the epidermal tissue and the underlying prominent vascular bundles will be referred to as the rind, and the region internal to the vascular bundles (the edible portion of a ripe cantaloupe) will be referred to as flesh. These divisions correspond closely to Sinnott's (23) designations as outer wall and inner wall, respectively.

Tissue slices were prepared by hand. The melons first were sliced in the equatorial plane into discs about 1.5 cm thick. The discs from the middle half to two-thirds of each melon were retained for slicing, and the remainder were discarded. Radial sections of tissue about 1.5 cm wide were then cut from the discs. The sections were freed of placental tissue, and the flesh portions were sliced in a plane tangential to the epidermis. Hand slicers designed to give slices 2, 4, and 6 mm in thickness were used. These thicknesses were chosen for preparing flesh slices containing approximately equal numbers of cell layers from melons harvested at 10 or 11, 15 to 22, and 30 days after anthesis, respectively. Tissue slices from different age fruits were, therefore, approximately comparable in the proportion of injured cells in each slice (13). The tissue that remained, after the flesh slices were cut from the radial sections, comprised the rind slices.

The slices were threaded onto 16-gauge stainless steel skewers; porcelain beads (4.7 mm × 6.3 mm) were used to space the slices, and a small piece of sterilized cork was used to retain the slices and beads in place on each skewer. Up to 12 loaded skewers were suspended on racks designed to fit into half-gallon, screw-top respiration jars, giving between 50 and 150 g of fresh tissue per jar. Wads of sterile cotton were inserted in the inlet and outlet tubes, provided for ventilation of the jars, to prevent entry of air-borne fungal spores and insects. Duplicate jars of slices were prepared, containing composites of tissue obtained from 3 melons of the same age. The respiration jars were held at 20° and were ventilated with humidified air at a rate sufficient to hold the effluent CO_2 concentration close to 0.5%.

CO_2 was measured by the colorimetric method of Claypool and Keefer (8). Ethylene production was measured by flame ionization gas chromatography (14). Ethylene treatments were administered by the continuous flow method of Pratt et al. (21), and the concentrations applied were monitored by the gas chromatograph.

Results

The results of 2 experiments are reported. In the first experiment, rind and flesh slices were compared as to respiration rate and the response to ethylene. The second experiment compared flesh slices with whole fruits with regard to respiration rate and ethylene production.

Comparison of Rind and Flesh Slices. Melons, ages 11, 15, and 22 days after anthesis, were sampled on 1 August 1961. Two jars each of rind and flesh slices were prepared during the afternoon of the same day, and initial respiration readings were made about 6 hours after cutting the slices. Ethylene (100 ppm) was applied continuously to 1 jar of each pair, beginning about 48 hours after harvest (fig. 1).

Respiration rates of the slices generally decreased after cutting; the decrease in respiration in flesh slices was greater than in rind slices and continued longer. Within 6 days after harvest, untreated rind and flesh slices showed a very large rise in respiration rate. The magnitude of the rise was greater in rind slices than in flesh slices. The ethylene treatment took effect more rapidly in slices from 22-day fruits than in those from the younger melons. While some increase in respiration rate was induced by ethylene, its main effect was to advance the onset of the rapid rise in respiration and the breakdown of the slices by 1 or 2 days, as compared to the untreated slices.

At the time of onset of the rapid rise in respiration, a large number of the slices became translucent and flooded with exudate. Microbial infection was seen at this time. During the first 2 days after cutting, the odor of the slices was pleasant and fruity, but it became unpleasant soon after the onset of the rapid rise in respiration. Traces of yellowing were seen in flesh slices treated with ethylene, and yellowing was marked in the rind slices at about the beginning of the respiration rise, especially in those treated with ethylene. If the breakdown of slices was not too severe, a decline in respiration rate was observed following the peak.

It was concluded from this experiment that flesh slices were more satisfactory for study; the observed differences between rind and flesh slices may have been inherent, but also could have been caused by injury to the rind during surface sterilization. Some water-soaked areas were observed in the rind of the youngest melons following the sterilization procedure, but this effect generally disappeared during the first day after cutting the slices.

