# Carbon Dioxide Effects on Fruit Respiration [1]. II. Response of Avocados, Bananas, & Lemons

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Most studies concerning the effects of the gas composition of the atmosphere surrounding fruit have been directed toward the response of the combined changes of oxygen and carbon dioxide. This was a result of the economic need for methods of prolonging the storage life by the use of modified atmospheres at temperatures above those which cause chilling injury. Several studies have been concerned with the effects of decreased oxygen alone. Parija (15) obtained minimum carbon dioxide production by apples at 5 % oxygen. Kidd and West (12) found an acceleration of the onset of the climacteric in apples by oxygen tensions higher than in air. Singh et al. (19) reported for mangos a critical oxygen level of 9.2 % below and above which carbon dioxide evolution increased. The studies of Claypool and Allen (6, 7) with apricots, plums, peaches, and pears indicated a decreased rate of $CO_2$ output at oxygen tensions below air. For a more comprehensive treatment of this subject the recent review by Biale (3) is suggested.

For the avocado, a fruit which exhibits a climacteric pattern of respiration, it was shown by Biale (2) that low oxygen reduced the respiratory activity during the preclimacteric period and the duration of the preclimacteric period was prolonged. Within the range of 2.5 to 21 % oxygen the time required to reach the climacteric peak was extended in proportion to the decrease in oxygen tension and the intensity of respiration at the peak was reduced. No significant stimulation of respiration was observed by concentrations of oxygen above 35 %. However, the cumulative carbon dioxide production from the time of picking until the climacteric peak was not changed by the treatment at any level of oxygen. In the case of the banana, another fruit with a climacteric pattern, it was found by Kidd and West (11) that storage in 2.5 and 5.0 % oxygen did not materially decrease the rate of ripening. On the other hand, Leonard (13) observed a reduction in $CO_2$ liberation by fruit stored in oxygen concentrations lower than air and no effect in concentrations higher than air.

Lemons did not exhibit the climacteric pattern of respiration after picking when stored in air or at oxygen tensions below that of air, as shown in the studies of Biale and Young (5). The respiratory activity under air decreased slightly during storage. Reduction of oxygen in the atmosphere surrounding the fuit reduced the rate of respiration in proportion to the oxygen concentration in the range of 21 to 5 % oxygen. Carbon dioxide evolution increased at oxygen levels below 5 %, indicating the similarity of the behavior of lemons with other fruits characterized by a critical oxygen concentration. The storage life was extended and the decomposition of chlorophyll in mature green lemons was delayed by lowered oxygen.

The effect of added carbon dioxide on respiratory activity of fruits at a particular oxygen concentration has been studied little, largely due to technical difficulties. Limited data are available on the banana. By the use of the katharometer method Gane (9) observed a suppression of the climacteric and reduction of respiratory activity in an atmosphere of 10 % carbon dioxide and 10 % oxygen. Wardlaw (20) subjected unripe bananas to different combinations of oxygen and carbon dioxide. On the basis of gas analysis he concluded that there was a 50 % reduction in rate of respiration over a wide range of $O_2$ and $CO_2$ concentrations as compared with air. His determinations were limited to green fruit and were done in a closed system in which the gaseous composition could not be kept constant. The use of analytical methods described in the first paper (21) of this series has enabled us to study the effect of carbon dioxide at several oxygen levels on the respiratory activity and storage behavior. The responses to $CO_2$ of avocados, lemons and bananas are reported at this time.

## Materials & Methods

Avocados of the Fuerte variety were picked in the orchard of the Plant Biochemistry department at the University of California, Los Angeles, or were obtained from commercial orchards in southern California. Fruit was always picked in the morning and put under treatment on the same day. Stems were cut close to the fruit and those with detached stems were not used. Fruit was selected for uniformity of size and 10 to 25 specimens were used per treatment.

Bananas of the Gros Michel variety grown in Central America were obtained with the cooperation of the Consolidated Fruit Co. Fruit was picked in the three-quarter full stage, shipped to Los Angeles by boat, and were still dark green when received.

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The transit period was normally 12 days, during which the fruit was subjected to a temperature of about 15 C and a relative humidity of about 90 %. Single bananas were separated from the hand and 15 to 20 bananas were tested per treatment. They were treated within one day after arrival of the boat.

