

Changes in Ethylene and CO₂ During the Ripening of Apples

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(Manuscript received 26 March 1973 and accepted 19 April 1973)

The internal concentrations and production of both ethylene and CO₂ were measured during the maturation and ripening of Cox's Orange Pippin apples. Within 12 hours of the onset of the respiration climacteric there was no measurable increase in the production of ethylene and the data indicate that the increase in ethylene production is synchronous with the increased CO₂ production that marks the start of ripening. It therefore appears that a factor other than a change in the rate of ethylene production determines the time at which apples commence ripening.

1. Introduction

Since the first demonstration of the efficacy of ethylene in artificially ripening fruits¹ and of its production by fruits that are already ripening,² this gas has been considered to play a hormonal role in the initiation of fruit ripening. The demonstration of increased production of ethylene before the onset of the respiration climacteric, which is crucial to such a view,³ has awaited the development of techniques sufficiently sensitive to measure the very low levels of ethylene present in unripe fruits.⁴ Using such techniques, Pratt and Goeschl⁵ have demonstrated such a sequence during the ripening of "Honey Dew" melons. They summarised previous work on other fruits by postulating three patterns of behaviour. In fruits such as the melon, the levels of ethylene in the fruit rise before the onset of the respiratory climacteric.^{4,6,7} In some other fruits, triggering levels of ethylene may be present some time before ripening but the response to ethylene appears to be inhibited until after the fruit is harvested,^{7–10} or until the fruit matures to a state where it is sensitive to its endogenous ethylene content.^{7,11}

Although the term "climacteric" was first applied to the respiration of the ripening apple,¹² the ethylene and CO₂ content of this fruit during the period immediately preceding ripening has not yet been studied in detail. Using several experimental approaches we have made such a study with Cox's Orange Pippin apples. The role of ethylene in initiating the ripening of apples is discussed.

2. Experimental

2.1. Materials

Cox's Orange Pippin Apples were grown on Malling IX stock at the Norfolk School of Horticulture, Burlingham, Norfolk. The petal fall date was noted and we were able

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to predict the probable date of the onset of the climacteric and ripening by comparison with previous seasons.

2.2. Methods

For measurements of their respiration and ethylene production, fruits were harvested from the tree and placed in ventilated respiration chambers at 12 °C. Constant flow rates were obtained by maintaining a constant pressure head with a barostat¹³ and inserting a capillary between this pressure head and the respiration chamber; in our experiments lengths of 0.01 inch i.d. stainless steel capillary column cemented in glass tubes were found to be ideal. Relatively fast flow rates (3 to 6 l/h determined with a bubble flowmeter) were maintained in order to keep external CO₂ and ethylene as low as possible.

Ethylene and CO₂ concentration in the effluent air stream were measured by gas chromatography, using commercially available equipment (Pye gas chromatogram, Pye Unicam, Cambridge, England). The electrometer for ethylene determinations was that described by Meigh.¹⁴ With careful adjustment of flow rates to ensure minimum flame "noise" and maximum sensitivity, we were able to measure routinely the ethylene contamination in air (approx. 0.005 parts/million).

Internal atmospheres were measured by direct sampling⁴ while each apple was held under water. A syringe needle (flat ended No. 30 cannula) was pushed through the pericarp into the core. A stainless steel wire in the bore prevented the needle from plugging with tissue during this operation. The wire was removed and the needle was connected to a 10-ml glass syringe with a piece of soft "Tygon" tubing closed with a spring clip. The clip was opened and a sample of the internal atmosphere withdrawn from the apple with the syringe. The clip was closed before the barrel of the syringe was released to return the sample to atmospheric pressure. Routinely, 6 ml of internal atmosphere was obtained by this procedure and its gaseous composition determined by gas-liquid chromatography (g.l.c.).

