THE RESPIRATORY METABOLISM OF McINTOSH APPLES
DURING ONTOGENY, AS DETERMINED AT 22° C.

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(WITH NINE FIGURES)

Introduction

Our knowledge of the respiratory metabolism of an apple fruit has been derived heretofore from examinations of continuous respiration records, and from the study of records so short, that they give nothing but the initial rates of respiration. The former are available only for apples in the later stages of their life (1, 3); the latter provide information for apples in all stages of their ontogeny (2).

A respiration record of a starving fruit may be looked upon as an expression of the physiological state, in which this fruit was present at the beginning of examination. By comparing a number of such records, produced by fruits in various stages of their ontogeny, one can follow the changes in the physiological state of a fruit during its development. By extrapolating the tendencies observed during ontogeny to the time of commercial picking in the fall, one gets a picture of the physiological drifts in a fruit at that time.

Such a method of attack, based on the knowledge of the past history, can give valuable information which cannot be easily obtained from direct studies of a fruit already in storage. Since a search through the literature failed to reveal any complete sets of such records for any variety of apple, it was decided to obtain them. The McIntosh apple was selected as experimental material since it is one of our most important commercial varieties.

Materials and methods

The work reported below was carried out in 1939, following the preliminary tests of the preceding year. A full grown McIntosh tree was selected in one of the largest commercial orchards near Kingston, and all of the apples studied came from this tree. Apple blossoms began to drop their petals on June 1st, and this date was taken as the time of fruit setting.

Samples of apples were taken throughout their ontogeny, beginning from two weeks after setting, and continuing until the death in storage of all the apples picked later in the fall. During the summer and early in the fall, fruits were picked directly from the tree. On October 2, all the remaining apples were gathered, and a hamper of ungraded fruits was brought to the same constant temperature room in which all the samples were kept for study. They were spread on shelves in boxes and a stream
of air was drawn over them to remove the CO₂ produced. All of the samples taken after this date were selected from these apples. Throughout the ontogeny samples were taken for respiration studies and starch determinations.

Samples taken for respiration studies on June 14, 16, 27, and 29 consisted of six, six, five, and two apples, respectively. All subsequent samples were of one apple each. Previous to the taking of each sample, all available apples were examined, and only average looking fruits were selected. As soon as a sample was taken, notes were made on the color of the apple. The fruit was then brought to the laboratory, weighed, and enclosed in a glass respiration chamber,¹ which in turn was placed in a constant temperature room kept at 22 ± 0.5° C. A stream of air drawn by a pump was passed over soda lime to free it from CO₂, over a weak solution of Ba(OH)₂ to bring it to a constant moisture content, over an apple in the respiration chamber and, finally, through a Pettenkoffer tube filled with 50 ml. of standard Ba(OH)₂. The Pettenkoffer tubes were changed daily, and the residual Ba(OH)₂ of each tube was titrated against standard HCl, using phenolphthalein as an indicator. The results have been expressed as milligrams of CO₂ produced per 100 grams of the initial apple fresh weight per hour.

Respiration of every sample was followed until the breakdown of the apple was apparent. With one exception, this appeared to have been caused by fungal infection spreading from the calyx. In only one case (sample 33), was it apparently due to some internal cause. One or two days previous to this apparent breakdown, there was a sudden and considerable increase in the CO₂ production, which apparently was produced while the internal organization of the apple was breaking down. In this work the respiration record was considered to have terminated immediately after the last low value, subsequent to which there was this increase in the CO₂ production, and one or two days later an apparent disintegration of the apple.

Samples of apples for the tests of their starch content with iodine were taken throughout the entire ontogeny of fruits. Each of these samples consisted of several apples, one of which was tested at once; the remainder were put in the same constant temperature room under conditions similar to those of the respiration samples. The starch content of these apples was determined later.

Results

Respiration records produced by different samples are shown in figures 1-4. Chronological number of each sample is given in the upper left-hand corner. At the bottom of each record there are letters, indicating the ground color of the apple at the time of its examination. Four colors were distin-

¹ Made by C. L. Muller, 6 Parton St., Red Lion Square, London, W. C. 1, England.
guished: green (G), yellowish-green (Y-G) greenish-yellow (G-Y) and yellow (Y).

