

PLANT PHYSIOLOGY

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RESPIRATORY ACTIVITY AND DURATION OF LIFE OF
APPLES GATHERED AT DIFFERENT STAGES OF
DEVELOPMENT AND SUBSEQUENTLY MAIN-
TAINED AT A CONSTANT TEMPERATURE¹

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(WITH TWENTY-ONE FIGURES)

In Part I of this series (9) it was shown that the respiratory activity of an apple gathered at "maturity" in the autumn and subsequently kept at a constant temperature was characterized by a rise followed by a fall. This rise in respiratory activity was attributed to a change of state in the protoplasm. It was considered to mark the onset of senescence and was termed the "climacteric." It is interesting to inquire whether the senescent rise occurs in fruit picked in an immature condition.

Outline of the main experiment, 1925

In 1925, Bramley's Seedling apples were taken at intervals during their period of growth from selected trees in an orchard near Cambridge. They were immediately brought into the laboratory and placed in glass containers held at a constant temperature of 12° C. and ventilated by a constant stream of CO₂-free air of constant humidity. Under these conditions the rate of CO₂ production was followed for as long a period as the fruit remained sound; *i.e.*, free from the attack of fungi. The details of the various gatherings are given in table I. The temperatures in the orchard are given in table II.

The glass containers used in these experiments were approximately 700 ml. in volume and were originally designed to hold one fully grown apple. Single apples were the experimental units in the case of the later gatherings. In the case of the earlier gatherings the experimental units consisted of more than one apple (table I). They were ventilated at a rate of about 4 liters an hour, the incoming CO₂-free air being passed through soda lime and through a wash bottle containing a 7 per cent. solution of sodium hydroxide. The number of fruits per container was so arranged that the rate of evolu-

¹ This is the second of a series of papers on the general subject of "Physiology of Fruit."

tion of carbon dioxide per container should be as far as possible the same in all experiments, and in any case not more than 1.5 ml. per hour.

The measurement of carbon dioxide was made by the Pettenkofer tube absorption method, using N/10 barium hydroxide as the absorbent. Subsidiary to the main plan of the research, as stated above, a number of additional experiments of an exploratory nature were conducted.

TABLE I
GATHERINGS OF 1925

GATHERING	DATE	HOURS FROM ZERO TIME	LOT. NO. OF SAMPLES	NUMBER OF APPLES IN SAMPLES	AVERAGE WEIGHT OF APPLE	REMARKS
I	May 26	0	1	6	0.76	
			2	9	0.65	
II	June 4	216	3	10	2.90	"Cleaned"
			4	10	3.18	
			5	10	3.00	
III	June 18	556	6	5	20.5	
			7	5	21.8	
			8	10	21.2	
IV	June 30	844	9	4	30.4	
			10	4	33.8	
			11	4	33.2	
			12	4	32.2	
V	Aug. 4	1684	13	2	76.3	
			14	2	70.2	
			15	2	66.5	
			16	1	129.0	
			17	1	80.0	
VI	Sept. 2	2380	18	1	89.1	
			19	1	140.8	
			20	1	124.6	
			21	1	107.8	
			22	1	115.8	
			23	1	110.0	
VII	Sept. 17	2740	24	1	244.7	
			25	1	113.8	
			26	1	118.7	
			27	1	120.3	
			28	1	93.4	
			29	1	55.3	

The measurement of oxygen intake was made by a manometric method. The closed container holding the apple was connected with a similar closed container by a manometer of capillary tubing filled with a solution of eosin in water. The two chambers were similar in size, close together, and shielded from air currents and from radiation. Each container had a tap opening to the air. The bottom of the apple container was covered with a 7 per cent. solution of sodium hydroxide while the sides of both containers were covered with filter paper soaked in this solution. The apple container was also connected by a capillary tube, provided with a stopcock, to a graduated burette filled with oxygen over a 7 per cent. solution of sodium hydroxide.

The whole apparatus was installed in a constant-temperature room at 12° C. In operation the carbon dioxide produced by the apples is absorbed by the sodium hydroxide; the oxygen consumed by the apples causes a fall in pressure. Oxygen is then passed in from the graduated burette until the manometer shows that the pressures in the two chambers are again balanced. In starting an experiment the first reading is taken after putting in the apple and allowing an hour to elapse before closing the taps connecting both the apple chamber and balance chamber to air. The barometric reading is taken at this time and the volume reading on the oxygen burette, with the manometer connecting the balance chamber and the apple chamber at zero. The tap connecting the oxygen burette and apple chamber is then closed. Every ten hours oxygen is run into the apple chamber in order to keep the manometer reading fluctuating about the positive and negative

TABLE II
TEMPERATURES IN THE ORCHARD

HARVESTS AND DATES, 1925	AVERAGE OF MEAN OF DAILY MAXIMUM AND MINIMUM
	°C.
Between gatherings I and II, May 26-June 4	10.9
Between gatherings II and III, June 4-June 18	16.9
Between gatherings III and IV, June 18-June 30	13.0
Between gatherings IV and V, June 30-Aug. 4	17.2
Between gatherings V and VI, Aug. 4-Sept. 2	16.1
Between gatherings VI and VII, Sept. 2-Sept. 17	11.4

sides of zero, corresponding to a fluctuation in oxygen concentration in the apple chamber between 18 and 22 per cent. At suitable intervals the manometer is slowly and carefully adjusted to zero and the second, third, etc., readings on the oxygen burette taken. Alternate sets of readings of intake of oxygen and of output of carbon dioxide are obtained with the same material, by transferring it at intervals from the apparatus for the measurement of oxygen intake to that used for the measurement of CO₂ production.

Results of the main experiment, 1925

All the primary data of the determinations of respiratory activity are presented as graphical records. In these records the rates of CO₂ production per unit fresh weight of apple at the time of gathering are plotted against time, every observation appearing as a point. The time scale is given in hours and in some cases also in days from zero time; namely, 9:30 a.m. on May 26, 1925, the hour and day of the first gathering.

FIRST GATHERING

These were "one-gram" apples gathered May 26, 1925. At this stage the fruits have only just begun to swell after the fall of the petals. The stamens

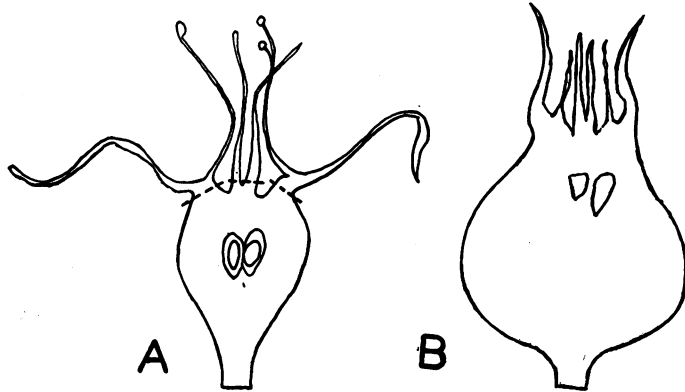


FIG. 1. Diagrammatic median longitudinal section through young apple fruits. Surface hairs not shown. Parts removed from "cleaned" fruits (see text) indicated by dotted lines. A, average weight of fruit 0.7 gm.; B, average weight of fruit 3.0 gm. The same parts were removed in both cases.

persist in a moribund state, dead at their tips, but still alive at their bases. The young fruits are covered with a soft downy coat of fine hairs. Parallel experiments were conducted (a) with fruits intact as gathered (Lot 1); and (b) with fruits from which the downy covering of hairs had been rubbed off and from which the calyx, stamens, and pistil had been removed (Lot 2, "cleaned"). The parts of the fruits removed are indicated in figure 1.

The production of carbon dioxide (fig. 2) was stimulated for a short period by the cutting and cleaning treatment. Subsequent to the first reading, however, the record of the cleaned fruit was 10 to 20 per cent. lower than that of the uncleaned fruit, but similar in form, and continued for a longer time.

The general higher level of the record for the uncleaned fruitlets is most probably due to the fact that in removing the calyx a certain amount of the

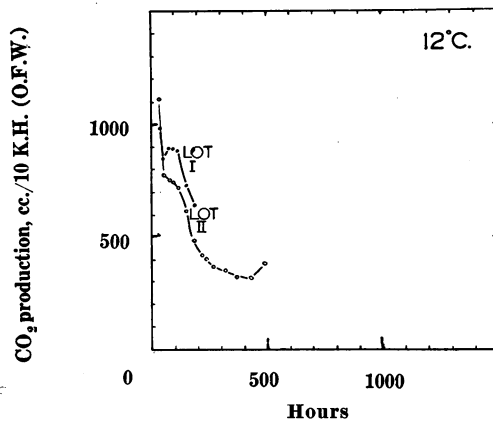


FIG. 2. CO₂ production of Bramley's Seedling apples. First gathering, May 26th. Lot 1, intact fruits; average weight 0.76 gm. Lot 2, "cleaned" fruits; average weight 0.65 gm.

youngest tissue of the growing fruit which is situated at the base of the calyx, was removed. The shorter record obtained in the case of the intact fruit was due to the fact that in their case growth of fungi on the stamen debris became excessive and invasion of the fruit by fungi occurred earlier than in the case of the "cleaned" fruits.

The record for the "cleaned" fruits was continued for 500 hours. The color of the fruits remained green throughout. The final point in the last 100 hours represents a rising trend in respiratory activity which is always associated with invasion by fungi. Several apples of the sample showed mould attack when examined after 500 hours.

This record appears to show two phases. The first phase lasts about 250 hours and is characterized by a rapid fall in respiratory activity broken in its course by a level period or even by a temporary rise. The second phase shows a slow and steady fall in the rate of CO_2 production.

SECOND GATHERING

These were "three-gram" apples gathered June 4, 1925. The fruits still possessed a downy covering of hairs and the sepals were inverted and enclosed the dead stamens. Parallel experiments were again conducted (a) with fruits intact as gathered (Lot 3) and (b) with fruits from which the hairs, sepals, and dead stamens had been removed (Lot 4).

A third lot (Lot 5) was used for the measurement of the CO_2/O_2 ratio. The hairs, stamens, and calyx were removed from this lot also.

The "cleaned" fruits (Lot 5) remained sound for about 3,500 hours, and during this period they showed no change of color from green to yellow. The respiratory activity of these "cleaned" fruits was again about 20 per cent. lower than that of the intact fruit (Lot 3). The intact fruit remained sound for only about 1,000 hours.

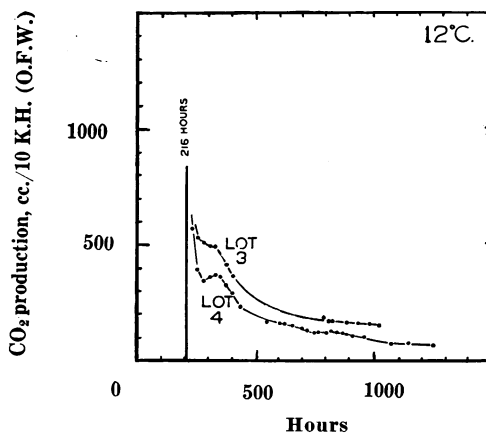


FIG. 3. CO_2 production of Bramley's Seedling apples. Second gathering, June 4th. Lot 3, intact fruits; average weight 2.91 gms. Lot 4, "cleaned" fruits; average weight 2.01 gms.

While the trend of respiratory activity with time is very similar to that shown by the first gathering, the rate throughout is lower at corresponding stages.

The measurements of oxygen uptake show that during the first phase the intake of oxygen closely corresponds to the output of carbon dioxide, the CO_2/O_2 ratio being about 0.9 (fig. 4).