Continuous determination of internal atmospheres was achieved by sealing a nylon tube (*ca.* 1/16 inch i.d.) into the apple core through the widest part of the pericarp with silicone grease. The nylon tube was conducted to a separate port on the respiration chamber, and samples could be taken independently of the measurements of respiration and ethylene production, without disturbing the apple. On each occasion, respiration and ethylene production were determined prior to analysis of the internal atmosphere, since depletion of internal gases would affect the diffusion rate to the flowing stream and hence the measurements of external production. This method is referred to later as the continuous sample method.

Most of the data on ethylene concentration and production are plotted logarithmically as suggested by Pratt and Goeschl⁵ to illuminate small changes at the low end of the scale.

3. Results

In a first series of experiments, apples were harvested from the tree and the internal ethylene and CO₂ were determined immediately and the orchard temperature noted.

The range of ethylene concentrations and the orchard temperatures are shown for each sampling date in Table 1. Wide variations in ethylene concentration on the tree even six weeks prior to the onset of the climacteric presumably relate to the wide variations in flesh temperature of the apples sampled and the large effect of such temperature variations on the rate of ethylene production by the fruit coupled with a relatively small effect on the rate of diffusion outwards of the ethylene produced.

TABLE 1. Changes in internal ethylene concentration of apples during maturation on the tree

Date (1972)	Mean internal ethylene concentration (parts/million)	Concentration range (parts/million)	Orchard temperature (°C)
13 Aug.	0.026	0.011–0.046	—
11 Sept.	0.086	0.04–0.120	—
14 Sept.	0.082	0.031–0.138	14.2
16 Sept.	0.035	0.006–0.020	13.4
20 Sept.	0.058	0.025–0.16	21.4
24 Sept.	0.041	0.025–0.058	16.6
27 Sept.	0.095	0.046–0.131	23.2
29 Sept.	0.098	0.024–0.25	16.2
2 Oct.	0.683	0.054–3.33	—
10 Oct.	2.32	0.097–10.8	13.8

Similar apples were taken from the tree and placed in a flowing air stream at 12 °C. After 24 h the respiration and ethylene production of the bulk sample and internal ethylene and CO₂ concentrations of individual apples were measured. The changes in respiration, ethylene production and internal ethylene concentration during maturation of the apples are shown in Figure 1. For at least six weeks prior to the onset of ripening on the tree (about 23 September) the internal ethylene concentration of equilibrated apples was constant with a mean value of 0.025 parts/million. In the sample taken on 24 September, the mean internal ethylene concentration had increased to 0.05 parts/million and in subsequent samples increased to a final figure of 6 parts/million—a 250-fold increase. When the ethylene concentration within the fruit had reached 0.05 parts/million, the respiration had also begun to increase but the production of ethylene, as measured externally, appeared to lag slightly behind the CO₂ production (respiration). Increases in internal ethylene concentration, respiration and external ethylene production therefore appear to be closely connected events.

The relationship between CO₂ and ethylene (production and internal concentration) was examined more precisely by studying individual apples passing through the climacteric in storage at constant temperature (12 °C). For this purpose, a large number of fruits was harvested at least 5 days prior to the onset of the climacteric on the tree and 24 of uniform weight were placed individually in respiration chambers. At intervals, the respiration and ethylene production of individual apples were measured and every day one apple was taken and its internal atmosphere analysed. The hole made during this operation was stopped with silicone grease and the apple was then replaced in the