Respiration of every apple from nos. 35 to 27 was represented essentially by the same gently declining line, which had been previously reported by several workers (1, 3) for the apples in storage. None of these apples were of pure green color, and all showed a transition from green to yellow. The most apparent point of difference among these records was that of duration: the earlier an apple was taken, the longer it lasted.

The respiration record of apple no. 25 was not of the same continuously declining type. It started with a low initial value, went up rapidly in the climacteric rise, after which it continuously sloped down, in the same man-

![Figure 1](image)

**FIG. 1.** Respiration records of apple fruits during starvation. The first date on the abscissa is the day of taking the sample.

ner, as did all the samples taken later. The climacteric rise for McIntosh apples was previously reported by PHILLIPS (4).

Apple no. 23, which was taken six days earlier, not only displayed the same climacteric as did no. 25, but previous to this it exhibited a brief preclimacteric period of several days duration.

The record of apple no. 21, though essentially of the same type as that of no. 23, showed for the first time a new feature. Following a climacteric rise, it did not slope continuously down, as was the case in all of the apples taken later; after a brief decline it again rose slightly, and then resumed its steady fall. This temporary rise on the declining arm of the respiration record might be passed unobserved, if it were not for the fact that apples
picked earlier had this hump developed to a considerably greater degree. In the record of apple 13a this hump was even larger than the climacteric itself. This additional rise and fall has been provisionally called a "post-climacteric hump," since it occurred after the climacteric.

In the record of apple 11a this postclimacteric hump was obscure. In that of apple 9c it was again quite apparent, though developed to a considerably lesser extent than the climacteric. In addition, this hump shifted its place from that shortly after the climacteric, to the end of the record. A postclimacteric hump was absent from the records of all the samples taken earlier than was 9c.

While in the records of apples from 23 to 19 the preclimacteric period was represented by a straight line more or less parallel to abscissa, the first deviation from this type was produced by apple 17a. In this sample, the respiration record started with a high value, declined rapidly at first, and flattened out later. This deviation is still more pronounced in the record of apple 15a, and especially in that of 13a. In the last record there was an appearance of another possible feature of a preclimacteric period: an indication of a slight hump in its flat portion. This hump was displayed even more by apple 11a, and in apple 9c it was developed only slightly less at its peak, than was the climacteric itself. Since this hump was present in the preclimacteric period of a respiration record, it has been provisionally called a "preclimacteric hump."

All of the apples, whose respiration records are shown in figures 2 and 3, were initially green in their ground color, and all of them began to turn
Fig. 3. Respiration records of apple fruits during starvation. The first date on the abscissa is the day of taking the sample.

Fig. 4. Respiration records of apple fruits during starvation. The first date on the abscissa is the day of taking the sample.
yellow after the beginning of the climacteric. We can consider, therefore, that one of the signs of the onset of a climacteric, is this change in color from green to yellow.

Respiration records of samples of 2 to 7b were all essentially of the same declining type—falling more rapidly at first and with a tendency to flatten out later. On this type of record there might be present either one (sample 3a, 3c), or several (sample 7b) humps.

Tests for the presence of starch in apples revealed that no starch was present in apples picked on June 28th. On July 6th the first traces of starch were observed; from July 11th and until August 8th there was an abundance of starch in both cortex and pith. From August 15th there began a continuous decrease in the starch content until apples examined on October 12th either had no starch at all, or mere traces of it.

As the place for the storage of starch, the cortex of the receptacle (outside the ring of vascular bundles) was found to be far more important than the pith. In the earlier stages of ontogeny the appearance of starch in the pith lagged behind its appearance in cortex. In the later stages starch disappeared at first from the pith and only later from the cortex. Disappearance of starch from the cortex was found to take place in the three following stages:

1. There was an appearance of clear areas without any starch in otherwise starch-containing tissue. This suggested at least a physiological heterogeneity of tissue; otherwise there should be a uniform decrease in the intensity of the blue color throughout the whole cortex.

2. Starch-containing areas changed their color from very deep blue to lighter shades.

3. There was a progressive disappearance of starch, starting from the center of an apple and spreading towards its periphery. The last traces of starch were always found under the skin.

Since exact quantitative data are lacking, no complete picture can be given now on the disappearance of starch from apples during starvation. The general trend of events is clear however. Starch is brought down from abundance to mere traces within two weeks, and when its initial amounts are low, this time may be shortened even more.

