

Role of Ethylene in Fruit Ripening^{1, 2}

Stanley P. Burg & Ellen A. Burg

Department of Physiology, University of Miami School of Medicine

There have arisen two schools of thought concerning the role of ethylene in fruit maturation: the classic view of Kidd and West (26) and Hansen (22) that ethylene is a ripening hormone, and a recent interpretation by Biale et al. (7, 3, 4) that it is a by-product of the ripening process. The original presentation of the by-product theory in this journal (7) was tempered with the reminder that 0.1 ppm ethylene may stimulate ripening, so that "in the absence of any information correlating the internal ethylene content with the rate of ethylene production, 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." The development of highly sensitive gas chromatographic instruments makes it feasible to appraise critically those instances in which fruits have been reported to produce ethylene not at all or only after the climacteric has started, and also to determine the content of ethylene within a fruit at the onset of the rise in respiration. Results of such experiments are reported in this communication, and they have a direct bearing on the problem of whether or not ethylene is a natural ripening hormone.

Materials

Mangoes (*Magnifera indica* L., cv. Kent & Haden) were harvested in local orchards. The fruits used for each experiment were picked from the same tree on the same day and were all of about equal size and apparent maturity. Bananas (*Musa acuminata* cv. Gros Michel) harvested at $\frac{3}{4}$ fullness were shipped from Ecuador. Pineapples [*Ananas comosus* (L.) Merr.] at various stages of maturity were flown from Honduras; they arrived in very satisfactory condition within a day of picking. The Citrus Experiment Station at Tampa, Fla. provided oranges [*Citrus sinensis* (L.) Osbeck] and passion fruits (*Passiflora edulis* Sims); other fruits were purchased in local markets.

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Methods

► Gas Analysis: Both ethylene and carbon dioxide were assayed by gas chromatography. For ethylene analysis a 2 foot aluminum oxide column (Burrell, activated alumina) was operated at 24 C and the column effluent passed through a Perkin-Elmer flame ionization detector. Amplification was accomplished with a Cary Model 31 Vibrating Reed Electrometer using a 10^8 ohm resistor and an applied source of 270/V.D.C. Except for the use of an adsorption column, the apparatus was similar in type to that described by Meigh et al. (30) and detected less than 0.02 ppm ethylene in a 5 cc volume of air. For a few measurements where the utmost in sensitivity was required, a 10^{10} ohm load resistor advanced the limit of detection to 0.005 ppm ethylene in a 5 cc sample. Because certain gases may chromatograph very near to ethylene even under the most favorable conditions (8), it was necessary in every case to confirm that the gas was ethylene by pretreating a sample with mercuric perchlorate reagent, bromine water, or aqueous base. Ethylene is removed completely by the first two reagents but not by the latter. Peak heights could not be accurately equated with ethylene quantity because both the size of the sample and the amount of ethylene contained in it affected the linearity of the calibration for ethylene. This difficulty was not experienced with peak areas which were determined with a planimeter. As described previously (10, 9), carbon dioxide was chromatographed on a silica gel column (Davison, Grade 923, 100-200 mesh) and determined with a high sensitivity katharometer. Peak height was an accurate measure of the quantity of gas. However, it was necessary to standardize the instrument daily as the silica gel gradually lost its absorbency, thus altering the calibration factor.

► Sampling Internal Atmosphere: Samples of the internal atmosphere, obtained by momentarily immersing the fruit in water while 1 to 5 cc of air were withdrawn from its interior with a hypodermic syringe, were directly chromatographed. The sampling operation was quite simple except with bananas whose internal air could only be obtained from the area separating the skin and pulp.

► Determination of Gas Production: To determine rates of ethylene and carbon dioxide production, fruits were placed in 2.3 liter chambers for 1 to 4 hours and samples of air withdrawn at intervals by inserting a syringe needle through a self-sealing rub-

ber port. The chamber accommodated either three bananas or two mangoes.

► **Artificial Application of Ethylene:** Mangoes were treated with ethylene in 40 liter glass containers by injecting the appropriate quantity of gas into each sealed vessel and gently warming one side in order to establish convection currents. Within 1 hour the mixing was complete as judged by the fact that samples of gas extracted through a rubber port with a syringe and analyzed by gas chromatography contained the expected content of ethylene: this concentration was maintained thereafter. Measurements of the ethylene content of fruits confined in chambers showed that the gas had equilibrated between the ambient environment and internal atmosphere within an hour. Each day the containers were opened and aerated completely until no ethylene or carbon dioxide could be detected. Since only two fruits were treated in each chamber the accumulation of gases was minimized; for instance at the climacteric peak less than 4% carbon dioxide was present at the end of a day, and prior to that time the average daily value approximated one percent.

Results

► **Scope of Ethylene Production:** Of the many fruits which have been investigated, the mango, citrus fruit, and pineapple are unique in that they have been reported not to produce any ethylene (7). These fruits evolve and contain ethylene in quantities just smaller than those which could have been measured by methods used prior to gas chromatography (table I). In fact among the more than dozen varieties examined, every individual fruit contained at least 0.04 ppm ethylene within its internal atmosphere whereas the content of ethylene in the external atmosphere was too low to be detected. However, the important point is not whether or not a fruit contains ethylene but rather whether the internal concentration of the gas is at a physiologically active level prior to the onset of the climacteric. In order to decide this issue it is necessary to determine both how the internal content of ethylene varies during the preclimacteric and climacteric periods and also what minimum concentration of ethylene is required for the induction of fruit ripening.