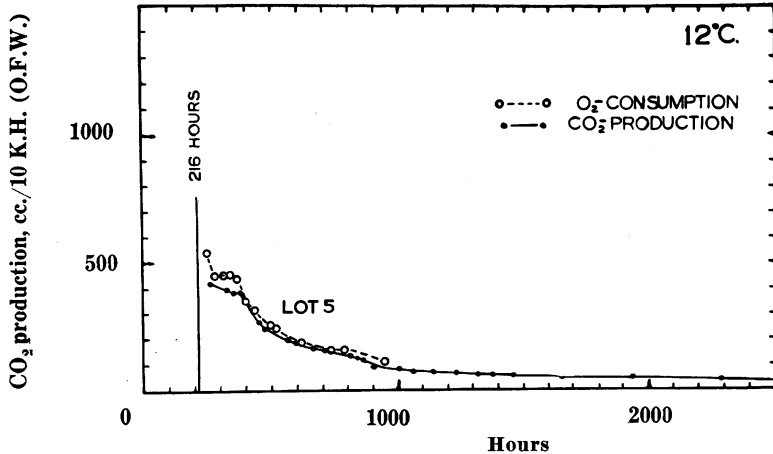


FIG. 4. CO_2 production and oxygen consumption of Bramley's Seedling apples. Second gathering, June 4, 1925; "cleaned" fruits. The measurements of oxygen consumption were discontinued after about 1,000 hours. The rate of CO_2 production showed no measurable change for 1,500 hours beyond the time shown in the figure.

THIRD GATHERING

These were "twenty-gram" apples gathered June 18, 1925. There was now no reason for "cleaning" the apples in any way. Two lots of five fruits (Lots 6, 7) were kept under observation. One of the apples in Lot 6 was attacked by a fungus after about 1,000 hours and was then discarded. All of the apples in the other lot remained sound for about 6,500 hours; *i.e.*, approximately nine months. The last of this group of five apples remained sound for at least 15 months. The apples of this gathering had the longest life of any gathering throughout the experiment.

The trend of respiratory activity at the outset was again very similar to that shown by the previous two gatherings. In the first phase the rise was more pronounced than that occurring in the case of the previous two gatherings. The second phase lasted about 1,200 hours; *i.e.*, until the end of August. A third phase now appeared, lasting $4\frac{1}{2}$ months, during which the steady falling rate was interrupted several times by small rises and falls, each lasting for a short time. At the end of this third phase the fruits were green and sound. The rate then continued to fall steadily (fourth phase) for a further 2,000 hours when a rise in rate of CO_2 production occurred which coincided with the infection by fungi of one of the apples. The loss in fresh weight at this point was 16 per cent. The fruits were still green. The other apples of this lot remained sound for many weeks longer.

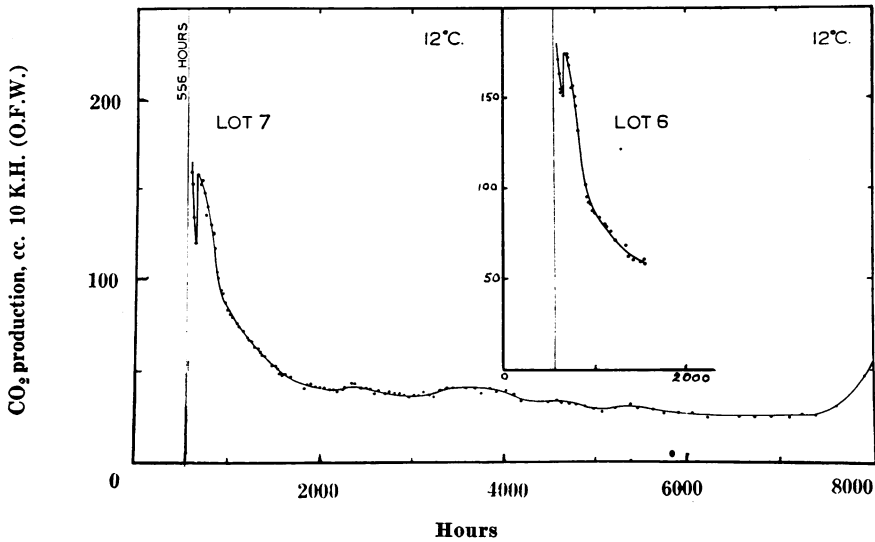


FIG. 5. CO₂ production of Bramley's Seedling apples. Third gathering June 18, 1925; intact fruits.

The series of rises which took place in the third phase are attributed, in the light of the results of later gatherings, to a more or less sudden increase in activity occurring in the case of each of the five individual apples at different times over the period.

As compared with the first and second gatherings the intensity of respiratory activity of the apples of the third gathering was again lower throughout at corresponding stages.

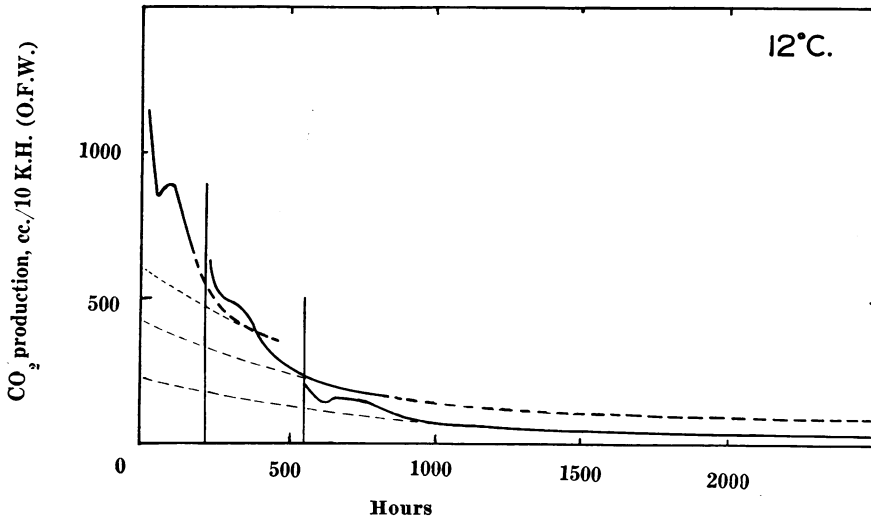


FIG. 6. Respiratory drifts of the first three gatherings (Lots 1, 3, and 7) set out for comparison (diagrammatic).

SUMMARY AND COMPARISON OF GATHERINGS 1 TO 3 OF MAIN EXPERIMENT, 1925

The results of the first three gatherings are brought together in figure 6, in which the plotting of the third gathering is not extended beyond the limits of the second phase. We have, in this figure, suggested by the use of "construction lines" a significant difference in character between the first and second phases in the downward drift of respiratory activity. We consider them to be distinct phases in a process of adjustment to starvation conditions.

A point of interest is that the CO_2 production (expressed as activity per unit fresh weight) of fruit growing normally on the tree, falls off at practically the same rate as that of gathered fruit, the growth of which ceases and which is kept under starvation conditions in the dark at 12°C .

FOURTH GATHERING

The apples have now increased in size to an average weight of about 30 grams; the gathering was made on June 30, 1925. Two lots of four fruits were kept under observation (Lots 9, 10). Lot 9 was used for analysis on August 5, 980 hours after starting the experiment. All apples of Lot 10 remained sound for about 6,500 hours.

A first phase lasting about 350 hours could again be distinguished, but compared with the previous gatherings, there was only a slight indication of a rise in rate during this phase. The second phase of steadily falling respiratory activity lasted about 1,100 hours. This second phase ended only a few days later than in the case of the previous gathering and was succeeded, as before, by a long third phase during which the steady fall in rate was interrupted by a succession of small rises. This third phase was rather shorter than before, lasting $3\frac{1}{2}$ months. A fourth phase of steadily decreasing respiratory activity followed. The first apple of the group to be attacked by molds became diseased about 6,500 hours from gathering. At this time all apples were still green. The loss of fresh weight was about 20 per cent., and the fruits were slightly wilted. Corresponding with the onset of attack by fungi the rate of CO_2 production began to rise.

As regards the intensity of respiratory activity, at the time of picking the fruits of the fourth gathering were less active than those of the third gathering. At the end of the first phase and throughout the second phase, the intensity of respiratory activity of the fourth gathering was practically identical with that of the third gathering at the same time.

Figure 8 shows the results obtained with two lots of fruit (Lots 11, 12) taken after gathering on June 30, 1925 to a temperature of 22.5°C . Lot 12 was first kept under observation. Respiratory activity fell off rapidly for about 140 hours and then increased again even more rapidly. After 350 hours, while the respiratory activity was still increasing, the fruits were discarded, three of the four apples being partly yellow and only one still green. At this time all apples of Lot 11 were still green, and the observation of respiratory activity was continued with these. The curve for the

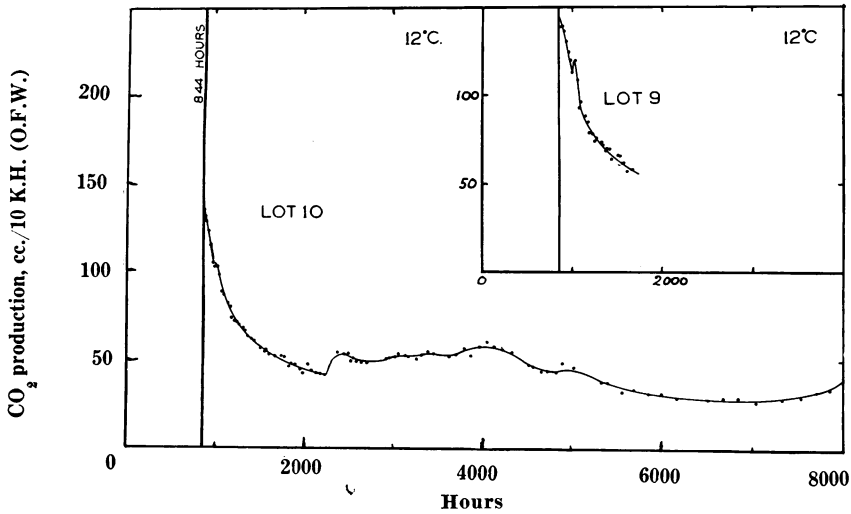


FIG. 7. CO₂ production of Bramley's Seedling apples. Fourth gathering, June 30, 1925; intact fruits.

values obtained fell on the extrapolation of the falling curve of Lot 12. Within a hundred hours or so, however, (550 hours from gathering) the respiratory activity began to rise at first slowly and then very rapidly to the same height as in the case of Lot 12. At the same time three of the apples were yellowing, one being fully yellow and two half yellow at about 1,000 hours from gathering. The remaining green apple was then separated from the others and its respiratory activity followed. Low values were obtained

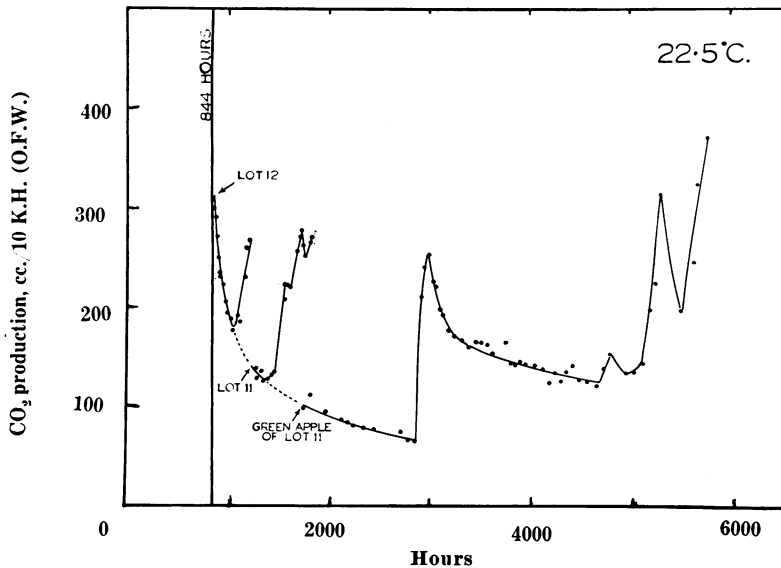


FIG. 8. CO₂ production of Bramley's Seedling apples. Fourth gathering, June 30, 1925; intact fruits (22.5° C.).

and for 1,100 hours a steady fall, appearing as a continuation of the previous falling curves, was registered. Then quite suddenly, within 48 hours, the respiratory activity rose 300 per cent. and continued to rise for another 60 hours to a value nearly four times that recorded before this sudden increase in respiratory activity. During the next 250 hours this outburst of activity partly subsided and the steady falling rate in activity was resumed, but at a higher level, the activity being about three times that which preceded the sudden rise. This fall continued for 1,500 hours before the final rise in CO₂ output, due to invasion by fungi, occurred. It is interesting to note that in this last phase the rate of CO₂ production did not rise steadily but in a series of waves, the maximum of each succeeding wave being higher than that of the previous one.

