Effect of Oxygen Concentration on Respiration of the Fuerte Avocado Fruit

Author(s): J. B. Biale

Reviewed work(s):

Source: *American Journal of Botany*, Vol. 33, No. 5 (May, 1946), pp. 363-373

Published by: Botanical Society of America

Stable URL: http://www.jstor.org/stable/2437124

Accessed: 09/02/2013 20:42

Frampton, V. L. 1942. On the size and shape of the tobacco mosaic virus protein particle. Science 95: 232–233.

Lauffer, M. A. 1944. The size and shape of the tobacco mosaic virus particles. Jour. Amer. Chem. Soc. 66: 1188–1194.

Rawlins, T. E. 1942. Recent evidence regarding the nature of viruses. Science 96: 425–426.

Spencer, E. L. 1942. Specific biological activity of tobacco mosaic virus as influenced by age of lesion and nitrogen supply. Plant Physiol. 17: 210–222.

Stanley, W. M. 1937. Chemical studies on the virus of tobacco mosaic. X. The activity and yield of virus protein from plants diseased for different periods of time. Jour. Biol. Chem. 121: 205–217.

———. 1938. Virus proteins, a new group of macromolecules. Jour. Phys. Chem. 42: 55–70.

———, and T. F. **Anderson**. 1941. A study of purified viruses with the electron microscope. Jour. Biol. Chem. 139: 325–338.

Wyckoff, R. W. G. 1937. Molecular sedimentation constants of tobacco mosaic virus proteins extracted from plants at intervals after inoculation. Jour. Biol. Chem. 121: 219–224.

EFFECT OF OXYGEN CONCENTRATION ON RESPIRATION OF THE FUERTE AVOCADO FRUIT [1]

J. B. Biale

Investigators in the field of fruit physiology and storage have devoted most of their attention to the effects of combined changes in the oxygen and carbon dioxide content of the atmosphere. This approach was prompted by the need to outline the best conditions for modified air storage. Little information is available on the response of fruit to variations in the oxygen concentration. Parija (1928), working with apples, found the lowest CO_2 production at 5 per cent O_2; it rose to higher levels below and above this critical concentration. Kidd and West (1934) succeeded in retarding the onset of the climacteric rise in apples by 5 per cent O_2 and in accelerating it by pure oxygen if the response to air was used as control. Singh (1937) found the critical oxygen concentration for mangoes to be 9.2 per cent. In some of these studies the anaerobic CO_2 production was found to be high over a considerable period of time, indicating the capacity of the tissue for making use of the fermentative enzyme system. In actively growing plant parts CO_2 evolution in pure nitrogen was very low as compared with mature fruits.

The avocado fruit occupies a unique position with respect to the relationship between the aerobic and the anaerobic process. Wardlaw and Leonard (1935) were able to depress respiration and delay maturation in some West Indian varieties of avocados by reducing the oxygen concentration and increasing the carbon dioxide level. However, their measurements were too few to illustrate the changes in the course of respiration of fruit subjected to a modified atmosphere. Biale (1941) found that in air the respiratory activity of Fuerte avocados at 15° C. was characterized by a rapid acceleration in CO_2 production followed by a decrease. This so-called climacteric rise did not take place in an atmosphere of nitrogen, nor did the fruit ever reach the soft, edible stage. From the standpoint of fruit storage, the question arose whether the physiological changes characteristic of the climacteric rise could be delayed without altering the nature of the respiration process. It was hoped that the onset of the climacteric and its actual value could be controlled by changes in oxygen tension. The effects of variation of oxygen concentration at different temperatures were studied in the experiments reported here.

Materials and methods.—The avocados for these experiments were of the Fuerte variety picked from 6 to 7 year old trees in the Horticulture orchard of the College of Agriculture, Los Angeles campus of the University of California. In the climatic zone in which this orchard is situated, the Fuerte variety normally attains horticultural maturity in December and may be harvested through the month of June. Size was the chief criterion employed in selecting uniform fruit since variability in the respiration behavior of fruits from one tree appeared to be as great as between specimens of different trees. When the carbon dioxide evolution of individual avocados was determined, it was found that the differences in time of the climacteric maximum were of the order of ten days. In the case of a composite sample the peak coincided with the earliest of the single fruits, and variabilities between different lots were much smaller. Hence each sample consisted of twenty avocados with the exception of experiments 8 and 13 in which 15 and 21 fruits were used respectively. The range in average fruit weight for samples of twenty Fuerte avocados was from 4552 grams in experiment 3 to 5964 grams in experiment 14. The variability was much lower for the several jars in each experiment.

For the studies of the effects of the oxygen variable, gas mixtures were prepared by combining the proper proportions of air and nitrogen for subatmospheric oxygen concentrations, and by mixing pure oxygen with nitrogen for oxygen tensions above those in air. The gas mixing board (fig. 1) consisted in its essential parts of carefully calibrated flowmeters, needle valves, and a mixing cylinder 18 inches long and 2½ inches in diameter. Air was delivered from a constant pressure compressed air supply line by means of a needle valve to the mixing board, where another needle valve and an air bleeding device made it possible to maintain

---

[1] Received for publication February 6, 1946.

Table 1. *The actual oxygen concentrations obtained from the gas mixtures.*

Per cent O_2 reported	Per cent O_2 found by gas analyses Exp. 3	4	6	7	8	9	12	13
2.5	2.25	2.54	2.52	...	2.59	...	2.62	2.48
5.0	5.00	5.35	5.13	4.73	4.91	5.07	5.01	5.35
10.0	9.81	10.37	10.29	9.58	9.85	9.83	9.38	9.95

reasonably constant flow rates. The oxygen and nitrogen cylinders were equipped with accurate pressure regulators which allowed for a constant gas supply of low pressure (3 to 5 lbs.) throughout the range of pressures in the cylinders. Here again the use of additional needle valves on the board facilitated fine adjustment of the flow. The flowmeters were calibrated by the method of Meuron (1941), displacing through them air from a 50 or 250 ml. burette which was being filled at different rates with water from a source of constant hydrostatic pressure. Calibration curves were prepared in which rates of flow in milliliters per minute (ml/min) were plotted against flowmeter deflections. The maximum error in oxygen concentration due to deviations in flowmeter readings was of the order of 1 per cent. Since the respective gases were delivered through flowmeters, the oxygen concentrations reported were on a volume percentage basis. The composition of the modified atmospheres was checked periodically by the Orsat method, and the results of the gas analyses for most of the experiments described here are given in table 1.

