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## FRUIT RESPIRATION AND ETHYLENE PRODUCTION<sup>1</sup>

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The primary objective of this study was to determine the causal relationship between ethylene production and the onset of the climacteric rise in respiration. The marked rise in oxygen uptake and carbon dioxide output, known to fruit physiologists as the "climacteric" rise, is a characteristic phenomenon of the ripening process. It marks a transition phase between development and the onset of functional breakdown, between ontogeny and senescence. The physiological and biochemical changes associated with the climacteric were reviewed recently by Biale (3), and the role of ethylene in fruit storage was summarized by Porritt (18).

One of the intriguing problems in fruit ripening is the question of whether ethylene induces the rise in respiration or is a product of this rise. In practically all cases ethylene was found to accelerate the onset of the climacteric if applied before the rise. Responses from external applications of ethylene are by themselves insufficient evidence that the naturally occurring climacteric is a result of the ethylene produced by the fruit. The information available on the relationship between ethylene production and respiration is limited and conflicting. Nelson (16) found that the sharp rise in ethylene production of McIntosh apples followed the rise in CO<sub>2</sub> evolution. In the case of bananas Nelson (15) observed an inverse relationship between ethylene and CO<sub>2</sub> evolution. His samples of both apples and bananas were on the climacteric rise at the start of the experiments. Hansen (8), working with pears, observed that the maxima in both processes occurred at the same time, but the relative increases calculated as the ratio of maximum rate to initial rate were much lower for respiration than for ethylene production. His data point to a close parallelism between the onset of the rise in CO<sub>2</sub> and in ethylene. A similar behavior was obtained by Pratt and Biale (19) for avocados. These authors employed a biological assay which at best is semi-quantitative. Since the initial values for ethylene production are intrinsic to this problem, a reliable and specific quantitative method is essential. The manometric technique developed in this laboratory (21) made it possible to obtain quantitative data on the avocado and to extend information on ethylene production to other fruits about which no

data were available. This report includes also, for the first time, evidence for the occurrence of the climacteric in several tropical and subtropical fruits. For purposes of comparison we included studies on a few of the temperate zone fruits, although the more extensive investigations were concerned with fruits from tropical and subtropical regions.

### MATERIALS AND METHODS

The fruit samples of the avocado (*Persea* sp.), Valencia orange (*Citrus sinensis*), sapote (*Casimiroa edulis*), and feijoa (*Feijoa sellowiana*) were obtained from the departmental orchard and placed under experimentation immediately after harvesting. The Navel oranges (*Citrus sinensis*), lemons (*Citrus limon*), and persimmons (*Diospyros kaki*) were received directly from a packing house. The cherimoyas (*Annona cherimola*) were grown in a commercial orchard in San Diego County and used within two days after picking. The mangos (*Mangifera indica*) were shipped from Florida by air express and were placed in the constant temperature chamber within two to three days from harvest time.<sup>2</sup> The bananas (*Musa sapientum*) originated in Central America, where they were picked "¾ full," shipped by refrigeration, and arrived in California in a fully green condition. The state of maturity of the pineapple (*Ananas comosus*) from Cuba and of the papaya (*Carica papaya*) fruit from the Hawaiian Islands was not as satisfactory as that of the other fruits. The results obtained with pineapple and papaya indicate only whether ethylene is produced in the ripe fruit, but they throw no light on the main objective of this study. The temperate zone fruits were either procured on the wholesale market or shipped from the University Farm at Davis.<sup>3</sup>

The methods employed consisted of measuring respiration by carbon dioxide evolution, and ethylene production by the manometric technique. A stream of air was passed at a constant rate through a flowmeter, over a jar of fruit, and then into a cylinder

<sup>2</sup> We wish to express our thanks to Miss Margaret Mustard of the University of Miami for procuring and shipping the mangos used in these investigations.