► **Relationship Between Accumulation & Evolution of Ethylene:** That the main causes of the gaseous gradient in fruits are the resistance to diffusion of the skin and the low surface to volume ratio can be demonstrated by a simple experiment. An apple containing 660 ppm ethylene and producing the gas at the rate of 51.6 $\mu\text{l/kg/hr}$ was cut into 16 segments; 4 sections were evacuated briefly with an aspirator while the remaining tissue was left untreated. Two hours later the evacuated tissue contained 148 ppm ethylene due to a production rate of 99.4 $\mu\text{l/kg/hr}$, while the unevacuated tissue contained 187 ppm ethylene with a production rate of 126 $\mu\text{l/kg/hr}$. Thus by increasing the surface to volume ratio and provid-

ing access to air via pathways other than across the skin, the gradient of 13 ppm ethylene per $\mu\text{l/kg/hr}$ which the intact fruit maintained was reduced to 1.5 in both the evacuated and unevacuated tissue. In experiments of this type it is not sufficient simply to measure the changes in internal gaseous content, because wounding fruits generally induces profound alterations in their rate of ethylene production and respiration. In order to evaluate properly the change in permeability, absolute values for the rates of gaseous production must also be considered.

The magnitude of the ethylene concentration gradient between a fruit's interior and the atmosphere is determined mainly by the rate of gaseous production and the geometry and structure of the tissue, and it is very little affected by the absolute amount of ethylene in the atmosphere. For instance, when a mango containing 3.14 ppm ethylene was placed in a sealed chamber and allowed to remain there until the ethylene content of the air within the container had risen to 0.8 ppm, the fruit now contained 3.89 ppm ethylene. In the presence of external ethylene the internal content of the gas was raised by a value nearly equal to the concentration of ethylene in the applied gas mixture, but the gradient of 3.1 ppm was maintained. This same behavior was observed when apples or oranges were placed in sealed chambers.

The relationship between the ethylene gradient (ppm) and rate of ethylene production ($\mu\text{l/kg/hr}$) of various fruits is indicated in table I. All values fall in the range between 1.8 and 13 ppm per $\mu\text{l/kg/hr}$.

Table I
Relationship Between Ethylene Production & Internal Concentration*

Fruit	Internal conc (ppm)**	Ratio (ppm per $\mu\text{l/kg/hr}$)
Apple (McIntosh & Baldwin)	25-2,500	2-13
Avocado (Choquette)	28.9-74.2	2.2
Banana (Gros Michel)	0.05-2.1	1.9
Green pepper	0.1	***
Lemon	0.11-0.17	1.8
Lime	0.30-1.96	3.4
Mango (Kent & Haden)	0.04-3.0	3.8
Nectarine	3.6-602	13
Orange (Valencia)	0.13-0.32	4
Passion fruit	466-530	9
Peach (Elberta)	0.9-20.7	5.2
Pear (Bosc)	80	5
Pineapple	0.16-0.40	2-7
Plum	0.14-0.23	3.9
Sapodilla	3.3	***
Summer squash	0.04-2.1	1.9
Tomato	3.6-29.8	3.2-4.4

* The internal concentration was determined with no detectable ethylene (<0.005 ppm) in the ambient atmosphere.

** Except in the case of bananas and mangoes these values do not necessarily represent the range of concentrations which may occur within the particular variety.

*** Production rate not determined.

When it is considered that these ratios were established for ethylene production rates varying from 0.02 to 200 $\mu\text{l}/\text{kg}/\text{hr}$ (a factor of 10^4) and with fruits differing markedly in geometry and state of maturity, it is remarkable that the absolute variation is less than a factor of seven. Several reports (2, 39, 19) indicate that clogging of air spaces or wax deposition causes a diminution in the permeability of fruits to gaseous exchange as they mature, enhancing the accumulation of carbon dioxide and the reduction of oxygen tension in the internal atmosphere. In table I no attempt has been made to correlate the magnitude of the ratio with the age of the fruit, and it is possible that some of the higher values are associated with post-climacteric tissues. Therefore, if an actual measurement has not been taken, it is suggested that a low value of 2 ppm per $\mu\text{l}/\text{kg}/\text{hr}$ be used to translate ethylene production rates into measurements of the internal concentration of ethylene within fruits entering their climacteric phase. Generally, this conversion factor may underestimate the internal ethylene content, but a conservative estimate is preferred since the point in question is whether or not at the onset of the climacteric sufficient ethylene exists to influence the process.

► Relationship Between Internal Ethylene Content & Fruit Respiration: *I. Mangoes*. The relationship between ethylene production, internal ethylene content and the rate of respiration of Kent and Haden mangoes is depicted in figures 1 and 2. A few hours after the fruits were harvested they contained detectable ethylene. The internal ethylene content rose to 0.04 to 0.05 ppm as the fruits entered their respiratory climacteric, and toward its peak there occurred an abrupt burst of ethylene evolution which coincided exactly with the period of rapid color development and fruit softening. Also evident was a characteristic dip in ethylene evolution almost concurrent with the respiratory maximum followed by the restoration of a high rate 1 day later. The internal concentration of ethylene is dependent upon the rate of ethylene production as would be expected (figs 1 & 2). For instance, with freshly harvested Kent mangoes the ratio ppm ethylene per $\mu\text{l}/\text{kg}/\text{hr}$ was 4.8; during the experiment it ranged from 3 to 7.7 and was at 2.8 on the last day. The magnitude of the variation in this ratio suggests that the values for the internal ethylene determined at the start of the experiment when the measurements had of necessity to approach the limit of sensitivity of the assay system were fairly accurate (and certainly could not be in error by a factor of 2). Therefore, it can be confidently asserted that between 0.02 and 0.08 ppm ethylene is present in the fruit at the onset of the climacteric, and it is important to determine if this range of concentrations has any physiological significance for mangoes.