Analysis and interpretation of data

The initial respiration rates plotted against the time of taking samples are shown in figure 5. The graph obtained represents the changes in the respiratory potentiality of apples during their ontogeny, and is an approximation to the respiration of apples on a tree and in storage, when corrected to the same temperature of 22° C.

During the early part of its course this graph declines rapidly, while
later its fall is more gradual. Shortly before the time of picking of apples in October it goes up in the climacteric rise, following which there is a gentle decline until death in storage. This rapid decline of respiration during the first month of growth (June) corresponds to the period of cell multiplication. Absence of starch during this month is probably due to a heavy demand on sugars for the formation of new cells. When, in the following stage of cell enlargement, this demand subsides, then the excess of sugars is condensed into starch.

Examination of figures 2 and 3 reveals that in each respiration record the minimal rates at the end of its preclimacteric and climacteric periods are usually not far apart. Figure 6 compares these two rates for the various samples of apples, and it is evident that these two are very close in each respiration record, and also that in different records they both vary in the same direction. Apples, taken after September 25, have no preclimacteric period; in such fruits only climacteric minimal rates are available.

Two explanations have been given as to the cause of the respiration rise in a climacteric. BLACKMAN (1) suggested that this is due to an increase

Fig. 5. Changes in the initial respiration rates of apple fruits during ontogeny.
in the hydrolysis facilities of tissues, as result of which there is an increase in the concentration of the respiratory substrate. On the other hand, Kidd (2) considered it to be connected with some change in protoplasm itself.

The mutual proximity of the minimal preclimacteric and climacteric rates in each record can easily be explained as caused by changes in the concentration of the respiratory substrate. On this ground the observed decrease in the respiration during the preclimacteric period is due to the progressively decreasing concentrations of the respiratory substrate. When the amount of this substrate broken down just fails to yield the amount of energy necessary for the maintenance of the proper protoplasmic organization, then there is an appearance of either a new substrate, or, if still the same, at least from a new source. The increased concentrations of this new substrate are causing an increase in the respiration rates resulting in the climacteric rise.

This new substrate, however, is eventually also brought down to such a low concentration that it fails to yield the necessary amount of energy. When this does happen, death occurs since there is no other source of the respiratory substrate to supply the deficiency. This mutual proximity of the minimal preclimacteric and climacteric respiration rates is due then to the fact that both represent the same thing; namely, the basal rate of metabolism for these apples under the described conditions. An increase of this rate in younger apples, and decrease in older, supports this view.

It was pointed out earlier in this paper that with the exception of one
sample, death appeared to have been caused by fungi. It is hardly a coincidence, that an apple always succumbed to a fungus at the time its climacteric respiratory rate dropped to the same low value, as it had been at the end of its preclimacteric period. A more probable explanation of this fact is that a fungal infection did take place only when an apple has reached a definite physiological state, represented by a definite rate of respiration. According to the view outlined above, an apple succumbed to a fungal attack when its respiration fell below its basal metabolic rate, and the final disintegration of its protoplasmic organization already was beginning to take place.
In other words a fungal infection was one of the consequences of death, and not the cause of it.

The preclimacteric and postclimacteric humps are two other peaks which were observed on some respiration records. The preclimacteric hump was present in samples 13a, 11a, 9b, and, probably, in 3a. The reason why the hump produced by sample 3a is considered to be preclimacteric, lies in the absence of color changes. It was pointed out earlier in the paper, that a climacteric period is associated with the change of color from green to yellow. Since sample 3a remained green throughout its entire life, in the absence of any better methods for the identification of this period, the stand is taken that this hump is a preclimacteric and not a climacteric one.

Figure 7 gives for various samples the total CO₂ produced by the whole record, as well as CO₂ produced during its preclimacteric and climacteric periods. The graph for the total CO₂ production starts with a high value, and falls rapidly during the month of June.⁵ It goes up from the beginning of July and reaches its peak by the end of the month; after this there is a continuous decline, rapid at first and slower later, interrupted only by a secondary peak at the time of the climacteric.