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containing alkali for CO<sub>2</sub> absorption, or into an absorber with mercuric perchlorate for ethylene absorption. Respiration measurements were made over a period of two to four hours each day, while ethylene was absorbed for a period of 20 to 22 hours. The ethylene method was developed in this laboratory and has recently been reported in full (21). Briefly it is based on the fact that ethylene forms a complex with mercuric perchlorate. The accumulated ethylene is released from the complex by the addition of hydrochloric acid, and its volume is measured manometrically. With this method 0.2 ml of ethylene can be determined with an accuracy of 5%. This value is equivalent to 0.33 ppm of ethylene in an air stream of 200 ml/min for a period of 50 hours. It is possible to measure smaller concentrations by using longer periods of time or by determining the gas evolved to a lower degree of accuracy.

### RESULTS

**THE AVOCADO, *Persea americana* × *Persea drymifolia*:** The Fuerte variety used in these studies is considered a cross between a Mexican and a Guatemalan race of avocado. Its season of maturation is relatively long. Fruits developed from the January to April bloom attain their maximum fat content of 15 to 25% (on fresh weight basis) in March of the following year. By that time sugars have declined to a value of 0.25 to 0.50% and the protein content has reached 2.0 to 2.5% of the fresh weight. The avocado is, then, a characteristically high fat, high protein, and low carbohydrate fruit.

In southern California coastal districts, fruits of the Fuerte variety may be harvested from October to August, though the commercial harvesting season is normally over by June. Fruit softening will not take place while the fruit is attached to the tree and while the stem is healthy. However, immediately after harvesting the processes leading to senescence are set into action and the typical climacteric course of respiration ensues, as shown in figure 1 for fruit placed at 20°C, and in figure 2 for fruit at 15°C. Late season Fuerte avocados reach the climacteric peak sooner and exhibit a sharper rise than early season fruit, but the actual values for CO<sub>2</sub> production at the peak remain approximately constant throughout the season and are a function of the storage temperature and of the atmospheric composition of the storage environment. At 20°C the rate of ethylene production follows closely the rate of respiration during the course of the climacteric, but no measurable quantities of ethylene were detected prior to the onset of the rise in CO<sub>2</sub> output. After the climacteric peak the rate of ethylene evolution decreased more markedly than that of CO<sub>2</sub> production. At 5°C no pronounced climacteric rise was observed and no significant ethylene values were determined. It should be noted that upon transfer from 5°C to 20°C after 34 and after 43 days, there was a rise in CO<sub>2</sub> output with no corresponding rise in ethylene evolution. This difference in the response of the CO<sub>2</sub> and of the ethylene reactions might serve as a basis for a new approach to the widely perplexing

problem of the effects of relatively low temperatures on the ripening processes in fruits and vegetables. It is known that prolonged exposure of a number of avocado varieties to 5°C or lower will cause physiological disorders. Discoloration of the flesh and the skin occurs after removal to higher temperatures and frequently normal fruit softening is inhibited. Apparently the suppression of the measurable climacteric rise at 5°C is responsible for the observed symptoms. However, one cannot exclude the possibility that certain physiological changes do occur at 5°C which are similar to those determined at higher temperatures. Pratt and Biale (19) reported the production of an active emanation by Fuerte avocados after exposure to 5°C for 57 days. They identified this emanation as ethylene by the triple response of pea seedlings. The concentration evolved was approximately 0.5 ppm. They obtained no pea response during the first eight weeks at 5°C, as was the case in this study.

The experiments with avocados at 5°C and at 20°C brought out several important points on the relation of ethylene to respiration, but failed to show whether ethylene induced the rise or was a result of it. The changes at 20°C were too rapid to show the causal relationship. We thought then that the responses at 15°C might be sufficiently slow to allow measurements in the initial stages of the rise. The results of one of the studies at 15°C are presented in figure 2.

It is evident from this figure that the rise in ethylene evolution started about the same time as the rise in CO<sub>2</sub> production. The ratios of peak to initial values are much higher for ethylene than for CO<sub>2</sub>. The rate of decrease after the peak is also considerably greater for ethylene than for respiration. While the actual magnitudes are lower at 15°C than at 20°C, the general behavior was essentially the same under those two conditions. The evidence from avocados neither supported nor refuted the idea of an ethylene induced climacteric. We turned our attention, therefore, to other tropical fruits.

