Ethylene Production, Respiration, & Internal Gas Concentrations in Cantaloupe Fruits at Various Stages of Maturity

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In studying the relationship between ethylene production and the climacteric rise in respiration exhibited by ripening fruits, it was not possible to determine when ethylene production begins until the advent of gas chromatography. This technique makes available quantitative measurement of ethylene concentrations at or below the threshold of physiological activity. Burg and Thimann (3, 4) were the first to apply the method to studies of fruit ripening: ethylene was determined with an especially sensitive thermal conductivity detector (2). In recent work, Meigh et al. (11, 12), using the more sensitive flame-ionization detector, measured ethylene concentrations as low as a few parts per billion.

Gas chromatography was used in this study of cantaloupe fruits (Cucumis melo L. var. reticulatus) to assess the rate of ethylene and carbon dioxide evolution and concurrent internal concentrations of ethylene, carbon dioxide, and oxygen. For the ethylene determinations, a highly sensitive flame-ionization detector was used, and the other gases were analyzed with a thermal conductivity detector.

Results from two experiments are reported. In the first experiment, analyses were performed on the internal atmospheres of cantaloupes harvested at different stages of development, and in the second, changes in the rate of production of ethylene and carbon dioxide and in the composition of the internal atmosphere were followed in harvested mature melons as they progressed from the preclimacteric through the climacteric stages of ripening. While a few values have been reported for the respiratory rate of cantaloupes (13), these data are the first to present a pattern of respiration and establish clearly the presence of the climacteric in this fruit.

Experimental Material & Methods

Plant Material. The fruit growth period in the cantaloupe (Cucumis melo L., cultivar Powdery Mildew Resistant No. 45) used in this study is clearly defined. It begins at anthesis and pollination, which occur on the same day (10), and ends with formation of an abscission layer at the point of attachment of the fruit to the pedicel. A well-defined stage in the development of the abscission layer, commonly referred to as "full slip" because the fruit will separate cleanly from the vine, occurs about 40 days after anthesis. Various degrees of slip may be described, depending on the extent of the development of the abscission layer, as shown by the nature of the break when the fruit is pulled from the plant (13). Growth studies on summer field-grown melons at Davis (McGlasson, unpublished data) have shown that the lengths of the growth periods of individual fruits within any one cycle of fruit set are very uniform, with estimated population standard deviations of less than ±1.8 days. Hence, chronological age provides a good basis for uniformity of sampling of fruit at different stages of development from plants grown under comparable environmental conditions.

Sample fruits were chosen from those tagged at anthesis or those obviously in the same setting cycle as tagged fruits. In this report, age and stage of development of fruits are designated in days from anthesis to harvest (e.g. 37-day fruit).

Gas Sampling. All samples for gas analyses were taken in tightly lubricated glass syringes fitted with long hypodermic needles (3 inch, 24 gauge). All analyses were made by gas chromatography. To determine carbon dioxide and ethylene production, the air stream from the fruit respiration jar was sampled, and for internal atmospheres, samples were drawn from the cavities of the melons. Simple precautions to eliminate diffusion errors allow gas samples to be handled in syringes with excellent accuracy: The sample drawn should be somewhat larger than that to be injected into the chromatograph: the syringe may be closed by pushing the needle into a soft rubber stopper, or the operator can slowly move the plunger to eject gas from the syringe as he moves toward the instrument, and the plunger is set at the final desired volume just before the needle is pushed into the injection port. It was not possible to withdraw gas samples by syringe directly from the 7-day melons: a modification of the mercury evacuation method of Ulrich and Marcellin (15) was used to obtain a sample for determining internal ethylene.

At the conclusion of the experiments, the melons were examined closely for evidence of decay at the sites of the needle punctures: none was observed. The storage life of fruits harvested at maturity (Experiment II, below) was terminated by decay, but it was randomly distributed on the fruits.