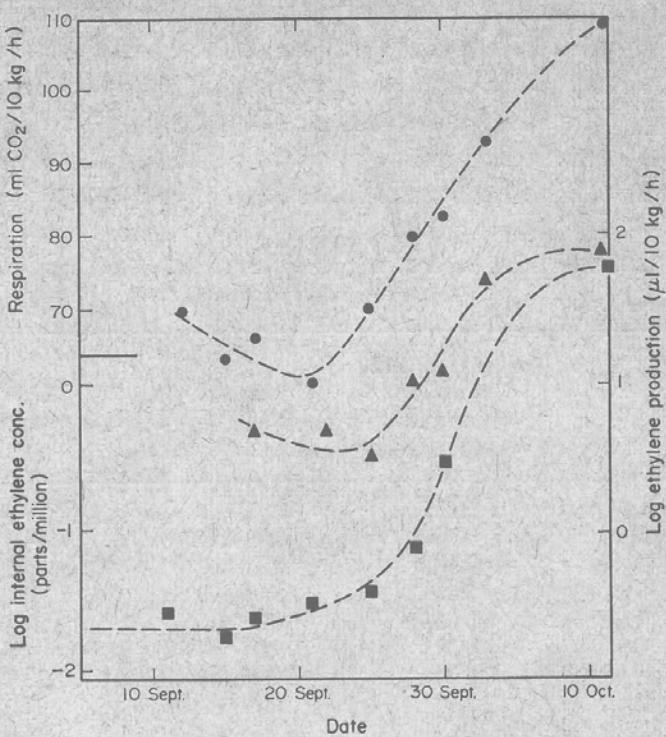


Figure 1. Respiration (●), ethylene production (▲) and internal ethylene concentration (■) of bulk samples of apples harvested at intervals during maturation and equilibrated at 12°C for 24 h.

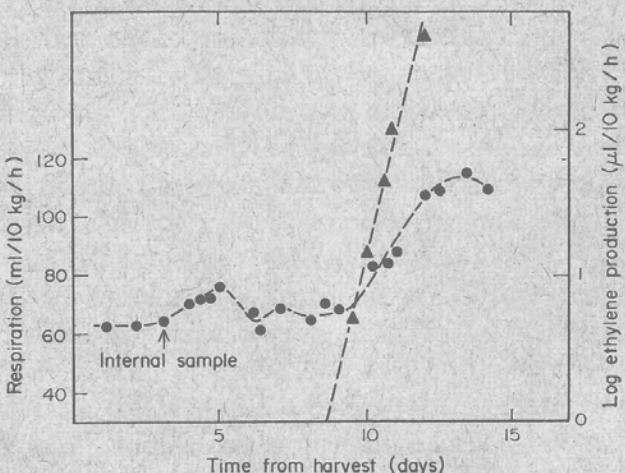


Figure 2. Typical pattern of respiration (●) and the log of the ethylene production (▲) of a pre-climacteric apple held at 12°C and sampled for internal atmosphere (↑) before the development of the respiratory climacteric.

jar. Further measurements of respiration and ethylene production were made until each apple had gone through its respiration climacteric.

The respiration and ethylene production of one such apple is shown in Figure 2. Following the insertion of the needle and the withdrawal of the sample, the respiration increased slightly, returning within three days to the control level and remaining there

until the apple commenced ripening some days later. This was typical of pre-climacteric fruit with no suggestion that the insertion of the needle started a continuous period of ethylene production. The sensitivity of our method for determining ethylene did not permit measurement of the very low amounts produced by pre-climacteric fruit, but the time of appearance of the first detectable amount of ethylene was very closely coincident with the start of the respiration climacteric (Figure 2).

The individual apples in this experiment ripened at random over a three-week period. This variation means that simple chronological plotting of the respiration and internal ethylene concentrations determined at daily intervals on individual apples would be meaningless. Valid comparisons might be made by reference to a standard physiological time in the ripening sequence of each fruit. Since ethylene production increases logarithmically for some time after ethylene is first detected (Figure 2), the time at which the plot of log of ethylene production against time intersects zero (equivalent to 1.0 $\mu\text{l}/10 \text{ kg/h}$) appeared to be a suitable reference point. The respiration and internal ethylene concentrations measured at the sampling time for each apple have been referred to this point. For the apple of Figure 2, for example, the time at which log ethylene production = zero was 8.7 days and the internal atmosphere was sampled at day 3 (arrow), which was therefore -5.7 days with respect to the reference point.