**THE BANANA, *Musa sapientum*:** The banana, unlike the avocado, is a high carbohydrate, low fat fruit. Changes occurring in bananas during ripening were described in a recently published monograph by von Loesecke (11). In the course of fruit ripening, starch decreases from about 20 to 1% and sugars increase from about 1 to 18%. The nitrogen content in the edible portion of the ripe banana amounts to about 0.2% of the fresh weight. Applied ethylene accelerates the chemical changes associated with ripening and causes a shift in the time axis of the onset of the climacteric rise. The question before us was whether the normally occurring ripening changes are brought about by ethylene produced by the fruit. A qualitative identification of ethylene in the gaseous emanations of bananas was reported by Niederl et al (17). We made a concurrent quantitative study of ethylene production and CO<sub>2</sub> evolution at 15°, 20° and 25°C. The results at 20°C are shown in figure 3.

The first significant value in ethylene evolution was observed on the fifth day and it corresponded with

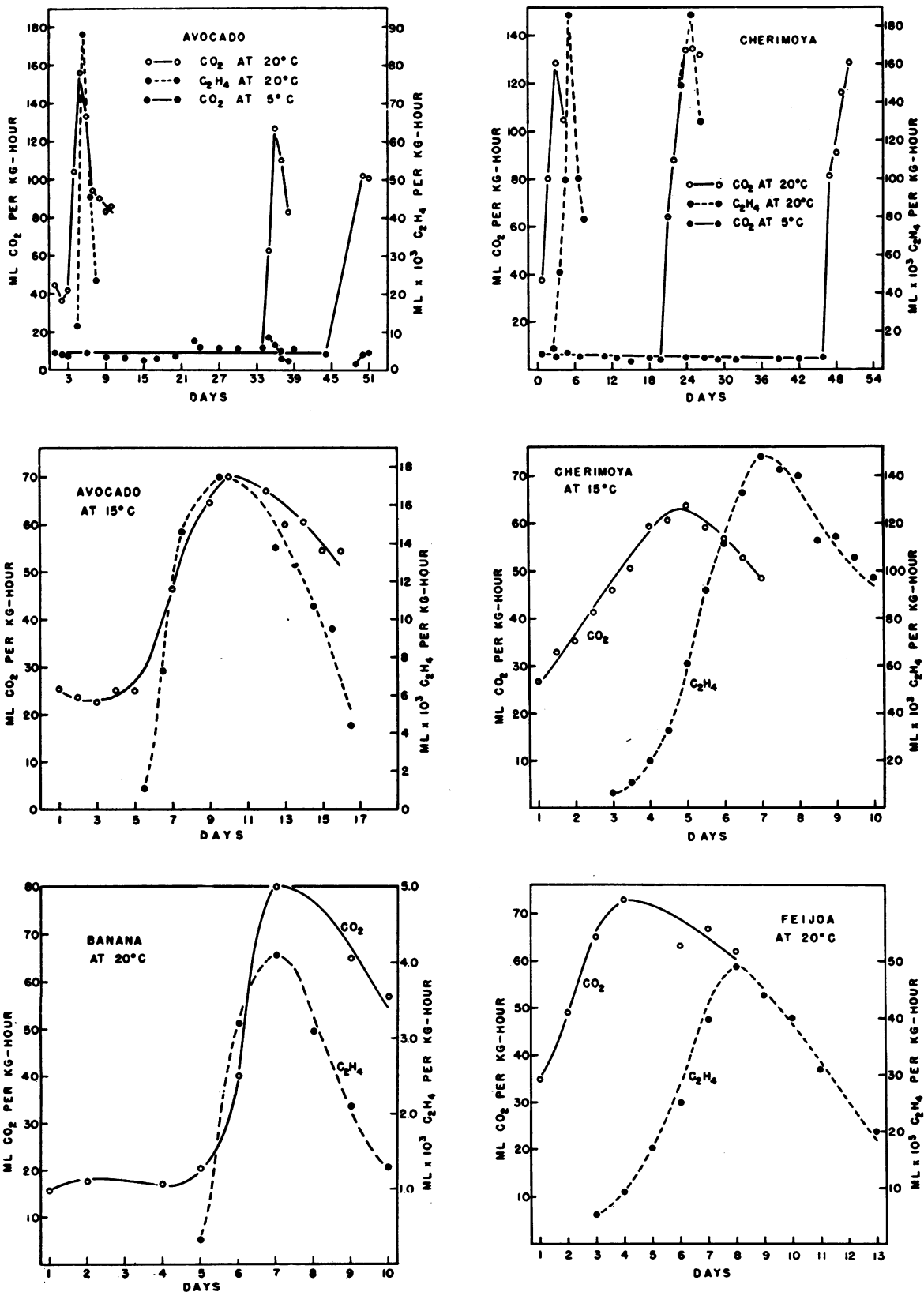


Fig. 1 to 6. Fruit respiration and ethylene production (solid line CO<sub>2</sub>, broken line C<sub>2</sub>H<sub>4</sub>). Fig. 1, 2—Avocado. Fig. 3—Banana. Fig. 4, 5—Chirimoya. Fig. 6—Feijoa.

the onset of the respiratory rise. While a similar curve was obtained for 15°C, the ethylene values were too low to be conclusive. Less than two  $\mu$ l of ethylene were produced by one kg of bananas for one hour during the peak of activity at 15°C. At 25°C the rate was four times higher than at 15°C, but the relationship between ethylene production and respiration was essentially the same as at 20°C. We have seen, therefore, that in the banana as in the avocado no clear conclusion can be drawn as to whether ethylene production precedes or follows the onset of the respiratory rise. We proceeded to extend our survey to fruits of lesser economic importance than those discussed up to this point.

**THE CHERIMOYA, *Annona cherimola*:** The cherimoya is considered to have originated in the highlands of tropical South America. It is grown on a very limited scale commercially. In describing this fruit Chandler (6) states that "at its best the custard-like flesh has a pleasant blend of sweetness and mild acidity combined with flavors suggestive of the pineapple or the nectarine, but a richness all its own." The ripe fruit is high in sugars (18% on fresh weight), high in proteins (1.8%), and low in acid content (0.06%). The Booth variety used for this study varied in size from 300 to 400 gm per fruit. The course of respiration and of ethylene production at 20°C and at 5°C is shown in figure 4.