Although numerous reports indicate that applied ethylene hastens the ripening process in mangoes (7, 36, 25), there have previously been no attempts to determine the quantity of ethylene required. About two hours after harvest five pairs of mangoes were

subjected to the following treatments with ethylene: A, complete aeration, B, 0.04 ppm, C, 0.4 ppm, D, 4 ppm, E, 40 ppm. Each day while the chambers were being aerated the respiration of the fruits was determined, care being taken to ventilate the tissue for at least an hour before the start of the determination in order to avoid an error due to outward seepage of accumulated carbon dioxide. The treatment with ethylene was terminated after 5 days, but respiratory measurements were continued until the last fruit had passed through its climacteric. All the concentrations of applied ethylene except 0.04 ppm stimulated the respiratory rate and hastened ripening (fig 5). Coloration and softening always occurred just prior to the peak in the climacteric, and were accompanied by an equally high endogenous rate of ethylene synthesis in every instance. Because of certain experimental details, the case in which the fruit was treated with 0.04 ppm ethylene requires special comment. The aerated mangoes were confined daily to determine their rate of ethylene production (see fig 1) and it was necessary in the course of the measurements to allow the fruits to raise the ambient ethylene concentration to a detectable level (0.04–0.07 ppm). On the other hand, during the treatment of fruits with 0.04 ppm ethylene some evolved gas accumulated, but at the end of the 1st day the gassing chamber contained only 0.05 ppm ethylene. The net result is that for at least a few hours each day the aerated tissue contained a higher internal concentration of ethylene than the fruits treated with 0.04 ppm. It should be noted in figure 5 that except for the first few days the fruits exposed to 0.04 ppm ethylene lagged behind the controls in their respiration rate. This may be due to the slight accumulation of carbon dioxide in the gassing chamber, although it is important to realize that even completely aerated pre-climacteric mangoes contain between 3 and 4 % of the gas in their internal atmosphere.

An indication of the absolute sensitivity of mangoes to ethylene can be derived from figure 5 by comparing the respiration rate of each lot of fruit on the 1st and 2nd days with that which it initially possessed when harvested. Throughout this period there is a direct relationship between the log of the internal concentration of ethylene and the effect of ethylene on the rate of carbon dioxide production (fig 6). The data for the 3rd and subsequent days is not included because by that time some of the fruits had passed over the peak of their climacteric making comparisons meaningless. In figure 7 the time between harvest and the climacteric peak has been plotted as a function of ethylene concentration, and again a log relationship is apparent. Although a value for the minimum dosage of ethylene affecting mangoes can not be derived from this presentation of the data, the fact that a log relationship holds to between 0.04 to 0.08 ppm makes it clear that these low concentrations influence the tissue's respiration.

The immediate response of mangoes to applied ethylene was studied by injecting a measured quantity

of the gas into one end of a 2.3 liter chamber containing two fruits. Air was sampled at the opposite end to determine carbon dioxide increments, thus eliminating the possibility that undiluted ethylene might be removed during the first measurements of the respiration rate. Ethylene was applied at a concentration of 100 ppm to Kent mangoes which had been maintained at 24 C for 3 days after harvest. These fruits had passed their preclimacteric minimum several days earlier and they contained approximately 0.2 ppm ethylene in their interior at the start of the experiment (fig 1). The fruit responded rapidly (fig 8), reaching a first respiratory maximum about 30 minutes after application of ethylene. This behavior is closely similar to that reported for bananas by Regeimbal et al. (37). It can not be attributed to a release of carbon dioxide contained within the tissue because within 15 minutes after application of ethylene the initial 3.2 % carbon dioxide gradient between the fruit and its environment had risen perceptibly to 3.5 %. By 100 minutes the rate of carbon dioxide evolution had declined to below the initial level, after which there ensued a secondary respiratory rise which undoubtedly represents the climacteric pattern. After 6 hours the tissue was removed from ethylene and when it was re-examined on subsequent days (see fig 8) the respiration rate appeared to be declining from a maximum and the fruits had colored. A second lot of mangoes was exposed to 100 ppm ethylene for only 15 minutes and (fig 8) the treatment resulted in an irreversible stimulation.

II. Bananas. The relationship in bananas between ethylene content and ripening was examined at two temperatures, 16 and 24 C (figs 3 & 4). In both experiments a single hand of fruit was investigated and measurements were carried out on three fingers which were excised each day. Because the 16 C fruit was maintained in an incubator having internal circulation but no provision for continuously venting the air, some accumulation of gas could have occurred. This may be reflected in the fact that

during the first 3 days at 16 C there occurred a slight increase of internal ethylene without a concomitant rise in the production rate. Under conditions of copious ventilation at 24 C there was a closer correlation between internal ethylene content and the production rate during the same period of time. At both temperatures the internal concentration of ethylene approximated 0.1 ppm until the day before the onset of the climacteric rise in respiration at which time the quantity doubled (at 24 C) or tripled (at 16 C). When the first respiratory increase was detectable the ethylene content had clearly surpassed 1.5 ppm, and this behavior has been confirmed in several subsequent experiments carried out at 24 C. As the carbon dioxide evolution increased there occurred a burst of ethylene production lasting for 3 days, during which time the major color changes ensued and the fruit softened. As was the case with mangoes, a temporary decline in ethylene production was associated with the respiratory maximum.