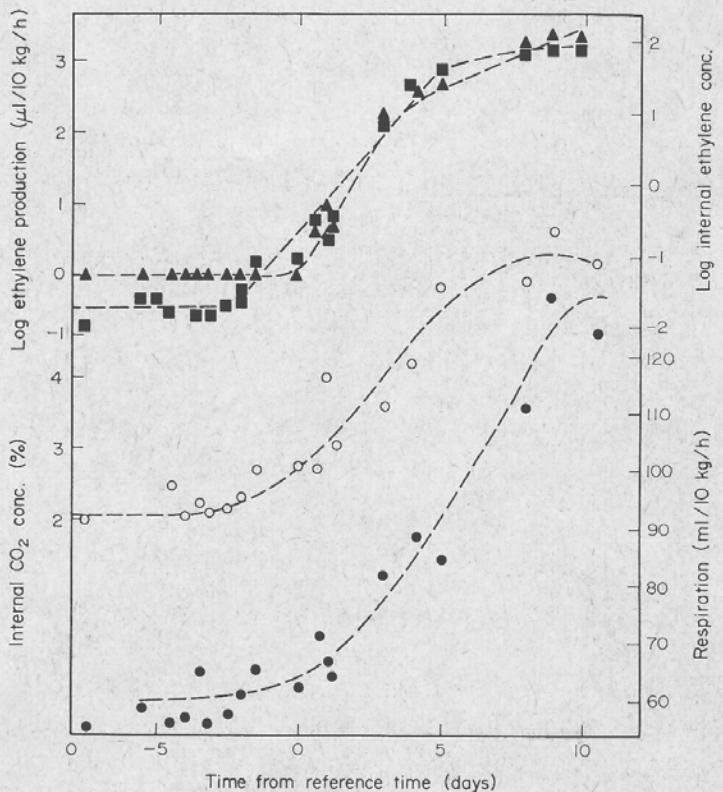


Figure 3. Composite graph showing the respiration (●), internal CO₂ concentration (○), ethylene production (▲) and internal ethylene concentration (■) of 24 apples harvested at one time, held in separate respiration chambers, and sampled individually over a period of three weeks. Data for each apple are referred (in time) to the date at which its ethylene production was 1 $\mu\text{l}/\text{kg/h}$ ($RT = 0$).

The ethylene production, internal ethylene concentration, respiration and internal CO_2 concentration at the sampling time are shown for all 24 apples as a function of the reference time scale (RT) in Figure 3. The internal concentration of ethylene and CO_2 remained constant until a few days before zero RT , after which both began to rise. The resolution of the method shows that the increases in ethylene and CO_2 occur within