Of the five phases that have been described in the respiratory history at the lower temperature (12° C.), the first two are not easily distinguishable from one another in this experiment at 22.5° C. A point of interest is that the rate of decrease in activity is identical with that occurring at 12° C. The two curves overlie completely when plotted on the same time scale but with the activity scales in the ratio 2 to 1.

The long third phase in which it was presumed that the respiratory activity of the apples increased individually and more or less suddenly at different times clearly has its counterpart at the higher temperature. Individual variation as regards the length of the period from gathering to this sudden rise was evidently large. The results suggest that in the case of Lot 12, three out of four apples entered this phase simultaneously 250 hours after gathering; that the same happened in the case of Lot 11, but very much later (500 hours after gathering); and finally, they show that the remaining apple of Lot 12 (isolated after 500 hours) did not enter the phase until 2,000 hours from gathering.

The fourth phase is interesting. A single apple only was under observation and showed clearly that the steady fall in respiratory activity of the second phase is continued in the fourth phase, but at a higher level. In the light of more recent work by the authors (12) it would appear that in the case of Lot 12 at 22.5° C. the onset of the climacteric in one apple was sufficient to stimulate the onset of the climacteric of others through the ethylene effect. In Lot 11, on the other hand, the first apple to develop its climacteric happened to do so somewhat later than the first apple to do so in Lot 12. Also, in the case of Lot 11, the stimulus failed to affect one of the fruits; this indicates that the concentration of ethylene produced under the conditions of the experiment was very near the threshold value for apples at this stage of development and at this temperature.

FIFTH GATHERING

In this gathering of August 4, 1925, four lots of apples were used. Three of these (Lots 13, 14, 15) consisted of a pair of apples of nearly the same size, each weighing about 70 grams. The fourth (Lot 16) consisted of a

single apple weighing 129 grams. This apple remained sound for 3,700 hours.

The apples of Lot 13 survived 9,500 hours, while those of Lot 14 were sound 7,300 hours after gathering. At this time their loss of fresh weight was 9.2 per cent. and there were signs of slight superficial scalding. Of the two apples of Lot 15, one was attacked by a slowly growing end-rot 5,400 hours after gathering.

The same phases in the drift of change in respiratory activity with time can be distinguished as in the case of the previous gatherings.

In the case of Lot 13 we find an almost continuous fall in the first phase (compare fourth gathering). Lot 16, on the other hand, shows a distinct rise in the middle of this first phase (compare third gathering). The other

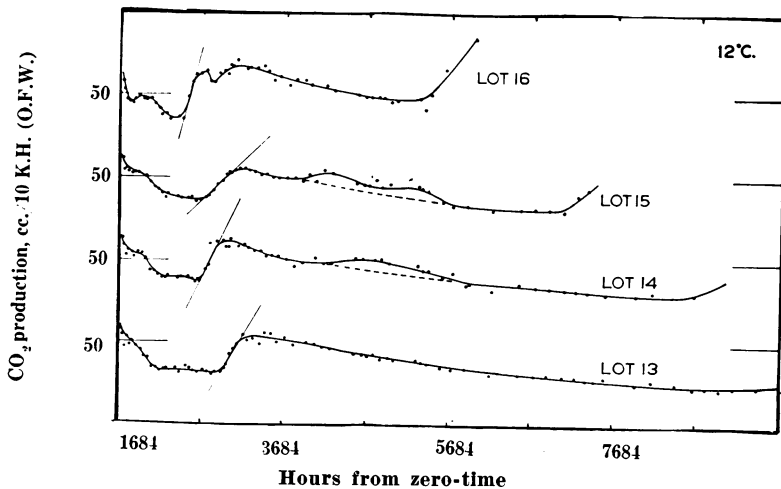


FIG. 9. CO₂ production of Bramley's Seedling apples. Fifth gathering, Aug. 4, 1925; intact fruits.

two lots are intermediate in behavior, showing an approximately level period in the middle of the phase.

The second phase is of much shorter duration than in the case of previous gatherings owing to the fact that the onset of the third phase, marked by a more or less sudden increase in respiratory activity, occurs about the same time as before in spite of the later gathering. Comparing Lots 13, 14, and 15 in which the average weight of the fruits was about 70 grams, there is a difference of about 400 hours between the time of the rise in Lot 13 and the time of the rise in Lot 14; Lot 15 is intermediate. In the case of the larger single apple (Lot 16), the onset of the third phase was considerably earlier (the second phase being almost nonexistent), and the rise in the rate of CO₂ production in the third phase was steeper. The slower rise in the case of Lots 13, 14, and 15 is, we suggest, due to there being two apples which did not synchronize with each other in the time of the occurrence of the rise.

In the third phase there appears to be a definite tendency for irregular

fluctuations in activity to occur. This is seen clearly in the case of Lot 16 (single apple). On general grounds it is not unreasonable to account for these fluctuations by the supposition that the cells of the apple show some variation as regards the time at which the rise in respiratory activity occurs.

When the results of the four lots (seven apples) are averaged and plotted and the resulting curve (fig. 12) compared with that obtained from apples of the third gathering (average of five apples) and the fourth gathering (average of four apples) it is seen that the third phase of respiratory activity, though starting at about the same time, lasts for a much shorter period; *i.e.*, about one month. In other words, there is now less variation among individual apples as regards the time of the onset of the third phase.

The trend of respiratory activity in the fourth phase is the same as in the case of previous gatherings. In the case of Lot 13 the fourth phase lasted over 5,000 hours (7 months). It probably reached its physiological limit, for at the end of this time, although there was no attack by fungi, the rate of respiration ceased to fall and began to rise very slowly with concomitant "scald" development.

In the case of Lots 14 and 15 the steadily falling rate of the fourth phase is interrupted by a temporary, though rather prolonged, period of somewhat higher activity in CO₂ production. This phenomenon is believed to be due to numerous local invasions by fungi the progress of which is checked, so that these invasions result in a surface spotting of the fruit. In the case of Lots 14 and 15 the final rise in rate of CO₂ production is due to the renewed activity of one or more of the checked invasions previously appearing as long-established dry spots.

The initial respiratory activity of the fifth gathering is lower than that of the fourth, but from the end of the first phase onwards the pitch of the respiratory activity of the fifth gathering does not appear to differ from that of the fourth gathering at corresponding points.

SIXTH AND SEVENTH GATHERINGS

These gatherings were made on Sept. 2 and Sept. 17, 1925, respectively; 2,380 and 2,740 hours from zero time. The sixth and seventh gatherings are considered together (figs. 10, 11). For the sixth gathering there are records for six individual apples (Lots 17-22) and for the seventh gathering records for seven (Lots 23-29). In the case of both gatherings a wide range of size of fruit was taken, but it appears from the records of respiratory activity that size did not affect the drift of respiration, which was determined solely by the age of the fruit.

In the sixth gathering, we again see in its entirety the characteristic first phase lasting 400-500 hours. The second phase has now almost disappeared, the first phase being followed immediately by the more or less sudden rise of the third, or climacteric, phase. In the seventh gathering it seems doubtful whether in any case, except possibly that of Lot 23, the first phase itself is completed before the onset of the third phase. On the average, the third phase begins only 300 hours after gathering.

The sixth and seventh gatherings are the only ones in which there is a full set of records of single apples, so that they are the only ones between which comparisons can be drawn with regard to the steepness and extent of the climacteric rise. There is little difference in the steepness of the rise between these two gatherings and moreover Lot 16 (a single apple) of the fifth gathering also shows the same steep rise. It seems probable, therefore,

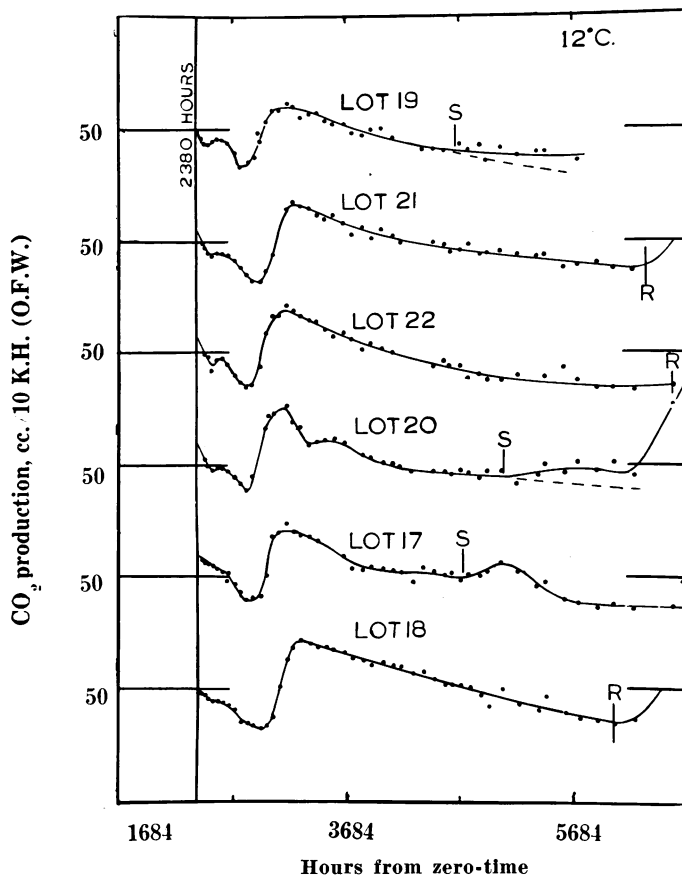


FIG. 10. CO_2 production of Bramley's Seedling apples. Sixth gathering, Sept. 2, 1925. S, skin spotting commenced; R, visible rotting.

that for a single apple the main climacteric rise is always steep. The extent of the rise in activity from the pre-climacteric minimum to the climacteric maximum is nearly 100 per cent. in the case of the sixth gathering as compared with about 70 per cent. for the seventh gathering. The smaller average rise of the seventh gathering may be due to the real extent of the rise not showing itself, because the countervailing causes responsible for the fall in the first phase are still operative at the outset of the third phase in apples of this gathering.

There is no distinct difference between the sixth and seventh gatherings as regards the scatter in time of the onset of the climacteric rise in individual

apples. In both gatherings the scatter is less than that which was shown by the apples of the fifth gathering. For the fifth, sixth and seventh gatherings the onset of the third phase (climacteric rise in respiratory activity) is progressively later in time, but not to an equal extent with the dates of gathering.