Discussion

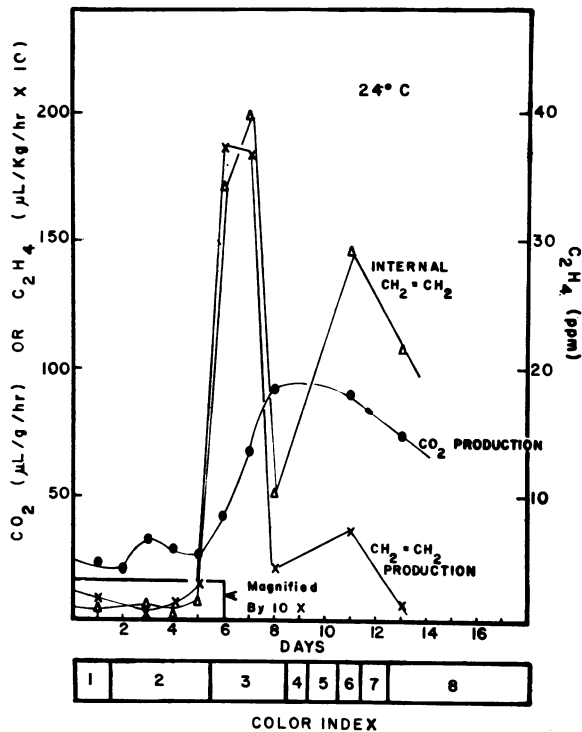
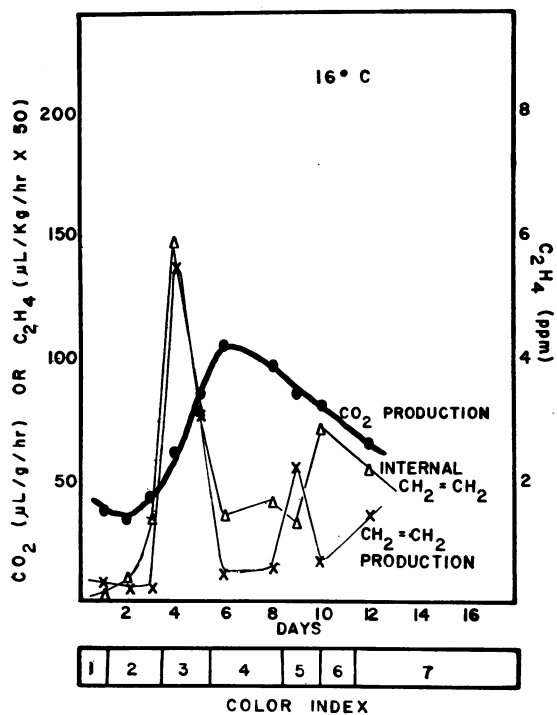
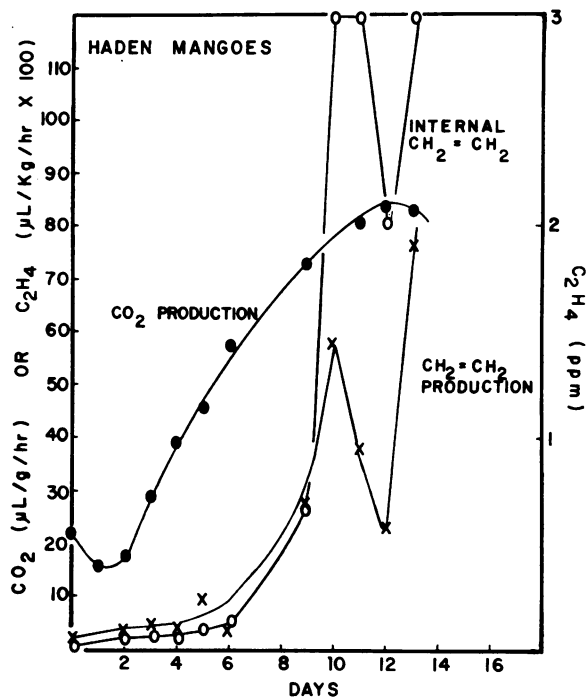
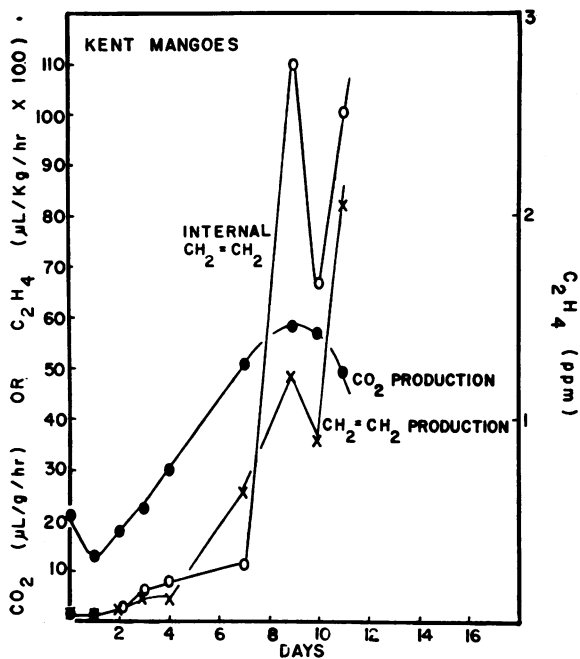
Results have been presented which establish two physiological relationships that contribute to an understanding of the role of ethylene in fruit ripening. A: The ethylene concentration gradient across a fruit's skin is related to the rate at which the tissue produces the gas by a factor conservatively estimated at 2 ppm per $\mu\text{l}/\text{kg}/\text{hr}$ (table I). The validity of this figure is further substantiated by the results of Lyons et al. (28), for a recalculation of their data shows that in cantaloupes the ratio varies from 2 to 3 ppm per $\mu\text{l}/\text{kg}/\text{hr}$. This behavior is in agreement with expectation since gases diffusing across a barrier must obey Fick's Law and, hence, the total amount of gas emitted and the concentration gradient must be directly related. B: The response of fruits to ethylene is related to the logarithm of the internal ethylene content. In addition to the results with man-

Fig. 1 (*upper left*). Relationship between ethylene production rate, internal content of ethylene, and rate of respiration during the ripening of Kent mangoes at 24 C. Measurements were initiated 2 hours after the fruits were harvested. Coloration and softening of the mangoes required several days, and reached completion just prior to the respiratory maximum.

Fig. 2 (*upper right*). Relationship between ethylene production rate, internal content of ethylene and rate of respiration during the ripening of Haden mangoes at 24 C. Measurements were initiated 2 hours after the fruits were harvested. Coloration and softening of the mangoes required several days and reached completion just prior to the respiratory maximum.