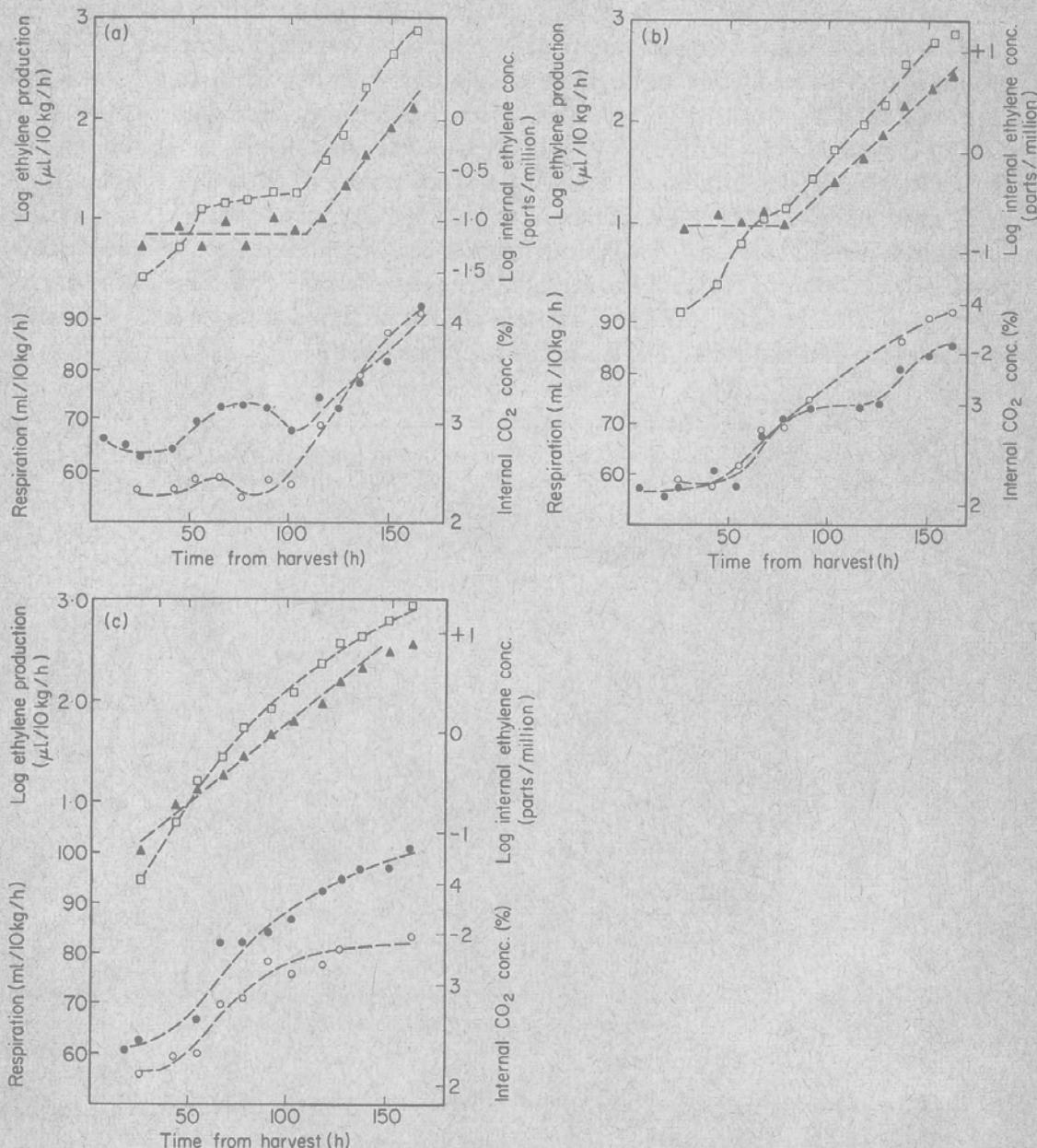


Figure 4. Typical patterns of respiration (●), internal CO_2 concentration (○), ethylene production (▲) and internal ethylene concentration (■) of individual apples held at 12°C and sampled by the "continuous sampling" procedure. (a) Preclimacteric apple, (b) apple entering the climacteric, (c) climacteric apple.

less than 12 h of each other. Accumulation of ethylene and CO₂ within the tissue occurs well before there is a measurable rise in ethylene or CO₂ production ($RT = 0$) as measured externally.

To investigate further the relationship between respiration and ethylene production, a number of apples were harvested immediately before the development of the climacteric on the tree. Respiration, ethylene production, internal ethylene concentration and internal CO₂ concentration were monitored through the climacteric using the continuous sampling method. Changes with time in these quantities are shown for three different apples in Figure 4. In apple (a) the respiration climacteric did not commence for 110 h from harvest and the shoulder previously noted in respiration rate due to sampling damage was correlated with an earlier rise in internal CO₂ and ethylene concentrations. The subsequent rise in the internal concentration of ethylene and CO₂ is clearly the development of the climacteric. In apple (b), which was more mature, the shoulder in CO₂ and ethylene concentrations caused by insertion of the nylon tube merged into the development of the climacteric. Finally, in apple (c) which was commencing ripening at the start of the experiment, the internal gas concentrations were increasing very rapidly from the first sample. As in the previous experiments, it was not possible, for any of the apples monitored, to distinguish between the time at which the ethylene concentration and the CO₂ concentration commenced rising. The frequency of sampling was such that the two gases must have started their increased evolution within 12 h of each other.