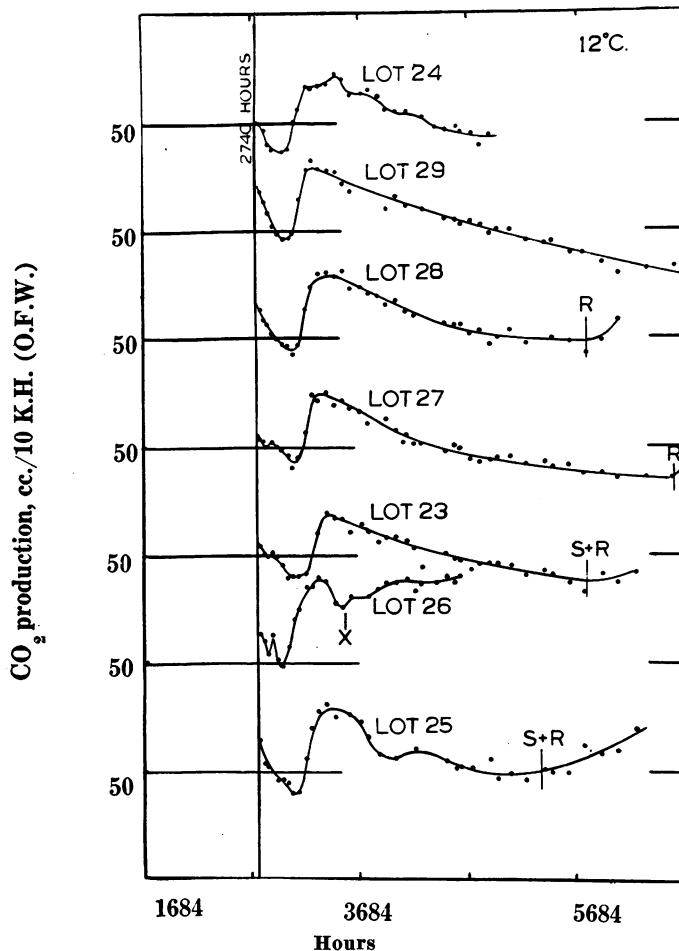


FIG. 11. CO_2 production of Bramley's Seedling apples. Seventh gathering, Sept. 17, 1925. S, skin spotting commenced; R, visible rotting.

Although in these records of single fruits the third phase is considered to consist of one main rise, in some cases there appear to be irregularities in the records both before and after the main rise and, as has been pointed out above, these may be due to variation within the apple as regards the time of the rise for different parts of the tissue. Comparing the one case of a single apple in the fifth gathering, the six cases in the sixth gathering and the seven cases in the seventh gathering, there does not seem to be any marked difference in the duration of the climacteric for a single fruit.

From what has been said above it is clear that the actual duration of this phase for a single cell is a matter of some interest. How far is the slope of the rise a statistical effect? It may well be that for any one cell the change in rate of CO_2 production is extremely rapid.

With regard to the fourth phase, there is no very marked difference between apples of the sixth and seventh gatherings. This phase is shorter in the later gathering. Yellowing of the skin takes place towards the end of this phase and the apples of the later gathering undoubtedly yellowed more quickly than those of the sixth gathering. The downward trend of the fourth phase appears similar to that of the second phase except that it proceeds at a higher general level of activity. The end of the fourth phase is marked by attack by fungi or by functional breakdown (confined to superficial scald in these experiments).

SUMMARY AND COMPARISON OF GATHERINGS 3 TO 7 OF
MAIN EXPERIMENT, 1925

The results of gatherings 3 to 7 are brought together in figure 12. In this figure respiratory activity is plotted on a more open scale than in figure 6. The record of the third gathering appears both in figure 6 and in figure 12 so as to illustrate the effect of the change of scale and to coordinate the two pictures. In figure 12, in order to avoid the confusion of overlapping

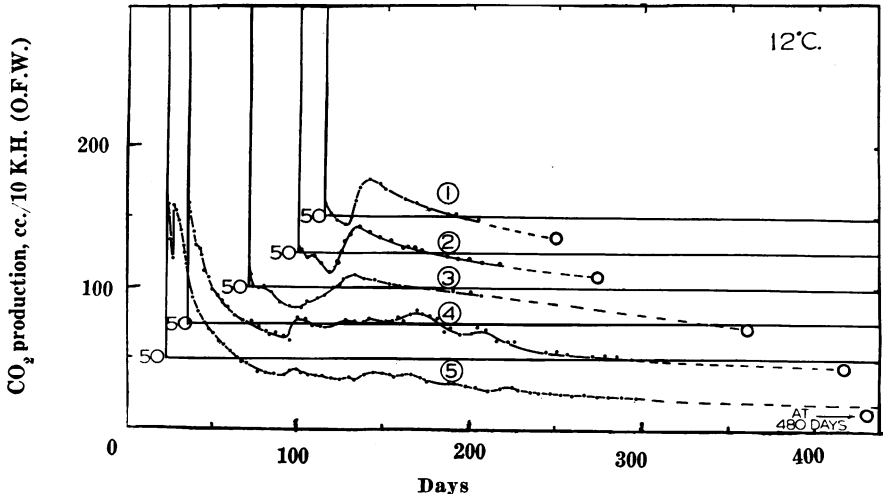


FIG. 12. Respiratory drifts of third to seventh gatherings set out for comparison (diagrammatic). Dotted extrapolations to point in time marked with circle indicate mean life of population. (1) Seventh gathering, average respiratory activity of samples of 6 fruits; (2) Sixth gathering, 6 fruits; (3) Fifth gathering, 7 fruits; (4) Fourth gathering, 4 fruits; (5) Third gathering, 5 fruits.

records, the base-line of zero respiratory activity for each successive gathering has been raised and accordingly horizontal lines corresponding to a respiratory activity of 50 ml. per 10 kilogram hours have been introduced for each gathering to guide the eye. By doing this we tend to obscure the

fact that activity (per unit fresh weight) in fruit growing on the tree, as indicated by the initial determinations of activity immediately after picking, falls in parallel with that of fruit which ceases to grow after picking and is kept under starvation conditions in the dark at 12° C. This fact, however, is brought out in figure 15, in which gatherings 3 to 7 are plotted to a common base-line.

The records are in each case continued to a point in time marked with a circle. This point corresponds approximately to the mean life duration in storage at 12° C. of the population of apples of the gathering in question. For convenience of reference the mean life-duration, the total carbon loss (in terms of hexose) during life after gathering, and the initial respiratory

TABLE III

TOTAL CARBON LOSS (IN TERMS OF HEXOSE) DURING LIFE AFTER GATHERING AND INITIAL RESPIRATORY ACTIVITIES

GATHERING	DATE	INITIAL RESPIRATORY ACTIVITY AT 12° C. CC. PER 10 K.H.	LIFE DURATION AT 12° C.		LOSS OF CARBON (AS GM. HEXOSE/100 GM. FRESH WEIGHT) FROM GATHERING TO DEATH AT 12° C.
			FROM GATHERING	FROM ZERO TIME	
I	May 26	1400	18	18	3.0
II	June 4	800	146	154	4.2
III	" 18	190	355	377	5.1
IV	" 30	140	333	368	5.0
V	Aug. 4	65	250	321	4.0
VI	Sept. 2	53	167	266	2.5
VII	" 17	62	133	246	2.4

activities of the whole seven gatherings are tabulated in table III and the duration of the different phases as they appear in figures 6 and 12 in table IV.

Progress curves of respiratory activity after gathering reflect an ontogenetic sequence or drift of change. They may be based on a single apple or on groups of apples. We use the term "phase" in the analysis of these progress curves, but it must always be borne in mind that the various characteristics of phases, their duration, are affected by variation in the timing of the sequence; e.g., as between apple and apple, and probably also as between cell and cell. The picture obtained does not therefore represent accurately the ontogenetic sequence of chemical and physical reactions in a single cell. It is the total result of a number of incompletely synchronized, though similar, sequences occurring together.

Figure 12 brings out the fact that in all gatherings, subsequent to the first and second, which did not reach the climacteric phase, the climacteric tended to occur at about the same point in time; and, further, that this point in time approximates to that at which the fruit in the orchard reached maturity. We have seen above that there is individual variation in the time of onset of the climacteric phase and that the younger the fruit when gath-

ered the greater the scatter in time of the onset of the climacteric. The records of figure 12 are records of average activity of groups of individuals, and the effect of the decrease of scatter with age of gathering is seen in the decreasing length of the third phase and the greater and steeper rise in the older fruit. The records of figure 12 show that the length of the period between gathering and the onset of the climacteric phase is for all the gatherings (3 to 7) about equal to the length of the climacteric phase itself; that is, to the extent of the period of individual scatter. Thus individual variation in total pre-climacteric activity as a percentage of the mean may not alter much with gathering age although the absolute scatter in time of the onset of the climacteric does.

The duration of life, whether reckoned from zero time or from time of gathering, markedly decreases from the third gathering (mid-June) to the

TABLE IV
DURATION OF DIFFERENT PHASES

GATHERINGS	TABULATED VALUES OF APPROXIMATE DURATION OF PHASES, FROM FIGURES 6 AND 12			
	1ST	2ND	3RD	4TH
	<i>hrs.</i>	<i>hrs.</i>		<i>days</i>
I	250
II	300
III	300-400	1200	4½ months	255
IV	350	1000	3½ months	210
V	400-500	200	1 month	220
VI	400-500	15 days	145
VII	300 +	10-12 days	110

seventh gathering (mid-September). This shortening of life duration with extension of the period of growth by cell enlargement seems to be due to a shortening of the fourth or post-climacteric phase. The yellowing of the skin takes place towards the end of this fourth phase and is not associated in time with the third or climacteric phase. Yellowing does not occur for months after the climacteric in the third and fourth gatherings; in the case of the sixth and seventh, it begins to occur much sooner. It thus appears that by remaining on the tree and continuing to grow in size the apple shortens its post-climacteric life. The amount of carbon oxidized from the time of gathering until the end of life also markedly decreases from third to seventh gathering.

CHEMICAL ANALYSES OF FRUITS USED IN THE MAIN EXPERIMENT, 1925

For present purposes, only the results of the determinations made of soluble and insoluble nitrogen need be given (tables V, VI).

The data show clearly that the nitrogen content per unit fresh weight of the apples fell during their growth; that the proportion of the soluble to the insoluble fraction did not vary greatly during growth; and that neither the total nitrogen content nor, in general, the proportion of soluble to insol-

TABLE V

NITROGEN CONTENT PER UNIT FRESH WEIGHT DURING GROWTH ON THE TREE.
BRAMLEY'S SEEDLING APPLES, 1925

GATHERING	NO. OF APPLES	AVERAGE FRESH WEIGHT PER APPLE IN SAMPLES ANALYZED	TOTAL NITROGEN PER APPLE	TOTAL NITROGEN CONTENT, PERCENTAGE FRESH WEIGHT	SOLUBLE NITROGEN CONTENT PERCENTAGE FRESH WEIGHT
		<i>gm.</i>	<i>mg.</i>	%	%
1	0.70	(3.1)*	(0.45)*
2	12	2.08	5.9	0.284	0.096
3	10	16.60	23.2	0.140	0.042
4	16	31.70	27.3	0.088	0.023
5	12	63.00	25.2	0.040	0.014
6	12	96.00	34.6	0.036	0.012
7	9	99.00	36.6	0.037	0.012

* Assumed on the basis of the results of ARCHBOLD (1), HOWLETT (5), and PFEIFFER (26).

uble nitrogen changed materially during storage at whatever stage the fruit is picked. In the case of the third gathering there was, however, a significant conversion of insoluble to soluble nitrogen during the period immediately after gathering.