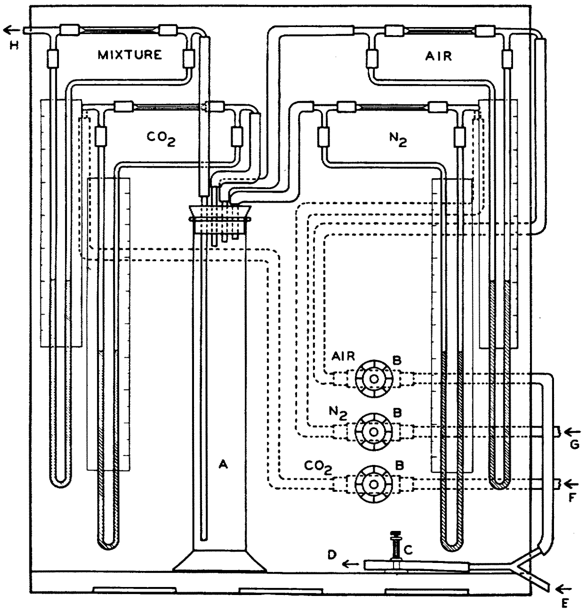


Fig. 1. Apparatus for preparing gas mixtures. ⅛ of original dimensions. A, mixing cylinder; B, ⅛ inch needle valves; C, screw clamps; D, outlet for excess air flow; E, from compressed air line; F, from CO_2 cylinder; G, from nitrogen cylinder; H, to respiration jar.

The figures given in the first vertical column will be used in this report for the sake of convenience, but reference may be made to this table for the actual compositions of the modified atmospheres. The percentage of oxygen in air, which was always used as the control, was found at different times to be approximately 20.8. The above indicated oxygen concentrations were employed at the following temperatures: 15, 10, 7.5, and 5°C. In experiments 7 and 8 at 15°C. the fruit was subjected to a wider range of oxygen concentrations, the analyses of which will be included below.

The method of determining CO_2 production was a modification of the procedure used by Claypool (1938) and consisted of passing air freed of carbon dioxide through the specially constructed respiration jar described by Biale and Shepherd (1941) and into a sintered glass bubbler immersed in a sodium hydroxide solution in a hydrometer cylinder. The quantity of 0.3 N base used varied from 25 to 75 ml., depending on the intensity of respiration. After a suitable period of time, usually 30 minutes for avocados, 10 ml. of 1.5 N $BaCl_2$ were added for each 25 ml. of base used, and the excess alkali titrated with 0.15 N hydrochloric acid to a thymolphthalein end point. This procedure will be referred to as method I to distinguish it from method II of measuring CO_2 evolution which was used in connection with the determination of oxygen absorption, and which will be described below.

The rate at which the gas mixtures were passed over the fruit had to be high enough to prevent significant changes in the composition of the atmos-

Table 2. *Effect of air rate on the respiratory course of Fuerte avocados.*

Jar number	Air rate ml/min	Mg CO_2 per kg-hour Initial	Minimum	Maximum	Number of days from initial to Minimum	Maximum
12	200	65.6	35.3	126.0	8	12
13	300	66.0	32.7	124.9	5	12
14	400	69.3	33.4	134.0	5	12
22	500	64.2	33.5	127.0	7	12
24	600	71.2	32.2	114.9	7	14

phere within the respiration jars. Two experiments were designed to determine the non-limiting rate of flow. In experiment 1, two jars with twenty fruits in each jar were placed at 15° C. under an air rate of 350 ml/min. The initial difference in carbon dioxide production between the two samples was less than 2 per cent. One of these samples was constantly subjected to the rate of 350 ml/min, while in the case of the second jar daily increases in the rate of flow of 50 ml/min were introduced until a rate of 500 ml/min was attained. Respiration determinations were carried out before and after the changes in flow. The results obtained indicated that there was an immediate rise in CO_2 evolution following increase in flow, but after one hour equilibrium was established and the rate of CO_2 evolution did not differ materially from that of the control jar which was maintained constantly at 350 ml/min.

In a second test on rates of flow (experiment 2), five composite samples picked on February 25 were placed at 15° C. under five different rates of flow. The course of respiration was followed until the post-climacteric stage; the results are given in table 2.

The uniform behavior of the several samples is evident from the results in this table. Close parallelism was observed also in the softening process in the five jars. On March 9 (12 days after picking), all the avocados were firm with the exception of several fruit that had barely started softening. Four days later most of the fruit in each jar were in the edible stage. It appears from this test that with respiration intensities occurring in air at 15°, a flow of 200 ml/min was sufficient to prevent excessive accumulation of CO_2 and significant depletion of oxygen in the jar. Since, however, larger respiration rates than those in air were expected with fruit subjected to 50 and 100 per cent O_2, it was decided to standardize on a rate of flow of 350 ml/min. With this rate, three jars could be treated simultaneously from each mixing board. Considering a peak value of 150 mg CO_2/kg-hour, the concentration of CO_2 in the container at equilibrium should not exceed 1.8 per cent at an air rate of 350 ml/min. No tests were made with rates lower than 200 ml/min because of previous findings with lemons, which have a much lower respiration intensity than avocados, that lower rates affect the CO_2 evolution of the material (Biale and Shepherd, 1941).

EXPERIMENTAL RESULTS.—*Effect of oxygen concentration on carbon dioxide production at 15° C.*—The decision to study extensively the various aspects of the respiratory activity of the avocado at 15° C. was based on observations made at temperatures varying from 5° C. to 25° C. The physiological changes characteristic of the senescent stage proceed at 15° C. at a rate at which they can be observed readily. They are neither too rapid nor too slow as compared with higher and lower temperatures. When, for example, fruit is placed under constant environmental conditions immediately after picking, there results a decrease in the rate of CO_2 evolution which may last for several days. This preclimacteric behavior is seldom observed at 25° C. On the other hand, at 5° C. the rise in respiration may escape detection because of limitation in the method of measuring low levels of CO_2 production. Therefore, most of the results in this study were obtained from tests at 15° C. conducted during the 1942–43 and 1943–44 seasons. Six representative experiments (numbers 3 to 8, inclusive) will be summarized here with each of these tests illustrating different aspects of the problem of oxygen tension effects.

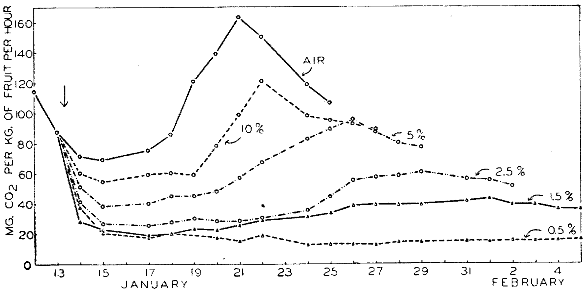
The avocados for experiment 3 were picked in the morning of April 19, 1943, divided into four lots, and placed the same day in a controlled room maintained at 15° C. ± 0.5° C. Though the variability in fruit weight of these samples was considerably higher than that in the later experiments, the initial respiration values taken in air and based on fresh weight were reasonably uniform. On April 20 the three semi-hourly respiration measurements gave rates of CO_2 evolution that differed by 2 per cent from the mean. In view of the uniformity in response of this material, the several jars were subjected to differential treatment on April 20 immediately after the respiration determinations. The effect of the different oxygen partial pressures was immediately observable, as shown in table 3.