Comparison of Flesh Slices with Whole Fruits. In this experiment, ethylene production and the effects of applied ethylene on the respiration rates of flesh slices and whole fruits were compared. Twelve fruits each, of ages 15, 20, and 30 days after anthesis, were obtained from a late planting in 1961. The experiment was done in 2 parts: the 15- and 20-day fruits were harvested on 6 September, and the 30-day fruits on 17 September. An initial respiration determination was made on each fruit 24 hours after harvest. On the afternoon of the second day, 6 of the fruits were sliced, and paired composited samples of the slices were taken, as previously described. Initial respiration and ethylene production determinations on the slices were made about 6 hours after cutting. Ethylene (100 ppm) was applied continuously to 1 jar

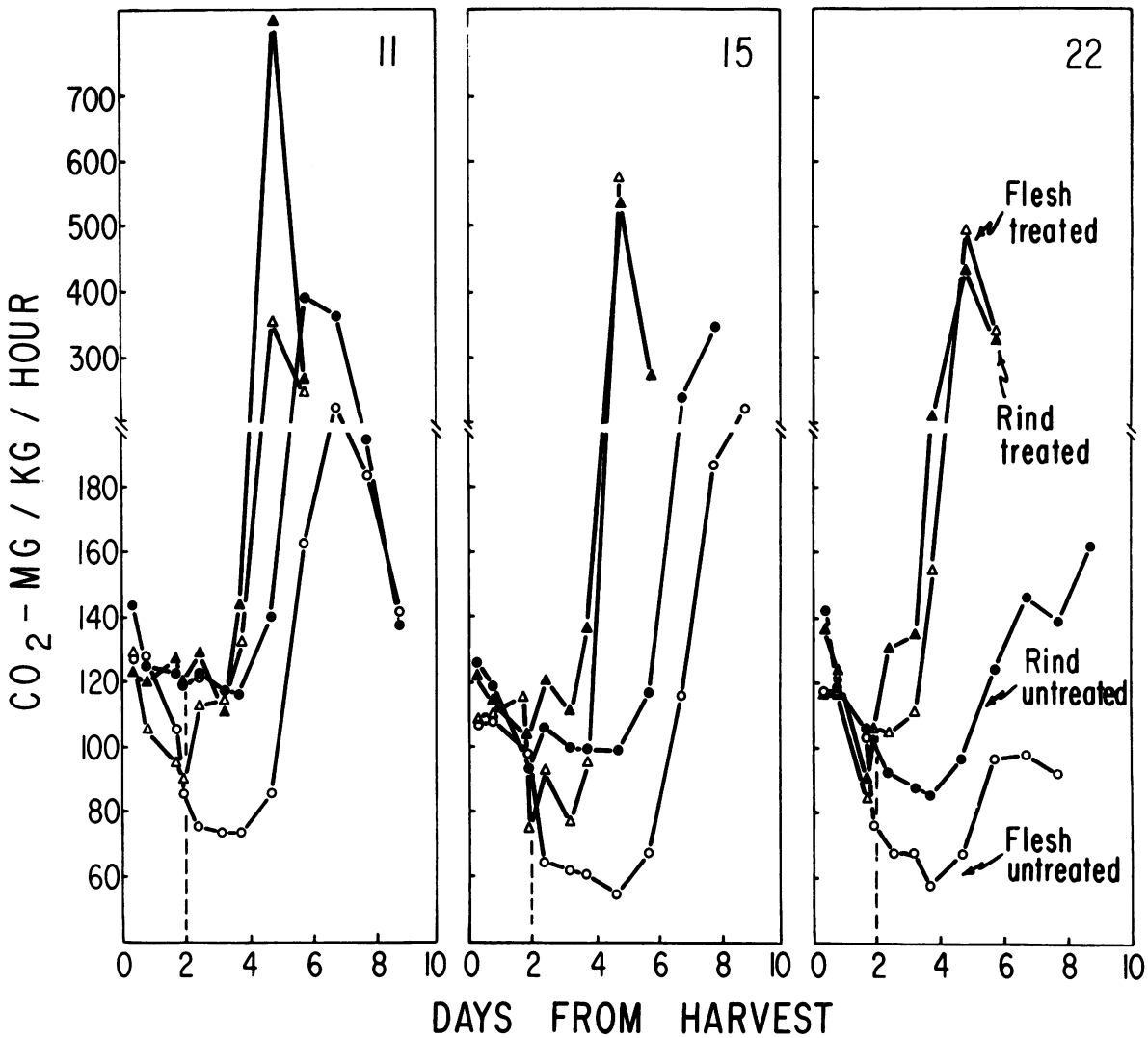

FIG. 1. Rate of CO_2 production (per kg original fresh weight) by duplicate samples of rind and flesh slices cut from cantaloupe fruits of 3 ages (11, 15, and 22 days after anthesis). Ethylene (100 ppm) was applied continuously to 1 sample of slices of each age, beginning 48 hours after harvest (broken vertical lines). The legends shown for 22-day fruits apply to the corresponding symbols of all ages.

of slices and to 3 of the 6 whole fruits of each age, beginning 48 hours after harvest (fig. 2).