Lemons were obtained from commercial packing houses in Los Angeles County. They were picked on the basis of size rather than color, and dark green fruit was selected for all experiments. Ordinarily one to two days elapsed between picking and the start of treatment. Fruit was washed, waxed, and sized at the packing house. In one experiment fruit waxed with water wax containing 2,4-D was used. To check the effect of the 2,4-D treatment a sample of the same pick as used in the experiment was removed from the belt ahead of the waxer. Respiratory activity and color changes were unaffected by the 2,4-D wax treatment. Button retention was somewhat better after 2,4-D treatment. Composite samples of 30 to 50 fruit, uniform as to size and color, were selected for each treatment.

Fruit was put in respiration jars described by Biale and Shepherd (4), and gas mixtures passed continuously as described in paper I of this series (21). Respiratory activity was measured as ml oxygen absorbed per kg fresh weight per hour. Oxygen uptake was measured on the Model G-2 oxygen analyzer as described previously (21). Oxygen uptake measurements were made every 2 to 8 hours during the experimental period, depending upon the number of experimental samples attached to the automatic sampling system. Where daily values are reported, the average of not less than four separate measurements is given.

For the chemical analysis of the lemons, three random samples of seven fruit each were taken from each treatment. Juice of seven was pooled for analysis and the average of the three aliquots is reported. Ascorbic acid was determined by the method of Ramsey and Colichman (17).

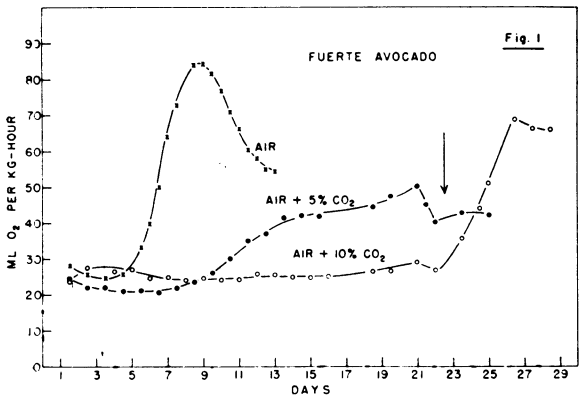
## Results

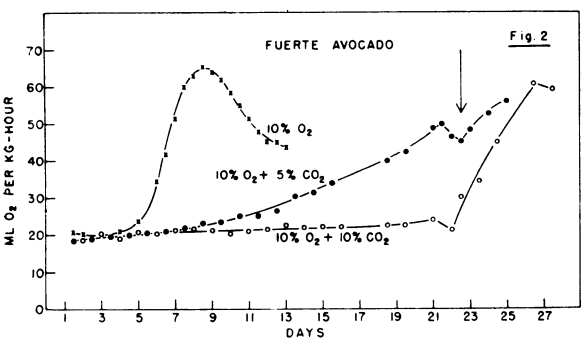
► Fuerte Avocado. Fruit used for the experimental data given in figures 1 and 2 was picked on May 20 and kept at 15 C throughout the experiment. Figure 1 shows a typical climacteric curve of respiration of avocados under air. The fruit remained hard during the preclimacteric period, which was 4 days for this experiment, started to soften as the respiratory activity increased, and became soft and edible about two days after the peak of respiratory activity. The effect on respiration of adding 5 and 10 % carbon dioxide to air is also shown in figure 1. As the addition of carbon dioxide reduces the oxygen concentration by dilution, sufficient oxygen was added to these mixtures to maintain the oxygen level at 21 % in all cases. When 21 % oxygen with 5 % carbon dioxide was used, the preclimacteric rate of oxygen uptake was affected only very slightly and the start of the climacteric rise was delayed by only about three days. Thereafter there was a gradual rise to a peak of respiration which was 40 % below the air control and was reached after 21 days compared to 8 days for the air treatment. The peak was followed by a decline and on the 23rd day the fruit were transferred to air. There was no definite increase in oxygen uptake after the transfer, indicating that the climacteric had really occurred and these fruit softened in the normal manner. When fruit was treated with a gas mixture containing 10 % carbon dioxide and 21 % oxygen, there was essentially no change in respiratory activity for 23 days and the rate was the same as that of the initial value of the control. Upon transfer to air there was an increase in respiration, indicating that the climacteric had not occurred under the carbon dioxide treatment but had occurred after transfer to air when the fruit softened normally.