Fig. 3 (*lower left*). Relationship between ethylene production rate, internal content of ethylene, rate of respiration, and color development during the ripening of Gros Michel bananas at 16 C. The fruits were warmed to 24 C just prior to each determination. The standard color index numbers for banana ripening are: 1 = all green; 2 = green-trace of yellow; 3 = more green than yellow; 4 = more yellow than green; 5 = yellow-green tip; 6 = all yellow; 7 = yellow flecked with brown; 8 = yellow-large brown areas. Softening of the fruit became noticeable toward the completion of stage 2.

Fig. 4 (*lower right*). Relationship between ethylene production rate, internal content of ethylene, rate of respiration, and color development during the ripening of Gros Michel bananas at 24 C. Softening became noticeable toward the completion of stage 2.



goes presented in figures 5 to 7, this conclusion is supported by considerable other evidence. Heulin and Barker's data for English potatoes (24) shows that in the range from 1 to 100 ppm ethylene (1,000 ppm is supra-optimal) the respiration rate 24 and 36 hours after treatment is proportional to the log of the quantity of ethylene applied to the tissue. Working with avocados, Biale (3) found that concentrations of ethylene between 0.1 and 1000 ppm shortened the time which elapsed before the onset of the respiratory rise. His data indicate that in the range from 0.1 to 10 ppm the time between harvest and the climacteric peak in oxygen consumption is a function of the log of the ethylene concentration (between 10 & 1,000 ppm there is little difference). The application of ethylene to navel oranges, a non-climacteric fruit, causes the oxygen consumption to increase to values which are related at their maximum to the log of the applied ethylene content throughout the range from 0.1 to 100 ppm. It was concluded (3,4) that the response of climacteric and non-climacteric tissues to applied ethylene is strikingly different. The climacteric fruits shift their time-axis to reach a maximum value of respiration which is the same regardless of the applied gas concentration, whereas in the non-climacteric fruit the magnitude of the respiratory peak is a function of the ethylene concentration. This interpretation should be appraised with a knowledge of the concentrations of ethylene which accumulate within climacteric fruits, because experimentally it is not easily possible to investigate their respiratory behavior in the continual presence of less than this amount of gas (see table II). For instance, in the case of avocados the dosage of ethylene can not be restricted to a final value of less than between 140 and 180 ppm. Since ultimately the respiratory capacity of a fruit must be limited by factors other than ethylene, the fact that quantities of ethylene in excess of those indicated in table II often fail to increase

Table II
Concentration of Ethylene in Fruits Prior to Climacteric Peak in Respiration*

Fruit	Ethylene conc (ppm)	Reference
Apple (McIntosh)	80**	32
Avocado (Fuerte)	140-180**	7
Banana (Gros Michel)	6-40	Figs 3-4
Cantaloupe (Powdery Mildew Resistant No. 45)	35-75	28
Cherimoya (Booth)	100-370**	7
Feijoa (Coolidge)	20**	7
Mango (Kent & Haden)	3	Figs 1-2
Passion fruit	140-220**	1
Pear (Bartlett)	250-500**	21
Tomato (Pearson)	4-6**	43

* In several instances the ethylene concentration rises to still higher values after the respiratory peak.

** Based on the conversion factor 2 ppm per $\mu\text{l}/\text{kg}/\text{hr}$.

the magnitude of the particular fruit's respiratory maximum is not proof that smaller concentrations are also without regulatory ability. The rates of ethylene production reported for non-climacteric tissues such as oranges (20) and potatoes (29) are very small. In these cases low concentrations of applied ethylene regulate the intensity of the respiratory maximum which they induce, and, therefore, it is unlikely that the respiratory rise is accompanied by a substantial increase in the rate of ethylene production. Possibly differences in the response of climacteric and non-climacteric fruits to applied ethylene reflect only their relative abilities to produce the gas.