Ethylene Determinations. The gas chromatography used in this study was a Perkin-Elmer model 900 gas chromatograph equipped with a flame-ionization detector. A 3% Carbowax 20M column was used as the stationary phase supported on 80/100 mesh Chromosorb W. The column was 3 feet by 1/8 inch and was maintained at 140°C. Helium was used as the carrier gas at a flow rate of 30 ml/min. The detector was set at a sensitivity of 0.01, and the injection port was 250°C. The injection port was a standard gas chromatography FIA-601 stopper, and the needle used to inject the sample was a 26 gauge stainless steel needle. Samples were injected into the instrument with a gas tight syringe. All samples were injected at the same time, and the first sample was always placed in the injection port to avoid possible contamination from the previous sample. The injection port of the chromatograph was equipped with a synthetic rubber stopper. The gas tightness of the injection port was tested by using the stopper to seal the port and applying a slight vacuum to the injection port. If the port was gas tight, it would be necessary to apply negative pressure to the injection port to displace the gas. If the port was not gas tight, the gas would be drawn through the stopper by the negative pressure.

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graph with flame ionization detector used in this study (Loenco Model 15F, Loe Engineering Co., Pasadena, Cal.) was modified to allow amplification of the detector output by a vibrating reed electrometer (Cary Model 31, Applied Physics Corp., Monrovia, Cal.). The amplifier utilized a $10^{-2}$ ohm input resistor and was connected to a strip chart recorder with a 10-millivolt range. The design of the flow system provides for mixing the nitrogen carrier gas and the hydrogen just prior to entering the burner jet; flow rates were 20 ml per minute for hydrogen and 60 ml per minute for nitrogen. This system makes a relatively large sample size possible; since the ratio of hydrogen to carrier gas or sample remains nearly constant at the burner tip, samples up to 20 ml can be injected without extinguishing the flame. Air to support combustion was supplied to the burner chamber at a rate of 44.5 liters per hour. The 18-inch analytical column was ¼ inch O.D. copper tubing packed with 110/120 mesh aluminum oxide. It was activated by passage of a slow flow of dry air during 4 hours heating in a 200 C oven. The chromatograph had no separate temperature controls but was operated in a constant temperature room at 20 C. With the above conditions it was possible to measure $4 \times 10^{-5}$ ml of ethylene in a 1.0 ml sample. Incidentally, by decreasing the ratio of carrier to combustion gas and decreasing the combustion air flow it has been possible with this instrument to detect qualitatively $3 \times 10^{-6}$ ml in a 1.0 ml sample.

**Oxygen & Carbon Dioxide Determination.** A standard gas chromatograph with a filament-type thermal conductivity detector (Aerograph Model A-90-C, Wilkins Instrument & Research Co., Walnut Creek, Cal.) was converted to two-column, two-detector operation, as described by Luh and Chaudry (9) and Vosti et al. (16). An injection port and a silica gel column were installed between the carrier gas supply and the reference side of the detector, and a polarity reversing switch was introduced into the recorder circuit. Helium was used as carrier gas at a flow of 50 ml per minute, the filament current was 270 milliamps, and the system was operated in a constant temperature room at 20 C.

In this scheme, the gas sample is introduced into the silica gel column (6 inches of Davison SA, 30-60 mesh, in ¼ inch O.D. copper tubing); this separates carbon dioxide from air and gives a peak using what is normally the reference side of the detector as the sample side. The sample side still contains only carrier gas and serves as the reference. The gases then pass into a molecular sieve column (10 feet of Linde 13X, 10-30 mesh, in ¼ inch O.D. copper tubing); it irreversibly absorbs carbon dioxide and separates nitrogen and oxygen into separate peaks. (Argon, normally 0.93 % in air, is not separated from oxygen under these conditions, and a suitable correction must be applied to the oxygen peak.) The molecular sieve column is followed by 10 feet of empty ¼ inch copper tubing, providing a time delay so that the latter peaks will follow the carbon dioxide peak on the chromatogram. The gases then enter the sample side of the detector. Meanwhile the reference side has been flushed with pure carrier gas and returns to its normal function. The recorder reversing switch is operated after completion of the carbon dioxide peak, maintaining all deflections in the same direction and allowing use of the full chart width. The complete analysis for carbon dioxide, oxygen, and nitrogen can be completed in 5 minutes for each sample.