TABLE 3. *Effect of oxygen tension on CO_2 evolution by Fuerte avocados (CO_2 in mg per kg of fresh weight per hour).*

Jar number	Treatment	April 20	Treatment % O_2	April 21	22	24	26	27	28	29	30
3	air	72.9	2.5	25.5	21.1	17.3	19.0	21.8	24.7	31.9	34.6
6	air	73.2	5.0	31.9	27.2	29.5	29.3	37.4	41.7	65.7	72.4
7	air	71.2	10.0	38.1	34.7	31.9	45.7	84.2	86.3	100.6	87.7
30	air	69.2	air	52.6	48.9	80.2	118.7	126.2	115.9	115.0	105.6

Jar number	% O_2	May 1	2	3	4	5	6	7	8	10	
3	2.5	44.6	44.0	48.0	48.8	47.0	50.5	42.7	40.1	37.7	...
6	5.0	81.4	73.6	66.7	61.3	63.3	57.3	50.6	46.9	41.1	...
7	10.0	64.6	51.6	62.0	61.4	...	...	56.5	54.5	50.7	...
30	air	88.6	82.7	78.9	66.4	69.7	70.0	64.4	60.1	60.0	...

All of these results are averages of three semihourly measurements. The readings on April 21 were taken 21 hours after the change from air to the several oxygen concentrations. At that time the CO_2 production in jar 3 under 2.5 per cent O_2 was 48.5 per cent of the respiration rate of control jar 30. In all cases there was a characteristic decrease as compared with the values obtained immediately after harvesting. The pre-climacteric minimum was followed by a sharp increase to a climacteric peak. The relative respiration values and the number of days from the pre-climacteric minimum to the peak are presented in table 4.



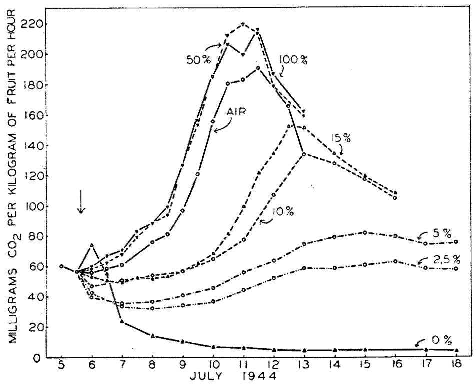


Fig. 2 (above). Effect of oxygen (0.5 per cent to air) on CO_2 production of Fuerte avocados at 15°C. (Arrow indicates beginning of differential treatment.)—Fig. 3 (below). Effect of oxygen (0 to 100 per cent) on CO_2 production of Fuerte avocados at 15°C. (Arrow indicates beginning of differential treatment.)

Table 4. *Relative respiration values and duration of the climacteric rise.*

Jar number	Treatment % O_2	Relative CO_2 production minimum	Relative CO_2 production maximum	No. of days minimum to peak
3	2.5	35	40	12
6	5.0	56	64	9
7	10.0	65	80	5
30	air	100	100	5

The post-climacteric period was characterized by a pronounced decline in CO_2 production accompanied by fruit softening. The chemical transformations that are responsible for the change in the firmness of the avocado flesh probably were set into motion before the peak was reached, but observable softening did not take place until after the maximum was attained.

Since softening is a function of the climacteric rise, there were marked differences between the several treatments in experiment 3 with regard to the storage life of the fruit. On April 30 all the avocados in jar 30 (air) were softening, while in jar 7 (10 per cent O_2) eight had barely started to soften, with the remainder still firm or hard. On May 2 when the control fruit was soft, in jar 7 there were five avocados in the nearly edible stage, eleven softening and four firm. In jar 6 (5 per cent O_2) eleven had barely started to soften, and nine were still firm. On that day the avocados in jar 3 (2.5 per cent O_2) had hardly changed in their texture, but on May 4 eight fruit began to soften, and three days later most were in the edible stage. On May 10 when the experiment was concluded, the fruit in jars 3 and 6 had the best appearance, while a good deal of mold was observed in the control jar and somewhat less in the jar subjected to 10 per cent O_2. Since the total duration of this experiment was twenty days, it is reasonable to conclude that the reduced oxygen content of 2.5 and 5 per cent had doubled the storage period of the fruit.

The end of the storage life of a sample of avocados is difficult to determine with precision. The best edible stage may vary from individual to individual. An objective measure of comparison might be arrived at by counting the number of days from the date of picking to the climacteric peak, as illustrated in table 5.

Table 5. *Number of days from picking to climacteric peak under different oxygen tensions.*

Exp. number	Date of picking	Per cent oxygen 2.5	Per cent oxygen 5.0	Per cent oxygen 10.0	Per cent oxygen Air
3	4–19–43	17	12	10	8
6	6–16–43	10	10	7	7
7	1–11–44	18	15	11	10
8	7– 4–44	12	10	9	7

It appears from a comparison of the early season experiment 7 with the late season experiment 8 that the time interval under consideration may be a function of fruit maturity. With the exception of experiment 6 the time lapse between picking and climacteric peak was nearly twice as long in the lowest oxygen treatment as compared to air. This relation was borne out by other experiments not reported here.

The general relationship between CO_2 production and the oxygen content of the storage atmosphere, as described above, was found to hold consistently in all the tests conducted throughout the picking season of the Fuerte variety. In some experiments a wider range of oxygen concentration was selected. In experiment 7 (fig. 2) the oxygen tension varied from 0.5 per cent to 20.8 per cent in air. Besides the treatments indicated in this figure, 1 per cent and 2 per cent oxygen were also employed, giving results intermediate between 0.5 per cent and 2.5 per cent O_2. The exact analyses of the gas mixtures included in this experiment and not shown in table 1 are 0.60, 1.25, 1.59, and 2.06 volume per cent for jars 9, 10, 14, and 16, respectively. The avocados for this experiment were picked on January 11, and placed the same day at 15°C. The weights of the samples varied from 5050 to 5295 grams, or a variation of 2.5 per cent from the mean. For the first two days all jars were under air. Because of close agreement in CO_2 evolution for the 8 jars, the averages only are presented for January 12 and 13. The first striking feature of these results is the initial high value of CO_2 production following picking. The magnitude of the initial rate may be ascribed to a respiratory condition of the fruit on the tree, the effects of which are carried over after picking. It is unlikely that it could be related to environmental factors, such as the temperature in the orchard at harvesting, because it was in the month of January that the initial rates were twice as high as in July. They varied from an average of 110.5 for experiment 7 to an average of 61.4 for experiment 8. To obtain a clearer insight into this problem it would seem advisable to follow the respiratory behavior of the fruit prior to, as well as following, harvesting.