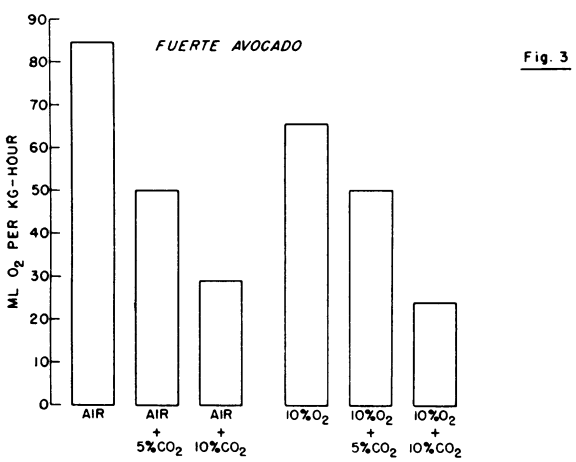
The effect of increased carbon dioxide at the 10 % oxygen level on the respiratory trend of avocados is illustrated in figure 2. Fruit for this experiment was from the same lot as that used for the experiment in figure 1. Treatment of 10 % oxygen alone reduced the climacteric peak value to about 65 ml/kg/hr as compared to 85 ml/kg/hr for air, but delayed the peak only very slightly. When 5 % carbon dioxide with 10 % oxygen was used, there was again a very gradual rise in respiratory activity to a peak value of 48 ml/kg/hr on the 21st day, which was the same as the peak value for 21 % $O_2$ with 5 % $CO_2$. The rise after transfer to air was probably due to the fact that one fruit was infected with a fungal rot. It should be noted that the rise in respiration started only about two days after the 10 % oxygen control, but the peak was delayed from 9 days for the control to 21 days for the carbon dioxide treatment. Again 10 % carbon dioxide in 10 % oxygen produced no change from the preclimacteric minimum of the control and showed no change in respiration up to the 23rd day. Ripening was normal after transfer to air. Fruit under all treatments was of good flavor, according to informal taste panels at the end of the storage period. There was some decay, particularly at the stem end of fruits stored under carbon dioxide. We believe this to be a function of the length of storage rather than due to the carbon dioxide treatment itself.

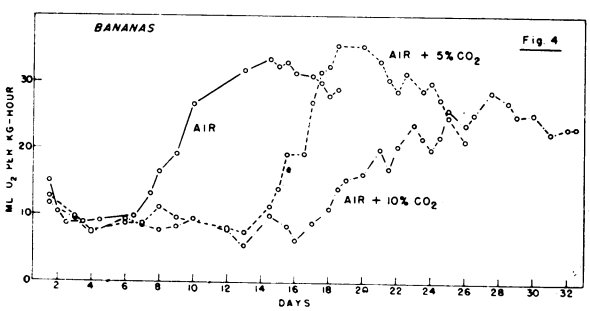
In figure 3 the maximum values for all treatments are compared. While the 10 % oxygen treatment reduced oxygen uptake at the climacteric compared to the air control, the peak values for 21 % oxygen with 5 % carbon dioxide and 10 % oxygen with 5 % carbon dioxide were about the same. Values at the 10 % carbon dioxide level do not represent climacteric peak values, and it is not known whether a climacteric would occur or not if more time had been allowed. It would appear, though, that when 5 % carbon dioxide was used it was the carbon dioxide which limited the reaction rate.

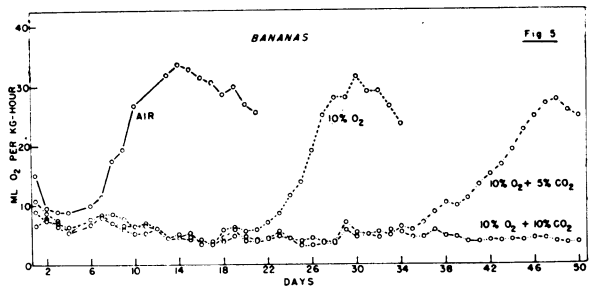
In other experiments, 5, 10, and 15 % carbon dioxide have been used with 5 % oxygen, and satisfactory ripening was obtained after 45 days' treatment