The sharp rise in ethylene production associated with the climacteric at 20°C suggests a general behavior similar to the avocado and the banana. There appears to be, however, an important difference. The onset of the rise in ethylene production is definitely later than the beginning of the rise in CO<sub>2</sub> evolution. This is reasonably clear at 20°C but definitely convincing at 15°C (fig 5). At 5°C no climacteric rise in respiration and no significant ethylene production were observed for 46 days.

The transfer from 5°C to 20°C after 20 and 45 days caused a rise in CO<sub>2</sub> evolution in both cases. However, the rise in ethylene production took place only in fruit transferred to the higher temperature after 20 days' storage at 5°C but not after 45 days. Apparently here, as in the avocado, a prolonged exposure to 5°C affected the ethylene reaction more markedly than the overall respiratory process. The cherimoya is doubtless subject to chilling injuries like most other fruits, but we are unaware of any study dealing with this problem. We do not have, therefore, sufficient information on the cherimoya to suggest an explanation for the difference between the first and the second transfer.

The evidence obtained from this fruit suggests that ethylene is a product of the respiratory changes peculiar to senescence rather than a causal agent. This observation made it necessary to extend the work to other fruits.

**THE FEIJOA, *Feijoa sellowiana*:** The feijoa, also known as the pineapple guava, is perhaps the least tropical of the fruits discussed thus far. It is more resistant to low winter temperatures than either the avocado or the cherimoya, and it tends to produce a

higher quality fruit in districts with cool summers. The fruit is relatively low in sugars (6 to 8% of fresh weight), average in proteins (0.8%) and rather low in acid. It is not suitable for shipment because of rapid deterioration of the central portion.

For this study the Coolidge variety was used. On account of the green color of the skin the state of maturity at harvesting could not be determined with precision. However, the sample appeared to be reasonably uniform if one may conclude from the regular respiratory behavior as shown in figure 6. Here, as in the cherimoya, the rise in ethylene followed the rise in respiration.

**THE MANGO, *Mangifera indica*:** For this study the Haden, Fascell, and Brooks varieties of mango were used. The fruit was relatively green when received. The immature mango has a considerable starch content which, upon ripening, changes into sugar. The ripe mango has a rather high sugar (15%) and high acid (0.5%) content, and is characterized by a turpentine-like flavor due to aromatic substances.