Differential treatment for experiment 7 was started on January 13. The rate of CO_2 evolution kept on declining in all cases until a preclimacteric minimum was reached which varied for the different oxygen concentrations. The magnitude of this minimum appeared to be more constant throughout the season than the initial rates. If we take as an example the control jars, the minimum respiration rate fluctuated from 48.1 in experiment 5 (5/29/43) to 69.1 in experiment 7 (1/15/44). For 5 per cent O_2 this value varied from 27.2 to 36.2 mg CO_2 per kg-hour. When the lowest rates of CO_2 production are considered, it was found that they bear about the same quantitative relation to oxygen tension as do the climacteric maxima. In the case of 0.5 per cent O_2, the increase was so gradual that one could not be sure that it was beyond experimental error. For all the other oxygen concentrations the peak respiration rates varied from 33.1 for 1.0 per cent O_2 to 163.0 for air, and the comparable interval between picking time and the peak was 18 days in the former and 10 days in the latter. As far as fruit softening is concerned, this experiment confirmed the previous one for the four highest oxygen concentrations. In the container subjected to 2 per cent O_2 only half of the fruit were edible on February 8, when the experiment was completed. The percentage of soft fruit was even smaller in the lower oxygen treatments.

In order to obtain a more complete picture of oxygen effects on avocado respiration, experiment 8 was designed to include oxygen concentrations higher than air. The results of this experiment are shown in figure 3, which also includes the respiration trends under previously employed conditions as well as the response to 100, 50, and 15 per cent O_2. Two jars were used for each of these new oxygen concentrations. The gas analysis of the pure oxygen cylinder showed 99.42 per cent O_2, the 50 per cent O_2 was actually 46.68 per cent and the 15 per cent amounted to 14.76 per cent. Because of rapid changes at high oxygen levels, the respiration determinations were carried on twice each day. In this test there were only 15 fruit per jar weighing from 3905 to 4110 grams per sample with a deviation of ±2.5 per cent from the mean. Since the material for this experiment consisted of late season fruit, the initial rates were a good deal lower than in experiment 7, and consequently the decrease in the rate of respiration from the initial to the minimum was less pronounced. The respiratory activities of the 50 per cent and 100 per cent oxygen samples were characterized by an immediate rise after differential treatment commenced. The rates for the two highest concentrations were almost identical, and the peak value was slightly but not significantly higher in 50 per cent than in 100 per cent oxygen. Neither of these treatments differed greatly from air. The climacteric peaks took place on the same day, and the fruit softening was only slightly delayed in air as compared with 50 per cent and 100 per cent O_2. On the ninth day, July 14, all the fruit in the 50 and 100 per cent oxygen jars were soft, edible, and in excellent condition, while in the air jar there were four avocados which were not quite edible. The behavior of the avocados under 2.5 per cent, 5.0 per cent, and 10 per cent O_2 was not much different from that in previous experiments. In 15 per cent oxygen the fruit response with respect to softening as well as respiration was intermediate between air and 10 per cent O_2.

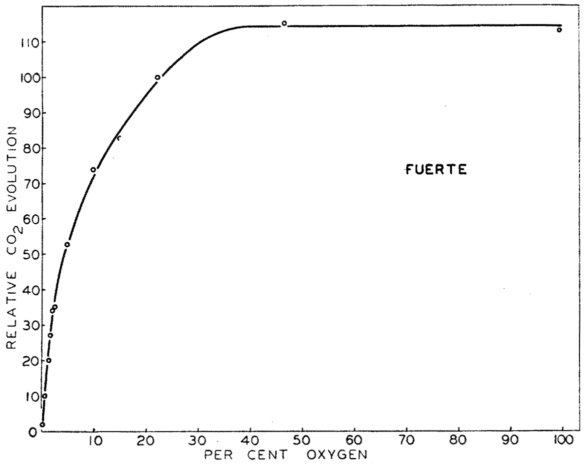
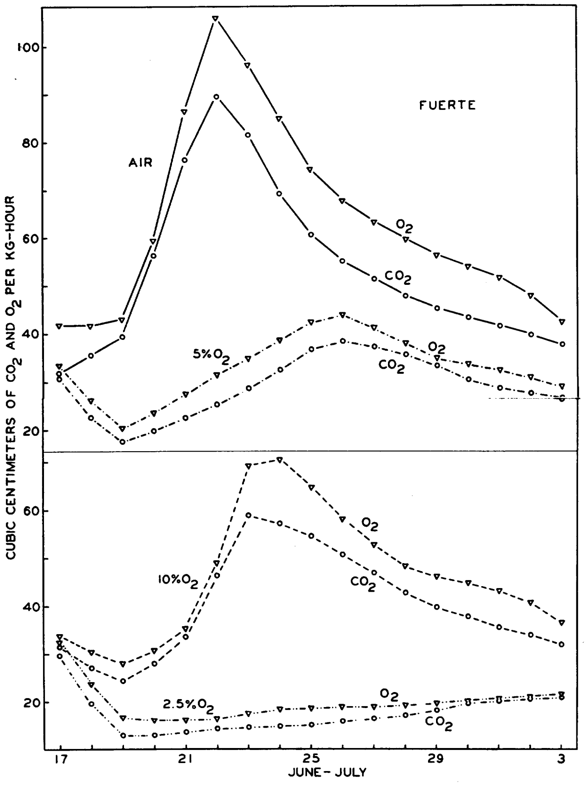


Fig. 4. Relative respiration rates of Fuerte avocados for the climacteric peak at 15°C. in relation to oxygen concentration. (Air value equals 100.)

It is of interest to examine briefly the carbon dioxide production of jar 8, which was kept in pure nitrogen freed of residual oxygen by passage over hot copper. An immediate rise in CO_2 evolution was followed by a sharp decline until the rate leveled off to a value of about 5 mg CO_2 per kg-hour. After 8 days, on July 13, a putrid odor emanated from this jar. After 14 days (on July 19) the fruit was transferred to air, resulting in a 3-fold increase in CO_2 production. The fruit rapidly turned dark brown, became subject to attack by fungi, and it never softened. Clearly, the avocado is highly sensitive to anaerobic conditions.



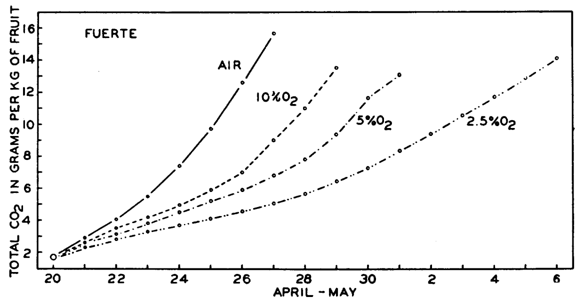


Fig. 5 (above). Simultaneous measurements of oxygen absorption and carbon dioxide evolution of Fuerte avocados at 15°C. in relation to oxygen tension.—Fig. 6 (below). Cumulative carbon dioxide production by Fuerte avocados at 15°C. as related to oxygen tension. Initial to climacteric peak values plotted.