The values for respiratory activity per unit nitrogen during growth are given in table VII. For this purpose the values taken for CO₂ production are those for the groups of apples used in each case. There is very little change in activity per unit nitrogen from the third to the seventh gathering. The activity per unit nitrogen of the young fruit, up to three grams weight, is higher.

In figure 13 are plotted the progress curves of CO₂ production per unit nitrogen after gathering of the first, third, fourth, and fifth gatherings, taking the initial activity as unity in each case. These curves are essentially the same in form as those per unit fresh weight since the content of nitrogen

TABLE VI

NITROGEN CONTENT DURING STORAGE

GATHERING	NO. OF APPLES	HOURS FROM ZERO TIME	NITROGEN CONTENT, PERCENTAGE ORIGINAL FRESH WEIGHT		INSOLUBLE NITROGEN, PERCENTAGE OF TOTAL
			INSOLUBLE	TOTAL	
		<i>hrs.</i>	%	%	%
Third	10	556 (gathered)	0.098	0.14	70
	10	1022	0.060	0.13	46
	10	1566 (Lot 5)	0.068	0.15	45
	5	2336	0.077	0.15	51
Fourth	8	844 (gathered)	0.065	0.09	72
	8	1370	0.060	0.10	60
	4	1825 (Lot 9)	0.065	0.12	54
Fifth	12	1684 (gathered)	0.026	0.040	65
	3	2860	0.032	0.044	72
	3	4010	0.031	0.039	79

TABLE VII

RESPIRATORY ACTIVITY (AT 12° C.) PER UNIT NITROGEN CONTENT DURING GROWTH ON THE TREE; GIVEN AS CC. CO₂ PER GRAM NITROGEN PER HOUR

Gathering	cc.
1	31
" 2	28
" 3	13
" 4	16
" 5	16
" 6	15
" 7	17

does not change during storage. When compared on this basis the results suggest that the fall in activity during the first phase after gathering (for duration of same see table IV) becomes progressively slower and smaller as the age at gathering advances. Just as the duration of the first phase increases, so the time to the end of the inflexion in the first phase likewise increases. In the case of the fifth gathering the climacteric phase occurs immediately after the last phase of adjustment. In the later gatherings which are not shown in figure 13 it supervenes while the first phase is still in progress.

In the earlier gatherings an increase in the sugar content took place during storage which was greater than could be accounted for by the hydrolysis of starch, thus indicating the presence of some other polysaccharide in considerable quantity.

Subsidiary experiments, 1928

Subsidiary sets of experiments were carried out in 1928 and in 1929. The object of those carried out in 1928 was to see whether similar results

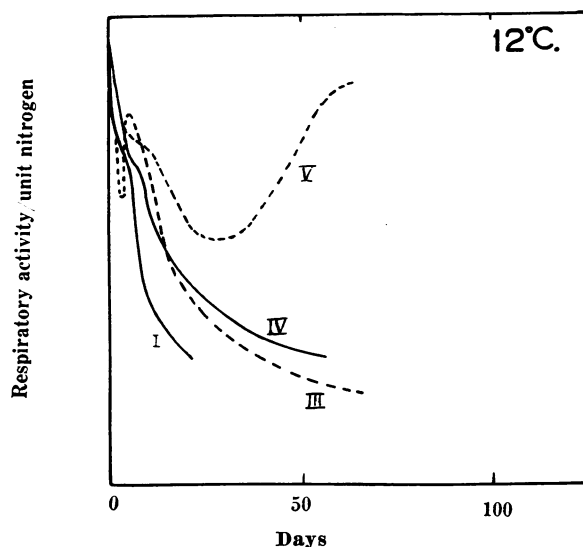


FIG. 13. First, third, fourth, and fifth gatherings compared on the basis of taking activity per unit nitrogen at gathering, as unity.

TABLE VIII

DETAILS OF, AND SAMPLES USED IN, RESPIRATION EXPERIMENT

GATHERING	DATE, 1928	LOT NO.	NO. OF APPLES	AVERAGE WEIGHT	NUMBER OF CONTAINERS	REMARKS
				<i>gm.</i>		
I	May 29	30	5	0.76	One	Air
II	June 4	31	8	2.2	Two	
III	June 12	32	4	6.4	Two	
IV	June 27	33	2	20.0	One	
V	July 11	34	2	29.0	One	(Nitrogen) (5% O ₂) (10% O ₂) (50% O ₂) (100% O ₂)
V	" "	35	4	Two	
V	" "	36	4	Two	
V	" "	37	4	Two	
V	" "	38	4	Two	
V	" "	39	4	Two	
VI	July 25	40	4	38.5	Two	Air
VII	Sept. 3	41a	2	72.5	One	
VII	" "	41b	2	72.5	One	
VII	" "	42	2	73.4	One	
VII	" "	43	2	75.4	One	
VII	" "	44	4	75.6	Two	
VII	" "	45	4	74.0	Two	

would be obtained if the fruit were held, after gathering, at a higher temperature of storage. The temperature employed was 18° C. At the same time experiments were included to study how far the characteristic course of respiratory activity after gathering was dependent upon the concentration of oxygen. The fruit came from the same source as that of the principal experiment.

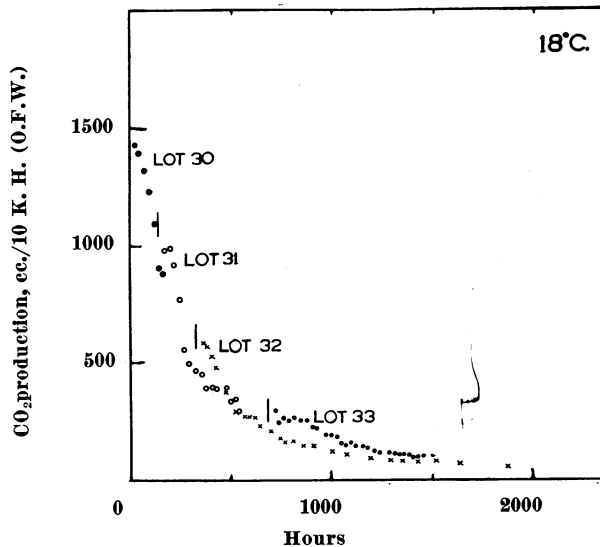


FIG. 14. Records of respiratory activity at 18° C. of fruits of the first, second, third, and fourth gatherings, 1928. The climacteric phase of the fourth gathering, Lot 33, is not included in this figure, but appears in figure 15.

The details of the experiments and of the samples used in the respiration experiments are given in table VIII. The samples of the different gatherings were not kept under observation as long as in the previous experiments and, therefore, the records end arbitrarily while the fruit is still sound. The records of CO_2 production in air for gatherings 1-4, Lots 30-33, are shown in figure 14. Those for gatherings 4, 6, and 7 are shown in figure 15, Lots 33, 40, and 41, and for comparison we have included in this figure a repetition of the 1925 results covering the same period of growth. The record for gathering 5 overlies those of gatherings 4 and 6, and for this reason is not included in this figure. It appears, however, in figure 16.

The identity in behavior as regards respiratory activity per unit fresh weight, of fruit that remains on the tree and increases in size, and of fruit

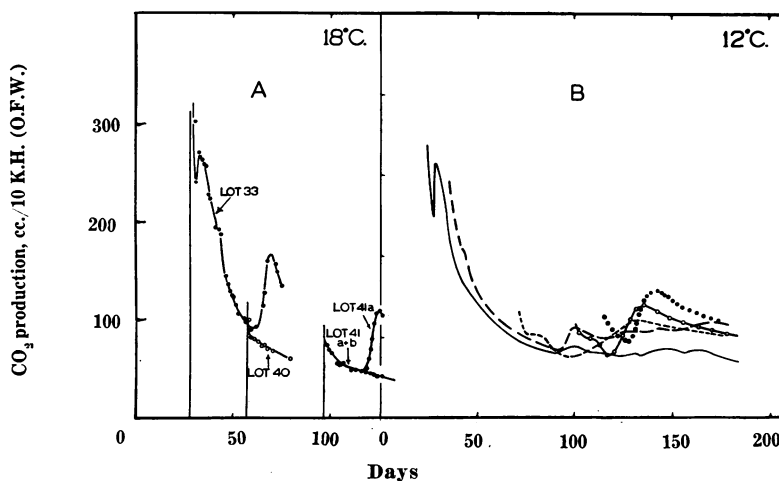


FIG. 15. A. Records of respiratory activity at 18° C. of fourth, sixth, and seventh gatherings, 1928. B. Records of respiratory activity at 12° C. of third, fourth, fifth, sixth, and seventh gatherings, 1925. See figure 12 for ordinate values for CO_2 production.

which is gathered and kept in the laboratory, is again seen. The higher temperature (18° C.) used in these experiments of 1928, as compared with 12° C. used in the previous experiments, does not appear to increase to any marked extent the rate of decrease in activity of gathered fruit. At the higher temperature, however, the downward drift in activity of the second phase appears to proceed towards a lower level.

In most of the gatherings there is an indication of two pre-climacteric phases; the first of these, as in the previous experiments at 12° C., lasting from 10 to 20 days. In the fourth gathering (Lot 33) the first phase appears, as in the previous experiments, to be one of rapidly falling rate interrupted for a time by the operation of some factor tending to cause a rise, before the steady drift of the second phase is reached.

The climacteric phase was not reached before readings were terminated in any of the gatherings except the fourth and the seventh. The time of the onset of the climacteric in the seventh gathering (Lot 41a; gathering date

3/9/28) was almost exactly the same as that of the sixth gathering of the 1925 experiments (gathering date 2/9/25). Lot 41b of the same gathering had not reached the climacteric when observations were discontinued. In the case of the fourth gathering there were only two apples in one container under observation at the time of the climacteric, which occurred about 30 days earlier than in the case of the corresponding gathering in 1925. In these experiments therefore there was no clear indication as to the effect of temperature upon the duration of the pre-climacteric phases.

An effect of one apple upon another in the same container, as regards the onset of the climacteric is again indicated. Clearly the two apples of Lot

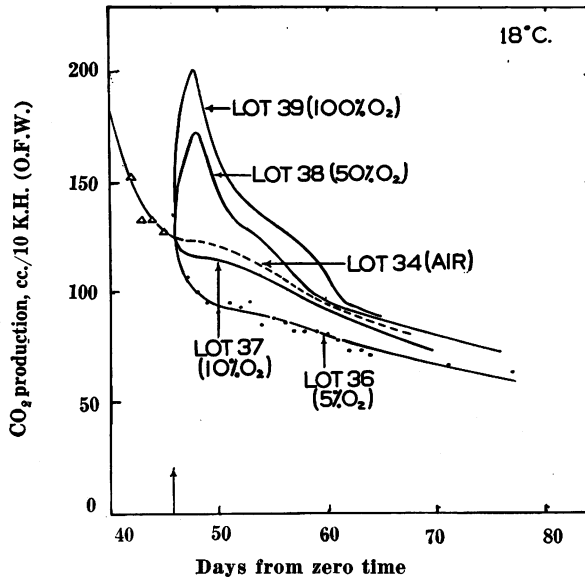


FIG. 16. Fifth gathering, 1928 (18° C.), transferred to atmospheres rich and poor in oxygen, 6 days from gathering. Points before transfer are average of all lots used; points after transfer shown for Lot 36 only.