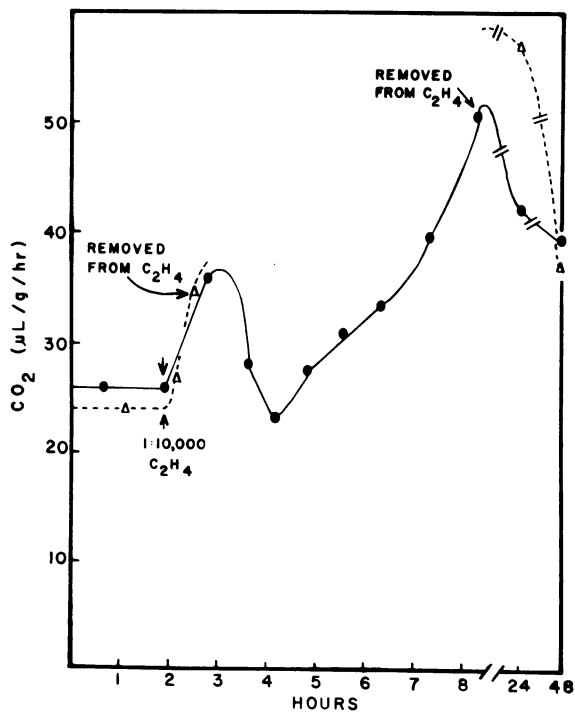
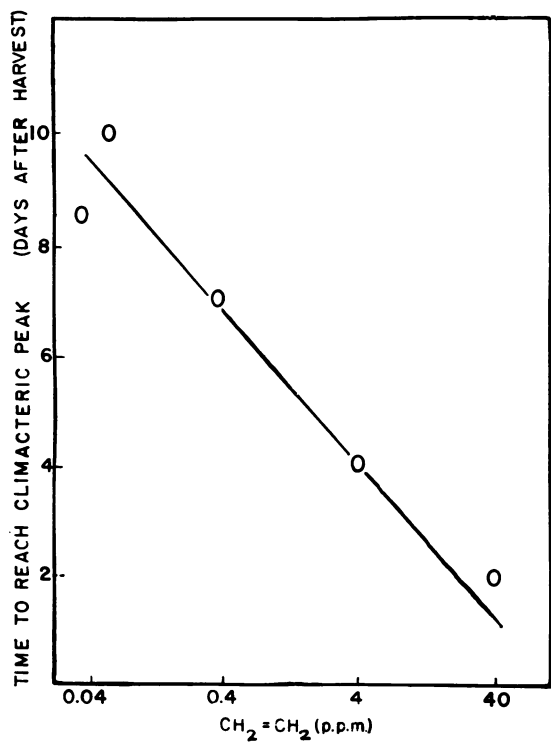
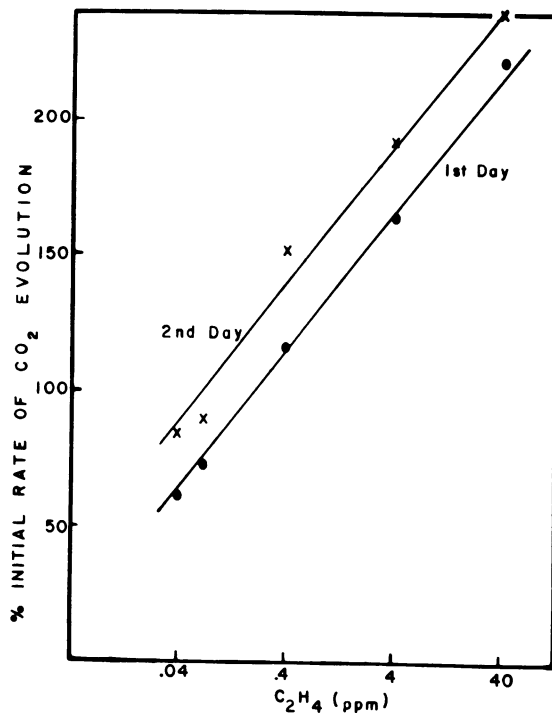
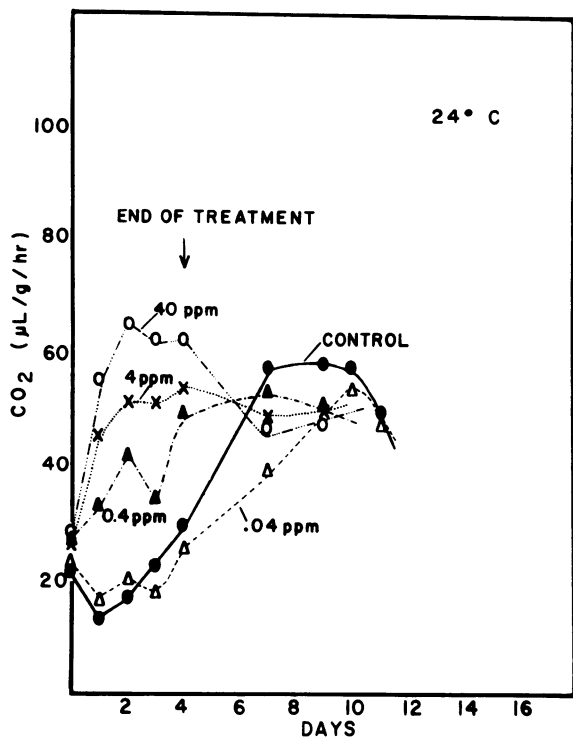
If ethylene accumulates within a preclimacteric fruit to a concentration which can be shown to hasten the respiration and ripening of that fruit, it follows that the gas functions as a natural ripening hormone. Clearly in the case of bananas this prerequisite is

Fig. 5 (*upper left*). Acceleration of respiration and ripening in Kent mangoes after application of ethylene at 24 C. Ethylene was applied to fruits a few hours after harvest, and the gas mixtures were replenished daily during a 5 day treatment. Each curve indicates the average behavior of a pair of fruits, and in every case coloration and softening reached completion just prior to the respiratory maximum. Coincident with each climacteric peak there occurred a high rate of endogenous ethylene production which was approximately equal to that of the control at the same stage.

Fig. 6 (*upper right*). Relative effectiveness of various concentrations of ethylene in accelerating the respiration of Kent mangoes. The carbon dioxide evolution of fruits continuously treated with ethylene is compared after 1 and 2 days to the initial rate of respiration 2 hours after harvest. By the 3rd day (not included) several of the fruits had reached or declined from their climacteric maximum so that the linear relationship between log ethylene concentration and respiration rate is no longer apparent.

Fig. 7 (*lower left*). Relative effectiveness of various concentrations of ethylene in accelerating the ripening of Kent mangoes. The time between harvest and achievement of the respiratory maximum is a function of the log of the concentration of ethylene within the fruits during the first few days of the experiment.