**Results**

**Experiment I.** In the first experiment, seven lots of fruit (7, 14, 23, 30, 37, 40, & 41 days after anthesis) were harvested at mid-morning on 26 August 1960. Each sample consisted of six fruits except the 40- and 41-day samples which consisted of four and two fruits, respectively. The 37-, 40-, and 41-day fruits corresponded to the following physiological stages: preclimacteric (half slip), climacteric peak (just full slip), and 1 day post-climacteric (full slip).

The melons were placed first at 20 C, because this temperature was close to the pulp temperature at time of harvest; effects due to change of temperature were minimized, and the internal atmosphere could be analyzed reliably the same afternoon. Internal levels of ethylene were low, so after the first gas sampling, the fruits were transferred to 30 C. This temperature approximates the daily mean pulp temperature of cantaloupes in the field at Davis and is reported to be about optimal for ethylene production by apple and tomato fruits (3, 5). After holding 24 hours at 30 C, the internal atmospheres were again analyzed. The 7-day melons were sampled only after holding at 30 C. During the 24-hour period at 30 C, the 37-, 40-, and 41-day melons were enclosed in respiration jars through which air passed at approximately 18 liters per hour. This air flow maintained the carbon dioxide concentration at about 0.5 % and permitted measurement of production rates of both carbon dioxide and ethylene just before sampling the internal atmospheres.

Internal gas concentrations of cantaloupes at six stages of maturity are shown in figures 1 and 2. Ethylene is produced by the fruits, even at a very early stage of development, at both temperatures. Because the values of internal ethylene concentration in the immature fruits, though measurable, are too small for the scale of the graphs, they are reported in table I. The data in both figures show the same general trend, with a fairly steady low level of ethylene concentration in 14- to 30-day fruits, followed by a rapid increase near the onset of the ripening process. Between the 30-day and 37-day stages of fruit development, the ethylene concentration increased about tenfold, and between 37-day and 40-day fruits it increased a hundredfold. The latter period corresponds to the rise between the preclimacteric minimum and the climacteric peak of carbon dioxide production. Internal oxygen steadily decreased as fruit maturity advanced, concomitant with an increase in carbon dioxide concentration.
rate of evolution is evident for both carbon dioxide and ethylene, with the same marked increase in ethylene production between the 37- and 40-day fruit stages.

**Experiment II.** Substantially mature cantaloupes can be obtained at a preclimacteric stage by harvesting at quarter to three-quarter slip. For the second experiment, six such melons were harvested at mid-morning on 31 August 1960 and placed at 20°C in respiration jars ventilated with air at approximately ten liters per hour. Measurement of the rates of production of ethylene and carbon dioxide and of the internal content of ethylene, carbon dioxide, and oxygen began 11 hours after harvest; measurements were repeated twice daily for the first 3 days and then daily.

Because the internal atmosphere of the 7-day fruits was sampled by the evacuation technique, these results (Table 1) are not comparable with the others. This technique probably removes ethylene from solution in the tissues, giving a higher value than would be obtained by syringe sampling of the gas phase. However, it is worth noting that ethylene is present internally at a very early stage of development.

In figure 3 is shown the rate of evolution of carbon dioxide and ethylene by 37-, 40-, and 41-day fruits after holding at 30°C for 24 hours (just before the corresponding samples of the internal atmosphere were taken). From a comparison of figures 2 and 3, a correlation between ethylene evolution and the rate of evolution of carbon dioxide is noted.

### Table 1

<table>
<thead>
<tr>
<th>Days from anthesis</th>
<th>Shortly after harvest 20°C</th>
<th>Held for 24 hr 30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0.04 ± 0.01</td>
<td>0.4 ± 0.1***</td>
</tr>
<tr>
<td>14</td>
<td>0.04 ± 0.01</td>
<td>0.1 ± 0.07</td>
</tr>
<tr>
<td>23</td>
<td>0.04 ± 0.01</td>
<td>0.07 ± 0.02</td>
</tr>
<tr>
<td>30</td>
<td>0.05 ± 0.01</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>37</td>
<td>0.3 ± 0.5</td>
<td>2.0 ± 0.8</td>
</tr>
</tbody>
</table>

* The values tabulated are those of the younger cantaloupe fruits of figures 1 and 2.
** The estimate of the standard deviation of the population is shown for each value (n = 6).
*** The gas sample was extracted from the 7-day fruit by a method not comparable with that used for the older fruit (see text)
until termination of the experiment by fruit spoilage. The fruits were removed from their containers just long enough to take the internal atmosphere samples.