33 entered the phase almost simultaneously. The same was true of Lot 41a; while Lot 41b, though comparable with Lot 41a in date of gathering and subsequent treatment, still showed no sign of entering the phase 10 days later.

Effect of oxygen concentration

Experiments with the fifth gathering (fig. 16) suggest that oxygen has a more marked effect in the first phase than in the second phase. Super-normal concentrations of oxygen (50 and 100 per cent.) markedly increased activity in the first phase and tended to make this phase contrast more obviously with the subsequent phase of downward drift. Subnormal oxygen concentration (5 per cent.) produced a general lowering of activity and rendered the first phase indistinguishable from the second. When oxygen is reduced to zero concentration the whole character of the phase of adjustment is altered (fig. 17).

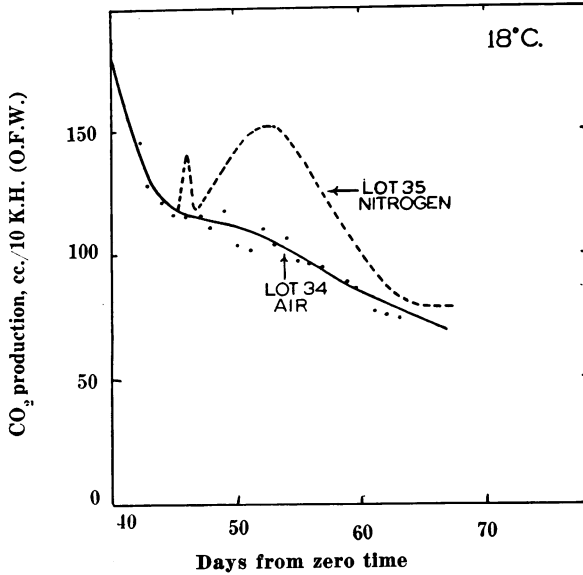


FIG. 17. Fifth gathering, 1928 (18° C.). Lot 35 transferred to atmosphere of nitrogen 6 days from gathering. Lot 34 in air throughout. Points shown for Lot 34 only.

Figure 18 presents the results obtained with the seventh gathering. Transference from air to various oxygen mixtures took place six days from gathering. The concentration of oxygen influenced the time of the onset of the climacteric rise. This began about ten days from gathering in those

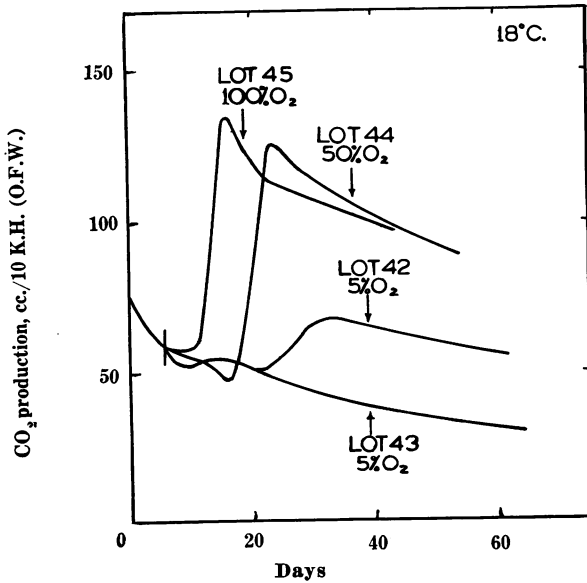


FIG. 18. Seventh gathering, 1928 (18° C.), transferred to atmospheres rich and poor in oxygen, 6 days from gathering.

apples treated with 100 per cent. oxygen; and about 16 days from gathering in those treated with 50 per cent. oxygen. In air, as stated above, one of the lots (41a) began to show a rise in 20 days; the other (41b) had not begun to show a rise by the thirtieth day. In 5 per cent. oxygen one of the lots (42) commenced its rise in 23 days; the other (43) had not shown any rise even after 65 days.

Four apples were treated with an atmosphere of 100 per cent. oxygen, two in each of two respiration containers. The same was the case with those treated with 50 per cent. oxygen. The onset of the climacteric was simultaneous in the two different containers in each case. It would appear, therefore, as if the oxygen was acting directly or indirectly as an over-riding stimulant.

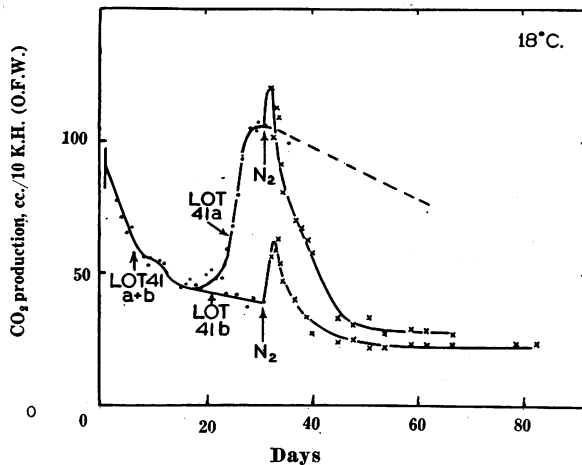


FIG. 19. Seventh gathering, 1928 (18° C.). Transfer from air to nitrogen of Lots 41a and 41b of the seventh gathering on the peak of the climacteric rise, and immediately before the climacteric rise respectively. The interrupted line extrapolating the record of Lot 41a represents the probable drift of activity in air which would have occurred if the transfer to nitrogen had not been made.

There were similarly two lots of two apples kept in air and two lots of two apples treated with 5 per cent. oxygen. Here the results were different. The climacterics were not simultaneous. In both cases it did not occur at all with one pair as long as the experiment lasted. In the case of those treated with 5 per cent. oxygen the form of the curve of the pair showing a climacteric rise suggests that only one apple of the pair entered the phase. Alternatively, if both apples entered the climacteric, it must be concluded that lowering the oxygen concentration to 5 per cent. has a marked effect on the rate of CO₂ production in the post-climacteric phase. That oxygen concentration between 5 and 100 per cent. has relatively little effect on the rate of CO₂ production in the pre-climacteric phase has been noted above in connection with the fifth gathering (fig. 16). This is confirmed by the results with the seventh gathering (fig. 18).

The two lots in air were transferred to nitrogen on the thirtieth day (fig. 19). At this time one was at the peak of its rise; the other, as stated above, had not begun to rise. Immediately on the exclusion of oxygen by the substitution of an atmosphere of nitrogen, CO₂ production in both cases showed a quick rise and thereafter a rather slower fall, this rise and fall being followed by a new steady falling drift. In the case of the pre-climacteric fruit, the new drift in rate of CO₂ production appears practically as a continuation of the drift obtaining before the exclusion of oxygen. In the case of the post-climacteric fruit the fall to the level of the new drift was greater, the level of the new drift in the post-climacteric fruit being in fact almost the same as that of the pre-climacteric fruit.

We have obtained similar results in other experiments (6) which were conducted on Sturmer Pippin apples. In these experiments an additional fact was established; namely, that post-climacteric fruit, on return to air after being kept in nitrogen for three or four weeks, quickly regains its post-climacteric activity.

TABLE IX
GATHERINGS OF 1929

GATHERING	DATE	LOT NO.	NUMBER OF APPLES	AVERAGE WEIGHT
I	June 24	46	5	<i>gm.</i> 9.5
	“ “	47	5	9.5
II	July 9	48	1	21.0
	“ “	49	1	23.4
	“ “	50	1	21.0

It may be inferred from this evidence that in the absence of oxygen the climacteric does not occur, and further that the exclusion of oxygen after the climacteric has occurred autogenously has the effect of eliminating the influence of the factor responsible for the climacteric rise. Increasing the oxygen concentration above that in air, on the other hand, intensifies the influence of this factor. In the light of more recent knowledge the results might be considered to suggest that the rate of production of ethylene by the apple is a function of oxygen concentration. On the other hand, it is also known that ethylene has no effect upon the rate of CO₂ production of pre-climacteric fruit in an atmosphere of nitrogen from which oxygen is excluded (12).

Subsidiary experiments of 1929

The experiments in 1929 were concerned with the question as to how far the striking difference between the third and fourth gatherings in 1925 in the course of respiratory activity in the first phase might be accidental. Fruit was obtained from another source and the details of the two gatherings made are found in table IX. The experiments were carried out at 10° C.

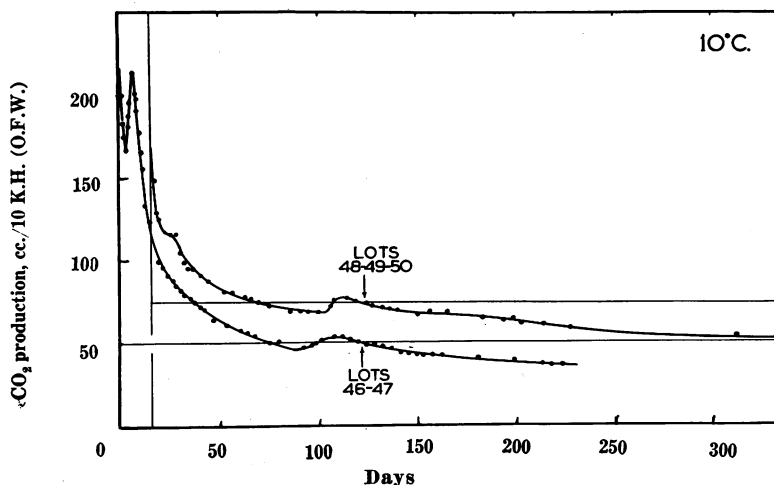


FIG. 20. Records of respiratory activity at 10° C. of two gatherings made on June 29 and July 9, respectively, 1929.

As will be seen from the data which are given in figure 20, close repetition of the results of 1925 was obtained. In the earlier of the two gatherings, the first phase is interrupted by a sharp and distinct increase in activity, while in the case of the later gathering the break in the falling rate of the first phase is marked only by a slight retardation in the fall in activity. It would appear, therefore, that it is only in the particular stage of development which occurs in the middle of June that the course of respiratory activity after gathering shows this marked rise in the first phase. In the 1928 experiments at 18° C. it was the fourth gathering (June 27th) which exhibited this rise.