Fig. 8 (*lower right*). Short term respiratory changes caused by brief exposures of Kent mangoes to 1:10,000 ethylene at 24 C. These fruits, harvested 3 days prior to the start of the experiment, had already passed through their preclimacteric minimum when one lot (*solid line*) was treated with ethylene for 6 hours and the other (*dashed line*) for 15 minutes. During the treatment and on 2 subsequent days the respiration rate was determined. When both lots of fruits were examined 24 hours after the ethylene had been applied all had colored and softened indicating that even the 15 minute treatment provided an irreversible stimulus. Note the immediate response to ethylene which subsides before the respiratory climacteric ensues.



satisfied and the gas is a normal and perhaps mandatory stimulus for the ripening process. The minimum dosage of ethylene which hastens ripening of Gros Michel bananas is between 0.1 and 1 ppm (29, 3). The measurements summarized in figures 1 and 2 reveal that the fruit maintains a minimum stimulatory level of ethylene (approximately 0.1 ppm) until the day prior to the climacteric rise at which time an increased rate of synthesis causes the concentration to exceed the maximal stimulatory level of 1 ppm. According to Lyons et al. (28) cantaloupes are similar to bananas in that their internal content of ethylene rises from a quasi-stimulatory preclimacteric level (about 0.05 ppm) to a stimulatory level (>0.1–1 ppm) prior to or coincident with the climacteric rise in respiration. While in both of these cases, optimal stimulation by ethylene occurs almost coincident with the onset of the climacteric, it is noteworthy that the ethylene contained prior to that time probably exerts an effect and may be an important factor determining when the climacteric is initiated. The relationship between ethylene and the ripening of certain tropical fruits is not so clearly defined. Biale et al. (7) noted that an abrupt increase in the rate of ethylene synthesis did not accompany the beginning of the climacteric in cherimoyas and feijoas, although eventually the ethylene production did increase rapidly to a high level. The patterns of ethylene production and respiration illustrated in figures 1 and 2 indicate that mangoes also are representative of this category of fruits. Although the approximately 0.05 ppm ethylene contained in a mango at the time of the preclimacteric respiratory minimum suffices to influence the fruit's metabolism, it is important to note that only slightly smaller amounts were present shortly after harvest. Recalling that the efficacy of ethylene is a log function of the gas concentration, it is difficult to ascribe the initiation of a metabolic change as profound as the onset of the climacteric in mangoes to less than a twofold increment in ethylene content. To do so would be to place extremely high demands on the processes controlling the rate of ethylene production in immature fruits, for otherwise any inordinately high fluctuation in some environmental factor such as temperature might prematurely initiate fruit ripening. The small amount of ethylene contained in freshly harvested mangoes must start the fruit toward its climacteric, and the additional ethylene which accumulates during the early stages of the respiratory rise may further accelerate the process. This hypothesis is supported by the demonstration that mangoes may be hastened to ripen by ethylene applied several days after the climacteric has begun. Similar behavior could not be demonstrated easily in those fruits whose rate of ethylene production increases abruptly coincident with the onset of the climacteric because they very quickly acquire an optimal dosage of ethylene due to their own metabolism. This almost certainly is the basis of the observation that ethylene is without effect on most fruits if it is applied after ripening

Table III
Minimum Effective Concentration of Ethylene

Tissue	Ethylene (ppm)	Response	Reference
<i>Vegetative plant</i>			
Buckwheat	0.05–0.1	Epinasty	13
Castor bean	0.1–1	Epinasty	23, 17
Pea	0.025–0.1	Epinasty	27, 35, 38
Rose	0.3	Epinasty	45
Sunflower	0.05–0.1	Epinasty	13
Tomato	0.05–0.1	Epinasty	13, 17
<i>Flower</i>			
Carnation	0.5	Closure	27, 12
Cattleya	0.002–0.1	Injury	14
Rose	0.3	Abscission	45
<i>Fruit</i>			
Avocado	0.1	Ripening	3
Banana	0.1–1 ppm	Ripening	18, 3
Cantaloupe	0.1–1	Ripening	28
Lemon	0.025–0.1	Injury	14
Mango	0.04–0.4	Ripening	...
Orange	0.1 ppm	Climacteric rise	3

has commenced, from which it has been inferred that a critical triggering concentration of ethylene is required to produce a response.

Attempts to measure the minimum dosage of ethylene which affects various tissues have yielded the results summarized in table III; without exception these values fall in the range between 0.025 and 1 ppm, with an average lower limit of about 0.1 ppm. However, the experimentally derived threshold for many fruits may be as indicative of the endogenous content of ethylene within the tissue as it is of the sensitivity of the tissue to the gas. Because the response of a fruit to ethylene is related to the log of the gas concentration, and since the internal content of ethylene will be augmented by approximately the concentration of gas maintained in the ambient atmosphere, it follows that a response can not be demonstrated easily unless the applied concentration equals or exceeds that within the fruit. This is borne out by the behavior of bananas, cantaloupes, and mangoes because in all three cases the minimum concentration of applied ethylene which effects the preclimacteric fruit is nearly equal to the internal concentration within the fruit at that stage of development. Estimating the threshold sensitivity to ethylene as 0.1 ppm and using the conversion factor 2 ppm per $\mu\text{l}/\text{kg}/\text{hr}$, it is possible to reappraise much of the existing information concerning the relationship between ethylene production and fruit respiration. In all cases it appears that a significant accumulation of ethylene precedes or coincides with the onset of the climacteric:

Nelson's data (32) for McIntosh apples does not include figures for the critical period when the climacteric rise is just beginning, but it indicates that within a few days after the respiratory rise, and long before the maximum rate of ethylene production has been attained, the internal content of ethylene is far in excess of 20 ppm. According to Biale et al. (7)