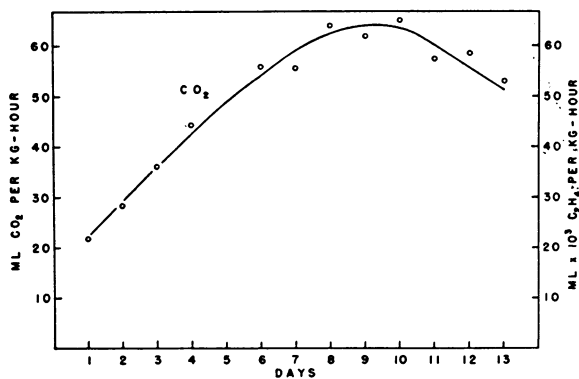


Fig. 7. Carbon dioxide production by Haden mango at 20°C.

Four experiments carried out with the mango gave essentially the same results. The data from one test with Haden are indicated in figure 7. Here we see a typical climacteric rise in respiration, but at no time were we able to detect measurable quantities of ethylene. The ripening process was normal with characteristic skin colors developing during the climacteric cycle. This is then a striking case with a typical climacteric unaccompanied by ethylene evolution into the gas phase. In one case a sample of fruit subjected to ethylene did show an accelerated rate of respiration. The ability of the fruit to respond to external ethylene is not correlated with its capacity for producing ethylene. A similar response was observed with citrus fruits, which are affected by ethylene but do not produce it.

**THE ETHYLENE STORY IN CITRUS:** The literature is replete with conflicting evidence concerning the problem of ethylene production by citrus fruits. Miller et al (14) reported that sound oranges caused epinasty of tomato plants when enclosed in a sealed jar. They also observed that fruit inoculated with the

common green mold, *Penicillium digitatum*, brought about an epinastic response. At the same time Biale (2) found that an active emanation, presumably ethylene, was produced by this fungus when grown on fruit or on agar medium. This active emanation was identified as ethylene by Young et al (20). At no time was there evidence published from this laboratory which would support the contention that citrus fruits free of mold produce ethylene. We were, therefore, incorrectly quoted by Miller (13), Von Loesecke (11), and by Bartholomew and Sinclair (1). On the basis of our experiences we consider the epinastic response in a closed system as insufficient evidence for the identification of ethylene.

Recently we carried on several studies with lemons and oranges in an attempt to settle this question. There was no response of pea seedlings to emanations of fruit kept in air at 15°, 20°, and 25°C. A response was obtained in some instances when fruit was placed under 100% oxygen. With the manometric method we obtained a maximum of 56  $\mu$ l per kg of Valencia oranges in 24 hours under pure oxygen. This was equivalent to approximately 1 ppm of ethylene in the gas stream. Under air no ethylene was detected. Our studies were in a moving stream as contrasted to a still atmosphere employed by Miller et al (14). Moreover, our evidence is based not only on a biological test but on a rather specific chemical method. Hall (7), using direct absorption in potassium permanganate, reported the production of five to six  $\mu$ l of ethylene by one kg of Valencia oranges in 96 hours. He found also that cut segments and fruit inoculated with *P. digitatum* produced larger quantities of ethylene than intact fruit. We question seriously these and all other findings of Hall in this paper on grounds of methodology. The only evidence which he brings in

support of the permanganate method is a comparison with the mercuric perchlorate absorption technique. He carried out this comparison on two different varieties of apples. He realized full well the great varietal differences, and he should have realized the properties of permanganate to react also with volatiles other than ethylene. Nelson (15), who was perhaps the first to use the permanganate method, took the precaution of absorbing the nonethylenic volatiles by sodamide, but there is no evidence that Hall (7) did. It is our contention that any proof for the production of ethylene by citrus fruits has to be based not only on the use of the proper methods but must be accompanied by convincing evidence that the sample was at all times free of ethylene-producing molds.