Data for the change in chemical composition of the fruit after gathering

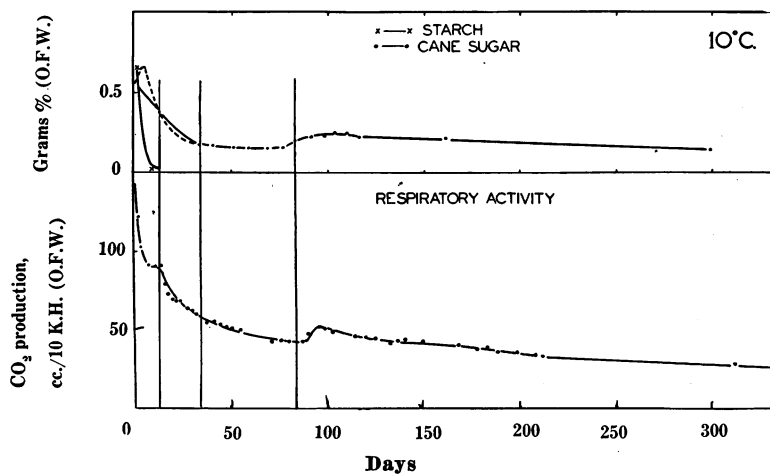


FIG. 21. Starch, cane sugar, and respiratory activity of fruit gathered July 9, 1929. Stored at 10° C.

were obtained for material gathered on July 9th. For the purpose of record, since no other published data for apples at this early stage of development are available, the results are presented in full in table X. For

TABLE X

CHEMICAL ANALYSES OF APPLES DURING STORAGE. BRAMLEY'S SEEDLING APPLES, 1929*

DATE OF ANALYSIS	DAYS IN	RELATIVE FRESH WEIGHT	DRY WEIGHT	TITRATABLE ACID (AS MALIC)	TOTAL SUGAR (AS INVERT) TS	REDUCING SUGAR (AS INVERT) RS	SUCROSE ST-SR	STARCH
STORAGE TEMPERATURE 10° C.								
	<i>days</i>	<i>gm.</i>	<i>gm.</i>		<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
10. 7.29	0	100.0	1.92	3.89	3.33	0.55	0.60
23. 7.29	13	97.2	11.50	1.96	4.46	4.08	0.38	0.03
12. 8.29	33	94.0	11.11	1.86	4.22	4.05	0.17	0.03
19. 8.29	40	93.5	10.74	1.72	4.20	4.03	0.17
30. 8.29	51	91.5	10.93	1.63	4.08	3.92	0.16
7. 9.29	58	90.3	10.63	1.53	4.02	3.85	0.17
14. 9.29	66	89.6	10.75	1.43	3.93	3.76	0.17
25. 9.29	77	88.1	10.72	1.37	3.97	3.80	0.17
2.10.29	84	87.3	10.35	1.31	3.89	3.69	0.20
8.10.29	90	86.5	10.45	1.32	3.94	3.72	0.22
16.10.29	98	85.6	10.37	1.36	3.75	3.52	0.23
22.10.29	104	84.7	10.02	1.38	3.63	3.39	0.24
29.10.29	111	84.2	9.74	1.09	3.90	3.66	0.24
5.11.29	118	83.7	9.65	1.16	3.64	3.42	0.22
20.12.29	163	78.9	9.37	0.98	3.48	3.27	0.21
6. 5.30	300	58.7	6.83	0.52	2.34	2.20	0.14
STORAGE TEMPERATURE 1° C.								
10. 7.29	0	100.0	1.92	3.89	3.33	0.55	0.60
12. 8.29	33	96.5	1.85	4.44	3.91	0.53	Trace
21. 8.29	42	94.8	1.78	4.38	3.84	0.54	Trace
30. 8.29	51	93.9	1.75	4.39	3.92	0.47	Nil
6. 9.29	58	93.0	1.66	4.08	3.63	0.45
14. 9.29	66	92.0	1.34	4.15	3.75	0.40
25. 9.29	77	91.0	1.47	4.19	3.78	0.41
2.10.29	84	90.1	1.51	4.06	3.68	0.38
8.10.29	90	89.3	1.49	3.88	3.50	0.38
16.10.29	98	88.1	1.45	3.97	3.62	0.35
29.10.29	111	87.4	1.46	4.05	3.70	0.35
26.11.29	139	83.1	1.22	3.67	3.42	0.25
7. 1.30	181	79.0	1.14	3.44	3.22	0.22
1. 4.30	266	72.5	0.83	2.87	2.66	0.21
5. 5.30	314	66.1	0.69	2.69	2.47	0.22

* Number of apples per sample, 50; average weight per apple, 23 gm. Results are means of analyses of duplicate samples expressed as percentage of fresh weight on July 10, 1929. Skin, core, and pips not included in analyses.

present purposes we shall refer only to those relating to starch, cane sugar, and hexoses at 10° C.

These are set out in figure 21 together with the curve for respiratory activity. Starch falls from its initial value of about 0.6 per cent. of the fresh weight to zero in about 12 days; cane sugar falls to a steady value in

about 30 days. This first period of 30 days corresponds approximately in the present case to the duration of the first phase of respiratory activity. The second phase of respiratory activity, the phase of steadily falling rate, lasts about 50 days, and during this period the content of cane sugar decreases only slightly. The climacteric rise which follows is accompanied by a corresponding increase in the content of cane sugar, and the slow fall in respiratory activity in the fourth phase is paralleled by a corresponding slow decrease in content of cane sugar.

In the light of results we have obtained since these experiments herein described were carried out, and which will be reported later, it is certain that during the first few days while starch is rapidly disappearing there was an increase in cane sugar as indicated by the broken line in the figure joining the initial observation to the subsequent observation made on the twelfth day.

The results both as regards content of starch and cane sugar, and as regards respiratory activity, are given in terms of grams per unit original fresh weight.

Discussion

In this discussion references have necessarily been made to observations briefly recorded in the Annual Reports of the Food Investigation Board, London, since the work fully described in the present communication was completed. This paper was written before the outbreak of war. The authors have not been able, until now, to devote the time and attention necessary to prepare it for publication. References to other published work are given up to January, 1940.

THE CLIMACTERIC

In the first paper of this series (9) a picture was given of the drift of change in the respiratory activity of Bramley's Seedling apples when gathered at maturity and subsequently stored at three temperatures; namely, 2.5°, 10°, and 22.5° C. This picture has in the present paper been enlarged so as to give a view of the respiratory activity of Bramley's Seedling apples gathered from the tree at various stages of growth and subsequently kept at 10°, 12°, 18°, or 22.5° C. until the onset of functional breakdown or attack by molds.

It is now seen that the climacteric which usually occurs immediately, or almost immediately, after gathering, in fruit gathered in the autumn, can also occur in fruit gathered at any stage during the main period of growth by cell enlargement, which begins about the middle of June when the apple weighs approximately 10 gm. (29).

Under the conditions of the present experiments the climacteric showed a tendency to occur about the same time; namely, in the autumn, in the case of all the gatherings which were stored at 10° or 12° C.; i.e., at about the average temperature of the orchard. At higher temperatures (18° or 22.5° C.) the onset of the climacteric usually occurred earlier.

The present writers have shown elsewhere (11) that apples which are gathered in the autumn and which have reached the climacteric after gathering, produce a gas which will immediately stimulate the onset of this phase in pre-climacteric fruit, and further that ethylene gas in extremely low concentrations (one part in 1,000) produces an exactly analogous effect. GANE (2), in this laboratory, has proved by critical methods of isolation and identification that post-climacteric apples produce small quantities of ethylene.

We have also shown (13, 20) that pre-climacteric apples, even when gathered very early during the main stage of growth by cell-division, produce a gas which, if allowed to accumulate, behaves exactly like ethylene. Thus, for example, Bramley's Seedling apples gathered on July 10th at the 30-gm. stage, when kept in a confined atmosphere of air, maintained as regards its composition by chemical absorption of carbon dioxide and by the addition of pure oxygen, immediately show the rise in respiratory activity characteristic of the normal climacteric of mature fruit or of immature or pre-climacteric fruit in a current of air containing traces of ethylene. Similar fruit, ventilated as in the present experiments, did not show the climacteric until the autumn. Measurements of the carbon of volatile carbon compounds evolved by the fruit were made by the authors' soda lime tube and combustion method. The results (20) indicated that small but definite amounts of volatile products containing carbon were continuously being produced by the fruit in the pre-climacteric phases and that during the climacteric phase the rate of evolution of such products greatly increased.

More recently various workers (3, 4, 25) on apples and pears gathered at about the normal time have carried out measurements of the rate of both CO₂ and ethylene production. Their results show that during the period of the climacteric the rate of ethylene production rises very markedly. Prior to the climacteric the amounts produced are very small.

A tentative conclusion to be drawn from the above facts would appear to be that throughout its growth the apple produces ethylene in very small quantities, but that a minimum or threshold concentration in the tissues is necessary before respiratory activity is affected.

In fruit on the tree or in fruit in storage after gathering, the autogenous climacteric in respiratory activity would then be regarded as ethylene stimulation brought about either by the result of (1) a fall in the threshold value for stimulation, (2) a rise in the rate of production of ethylene, (3) a factor or factors influencing the escape from the fruit of this gas, or (4) a combination of these factors. It is clear, however, that once an autogenous climacteric has occurred, ethylene is produced much more rapidly than before, and for this reason the phenomenon of the normal climacteric appears as an irreversible one.

The results of the present experiments suggest that when small groups of very immature apples were held together under conditions of ventilation with a slow current of air at moderate temperatures (12° C.), an autogenous climacteric occurring in one did not produce sufficient ethylene to stimulate

the others (third, fourth, and fifth gatherings, 1925). At higher temperatures, however [see experiment conducted at 22.5° (1925) and at 18° (1928)] it appeared that the climacteric occurred (with one exception) more or less simultaneously in the case of small groups of immature apples held together in a slow current of air in respiration containers. Hence we may reasonably suppose that at the higher temperatures sufficient ethylene was produced by one apple to stimulate a neighboring apple under the conditions of ventilation employed. We have found, as recorded elsewhere (12) with more mature apples of a different variety (Sturmer Pippin at 15° C.), that the climacteric phase appeared earlier, that the rising curve of respiratory activity was steeper, and that the peak reached was higher in the case of a group held together in a single respiration container, than in the case of a corresponding group ventilated at the same rate per apple, each individual of which was isolated in its own small respiration chamber. In the case of the group held together, we appeared to be dealing with conditions under which the ethylene produced by the first apple to reach its climacteric was sufficient to stimulate at once the onset of the climacteric in the other members of the group.

It seems possible to suggest an analogy between the behavior of apples confined in a group and the behavior of cells making up the tissue of a single apple. When one cell undergoes autogenous stimulation and its rate of ethylene production is thereby increased, the concentration of ethylene will correspondingly be raised around it. This in turn may bring about the stimulation of other cells which will then produce more ethylene, until all the tissues of the apple are affected. The observed acceleration of carbon dioxide production, or the duration of the climacteric phase, may, therefore, depend partly on the complex of factors which determine the spread of ethylene stimulation through the tissue; *i.e.*, the degree of ventilation of the internal atmosphere, the variation in the susceptibility of cells to ethylene stimulation, and the extent of increase in rate of ethylene production following stimulation.

If the above picture is a true one the duration of the climacteric phase must be affected by the degree of ventilation of the intercellular spaces of the apple. In the previous paper of this series (9) it was shown that the climacteric was very prolonged at low temperatures as compared with high temperatures taking, for example, approximately 190 days at 2.5° C. as compared with 7 days at 22.5° C. The same has since been shown for pears (16). At low temperatures the degree of ventilation relative to the rate of production of carbon dioxide and, presumably, of ethylene is greater than at higher temperatures as is shown by the recognized fact that the concentration of carbon dioxide in the internal atmosphere is much lower at low temperatures. The duration of the climacteric at low temperatures may therefore be affected not only by temperature directly, but also by a scatter in time in the stimulation of the individual cells of the apple; and this may be a possible explanation of the high temperature coefficient we have ob-

served for the rate of rise in respiratory activity both in apples and in pears. Our previous view had been that the high temperature coefficient of the climacteric might be accounted for by assuming that some process with a high temperature coefficient, such as protein denaturation, was limiting.

In an attempt to confirm the present suggestion we have shown (18) that if single apples (Sturmer Pippins) were taken to a low temperature (1° C.) before the onset of the climacteric phase and were then treated for three days with ethylene (1 in 500 by volume), the climacteric was completed within a much shorter time and the respiratory activity reached a higher maximum value than in similar fruit allowed to develop a normal climacteric at this low temperature.

From what has been said above it follows that the greatest prolongation of pre-climacteric life, other things being equal, might be expected under the conditions most favorable to the escape of ethylene from the fruit. Such conditions would appear to be those of free exposure on the tree. Neither the present experiments nor those recorded in the previous paper of this series include an example of apple left on the tree beyond the time of normal picking maturity, which in the case of the Bramley's Seedling variety grown in England falls definitely in the pre-climacteric phase. It is interesting to inquire, therefore, whether in the case of the Bramley's Seedling variety the climacteric ever occurs on the tree. The impression to be gained from results of experiments covering this point, which have been published in outline elsewhere (10), is that the climacteric rise in Bramley's Seedling apples on the tree is a slow, drawn out phenomenon which is accelerated immediately after the fruit is gathered and enclosed in a container for measurement of respiration.