Fuerte avocados ripened at 15 C produce about 1 $\mu\text{l/kg/hr}$ ethylene one-half day after the start of the climacteric. No figures are available for the ethylene production prior to that time, but it is evident that at least 2 ppm ethylene must be contained within the tissue only very shortly after the respiratory rise begins, and 0.1 ppm is known to stimulate the fruit (3). Fairly complete information is available for tomatoes. The internal ethylene concentration just prior to the respiratory rise is more than 2 ppm for Vitamold 12 variety according to Spencer's data (40), and about the same for Pearson tomatoes (43), although in the latter case a few instances were noted in which the sensitivity of the mercuric perchlorate assay was insufficient to detect ethylene at that stage of development. We have found the internal ethylene content of green tomatoes to be 3.55 ppm just prior to the time that the first color changes became apparent. Hansen's study (21) of the relationship between ethylene production and the respiration of pears also indicates that substantial amounts of ethylene are present at the onset of the respiratory climacteric. According to his data there must exist a concentration of at least 20 ppm before the climacteric rise in Bartlett pears, and more than 0.3 ppm somewhat in advance of this stage in Anjou pears. On the 3rd day of Biale's experiments (7) with both cherimoyas (at 15 C) and feijoas (at 20 C) a rate of 4 $\mu\text{l/kg/hr}$ ethylene production was noted, which corresponds to an internal concentration of at least 8 ppm ethylene. The shape of the ethylene production curves suggests that when the climacteric was initiated prior to the start of the experiment the internal concentration of ethylene was not an order of magnitude lower than 8 ppm. The relationship between ethylene production and respiration in the purple passion fruit has been studied in detail by Akamine et al. (1); their data at 20 C indicate that at least a few ppm ethylene are present in preclimacteric fruits (assuming 2 ppm per $\mu\text{l/kg/hr}$), and that this value has increased several-fold by the day prior to the start of the climacteric rise. At 25 C smaller amounts of ethylene were produced at the onset of the climacteric, but the internal content must still have been in excess of 1 ppm. At both temperatures the ripening of the fruit was accelerated by application of 500 ppm ethylene, but there is no indication that lower concentrations were tested. The conclusion by Akamine et al. that 500 ppm are much higher than a physiological content is not valid (see table I). At least this amount of ethylene must be contained in passion fruits (20 C) only a few days after the start of the climacteric, and the ratio established in table III suggests that as much as 50 to 100 ppm ethylene may be present when the climacteric starts, and 3,330 ppm at the peak of the ethylene evolution.

Measurement of the ethylene contained in various citrus fruits is of particular significance in view of the past disagreement concerning the ability of lemons and oranges to synthesize the gas. An early report that gaseous products from oranges accelerate the

ripening of bananas (11) was followed by the demonstration that several types of citrus fruits cause epinasty of test plants (31), and Hall (20) found Valencia oranges to produce ethylene at a rate of 0.05 to 0.06 $\mu\text{l/kg/hr}$. However, neither Gane (16) nor Biale et al. (7) could find any indication using a bioassay that sound oranges produced ethylene in air, although Valencia oranges kept in 100 % oxygen produced the gas at a rate of about 2 $\mu\text{l/kg/hr}$ (7). It was suggested (7) that the bioassay is unreliable in a closed system such as that used by Miller et al., and that Hall's permanganate reduction method is non-specific, so that in neither case was there an adequate demonstration of the production of ethylene by intact citrus fruits in air. Moreover it is essential to prove that the fruit is free of mold since at least one common contaminant, *Penicillium digitatum*, produces abundant quantities of ethylene (5, 31, 44), and citrus fruits infected with this organism evolve large amounts of the gas (20, 31). Measured by gas chromatography, the rate of ethylene production of Valencia oranges in air was 0.02 to 0.06 $\mu\text{l/kg/hr}$, which agrees well with the values reported by Hall. As the oranges were discarded in a sound condition 1 week after the experiment was terminated, and since they maintained a fairly uniform rate of ethylene production over the course of several days, it is unlikely that fungal contamination contributed to the results. Since both fully mature lemons and limes also contained and evolved ethylene, the gas is a common metabolic product of citrus fruits. Lemons maintained in oxygen concentrations higher than air pass through a respiratory climacteric, whereas in air or lower oxygen concentrations the respiratory rise is suppressed (6). This is a significant observation in view of the inordinately high rate of ethylene evolution reported for oranges kept in 100 % oxygen (7). Since as little as 0.025 to 0.05 ppm ethylene will hasten ripening and induce a climacteric in lemons, the fruit must normally contain even lower concentrations of the gas. However, if the lemon, like the orange, responds to elevated oxygen tensions by increasing its ethylene production it follows that such treatment might easily raise the internal content of ethylene to a stimulatory level.

Conclusions

Porritt's comprehensive review (33) of the role of ethylene in fruit ripening cited numerous examples in support of the concept that ethylene is a ripening hormone, and it is noteworthy that at the time (1951) there was no mention of the converse point of view. Shortly thereafter the behavior of several fruits was reported to be in discord with the hormone theory (7) and it was concluded that ethylene might be a by-product of fruit metabolism (7, 3, 4). Subsequently, this controversy has been a recurrent theme in discussions of post-harvest physiology (41, 3, 4, 34). Because the original objections to the hormone theory can no longer be supported or are inconclusive, the proposal that ethylene is a metabolic by-product is

without substantial basis. Contrary to the original report (7), mangoes have a typical pattern of ethylene production, citrus fruits and pineapples produce small amounts of the gas, and there is no evidence that the ethylene contained in preclimacteric feijoas and cherimoyas is without physiological activity. Therefore, the facts which originally gave rise to the theory that ethylene functions as a ripening hormone should once again receive the consideration due them. These have now been complemented by a demonstration that stimulatory quantities of ethylene accumulate prior to the onset of the climacteric in several fruits.

Summary

Internal ethylene contents and the rates of ethylene production were determined for a variety of fruits including several which had previously been described as devoid of ethylene; e.g., the mango, pineapple, orange, and lemon. Mangoes were found to have a normal pattern of ethylene evolution coincident with their respiratory climacteric. Applied ethylene hastens ripening of mangoes even after the climacteric has begun; the effect is related to the log of the gas concentration and becomes apparent within 15 minutes. The ratio between the internal-external ethylene gradient and the rate of ethylene production varied by less than a factor of ten among more than 17 varieties of fruits, and can be conservatively estimated as 2 ppm per $\mu\text{l}/\text{kg}/\text{hr}$. Direct measurements indicate that accumulation of a stimulatory concentration of ethylene precedes the onset of the climacteric in both mangoes and bananas. A similar conclusion is indirectly derived for several other fruits.

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