COMPARATIVE RATES OF ETHYLENE PRODUCTION: Table I summarizes the results obtained with the fruits discussed up to this point and includes findings for several additional fruits. Wherever a definite climacteric peak was observed the maxima reported correspond to the values for the peak. In the case of the sapote, the rise of ethylene production appeared to start three to four days later than the rise in CO<sub>2</sub> output at 20°C. This could not be ascertained with precision because the samples of sapote were not as uniformly mature as most of the other fruits. Our evidence is not clear-cut about the occurrence of the climacteric in the persimmon. There is no question that the citrus fruits do not exhibit the climacteric rise at ordinary temperatures and under normal atmospheric conditions. On the other hand, a respiratory rise was reported by Biale and Young (4) for lemons subjected to oxygen levels higher than air. It was not clear, however, that this rise was the typical climacteric rise, since the citrus fruits apparently do not undergo chemical changes unique to the ripening

TABLE I  
CONCURRENT VALUES FOR ETHYLENE AND CARBON DIOXIDE PRODUCTION

FRUIT	VARIETY	TEMP. °C	C <sub>2</sub> H <sub>4</sub> ML/KG · HR × 10 <sup>3</sup>	CO <sub>2</sub> ML/KG · HR	$\frac{C_2H_4}{CO_2} \times 10^3$
<i>Tropical</i>					
Banana	Gros Michel	20	4	80	0.05
Mango	Haden	20	0	65	0.0
Papaya	.....	25	37	44	0.84
Pineapple	.....	25	0	42	0.0
<i>Subtropical</i>					
Avocado	Fuerte	20	88	156	0.56
Cherimoya	Booth	20	186	129	1.44
Feijoa	Coolidge	20	50	73	0.69
Lemon	Eureka	25	0	6	0.0
Orange	Valencia	25	0	8	0.0
Orange	W. Navel	20	0	8	0.0
Persimmon	Hachiya	20	2	17	0.12
Sapote	Pike	20	129	43	3.0
<i>Temperate</i>					
Apple	McIntosh	20	112	12	9.3
Pear	Bartlett	20	122	42	2.9
Pear	Bosc	20	29	14	2.1
Peach	Hale	20	36	37	0.97

process in other fruits. With the pineapple, we observed no respiratory rise but our experiments were too few to be conclusive.

The last column in table I was designed to be used for comparing the ethylene activity values in relation to the respiratory capacities of the various fruits. Generally a low rate of CO<sub>2</sub> production is associated with low or no ethylene evolution. The McIntosh apple is a notable exception with an unusually high rate of ethylene evolution. Our findings with this variety agree with those of Nelson (16). There seems to be also some correlation between high rates of ethylene production and high rates of respiration. Of the fruits which produce ethylene the banana is the only exception to this direct correlation. It is interesting to note that the temperate zone fruits show a high ethylene to CO<sub>2</sub> ratio. Our results for Bartlett pears correspond closely with those of Hansen (8), who used the micro-bromination method. Compared to the Bartlett, the rate of ethylene evolution of the Bosc pear was reduced to about the same extent as the rate of respiration. It would be of interest to have data on other varieties and species of temperate zone fruits. The information available for all the species reported here suggests, however, that the ethylene-producing mechanism might be affected by factors which leave the overall respiration intact.

#### DISCUSSION OF RESULTS

The fruits under discussion in this paper might be classified into the following three groups with respect to the relation of ethylene production to respiration.

(A) Fruits with a pronounced climacteric capable of producing significant quantities of ethylene. Examples: avocado, banana, cherimoya, feijoa, sapote.

(B) Fruits with a pronounced climacteric but devoid of ethylene emanation. Example: mango.

(C) Fruits not exhibiting a climacteric and not producing ethylene under ordinary conditions. Example: lemon and orange.

We do not know of any case in which fruit fails to exhibit a climacteric under any ripening temperature but does produce physiologically important quantities of ethylene. The minimum external concentration of this gas required to accelerate the ripening process is probably of the order of 0.1 ppm, though conclusive evidence on this point is lacking. We do not know the relation between external and internal concentration in the fruit tissue. Until this is established, one can advance the argument that small quantities sufficient to induce ripening are produced prior to the rise of respiration, but measurable amounts are detected only after the onset of the climacteric. Hansen (9) working with premature pears postulated the hypothesis that when the concentration of ethylene is sufficiently high the climacteric rise ensues. His results show, however, a close parallelism between the increase in CO<sub>2</sub> and ethylene production, similar to our findings with the avocado and the banana. The delay in the onset of the climacteric in a highly aerated system can be explained in terms of ethylene dilution, but the evidence is certainly indirect. The