In order to relate respiration rates to oxygen tension, the peak values of CO_2 production were selected from the several experiments and plotted against volume per cent of O_2 (fig. 4). Respiration in air for each test was taken arbitrarily as 100. For the anaerobic condition, the CO_2 value chosen was the one arrived at after the rate leveled off, since no climacteric rise took place in nitrogen. If that value were obtained within twelve hours or even one day after transfer from air to nitrogen, the curve would have assumed a different shape. We would observe then a critical oxygen concentration of 0.5 or 1.5 at which the CO_2 evolution is at a minimum. Before a final decision is reached on this point it may be necessary to find out what time period must be allowed for the change from one environmental condition to the next one. If this interval is prolonged, certain toxic substances may accumulate due to the absence of oxygen and suppress the CO_2 evolution below the rate characteristic for the anaerobic process. Insufficient data are available to indicate whether this was the case with the material under consideration, but by comparison with other fruits one is justified in selecting the value for CO_2 evolution after the rate became constant. However, irrespective of the actual magnitude of the anaerobic CO_2, the respiration rate rises sharply in the low oxygen tensions until about 5 per cent O_2. Above that concentration the slope of the curve decreases until a value of approximately 35 per cent O_2 is reached, above which there is no change. Actually the difference between air and the higher oxygen tensions is hardly significant. While the curve was selected for the maximum respiration rates, a similar relation was obtained for the preclimacteric minima whenever they could be detected. In the high oxygen atmospheres the curves rise almost immediately after the commencement of differential treatment. A continuous recording of CO_2 production might facilitate detection of the minima even where the changes are rapid. Another possibility would be to conduct the experiment at a somewhat lower temperature, but in this case the measurements for the low oxygen tensions may be too small to show significant differences. However, in comparing the respiration-oxygen tension curve with the curves for respiration-time at different oxygen concentrations (fig. 2, 3), it is readily seen that the former represents the trends reasonably well, not only for the climacteric peak but throughout the course of respiration.

Effect of oxygen concentration on the respiratory quotient at 15°C.—In the experiments described thus far, the carbon dioxide evolution was used as an index of the respiratory activity of the fruit. The question arises as to what extent this index represents the aerobic process, and whether a certain percentage of the CO_2 measured may be accounted for by reactions other than complete oxidation. A definite answer to this question can be obtained by parallel determinations of the gas exchange and the chemical analyses of the end products formed. However, a suggestion as to the nature of the respiratory process may be gained by simultaneous measurements of CO_2 evolution and O_2 absorption. For this purpose the procedure worked out by Haller and Rose (1932) was adopted with some modifications (Masure, 1938; Hansen, 1942). The respiration jar was provided with a 200 ml funnel inserted through a hole in the bottom. This funnel was filled with 100 ml of 2 N KOH through one of the glass tubes in the glass top of the jar. Then all the open ends were closed with the exception of one of the tubes, which was connected to a 500 or 1000 ml graduated cylinder of oxygen under constant hydrostatic pressure. At the end of the measurement period, the volume of oxygen displaced was recorded, and the funnel of alkali was emptied into a 500 ml volumetric flask and rinsed twice with 170 ml H_2O. After making up to volume, 15 ml aliquots were pipetted into hydrometer cylinders, to which 200 ml H_2O and 10 ml of 1.5 N $BaCl_2$ were added and titrated with 0.15 N HCl. This method of measuring CO_2 evolution in a closed system (referred to as method II) was compared with the previously described method I, in which a continuous stream of air was passed over the fruit and through the absorbing solution. The comparison made for air, 10, 5, and 2.5 per cent O_2 showed close agreement with deviations of the order of 2 per cent.

This simultaneous measurement of CO_2 evolution and O_2 absorption was used in several tests, of which experiment 6 can be cited as representative. In this experiment (fig. 5) the fruit was picked on June 16, 1943, and placed for one day in air at 350 ml/min. Differential treatment started on June 17 after the first CO_2 determination. While oxygen absorption was measured, the air and gas mixtures were discontinued and the jars connected to the oxygen graduates. Length of test varied from one and one half to four hours, depending on the intensity of respiration. Figure 5 shows that the nature of the curve for O_2 absorption did not differ materially from that for CO_2 evolution. The minimum and climacteric values as well as the slopes of the curves were approximately the same. The actual magnitudes were plotted in ml instead of mg of CO_2 so that comparisons might be drawn more readily. The O_2 rates were consistently higher than the CO_2 rates, resulting in a respiratory quotient (R.Q.) of less than one. In the case of the fruit in air there was a slight tendency for the R.Q. to increase until June 21, that is, until two days before the climacteric peak when the maximum R.Q. of .948 was reached. The subsequent course is that of a gradual decrease. A similar pre-climacteric condition was observed for the 10 per cent O_2 treatment. With the 5 per cent O_2 there was a decrease from .922 on June 17 to .806 on June 22, followed by an increase to .960 on June 29 after the climacteric peak. In 2.5 per cent O_2 there was a consistent rise from .784 on June 19 to .985 on July 1. Generally, there appeared to be somewhat higher R.Q. values in the lower oxygen concentration, but the differences were not large enough to suggest basic deviations from the normal respiratory process.

Total carbon dioxide production as affected by oxygen concentration.—In all the experiments on avocado fruit respiration it has been observed that under aerobic conditions the climacteric rise is a constant feature. The slope of the curve and the magnitude of the maximum can be altered by external factors, but they cannot be eliminated. It is intriguing to conjecture as to what it is that induces the sudden rise and what determines the time for the peak. If the highest rate of respiration coincides with the disappearance of certain substrates, then all the fruit samples in one lot may be expected to produce the same quantities of the end product irrespective of treatment. Attempts were therefore made to find out what are the total amounts of CO_2 formed between the first reading and the climacteric peak. The figures obtained will be referred to as cumulative CO_2 values. In experiment 3 (fig. 6) the hourly rates shown in table 4 were multiplied by 24 and each value added to the previous one, so that the final points on each curve give the total amount of CO_2 evolved up to that date. For those days in which no respiration measurements were available, interpolated rates were found on the respective graphs. The average CO_2 accumulated for 2.5, 5, and 10 per cent O_2, and for air was 14.10 grams per kg of fruit with a deviation of 11 per cent from the mean. This may appear to be high variability, but it should be remembered that the respiration of the last day, when the climacteric peak occurred, was at a maximum and could contribute greatly to the error. This was particularly true in the case of the control, because of the short time interval between the initial reading and the climacteric maximum. If the peak occurred early in the day and the CO_2 was calculated for the entire 24 hour period, the error could well be considerable. The fruit in the air jar, for example, produced on the day of the peak (April 27) 3.03 grams CO_2, which was 19.3 per cent of the total accumulated up to that day. This source of error doubtless could be eliminated by a continuous recording of the respiration results.