4. Discussion

Our results illustrate the difficulties that may be encountered in determining the precise sequence of events at the initiation of ripening of fruits. In the melon,⁵ the high level of ethylene required to trigger ripening (3 parts/million), the uniformity of the fruits with respect to the date of ripening and their insensitivity to repeated internal sampling, enabled Pratt and Goeschl to obtain convincing evidence of a gradual rise in ethylene content of the tissue for 10 days prior to the onset of the respiration climacteric. In contrast, the apple has constant low levels (*ca.* 0.02 parts/million) of ethylene in its tissue to within hours of the start of the climacteric; individual apples, apparently uniform, may ripen at times separated by as much as three weeks; and apples show marked respiration responses to internal sampling procedures. Demonstration of the gas exchange behaviour during initiation of ripening in such fruits requires careful elimination of artifacts of technique. Meigh, Jones and Hulme¹⁵ demonstrated a rise in the production of ethylene by apple fruits up to 1 day before the onset of the respiration climacteric. Our data on the range of ripening dates for individual apples suggest that their observation probably reflects one early-ripening apple triggering the onset of the respiration climacteric of all other apples in their bulk samples. Likewise, experiments where the respiratory rise has been shown to occur some time after a rise in internal ethylene concentration⁹ must be re-examined in the light of the "lag" between rising internal CO₂ and increased external CO₂ production that has been shown in our experiments. While none of the techniques used in the present study is entirely free

from criticism, we have adopted several experimental approaches to eliminate any bias introduced by use of a single technique.

Within the limits of resolution of our methods (*ca.* 12 h), the ethylene content of apples increases coincidentally with the onset of the climacteric as evidenced by increased internal concentration of CO₂. The lag-phase for the action of ethylene in fruits and other plant organs is such^{16,17} that one can conclude that the onset of ripening in apples is not induced merely by prior increase in the production of ethylene. Kidd and West¹⁸ suggested that the climacteric was stimulated by ethylene "... either by the result of (i) a fall in the threshold value for stimulation, (ii) a rise in the rate of production of ethylene, (iii) a factor or factors influencing the escape from the fruit of this gas or (iv) a combination of these factors". Our data permit us to choose the first of these postulated alternatives as the most likely means by which ethylene stimulation occurs. Increased ethylene production accompanying the climacteric may therefore be merely one of the "symptoms" of ripening, along with many other profound metabolic changes.¹⁹ It is the ethylene *already present* at the pre-climacteric minimum which is involved in the initiation of ripening.

Such an hypothesis would require that it is the balance between ethylene and some other "factor" which controls the time of the initiation of ripening. It is already clear that, in fact, other factors do participate in the induction of ripening: avocados will not ripen unless taken from the tree, although their ethylene content is sufficiently high to initiate ripening while attached to the tree; apples have been shown to have variable sensitivity to ethylene as they mature—an immature apple requires much more ethylene to induce ripening than one at full maturity;¹⁷ we have shown that the levels of ethylene present in fruits on the tree may be many times the pre-climacteric level at 12°C depending on the flesh temperatures—weeks before the start of ripening; apples detached from the tree at maturity ripen more rapidly than fruit left on the tree. These observations argue for the presence of a substance translocated from the tree which prevents the action of ethylene in immature fruits. In young fruits this compound would be present in high amount and as the fruits mature the amount would decrease. In the case of the avocado, which never ripens on the tree, this factor would always be present in amounts sufficient to prevent ripening; in the apple, which is very sensitive to ethylene at maturity, fruits would ripen immediately the level of this other compound falls below a level sufficient to prevent the action of ethylene.

Thus, although ethylene is undoubtedly the hormone which initiates fruit ripening, its action appears to be controlled by another factor, the elucidation of the nature of which will further advance our understanding of the control of the *time* of ripening.

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