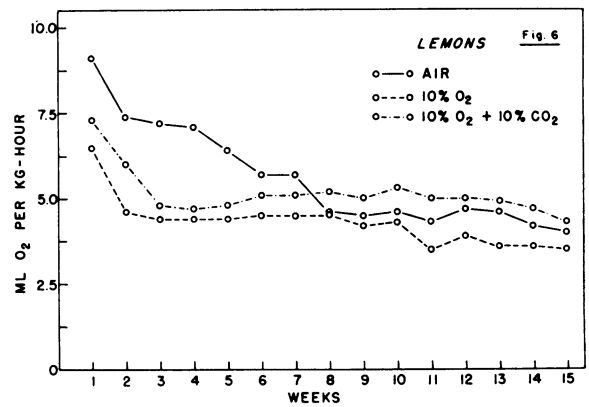

FUERTE AVOCADO
Fig. 1
ML O2 PER KG-HOUR
AIR
AIR + 5% CO2
AIR + 10% CO2
DAYS

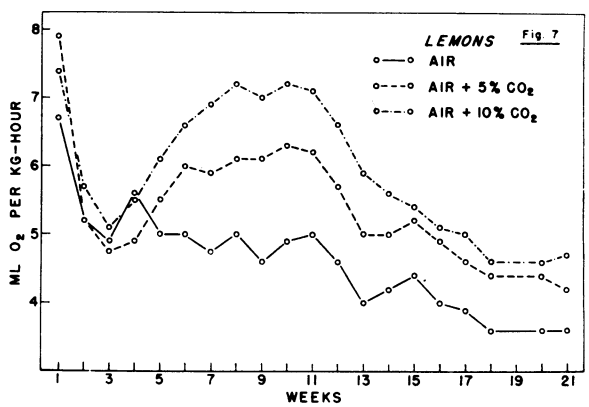

FUERTE AVOCADO
Fig. 2
ML O2 PER KG-HOUR
10% O2
10% O2 + 5% CO2
10% O2 + 10% CO2
DAYS

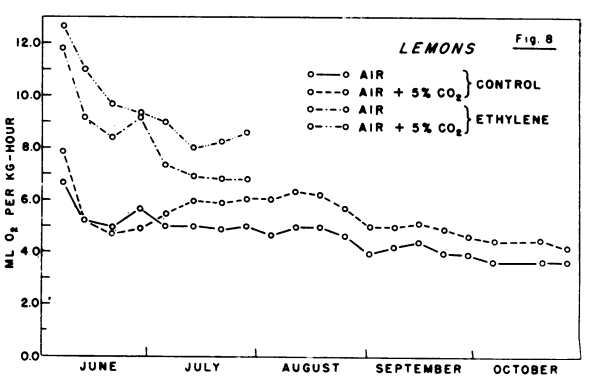

FUERTE AVOCADO
Fig. 3
ML O2 PER KG-HOUR
AIR
AIR + 5%CO2
AIR + 10%CO2
10%O2
10%O2 + 5%CO2
10%O2 + 10%CO2


BANANAS
Fig. 4
ML O2 PER KG-HOUR
AIR
AIR + 5% CO2
AIR + 10% CO2
DAYS


BANANAS
Fig. 5
ML O2 PER KG-HOUR
AIR
10% O2
10% O2 + 5% CO2
10% O2 + 10% CO2
DAYS


LEMONS
Fig. 6
AIR
10% O2
10% O2 + 10% CO2
ML O2 PER KG-HOUR
WEEKS


LEMONS
Fig. 7
AIR
AIR + 5% CO2
AIR + 10% CO2
ML O2 PER KG-HOUR
WEEKS


LEMONS
Fig. 8
AIR
AIR + 5% CO2
CONTROL
AIR
AIR + 5% CO2
ETHYLENE
ML O2 PER KG-HOUR
JUNE
JULY
AUGUST
SEPTEMBER
OCTOBER

at 15 C when early season fruit were used. Fifteen per cent carbon dioxide appeared to produce some injury to the fruit.

► Bananas. The banana is another fruit which exhibits the climacteric pattern of respiration associated with ripening. The results of respiratory measurements of fruit stored in air, 21 % oxygen with 5 % carbon dioxide, and 21 % oxygen with 10 % carbon dioxide at 15 C are shown in figure 4. Here we see that under all three conditions there was a definte climacteric, that the peak values of oxygen uptake were essentially the same, and that the shapes of the curves of oxygen uptake are similar. While the fruit under 21 % oxygen with 10 % carbon dioxide actually showed a slightly lower peak, the slope of the increase was lower and more irregular. We believe this to be an indication that the fruit ripened less uniformly. If only a few fruit start to increase their respiration on particular days, it would be expected that the curve would be broader and more irregular. The change in color from green to yellow which is coincident with the climacteric was less uniform for this treatment. The important point to note is that the only essential difference between the three treatments is the time of onset of the climacteric and the day that the climacteric peak occurred. The air control treatment reached the peak on the 14th day, 21 % oxygen with 5 % carbon dioxide on the 19th day, and 21 % oxygen with 10 % carbon dioxide after 27 days.