Respiration rates of the untreated and treated whole fruits showed the trends expected from the studies reported previously (17), but within 6 hours after cutting, the rates shown by the tissue slices were at least double those of the whole fruits. Respiration rates of slices from 15- and 20-day fruits declined subsequently, and then showed a rise, accompanied by tissue breakdown. Untreated slices from 20-day fruits survived a remarkably long time after cutting; in fact, they did not break down until over a week after breakdown of the untreated whole fruits. The respiration rate in these slices declined gradually to a rate similar to that shown by whole fruits. The reason for their unexpected longevity was not apparent; microscopic examination of unstained hand sections, from the soundest slices at the end of the experiment, revealed no evidence of wound healing. Ethylene treatment of slices from 15- and 20-day fruits induced changes similar to those described in the first experiment, but respiratory behavior of slices from 30-day fruits was very different from that of slices from the younger fruits. The respiration rate continued to increase after cutting, reaching a maximum on the third day after harvest, declining to a minimum on the sixth day, and finally again increasing as the tissue slices broke down. Ethylene treatment did not influence this pattern.

Striking differences in rates of ethylene production were found between tissue slices and whole fruits. Ethylene production by whole fruits remained at a very low level until the onset of the climacteric, and the maximum rates of production were comparable in

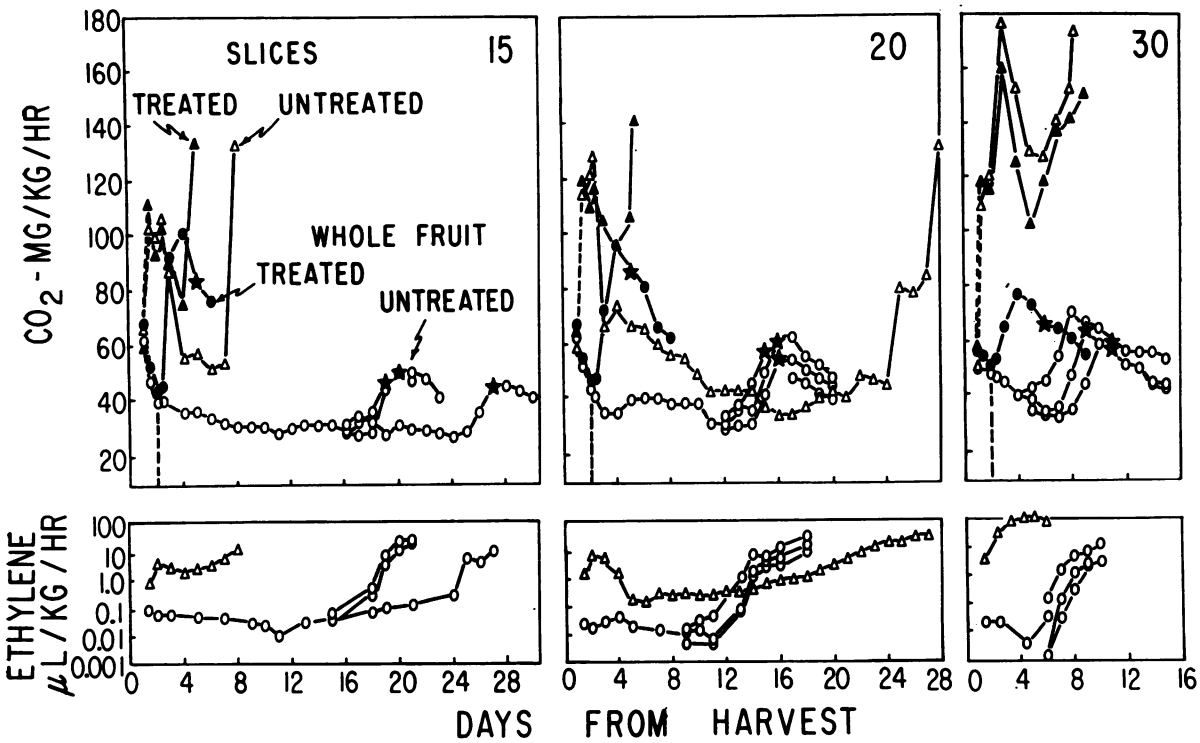

FIG. 2. CO_2 and ethylene production rates (per kg original fresh weight) of flesh slices and comparable intact fruits of 3 ages (15, 20, and 30 days after anthesis). The first respiration readings were taken before cutting, and the broken respiration line connects these readings with the first readings made on the cut tissue. The ethylene production rate is a log plot because of the wide range of values encountered. The start of the ethylene treatment is indicated by the broken vertical line. Each curve for whole fruits represents the average of 3 individuals; data for the untreated fruits were plotted individually after marked differences in respiration trends appeared. Stars indicate the beginning of yellowing of whole fruits. The legends shown for 15-day fruits apply to the corresponding symbols for all ages.

fruits of all 3 ages. The ethylene production rate of the tissue slices from 15-day fruits, 6 hours after cutting, was at least 10 times greater than that of whole fruits. This disparity was even greater for the 20-day and 30-day fruits. Ethylene production by slices from 15-day fruits increased to a maximum on the second day after harvest and remained at a level comparable to that attained by whole fruits at the climacteric. In slices from 20-day fruits, ethylene production attained a maximum 2 days after harvest, declined to a minimum on the sixth day, and then gradually increased during the remainder of the storage life. During the final rise in respiration, ethylene production reached about the same rate as that of whole fruits at or past the climacteric. Ethylene production by tissue slices from 30-day fruits increased after cutting to an approximately constant rate which was maintained even though the respiration rate was decreasing. The ethylene production rate by these slices exceeded the rate shown by untreated whole fruits at or past the climacteric peak.