findings of Kidd and West (10) have some bearing on the question of the induction of the respiratory rise. They subjected pre-climacteric apples to ethylene for different exposure periods. The fruit treated for one or five hours exhibited temporary stimulation which was soon followed by recovery to the level of the controls. The climacteric did not take place as a result of stimulation. The action of ethylene as an inducing agent was insufficient. It was necessary to maintain the ethylene application in order to continue the respiratory rise. We think that this study of Kidd and West supports the idea advanced here that ethylene is not necessarily the primary causal agent for the onset of the climacteric when normal ripening takes place. Changes in metabolic reactions of a more universal nature are presumably associated with the chemical transformations characteristic of the climacteric. Some attempts were made to describe these reactions (5, 12), and more studies are in progress dealing with oxidative phosphorylation of the cytoplasmic particles obtained from fruit at different stages of the climacteric cycle.

#### SUMMARY

1. Fourteen species of fruits of tropical, subtropical, and temperate climatic zones were investigated for the relationship between ethylene production and respiration.

2. The occurrence of the climacteric rise in CO<sub>2</sub> production was established in several species and confirmed in others.

3. The fruits with a marked climacteric showed appreciable to high rates of ethylene production with the mango as the sole exception.

4. The ratio of ethylene evolution to CO<sub>2</sub> output was the highest for the apple, followed by the sapote, pear, cherimoya, peach, papaya, feijoa, avocado, persimmon, and banana.

5. Oranges and lemons exhibited no climacteric in air and produced no ethylene.

6. The hypothesis was advanced that native ethylene is a product of the ripening process rather than a causal agent.

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## THE EFFECT OF X-RAYS ON UPTAKE AND LOSS OF IONS BY POTATO TUBER TISSUE<sup>1</sup>

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Alteration in permeability has frequently been cited as an effect of x-rays on cells (1, 2, 7). X-rays have been found to induce changes in permeability, both increased and decreased permeability having been reported in various plant and animal tissues (1). Since it is now well known that ion uptake or loss in living cells is largely regulated by metabolic activities, such changes may well be referable to alterations in an active transfer or transport system. The present investigation was undertaken to ascertain the effect on permeability, or on an active transfer system, of x-irradiated, mature, plant parenchyma tissue by measuring rates of ion uptake and loss. Emphasis was placed on the immediate response in an attempt to distinguish between the physiologic function specifically concerned with salt relations and the various genetic changes which may be harmful to the tissue but are probably not closely related to salt movements.

### MATERIALS AND METHODS

The tissue used in all experiments was that of mature potato tubers (Red Triumph or California Long White). In preparing discs of tissue for uptake or loss tests, a cork borer was used to cut out a cylinder of tissue along the longitudinal axis from the

central portion of a potato. This cylinder was subsequently sectioned into thin discs. The dimensions of the discs were 1 mm thick and 22 mm in diameter, except in certain tests as noted below. The discs were washed thoroughly in at least two changes of distilled water before use.

X-irradiation was done using an x-ray tube equipped with a tungsten target and operated at 15 milliamperes at 200 or 250 kvp; no filter was used other than the glass wall of the tube. The dose-rate in various tests was between 900 to 1100 roentgens per minute except as otherwise noted. During exposure, discs, typically, were placed on moist filter paper and covered by cellophane in an open Petri dish. Some treatments of submerged discs and of intact potatoes were at dose-rates as low as 340 roentgens per minute.

Uptake was measured as the rate of accumulation of radioactive rubidium by discs of potato tuber tissue from Rb<sup>86</sup>-labelled RbCl solution. Counts of fresh moist discs—as selected for uniform weight in the experiments reported here—were found to represent approximately 75% of the Rb<sup>86</sup> present as shown by the assay of ashed discs at the end of the tests. Thus the proper correction for self-absorption was made in calculating actual concentrations as in figure 1. Loss of ions from potato discs was measured by the increase in conductance of the external solution.

Some additional details of techniques are given in the descriptions of particular experiments below.

**RUBIDIUM UPTAKE:** Previous work has shown that

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