In figure 5 is shown the results of treatment with 5 and 10 % carbon dioxide added at the 10 % oxygen level. Here again peak values at 10 % oxygen and at 10 % oxygen with 5 % carbon dioxide are essentially the same as the air treatment, but the 10 % oxygen treatment required 30 days to reach the peak while 10 % oxygen with 5 % carbon dioxide required 47 days. Fruit treated with 10 % oxygen with 10 % carbon dioxide showed no increase for 50 days and were still dark green. Upon transfer to air, these fruits ripened normally and were of good flavor and quality. We have not established whether bananas maintained under this treatment for more than 50 days would undergo a climacteric, but there appeared to be no injury at least for this period. These data show that avocados and bananas with typical climacteric patterns of respiration responded to modified atmospheres in different ways. For bananas, both decreased oxygen and increased carbon dioxide caused a delay in the time of onset of the climacteric, but once the climacteric was induced the treatment seemed to have no effect on the rate of respiration. On the other hand, the rate of respiration of avocados during the climacteric was affected by the same treatments.

► Lemons. It has been shown by Biale and Young (5) that lemons did not exhibit a climacteric pattern of respiration, at least at the oxygen tension of air or below. All other citrus fruits which have been examined behaved in a similar manner. The respiratory activity declined steadily from picking until the onset of decay by fungi. The respiratory quotient (RQ) of lemons is just a little less than one and changes only slightly with oxygen tension between 2.5 and 100 % oxygen (5), so that either oxygen uptake or carbon dioxide production may be used to measure respiratory activity.

Earlier experiments (5) had shown that 5 % oxygen was a desirable oxygen concentration for the storage of lemons. In our first experiments we used 5 % oxygen with 0, 5, and 10 % carbon dioxide at 20 C. Respiratory activity of these samples was essentially constant after the first 2 weeks of storage. Average values for oxygen uptake of these treatments are shown in table I. While 5 % oxygen did bring about a decrease in respiratory activity, the addition of 5 % carbon dioxide caused little further decrease, while at the 10 % $CO_2$ level a slight increase in respiratory activity was noticed.

Similar experiments at the 10 % oxygen level were carried out at 20 C. One air treatment was always used as a check on the reproducibility of behavior of the fruit. Curves of the oxygen uptake are

**Table I**

Respiration of Lemons in Relation to Carbon Dioxide Tension

| Week | ml $O_2$ per kg-hour | | | |
|---|---|---|---|---|
| | Air | 5 % $O_2$ | 5 % $O_2$ + 5 % $CO_2$ | 5 % $O_2$ + 10 % $CO_2$ |
| First | 4.7 | 1.8 | 1.6 | 2.0 |
| Second | 4.5 | 1.7 | 1.5 | 2.0 |
| Fourth | 4.7 | 1.7 | 1.6 | 2.1 |
| Sixth | 4.8 | 2.5 | 2.0 | 2.4 |
| Eighth | 4.4 | 2.9 | 2.2 | 3.0 |

Fig. 1. Oxygen uptake by Fuerte avocados in air and in 21 % $O_2$ with 5 and 10 % $CO_2$ at 15 C. Arrow indicates transfer from $CO_2$ treatment to air.

Fig. 2. Oxygen uptake by Fuerte avocados at 15 C in 10 % $O_2$ and in 10 % $O_2$ with 5 and 10 % $CO_2$ added. Arrow indicates transfer from $CO_2$ treatment to air.

Fig. 3. Maximal respiratory rates of Fuerte avocados in relation to $O_2$ and $CO_2$ concentration.

Fig. 4. Oxygen uptake by bananas at 15 C in air and in 21 % $O_2$ with 5 and 10 % $CO_2$.