No separation of respiration records into the preclimacteric and climacteric periods can be seen in samples taken during the month of June. But when in later samples, such a separation becomes established, the climacteric period then contributes the bulk of CO₂. The graph for the CO₂ produced in the climacteric is at first closely parallel to that of the total CO₂; from the end of September, when the preclimacteric period disappears entirely, these two graphs merge into one.

The ground on which the hump exhibited by sample 3a is considered to be preclimacteric and not climacteric, has been given above. Other samples taken in June produced continually declining respiration records; humps, if present, were not followed by death; and, finally, apples did not show color changes from green to yellow.

On all these grounds respiration records of June samples have to be considered as represented by the preclimacteric periods only. On the basis of this conclusion figure 8 gives the total CO₂ of both preclimacteric and climacteric periods of respiration, produced by apples in various stages of their ontogeny. In June, when apples are present in the stage of cell multiplication, all their CO₂ is produced in the preclimacteric period. The onset of cell enlargement brings a steady decline of the preclimacteric, interrupted only by two secondary peaks, and by the end of September this

⁵ The two last values of this month, while placed on this graph correctly chronologically, ontogenetically should be plotted earlier. Samples 7a and 7b, taken on June 29th, both were characterized by higher initial respiration rates, than sample 5 taken on June 27th.
period disappears completely. On the other hand the climacteric period does not appear until apples have entered the stage of cell enlargement. But once established it gains rapidly in magnitude, reaching a peak a few weeks later, and then declines until the complete extinction of the pre-

climacteric period. From this time on the climacteric is the only period represented in respiration.

Figure 9 presents, for the various samples, the length in days of total respiration records, and the respective preclimacteric and climacteric periods. A careful examination of this figure, and its comparison with
figure 7, reveals a considerable similarity between the two. The most apparent difference is the short life of apples picked in June, in spite of the large amounts of CO₂ produced by them. But from the beginning of July onward, the total life of an apple, and the duration of its preclimacteric and climacteric periods are all directly proportional to the total amounts

Fig. 9. Duration in days of the total respiration records, and of their respective periods, as observed in apple fruits present in various stages of ontogeny.
of CO₂ produced in them. Since during this time CO₂ is produced either mainly or exclusively in the climacteric period, the duration of life depends now on the total amounts of the substrate for this period present in an apple. The larger these amounts, the longer an apple will last. The practical importance of more work for the elucidation of the nature of this substrate and of the conditions favoring its accumulation is obvious.

The work reported above was actually carried out using as experimental materials, not only McIntosh but Northern Spy apples as well. The data obtained for both apples, while different quantitatively, were so alike qualitatively, that in some cases data for the Spy apples helped in the interpretation of those for the McIntosh. The only important point of difference between the two varieties is the greater sensitivity of Spy apples to higher temperatures in later stages of their ontogeny. Thus while early Spy samples produced respiration records strikingly similar to those of McIntosh, samples studied in October began to disintegrate before their climacteric rate reached a value comparable to their minimal preclimacteric value. Duration of life in such Spies was considerably less than in McIntosh apples.

Summary

1. A number of continuous respiration records of McIntosh apples is presented. These were produced by samples of apples taken throughout the whole ontogeny of the fruits, and stored at 22° C.

2. A complete respiration record of a fruit is found to consist of a preclimacteric and a climacteric period. The relative importance of these two periods in various stages of fruit ontogeny is described. It is shown that the earliest records are represented by the preclimacteric and the latest by the climacteric periods only.

3. A tentative explanation is given of changes in a complete respiration record, based on the assumption that these are due to variations in the concentration of the respiratory substrate.

4. From the data presented it is concluded that a fungal infection did take place only after an apple had reached a definite physiological stage of its starvation, and when disintegration of its protoplasmic organization already was taking place. Fungal infection, consequently, was one of the consequences of death, not the cause of it.

5. The life in days is given for the apples in various stages of their ontogeny. From the beginning of July and onward, a direct proportionality is observed between the duration of life, and the total amounts of the CO₂ produced by apples in the climacteric period of their respiration.

6. The value of the reported work is considered to be two-fold: first, it gives a general idea of the respiratory metabolism of McIntosh apples during their ontogeny; second, it may serve as a kind of time table, so that if in
the future it is decided to investigate in detail any particular stage of metabolism, one can choose for this purpose the best suited age of apples, and one knows how far apart samples for analysis should be taken.

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