Of the six fruits used in the experiment, one fruit had entered the climacteric at the time of the first sampling, and a second fruit decayed prematurely. The remaining four fruits showed typical and complete climacteric patterns of respiration (fig 4). The changes in external production of carbon dioxide were correlated with changes in internal concentrations of oxygen and carbon dioxide. Internal oxygen concentrations fell from about 17.5% to about 13.5% at the climacteric peak and then rose again gradually as the respiratory rate fell during the post-climacteric phase. Internal carbon dioxide concentrations rose from a pre-climacteric minimum of about 4.5% to a maximum of about 10%, and then gradually decreased as the respiratory rate declined.

Changes in evolution and in internal concentration of ethylene appeared to follow about the same time course as the changes in oxygen and carbon dioxide. Maximum ethylene production was reached at the climacteric and then fell slightly in the post-climacteric phase until the onset of decay. Rates of production of ethylene and the maximum internal concentrations reached, varied somewhat more among

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**Fig. 4.** Rate of ethylene and carbon dioxide production and internal concentration of ethylene, carbon dioxide, and oxygen, in individual cantaloupe fruits harvested mature but pre-climacteric and held to ripen at 20°C. Note that the respective ordinate scales apply across both pairs of graphs.
fruits than did the values for carbon dioxide. While the rate of carbon dioxide production approximately doubled during the climacteric, the rate of ethylene production increased 60- to 160-fold. Data on the concentration ratios of ethylene and carbon dioxide, between the internal and ambient atmospheres, are shown in table II.

**Discussion & Conclusions**

The study reported here demonstrates the application of gas chromatography for measuring ethylene evolved and the naturally occurring intercellular concentrations of ethylene, carbon dioxide, and oxygen in single intact fruits. Fruits such as the cantaloupe, which have a distinct internal cavity except at very young growth stages, are especially suitable for study, since small gas samples can be taken easily by hypodermic syringe. It is interesting to note the similarity in the changes in the internal concentrations of carbon dioxide and oxygen found in cantaloupe fruits (figs 1, 2, & 4) and those reported earlier by Wardlaw and Leonard (17) for the papaya. They withdrew successive 10 ml gas samples from the fruit, through a glass tube inserted into the seed cavity, for analysis with a Haldane apparatus.

Our data show that the cantaloupe has the climacteric respiratory pattern expected in most fleshy fruits as they ripen, and that ethylene is present in immature cantaloupe fruits, two observations which have not been previously recorded. From studies on the ethylene treatment of 30-day cantaloupes (McGlasson, unpublished data) it is suggested that the minimum externally applied concentration of ethylene required to accelerate the ripening process is between 0.1 and 1.0 ppm at 20 C. Hence, from the data presented here it is suggested that the intercellular concentration of ethylene only reaches a physiologically active level at the immediately pre-climacteric stage. The data in experiment II, based on readings made at intervals of about 12 hours, show that the increase in ethylene concentration to the physiological level coincides with or immediately precedes the rise in carbon dioxide production. This general relationship is in agreement with observations on other fruits (1, 8). Measurements made at very short time intervals, however, will be necessary to establish exactly when the increase in ethylene production occurs, relative to the rise in carbon dioxide production.

Another aspect of this problem is the comparison of ethylene concentrations within the tissue to those in the ambient atmosphere. In table II, ratios of internal to ambient concentration for the melons of figure 4 are given for both ethylene and carbon dioxide. The lower ratios for carbon dioxide reflect the higher ambient concentrations of carbon dioxide which were held close to 0.5% by adjustment of the air flow. Both ratios are quite constant once ripening is well started, and for three of the four fruits are very similar. The exception is the fruit (lower left) shown in figure 4 which also had higher internal ethylene concentration and production than the other fruits.