Discussion

Measurements made within 6 hours showed that cutting cantaloupe fruits into tissue slices could increase the respiration rate by a factor of 2 and ethylene production by a factor of 10 or more. The rate of ethylene production by slices from 15-, 20-, and 30-day cantaloupe fruits approached or exceeded the rates reported by Lyons et al. (14) for intact ripe melons.

Although the preclimacteric cantaloupe fruit is naturally better ventilated (14) than the fruits studied by others, further improvement in ventilation may be a factor in the increased respiration rate of tissue slices. Although the temporarily increased rate of ethylene production (during the first hour), which has been observed by others after cutting fruits into segments (6, 18), has been attributed (5) to release of a relatively high content of ethylene already present in the fruit, the ethylene content of developing cantaloupe fruits (14) is much lower than that reported for apples in the other studies cited. A clear distinction between ventilation and wounding effects is not possible, but it seems likely that the high rate of ethylene production by cantaloupe tissue slices is, at least in part, a direct response to injury.

Tissue slices from 30-day fruits produced ethylene at a faster rate than did slices from the 15- and 20-day fruits. The higher rates in slices from the 30-day fruits were apparently great enough to cause the onset of a respiratory climacteric in the slices (fig. 2),

in spite of the reduction in their ability to retain ethylene. Similarly, the increase in endogenous ethylene in slices from younger fruits may have made the applied ethylene treatments relatively ineffective with respect to respiration rate, although the onset of tissue breakdown was hastened. The differences in respiratory patterns, ethylene production rates, and effects of applied ethylene shown by slices from cantaloupe fruits of different ages can be related to previously reported observations for intact fruits (17); the physiological changes leading to natural ripening, which appear to be initiated about 20 days after anthesis, must make possible the greater response to endogenous ethylene shown by slices from 30-day fruit. On the other hand, ability to produce ethylene existed even in the youngest tissue, and wounding apparently overcame whatever factor limits ethylene production in the intact fruits.

Summary

Tissue slices were cut from cantaloupe fruits (*Cucumis melo* L., var. reticulatis Naud.) harvested at various stages of development. The fruit wall was separated into rind and flesh on the basis of gross morphology. Differences in behavior between rind and flesh slices were observed, but these may be attributable to surface sterilization of the fruit prior to cutting.

Cutting caused an immediate increase in respiration of the flesh slices, compared to that of intact fruits. The rates subsequently declined until the onset of tissue breakdown, when respiration again increased. Ethylene production by tissue slices was at least 10 times that by intact fruits. Only slices from cantaloupe fruits harvested more than 20 days after anthesis showed a climacteric pattern of respiration.

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