Fig. 5. Oxygen uptake by bananas at 15 C in air, 10 % $O_2$, and in 10 % $O_2$ with 5 and 10 % $CO_2$

Fig. 6. Oxygen uptake by lemons at 20 C in relation to $O_2$ and $CO_2$ concentration.

Fig. 7. Oxygen uptake of lemons at 20 C under air and 21 % $O_2$ with 5 and 10 % $CO_2$.

Fig. 8. Oxygen uptake by lemons at 20 C in relation to $CO_2$ and ethylene treatment.

shown in figure 6. During the first 6 weeks of the experiment the respiratory activity under both treatments was markedly lower than in the air control. After 3 weeks, the rate of respiration in the 10 % oxygen treatment leveled off while the activity of the fruit under 10 % $O_2$ with 10 % $CO_2$ actually increased slightly. At 8 weeks, the respiration of the $CO_2$ treated lot exceeded that of the control fruit and remained higher for the final seven weeks of the experiment.

Treatment in atmospheres containing 21 % oxygen with 5 % carbon dioxide and 21 % oxygen with 10 % carbon dioxide showed much more striking stimulation of respiration (fig 7). For the first 3 weeks oxygen uptake of all samples decreased. Then while the rate of respiration in air continued to decline slowly for the next 21 weeks, both the 5 and 10 % carbon dioxide treatments brought about an increase in respiratory activity, reaching peak values at about nine weeks and declining thereafter, but always remaining above the air control. At the 10th week, the oxygen uptake in the 5 % carbon dioxide treatment was about 31 % above the air control and the 10% carbon dioxide treatment was 50 % above the air control.

Denny (8) demonstrated that ethylene increased the respiratory activity as well as hastened the decomposition of chlorophyll in the rind of mature green lemons and accelerated the onset of decay. In figure 8 is illustrated the effect of adding 10 ppm of ethylene to air and to 21 % oxygen with 5 % carbon dioxide. The addition of ethylene to the air treatment nearly doubled the respiratory activity, but addition of ethylene to the 21 % oxygen with 5 % carbon dioxide treatment caused an additional small but significant stimulation. It is not possible to pick comparable time periods to calculate the exact percentage stimulation. The stimulation by both ethylene and $CO_2$ is greater than that caused by either carbon dioxide or ethylene separately. It would appear that the stimulation of respiration by ethylene is brought about by a different mechanism than the stimulation due to carbon dioxide.

On the basis of the relationship established between respiratory activity and storage life as measured by the occurrence of physiological breakdown, invasion of fungal decay, color changes, and abscission of the calyx (5), one would expect that the carbon dioxide enhanced respiration would shorten the storage life, hasten chlorophyll decomposition in the rind of mature green lemons, and accelerate abscission of the calyx. Actually, however, there was a marked delay rather than acceleration of decomposition of chlorophyll, as is shown in table II, and of abscission of the calyx. Observations recorded in this table were made after 10 weeks of storage when the best color contrasts occurred. In all three experiments there was a definite delay in chlorophyll decomposition due to the $CO_2$ treatment. It was true, however, that there was more variability under the carbon dioxide treatment than under air or 10 % $O_2$. This

**Table II**

Color Transitions of Lemons in Relation to Carbon Dioxide Treatment

| Exp. | Gas mixture $O_2/CO_2$ | % in Color class | | | |
|---|---|---|---|---|---|
| | | Yellow | Silver | Light green | Dark green |
| A | 21/0 | 90 | 10 | 0 | 0 |
| | 5/0 | 0 | 40 | 37 | 23 |
| | 5/5 | 0 | 3 | 87 | 10 |
| | 5/10 | 0 | 0 | 79 | 21 |
| B | 21/0 | 100 | 0 | 0 | 0 |
| | 10/0 | 0 | 100 | 0 | 0 |
| | 10/10 | 35 | 28 | 33 | 4 |
| C | 21/0 | 26 | 70 | 4 | 0 |
| | 21/5 | 0 | 90 | 10 | 0 |
| | 21/10 | 0 | 76 | 20 | 3 |

was particularly noticeable in experiment C for 10 % carbon dioxide with 10 % oxygen, in which case 35 % of the fruit were yellow and 4 % were still dark green, while under 10 % oxygen with no carbon dioxide all fruit were silver.