With a late variety (Rome Beauty) (19) and with a mid-season variety (Cox's Orange Pippin) we have found that with single fruits, still attached to the tree enclosed in a respiration container through which a current of air was passed, the complete climacteric phenomenon occurred rapidly, just as it did with gathered fruit.

It is interesting to compare the Bramley's Seedling, a late English variety, with early and mid-season English varieties such as the Gladstone and James Grieve. With these varieties results have been obtained (22) which show that if the apples are allowed to remain on the tree beyond their normal picking date they reach the post-climacteric stage in respiratory activity. In the case of the McIntosh apple, an American variety, the climacteric occurs just before the normal time of gathering (27). The difference in behavior between early and late varieties as regards the occurrence of the climacteric on the tree may possibly be related in part to the larger production of ethylene in the case of the earlier varieties (24) which also show greater activity in the production of carbon dioxide (28).

As to whether there is any change with age in the threshold value for ethylene stimulation we have at present no direct evidence, and further work dealing with this problem is in progress. There is, however, indirect evi-

dence from the fact that immature pre-climacteric fruit shows recovery from short exposure to ethylene stimulation. After longer exposure there is no recovery (18).

The curves of respiratory activity following short period stimulation are extremely interesting. A rapid rise takes place while the stimulus is being applied. As soon as it is removed the curve for respiratory activity falls, but not to the previous level, and finally the autogenous climacteric occurs sooner than it would otherwise do.

It would appear as if different groups of cells had been affected in different degrees; some had been stimulated and had then recovered completely, while others, on the removal of the stimulus, had not recovered. If the stimulus is applied for a sufficiently long time all the cells are affected, and there is no recovery. If these deductions are correct, it follows that there must be a variation in the susceptibility of different cells to ethylene stimulation. Further, the behavior under artificial stimulation in the pre-climacteric phase, contrasted with that shown in the autogenous climacteric, indicates that there must be either a progressive increase with age in the rate of production of ethylene subsequent to stimulation or a fall in the threshold value for stimulation.

One of the most striking results of the present experiments is the fact that there is no marked difference in the time of the onset of the climacteric as between apples gathered when weighing only a few (20 or more) grams and subsequently kept under starvation conditions at moderate temperatures (comparable with those obtaining in the orchard) on the one hand, and on the other corresponding fruit allowed to grow to full size on the tree. It seems obvious to look for some relation between this fact and the equally striking one that there is no marked difference in the rate of decline of respiratory activity per unit fresh weight as between the gathered apples and those remaining on the tree. The two ontogenetic systems appear to be in almost every respect different, physically and chemically. They have, however, two characteristics in common. First, the gross concentration of acid per unit fresh weight declines in both cases to an approximately equal extent, as can be judged by comparing the results of ARCHBOLD (1) with those here reported. Secondly, the total production of carbon dioxide per unit fresh weight of tissue is the same in both cases so that one could conceive of a parallel accumulation per unit fresh weight in both cases of some unknown substance produced in the course of respiratory activity. If the climacteric is related either to the fall in acidity or to the accumulation of some unknown substance it would seem most reasonable at present to suppose that these produce their effect by lowering the threshold values of ethylene stimulation. In this connection reference may be made to our experiments (14) in which pre-climacteric Sturmer Pippin apples were treated at 20° C. with HCl gas (50 parts per million by volume) and gaseous ammonia (400 to 1,000 parts per million) for two or three days. The ammonia treatment stimulated an immediate climacteric rise; the hydrochloric acid treatment was without effect.

There are other facts with regard to the duration of the pre-climacteric phase in gathered fruit worth noting in this discussion. The first is that with fruit gathered in a very immature condition (8, 15); higher temperatures of storage do not always have a clear-cut effect in shortening the pre-climacteric phase. The other is one we have noted both for apples and for pears; namely, that when dealing with commercially gathered pre-climacteric fruit remarkably little difference is observed in the time elapsing before the onset of the climacteric as between fruit kept at low, medium, and high temperatures. The full explanation of these facts is still to be sought, but we feel that there is sufficient evidence to indicate that the explanation may be found in the interplay of the various factors we have indicated as probably operative in determining the onset of the climacteric. As regards the effect of temperature, it may be noted that our results for pears (21) have suggested an optimum at 10° C. for the rate of production of ethylene in fruit during the climacteric phase.

Finally, the data on the influence of oxygen and of carbon dioxide upon the climacteric are noteworthy. Investigations here and elsewhere (7, 12, 23) reported by us establish that in the absence of oxygen the climacteric does not occur, and suggest the possibility that in the absence of oxygen ethylene is not produced. If the climacteric has occurred, exclusion of oxygen causes a reversion to the pre-climacteric state of activity with the difference, however, that, if oxygen is again given, rise of activity to the post-climacteric level begins at once. This observation could be interpreted on the assumption that, in the absence of oxygen, ethylene continues to be produced by the fruit but cannot exert its stimulatory effect. It has in fact been proved experimentally that the rate of CO₂ production of pre-climacteric apples in an atmosphere of nitrogen is not affected by ethylene. On the other hand, the observation is equally well explained by assuming that in nitrogen the fruits cease to produce ethylene and so revert to a pre-climacteric state. In either case it is implied that the post-climacteric state requires the continuous presence of ethylene for its maintenance.

By raising the oxygen concentration above air values the autogenous climacteric is brought on earlier and the stimulus of one fruit on another is increased. These observations suggest that the rate of ethylene production by the cells of the fruit is a function of the oxygen concentration.

The facts with regard to the effects of carbon dioxide are that moderate concentrations of carbon dioxide (about 10 per cent.) in the atmosphere postpone the onset of the climacteric but do not render the fruit insusceptible to stimulation by ethylene (12, 17, 18). In the presence of carbon dioxide the postponed autogenous climacteric is more drawn out and does not show so marked an increase in activity as in the case of a normal climacteric in air, thus indicating a wider scatter in time of the reaction to the stimulus of the different cells of the tissues. These two effects of carbon dioxide suggest that it may act by raising the threshold value of ethylene stimulation.

Carbon dioxide in moderate concentrations in the atmosphere affects the

rate of CO₂ production. In post-climacteric fruit it depresses respiratory activity, but when applied to pre-climacteric fruit during the first phase after gathering, it has the opposite effect, that is, one of increasing the rate of CO₂ production. In the second phase the respiratory activity in the presence of 10 per cent. carbon dioxide is depressed well below the corresponding values in air. Thus in a case of Bramley's Seedling apples gathered on July 10, 1934, with an average weight 30 gm. and placed immediately in an atmosphere containing 10 per cent. of carbon dioxide and 20 per cent. of oxygen at 10° C., the first effect was to increase the rate of CO₂ production above that shown by controls in air. The full depressive effect of carbon dioxide was not shown until 20 days after gathering.

In this experiment the controls in air entered the climacteric phase after about 50 days, while those in the presence of carbon dioxide had not shown any climacteric rise after approximately 120 days. These experiments formed part of an extensive examination of the chemical changes occurring during storage at various temperatures of fruit gathered at a very immature stage of development. The results of this work have not yet been fully published, but it may be put on record here, as it is of interest in relation to what has been said above with regard to acids, that there was no difference in the rate of decrease in the acid content of the tissue as between apples gathered on the above date and stored in air at 10° C., and comparable apples stored in the presence of 10 per cent. carbon dioxide and 20 per cent. oxygen. (See also KIDD and HANES (7) who record hydrogen-ion concentrations of sap in this experiment.)

To sum up this discussion of the climacteric phase in the apple, it is suggested that one is dealing with a very subtly balanced phenomenon depending on (1) a slow and possibly steadily increasing rate of production of ethylene during growth, (2) a slow fall with age in the threshold value for stimulation, and (3) a number of variables which affect the rate of escape of ethylene from the fruit. The course of events can be altered by quite small changes in the ventilation of the fruit or by "massing" the fruit. Under extreme conditions cases might be expected where the critical stimulatory effect is never achieved. One such case is recorded elsewhere (15), the fruit in question being Comice pears, which not only failed to produce a climacteric but also failed to ripen normally.

CO₂ PRODUCTION, SUBSTRATE, AND ENZYME

In Part I of this series (9) the conclusion was reached that the changes in respiratory activity over the climacteric could not be explained in terms of parallel changes in the observed gross concentration of sugars and acids. When viewing the wider picture covering the growth period of the fruit, we must take into consideration changes in amount of cytoplasm present per unit fresh weight as well as changes in the concentrations of substrates. As an approximate measure of cytoplasm the nitrogen fractions of the fruit were estimated. The interesting fact emerged that over the main growth

period of the fruit, that is, the period when growth takes place by cell-enlargement, the respiratory activity per unit nitrogen is a constant. On the other hand, the respiratory activity per unit nitrogen falls sharply immediately the fruit is gathered and separated from its source of supply. On the basis of the classical conceptions of enzyme and substrate it is difficult to bring these two observations into line except by assuming that during growth the concentration of substrate as determined by supply must remain relatively constant. If so, it suggests the operation during growth of an auto-regulatory mechanism. For example, if growth in size of cells and synthesis of protein are functions of respiratory activity, any tendency to increase the concentration of substrate from supply must lead to an increase in R/N and hence to the formation of more protein and larger cells. These results immediately set up counter tendencies towards a lower R/N as the result of consumption of substrate, its dilution by uptake of water, and the increase in nitrogen.

As regards the nature of the substrate, it is sufficient here to state that in gross concentration none of the main components of the fruit, such as glucose, fructose, malic acid, etc., is constant or even approximately constant, during growth (1); while, on the other hand, the changes in R/N after gathering do not correspond to the changes in concentration of any of these possible substrates. Between gross concentration of cane sugar in the fruit after gathering, however, and the course of respiratory activity a parallelism exists which breaks down only during the short period of starch hydrolysis when cane sugar is increasing and respiratory activity is decreasing.

Summary

The respiratory activity at normal temperatures of Bramley's Seedling apples gathered at various stages of growth has been examined and recorded.

There is a marked fall in respiratory activity per unit fresh weight during the early stages of the development on the tree—the period of cell division. The fall continues, but much more slowly, during the main growth period—the period of cell enlargement.

The respiratory activity per unit nitrogen, and probably also per unit protein, remains approximately constant during the main period of growth by cell enlargement. During the early stages of growth by cell division the respiratory activity per unit nitrogen is somewhat higher. It is suggested that respiratory activity on the tree is not limited by the effective amount of enzyme present but remains constant as a result of an auto-regulation of substrate concentration, through a linkage between respiratory activity, formation of new cytoplasm, uptake of water, and increasing size of cell.

During both growth periods the respiratory activity, after gathering, per unit fresh weight and also per unit nitrogen, falls.

The drift in respiratory activity per unit fresh weight after gathering shows in general four main phases. These have been discussed in relation to their causation. Particular attention has been devoted to the third or

climacteric phase, and to the effect of temperature, oxygen supply, and ethylene upon its onset.

The climacteric rise in respiratory activity has been shown to occur in fruit on the tree as well as in fruit gathered at any stage of maturity. Evidence is produced and discussed which indicates that the climacteric occurs as a result of the production of ethylene by the fruit and of an auto-stimulation produced by this gas when present in the tissues in amounts above critical threshold values.

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