The cumulative CO_2 values for the experiment just described were compared with those from several other tests and are presented in table 6.

In experiment 8 the cumulative CO_2 for several additional oxygen concentrations was calculated and found to be 14.39, 15.98, and 17.55 for 15, 50, and 100 per cent O_2. Here again the deviations from the mean are most conspicuous for the higher oxygen tensions, and the explanation for this behavior may be the same as presented above in connection with the discussion of figure 6. There is an indication in this table that the cumulative CO_2 from initial to climacteric peak decreased as the season advanced. This is particularly true for the 1944 season, because an early sample of ex-

Table 6. *Cumulative CO_2 values in grams per kilogram of fruit.*

Exp. number	Date of initial reading	Per cent oxygen 2.5	5.0	10.0	Air	Average
3	4–20–43	14.10	13.12	13.54	15.65	14.10
5	5–26–43	11.95	....	....	13.78	12.87
6	6–17–43	11.89	12.70	11.33	13.87	12.45
7	1–12–44	19.19	23.36	20.62	23.77	21.74
8	7– 5–44	13.88	14.64	15.56	15.03	14.79

periment 7 could be compared with the late sample of experiment 8. Whether this difference can be accounted for by the depletion of some respiratory substrate cannot be determined at the present time. It is known that the sugar content of avocados decreases with the advance of the season, but it will be pointed out in the discussion that free sugars cannot account for the total CO_2 produced.

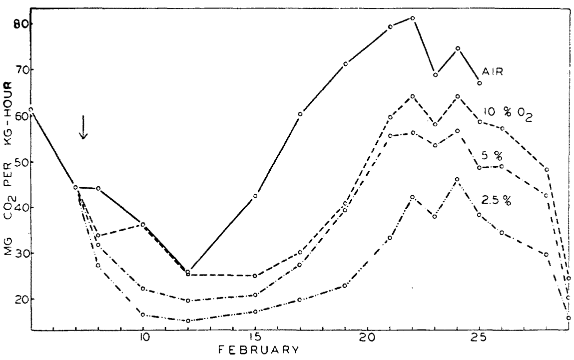
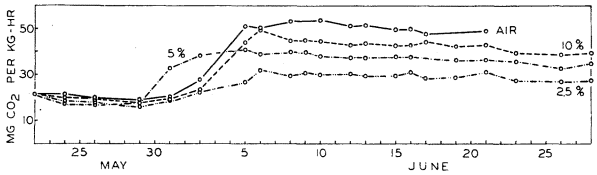


Fig. 7. Effect of oxygen tension on respiration of Fuerte avocados at 10°C. (Arrow indicates beginning of differential treatment.)

Oxygen tension effects in relation to temperature.—The results of experiments at 15°C., as described above, were compared with observations on the effects of oxygen on avocado respiration at 10°, 7.5°, and 5°C. Two or more tests were conducted at each of these temperatures. In experiment 9 the fruit was kept at 10°C. until it passed the climacteric stage and became edible, while in experiment 10 the fruit was placed under air at 15°C. after two weeks of storage in different oxygen concentrations at 10°C. The avocados for experiment 9 were placed in air on February 3, 1944, and differential treatment started on February 7, following the respiration measurements. The results in figure 7 indicate an initial value as high as in most experiments at 15°C. The rate of decrease from the initial is very marked with a slope steeper than that for the climacteric rise. The time interval between the initial and preclimacteric minimum was from eight to eleven days, while at 15°C. it varied from two to four days. The period from picking to the peak in respiration in the case of air and 10 per cent O_2 was twice as long at 10°C. as at 15°C. However, the differences between treatments were slight as far as the time required to reach the peak was concerned. At the minimum stage the actual respiration magnitudes were significantly different from the control for 2.5 and 5 per cent O_2, but not for 10 per cent O_2. The relatives rates of CO_2 production at the peak were 100, 79, 70, and 55 for the progressively decreasing oxygen concentrations. Reduced oxygen also had a marked influence on the softening process. On February 25 (after 22 days) most of the fruit in the control jar was softening, with some in the soft edible stage. On March 4 (after 30 days of storage) the avocados in jar 18 (2.5 per cent O_2) were found to be only slightly soft. Fruit quality was good in all cases.

The effects of change of temperature coupled with transfer to air were observed in experiment 10. Since the avocados for this experiment were picked six weeks later than for experiment 9, the onset of the climacteric rise took place after eight days in air, 10, and 5 per cent O_2. The rise in 2.5 per cent O_2 was very slight during the two weeks at 10°C. Hence the three highest O_2 concentrations exhibited a generally decreasing rate of CO_2 evolution when transferred to air at 15°C., while the fruit in the 2.5 per cent O_2 showed the typical rise followed by a drop. When the experiment was com-



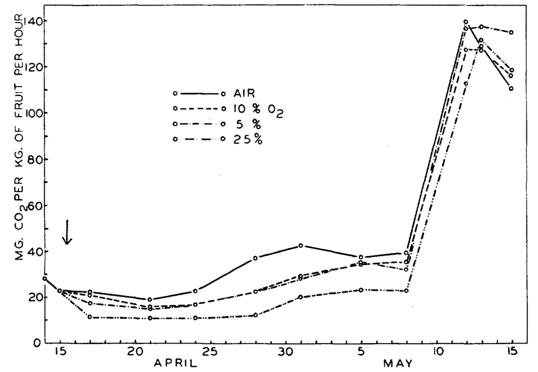


Fig. 8 (above). Effect of oxygen tension on respiration of Fuerte avocados at 7.5°C.—Fig. 9 (below). Effect of oxygen tension on respiration of Fuerte avocados at 7.5°C. (Arrow indicates beginning of differential treatment. On May 11 all jars transferred to air at 15°C.)

TABLE 7. *Respiration in air at 15°C. after storage in different* O_2 *at 5°C. (a = 49 days at 5°C., b = 25 days at 5°C.).*

Condition of storage % O_2	Days after transfer from 5°C.					
	First		Second		Third	
	a	b	a	b	a	b
2.5	129.3	105.8	147.4	121.7	147.6	130.7
5.0	154.7	116.6	166.1	133.3	130.1	139.8
10.0	148.8	114.3	149.7	126.8	140.7	125.3
air	131.3	111.4	136.7	115.2	120.6	127.6
	Fourth	Fifth	Sixth		Seventh	
	b	a	a	b	b	
2.5	136.6	136.1	122.1	116.5	112.5	....
5.0	145.9	....	110.1	111.3	104.9	....
10.0	134.6	129.8	108.8	110.6	102.4	....
air	138.3	112.9	89.4	115.9	107.4	....

pleted on April 4, the fruit in the containers with the two lowest oxygen concentrations were in edible state and of much better appearance than the control, in which brownish discoloration of the skin was recorded.