That the tissues retain a physiologically active concentration of ethylene against a gradient to the surrounding air is of considerable interest. Previous reports and conclusions on the physiology of ethylene in ripening fruits (1) have been based on measurements of ethylene evolved by the fruit, using methods which were not sufficiently sensitive at threshold concentrations for physiological activity. These data for cantaloupe show that evolution of ethylene does not provide an adequate test for possible physiologically active concentrations within the tissues. Since the values of table II refer to the central cavity versus the external atmosphere, the same ratios could not be expected at all levels in the tissues. The ratio could be expected to decrease toward the skin of the fruit. Perhaps this is related to the observation that ripening starts in the center of many fruits.

The higher concentration found by evacuation in the very immature melons, compared to that in gas samples withdrawn by hypodermic, may be significant if such methods are used to measure ethylene concentrations. It suggests the presence of other gases or a higher concentration not measured during withdrawal of gas samples. This is suggested by the lower ratio of ethylene to carbon dioxide found in the unpierced fruits.
tissues, with a higher total amount present in the fruit than is found by analysis of the gas phase only. It will be of interest to establish this comparison for melons at various stages of development and also to establish the amount of adsorbed ethylene required for physiological effect. There is, no doubt, an interaction, in ethylene treatments, between concentration and duration of treatment which could be related to the establishment of an effective adsorbed amount.

It is possible that accumulation of ethylene in lipid material of the cell may be the critical factor in relating concentration to physiological activity. Using olive or cotton seed oils as representative lipids, the oil/water solubility ratio for ethylene was approximately 14 to 1 at 37°C (6). This finding, although not directly applicable to the lipid phase in living plant cells, is meaningful when one considers the apparent location and role of lipids within plant cells. According to Sjöstrand (14), considerable indirect evidence suggests that cell membranes consist of lipoproteins forming a double layer of lipid molecules sandwiched between two layers of protein molecules. Thus the plasma membranes, mitochondrial membranes, nuclear membrane, endoplasmic reticulum, etc., would be rich in lipid material which could be expected to dissolve or adsorb ethylene. An analogy can be found in studies on toxicity of hydrocarbon oils employed as herbicides. Currier and Peoples (7) found that differences in physical properties of the hydrocarbon oils as related to toxicity could be correlated with the distribution coefficients between oil/air and to a lesser extent to the oil/water or water/air values. They proposed that it is the lipid/air distribution that determines equilibrium concentrations in the ectoplast. These ideas offer an attractive approach in the search for an understanding of the role of ethylene in fruit ripening and are in keeping with the notion which has been expressed from time to time that ethylene effects membrane permeability. Studies are being continued with developing cantaloupe fruits to determine possible relationships between fruit respiration and ethylene-related phenomena, including rate of ethylene production, intracellular concentration, and possible membrane effects, to further elucidate the processes which precede the climacteric.

**Summary**

The compositions of the internal atmospheres (carbon dioxide, oxygen, & ethylene) of cantaloupe fruits harvested at different stages of development were measured with sensitive gas chromatographs. Traces of ethylene appeared in melons harvested as young as 7 days from anthesis. In studies of harvested mature melons, a climacteric rise in respiration was demonstrated during ripening. Coinciding with, or immediately preceding, this rise in carbon dioxide evolution was a great increase in both internal and evolved ethylene, a decrease in internal oxygen, and an increase in internal carbon dioxide. A high but relatively constant ratio of gas concentrations between the central cavity of the fruit and the ambient atmosphere was observed for both ethylene and carbon dioxide, showing that a physiologically active ethylene concentration may exist in tissues, while the apparent rate of production is almost too low to detect. The role of ethylene remains obscure, but its concentration appears to be closely related to the ripening process in cantaloupes (*Cucumis melo* L. var. reticulatus, cultivar Powdery Mildew Resistant 45).

**Literature Cited**