Abscission or drying of the calyx or button of lemons is used by the citrus industry as an indication of the keeping quality of a sample of fruit. In the experiments listed in table II the buttons were green and normal after 10 weeks storage for all treatments. However, fruit under the same oxygen and $CO_2$ treatments but in the presence of 10 ppm ethylene was yellow after 10 weeks storage. The buttons of the fruit kept in air with ethylene were all dry or had abscised. Approximately 50 % of the buttons wilted or abscised after 10 weeks on fruit treated with ethylene in the presence of 10 % oxygen or in 21 % $O_2$ combined with 5 % $CO_2$. When ethylene was added to 21 or 10 % oxygen with 10 % $CO_2$, buttons of essentially all fruit remained healthy for the 10 week period.

Lemons used in experiment C were analyzed for total soluble solids (sugars), total titratable acidity (as citric acid), and for ascorbic acid at the time the

**Table III**

Chemical Analysis of Lemons Before & After Storage

| | Initial | Final | | | | |
|---|---|---|---|---|---|---|
| % $O_2$/% $CO_2$ | | 21/0 | 21/5 | 21/10 | 10/5 | 10/10 |
| Brix | 9.6 | 7.7 | 7.0 | 6.8 | 7.2 | 6.7 |
| Acid | 5.58 | 5.38 | 4.63 | 4.42 | 4.15 | 3.39 |
| Ascorbic acid | 67.1 | 45.6 | 40.8 | 34.4 | 34.5 | 30.2 |

Brix refers to total soluble solids on Brix hydrometer scale.
Acid expressed as mg citric acid per 100 ml juice.
Ascorbic acid as mg per 100 ml juice.
21 % oxygen levels stored for 28 weeks.
10 % oxygen levels stored for 41 weeks.

experiment was started, and again as the fruit was approaching the end of their storage life. Data for these analyses are shown in table III. Samples of lemons held under 21 % oxygen and under 21 % oxygen combined with 0, 5, and 10 % carbon dioxide were analyzed after 28 weeks of storage. Sugars, acids, and ascorbic acid all decreased as the carbon dioxide concentration was increased. Data for 10 % oxygen treatment were not available for this experiment, but these same three analyses yielded lower values for 10 % oxygen with 10 % carbon dioxide than for the 5 % carbon dioxide treatment at 10 % oxygen when analyzed after 41 weeks. It appears, therefore, that carbon dioxide-stimulated respiration does bring about significant decreases in sugar, acid, and ascorbic acid in the juice of lemons.

Determinations were also made of the volume of extractable juice, fresh and dry weight of the rind, thickness of the rind, and change in total weight of the fruit. There was no correlation between these measurements and the treatments.

## Discussion

While most fruits respond to reduced oxygen in the atmosphere by reduced respiration and a delay in induction of the climacteric, these data show that avocados, bananas, and lemons each reacted in different ways to the addition of carbon dioxide to the atmosphere surrounding the fruit. Carbon dioxide depressed the rate of respiration in avocados, delayed the induction of the climacteric without affecting the rate of respiration in bananas, and stimulated the respiratory rate of lemons.

Carbon dioxide appeared to have no effect on the rate of respiration of either avocados or bananas prior to the induction of the climacteric rise. Avocados treated with 5 % carbon dioxide at either 21 or 10 % oxygen showed a delay in the onset of the respiratory rise of not more than 2 or 3 days, but the peak activity occurred 12 days after the controls. Five per cent carbon dioxide with 21 % oxygen reduced the peak activity by 40 %, and at the 10 % oxygen level 5 % carbon dioxide reduced the respiratory rate at the peak by 25 %. The actual value of oxygen uptake at the climacteric peak was the same under both treatments.

These data are consistent with the idea that when 10 % oxygen is used with no carbon dioxide added, the rate of respiration is limited by availability of oxygen as an electron acceptor. Experiments dealing with oxygen effects alone reported earlier (2) showed that the rate of respiratory activity of avocados was not changed materially by increasing the concentration of $O_2$ from that in air to 100 %. It is reasonable, therefore, to suggest that when $CO_2$ is added to 21 % oxygen the rate of oxidation is not limited by availability of oxygen as an electron acceptor. Conceivably during the climacteric and not prior to its onset, a different metabolic pathway is superimposed on the basic pathway. This might be accounted for by a $CO_2$ induced inhibitor of the new pathway such as malonate, the inhibitor of the citric acid cycle. Alternatively, the partial reversal of a decarboxylation reaction necessary to permit further dehydrogenase activity could limit the availability of electrons to the electron transport system. In avocados and bananas under 10 % $CO_2$ there was no evidence for a climacteric and we presume that here the new pathway is completely suppressed. If avocados are held much longer than 23 days at 15 C under 21 % oxygen and 10 % $CO_2$, physiological breakdown occurs which suggests that some inhibitor or product has accumulated to the point that normal metabolism can no longer take place.