Since marked differences were observed between the respiratory behavior of avocados at 5°C. and at 10°C., it became advisable to study oxygen effects at narrow intervals of temperature. Three experiments were conducted at 7.5°C. In experiments 11 and 12 the fruit was transferred to 15°C. after 26 and 24 days of storage, respectively, while in experiment 13 softening took place at 7.5°C. The avocados for the last test were subjected to differential treatment on May 22. The trends in respiration are illustrated in figure 8, showing a somewhat different picture than in previous experiments. The rise in CO_2 evolution started one day earlier in the 5 per cent O_2 jar than in the other treatments. This anomalous behavior may be explained by the presence of one or more avocados that were considerably more advanced in their softening process. Since there is no sharp peak in the respiration curves at this temperature, comparisons can be made only for the different levels of CO_2 production. The relative maximum rates were found to be 100, 84, 72 and 60 for air, 10, 5, and 2.5 per cent O_2 respectively; the differences recorded for the minima were hardly significant. The softening process was prolonged markedly as compared with previously described tests at 15°C. and 10°C. There was a time lapse of a week to 10 days in softening between the treated samples and the control. After 41 days almost all the avocados in 2.5 per cent O_2 were edible and of better quality than the fruit subjected to air. However, the differences between the several oxygen concentrations were less marked.

In two other experiments the fruit was transferred from 7.5°C. to air at 15°C. In experiment 12 (fig. 9) this happened on May 11, 25 days after picking. The response of the fruit to this change differed with treatment. In the control the rate of CO_2 evolution was on a definite decline; in the 5 and 10 per cent O_2 there was little difference in rate between May 12 and May 13, followed by a decided decrease. On the other hand, the fruit subjected to 2.5 per cent O_2 went through the climacteric cycle. These trends of the several jars can be traced to their behavior at 7.5°C. prior to the transfer. The control was the only fruit which appeared to have a somewhat more defined peak in respiration on May 1, while in the remainder the increase was gradual without any typical climacteric. When the experiment was discontinued on May 16 (after 33 days) the fruit subjected to 5 and 10 per cent O_2 was in best condition, uniformly soft and edible, with excellent flavor, color, and texture. There was slight browning in the 2.5 per cent O_2 treatment, while the control avocados did not soften as uniformly as the rest and showed more of the brownish discoloration.

From the tests at 10°C. and 7.5°C. it seems evident that the differences between the various oxygen concentrations are not so pronounced as at 15°C. This behavior was borne out even more strikingly in the studies at 5°C. In experiment 14 the fruit was picked on April 6, 1943, subjected to different oxygen concentrations on April 10, and left for 49 days under this treatment at 5°C. On May 26 all the jars were transferred to air at 15°C. In experiment 15 the fruit was received on May 26, 1943, and placed in air at 5°C. for two days. Differential treatment lasted from May 28 to June 22, that is, for 25 days, after which time all jars were transferred to air at 15°C. The rate of CO_2 evolution was of the order of 10 to 20 mg per kg-hour, showing little or no response to oxygen tension. When the fruit was placed under air at 15°C., there was a considerable difference in the course of respiration for these two experiments, as shown in table 7.

In experiment 15 (designated by "b" in the table) all the avocados irrespective of treatment showed upon transfer an increasing rate of respiration, while in experiment 14, with twice as long a storage period, the 2.5 and 5 per cent O_2 fruit behaved similarly. The fruit subjected to 10 per cent O_2 and air had a declining rate of CO_2 evolution, indicating a post-climacteric behavior. They apparently had undergone physiological changes at 5°C. that are characteristic of the climacteric phase, even though this could not be detected by the respiration measurements. Pratt and Biale (1944) have shown by the triple response of pea seedlings that such changes may be observed at 5°C. The condition of the fruit in experiments 14 and 15 at the end of the softening period was not satisfactory. A large percentage of the avocados did not soften properly, with discoloration occurring both on the surface as well as inside the flesh. Treatment with decreased oxygen had not materially improved the condition of the fruit when subjected to 5°C.

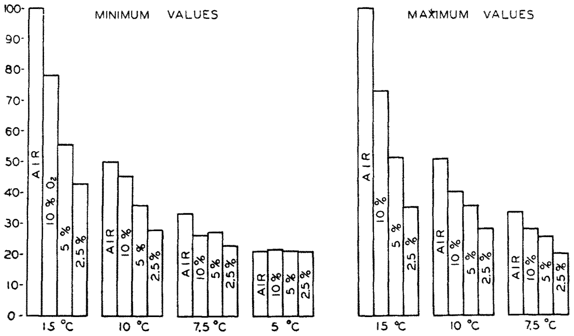


Fig. 10. Relative respiration rates of Fuerte avocados under different oxygen pressures in relation to temperature.

By way of summary, figure 10 is included to show a diagrammatic comparison of the effects of oxygen for the several temperatures. The lowest and the highest respiration values were selected. The preclimacteric minimum and the climacteric maximum magnitudes for air at 15°C. were taken as 100 and all other values computed with reference to those rates. Since no definite increase took place at 5°C., only minimum rates are given for that condition. This diagram illustrates clearly that with a decrease in temperature, oxygen tensions of the range used cease to be the determining factors for the rate of CO_2 evolution of Fuerte avocados.

Discussion of results.—The results of the investigations presented in this paper point to a certain relationship between the effects of oxygen and temperature on the respiration rates of Fuerte avocados. At low temperatures the effects of oxygen concentrations are minimized with the consequence that the temperature coefficient (Q_{10}) is markedly different for the several oxygen levels. Thus at 2.5 per cent O_2 the Q_{10} for the minimum respiratory rates in the range of 5°C. to 15°C. has a value of two, while the corresponding Q_{10} magnitude in air is two and one half times as high. The temperature coefficient in this range for the peak respiration rates cannot be determined with precision because of the uncertainty in establishing the exact date of the climacteric maximum at 5°C. However, an approximation can be obtained by following the effects of avocado emanation on pea seedlings along with the CO_2 measurements. As indicated above the maximum response of the etiolated seedlings coincides with the climacteric rise and takes place at 5°C. as well as at higher temperatures. If the respiration rates corresponding to the maximum pea response are selected, we find the Q_{10} in the range of 5°C. to 15°C. for the peak values to vary from approximately four in 2.5 per cent oxygen to about eight in air.

From the standpoint of fruit storage this interrelation between temperature and oxygen tension effects suggests the advisability of using temperatures higher than 5°C. for storing Fuerte avocados in subnormal oxygen atmospheres. The use of fairly high temperatures is often correlated with better fruit appearance after removal from storage. It may also mean considerable reduction in chilling injuries to sensitive varieties. Wardlaw and Leonard (1935) reported marked chilling effects for some West Indian avocados when exposed for fifteen days to 53°F. (11.7°C.). These effects are frequently associated with browning of the skin and discoloration of the flesh. They ascribed the skin symptoms in certain varieties to subnormal oxygen, while the condition of the flesh was thought to be due either to high CO_2 or to a combination of high CO_2 and low O_2. These physiological reactions were found to vary for different varieties ranging from high resistance to complete sensitivity to subnormal oxygen or modified air storage. In the case of the Fuerte variety as grown in Southern California the available results appear to show that chilling injuries are limited to temperatures below 7.5°C.

These low temperature effects introduce complications in determining the storage life of avocados. In this work we have used the course of respiration as a criterion for the condition of the fruit. Softening followed the peak in CO_2 production in air as well as in the different oxygen atmospheres employed. Wardlaw and Leonard (1935) claimed that the climacteric rise is not correlated with the color change in coloring varieties or with softening of the flesh. They based this conclusion on the fact that the above manifestations occurred after the peak in respiration was attained. We know that at least softening in the non-coloring Fuerte variety is always a function of the incidence of the climacteric. The fact that complete softening occurs after the respiration maximum does not preclude the possibility of an earlier initiation of the process. As far as storage behavior is concerned, the relevant point is that delayed climacteric rise means delayed maturation, and that this condition can be brought about by reduced oxygen.

In addition to respiration rates some workers have used total or cumulative CO_2 as a criterion for storage life. Kidd and West (1930) calculated the total amount of carbon dioxide produced by apples between picking and death by fungal attack. They found this quantity to be reasonably constant for several fruit samples stored at three different temperatures. When converted to equivalent sugars oxidized, they observed that only 16 to 20 per cent of the reserve carbohydrates were used up. Similarly, Smock (1942) investigated the total amount of

CO_2 evolved by McIntosh apples in ordinary cold storage as compared to controlled atmosphere storage. The cumulative CO_2 values between picking and the end of storage life (based on marketability) were approximately constant under the two treatments, but they varied somewhat from one season to the next.

In the case of the Fuerte avocado we found a condition similar to that for apples, but insufficient data are available to recommend the cumulative CO_2 values rather than the rate of respiration as a storage criterion. By using the climacteric peak as the index of storage termination a more objective evaluation was employed. One of the reasons for calculating cumulative CO_2 was to ascertain whether sugars could account for the CO_2 evolved. If the value of 21.74 grams of CO_2 is selected from table 7 for a sample of avocados picked in January, and if the assumption be made that the process involved is that of complete oxidation, the sugars consumed would amount to 14.8 grams or approximately 1.6 per cent of the fresh weight of the flesh. This is a much higher sugar content than that reported by Church and Chace (1921) for January fruit. Moreover, they found that sugars were not depleted during storage. There is also some evidence available that fats could not account for the respirable substrates either. It appears that more complete biochemical data are needed to explain the physiological changes described in this paper.

SUMMARY

The effects of oxygen on CO_2 evolution by Fuerte avocados were studied in the range of 0 to 99.4 per cent O_2 at 15°C. At 10°, 7.5°, and 5°C. the response to 2.5, 5.0 and 10.0 per cent oxygen was compared with the behavior in air. Apparatus for preparing the gas mixtures is described, as is the effect of different rates of flow.

When the oxygen concentration is reduced to values below that of air, the rate of CO_2 evolution is markedly affected, with the climacteric peak delayed and suppressed in magnitude. At oxygen tensions higher than air no significant differences occurred as compared with the controls. Under anaerobic conditions the rate of CO_2 production is markedly inhibited with the climacteric rise completely lacking. When the oxygen concentration affected the respiratory course, there was also a pronounced influence on fruit softening. At 2.5 per cent O_2 it took approximately twice as long for the fruit to soften as in air.

The rate of oxygen absorption by the fruit was studied at 15°C. under 2.5, 5.0, and 10.0 per cent O_2 and in air. It was observed to follow closely the rate of CO_2 production with some tendency for a higher respiratory quotient at low oxygen tension.

The total amount of CO_2 given off by the fruit during the period ending with the climacteric peak was found to be approximately constant for the several modified atmospheres at 15°C.

The effects of oxygen tension on respiration were markedly modified by temperature. Differences in CO_2 evolution of the fruit due to the several treatments were most pronounced at 15°C. and least at 5°C.; at 10°C. and 7.5°C. the respiration rates of the avocados were less affected by oxygen than at 15°C. but considerably more than at 5°C. Fruit softening was found to be closely related to the respiration trends.

Division of Horticulture,
University of California,
Los Angeles 24, California

LITERATURE CITED

Biale, J. B. 1941. The climacteric rise in respiration rate of the Fuerte avocado fruit. Proc. Amer. Soc. Hort. Sci. 39: 137–142.

———, and A. D. Shepherd. 1941. Respiration of citrus fruits in relation to metabolism of fungi. I. Effects of emanations of *Penicillium digitatum* Sacc. on lemons. Amer. Jour. Bot. 28: 263–270.

Church, C. G., and E. M. Chace. 1921. Further studies on maturity of avocados. Calif. Avocados Assn. Ann. Report for 1920–21: 45–58.

Claypool, L. L. 1938. Internal gas in fruits as influenced by external treatments. I. Carbon dioxide. Proc. Amer. Soc. Hort. Sci. 36: 374–378.

Haller, M. H., and D. H. Rose. 1932. Apparatus for determination of CO_2 and O_2 of respiration. Science 75: 439–440.

Hansen, E. 1942. Quantitative study of ethylene production in relation to respiration of pears. Bot. Gaz. 103: 543–558.

Kidd, F., and C. West. 1930. Physiology of fruit. I. Changes in the respiratory activity of apples during their sensecence at different temperatures. Proc. Roy. Soc. London B 106: 93–109.

———, and ———. 1934. The influence of the composition of the atmosphere upon the incidence of the climacteric in apples. Dept. Sci. Ind. Res. Food Inv. Board Rept. for 1933: 51–57.

Masure, M. P. 1938. Some comparisons of methods of measuring fruit respiration. Proc. Amer. Soc. Hort. Sci. 36: 223–229.

Meuron, H. J. 1941. Rapid method for calibration of flowmeters. Ind. Eng. Chem. Anal. Ed. 13: 114.

Parija, P. 1928. Analytical studies in plant respiration. II. The respiration of apples in nitrogen and its relation to respiration in air. Proc. Roy. Soc. London B 103: 446–490.

Pratt, H. K., and J. B. Biale. 1944. Relation of the production of an active emanation to respiration in the avocado fruit. Plant Physiol. 19: 519–528.

Singh, B. N. 1937. The response of the respiratory system in mango and guava to alteration in the concentration of oxygen and nitrogen. Annals Botany n. s. 1: 311–323.

Smock, R. M. 1942. Influence of controlled atmosphere storage on respiration of McIntosh apples. Bot. Gaz. 104: 178–184.

Wardlaw, C. W., and E. R. Leonard. 1935. The storage of avocado pears. Imperial College of Tropical Agriculture, Low Temp. Res. St. Memoir No. 1.