The rate of respiration of bananas is not affected by the addition of 5 or 10 % carbon dioxide either before or after the climacteric has been induced, but only the time of induction is affected. Low oxygen also appears to delay the induction of the climacteric, but in addition it reduces the pre-climacteric oxygen uptake. The effect of low oxygen and increased carbon dioxide appears to be synergistic in delaying the induction of the climacteric. Five per cent carbon dioxide in air delays the climacteric peak by 5 days. Ten per cent oxygen causes a delay of 16 days. The combination of 10 % oxygen and 5 % carbon dioxide brings about a climacteric peak after 34 days, or 13 days later than the sum of the delays caused by either reduced oxygen alone or increased carbon dioxide alone.

Evidence of Hulme (10), Pearson and Robertson (16), and of Rowan et al. (18) indicate that in three different fruits, the apple, tomato, and avocado, the climacteric is accompanied by formation of more protein, which suggests the possibility of synthesis of new enzymes and the introduction of new metabolic pathways. Some support for this contention is supplied in the report by Allentoff (1) in which $C^{14}O_2$ fixation into amino acid residues of protein of detached apples was demonstrated. Specific activity of four amino acids in the bound and free states was measured. Two had much higher and the other two, lower specific activity in the protein than in the free form. If this is true for the banana, reduced oxygen might be expected to delay the induction of the climacteric by decreasing the available ATP for synthesis, and increased carbon dioxide might delay the formation of a specific amino acid necessary for synthesis of a specific enzyme or delay decomposition of an inhibitor of either new enzyme synthesis or another pathway. Although the above mechanisms are completely speculative, the divergent responses to $CO_2$ suggest that the climacteric in the banana and avocado represent different biochemical phenomena in contrast to the general assumption that the climacteric has as its basis a single specific reaction common to all fruits which exhibit this phenomenon.

Neal and Hulme (14) have shown that apple skin slices produce carbon dioxide when fed malic acid without equivalent oxygen uptake. He suggests that the climacteric in apples may be due to this malic decarboxylase. If this malate decarboxylase is responsible for the climacteric in apples as shown by carbon

dioxide production, no climacteric should appear by oxygen uptake measurements and this would represent a third type of climacteric.

Lemons do not exhibit a climacteric at the oxygen tension of air or below. When subjected to added carbon dioxide, the curve of respiratory activity appears somewhat like a climacteric but actually is quite different from the rise which normally occurs in avocados and bananas. All fruits which exhibit a climacteric can be induced to a climacteric by treatment with low concentrations of ethylene, but once the climacteric is induced ethylene has no additional effect. Lemons, on the other hand, which are induced to a stimulated respiration by carbon dioxide are further stimulated by treatment with ethylene, and the ethylene stimulated respiration persists for the duration of the treatment. It would appear that these two stimuli act at different sites of the metabolic pathway. It will be shown in paper three of this series that carbon dioxide probably acts in the lemon by accelerating the turnover of a rate limiting step in the metabolic pathway rather than induction of a new pathway.

## Summary

Avocados, bananas, and lemons were subjected to 0, 5, and 10 % carbon dioxide at 5, 10, and 21 % oxygen.

Carbon dioxide delayed the onset of the respiratory rise in the avocado, reduced the rate of oxygen uptake at the climacteric peak and prolonged storage life.

In the banana the induction of the climacteric was postponed but the rate of respiration at the peak was unaffected by $CO_2$ treatment whenever the peak occurred; the suppression of the climacteric pattern and the extension of storage life was particularly pronounced in an atmosphere of 10 % carbon dioxide combined with 10 % oxygen.

Carbon dioxide caused an unprecedented stimulation of respiration in lemons. This phenomenon was more striking with 10 than with 5 % $CO_2$ and more in combinations of carbon dioxide with air than with oxygen concentrations below air. There appeared to be an increased utilization of sugars and acids as a result of carbon dioxide treatment. In general, the life of lemons was prolonged by carbon dioxide, especially at the lower levels of oxygen.

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