

Some Concepts of the Respiration of Seed Plants

RAYMOND E. GIRTON,* Purdue University

In elementary botany and biology courses one occasionally hears the questions asked: "Do plants breathe?" "Is respiration the same as breathing?" "Are stomata breathing pores?" These and similar questions reveal widespread ignorance about the import and true nature of plant respiration.

Meaning of Respiration.

If we look into the meaning of the term "respiration" we find that it has its origin in the Latin prefix "re-" meaning back and the Latin verb "spiro" meaning to breathe. To respire, therefore, in the original meaning, is to breathe back, or to breathe in and out—that is, to inhale and exhale. In other words, respiration, in this usage, is synonymous with breathing.

Plants, however, do not breathe in the true sense of the term. It is conceivable that the nearest approach to actual breathing in seed plants is the result of wind which produces a swaying and bending of the stems and of the leaves also. This compression and expansion of stem and leaf tissues may produce a slight bellows action and cause a forceful exhalation and inhalation through lenticels and stomata, similar to the forced breathing in man when artificial respiration, or resuscitation, is used. Aside from this instance, the exchange of gases in plants is a diffusive process and cannot be catalogued as "breathing."

What, then is the modern usage of the term "respiration" as applied to plants? Meyer and Anderson (1939) in their text *Plant Physiology* have a clear answer to this question. They state that "plant physiologists use the term respiration primarily to refer to the oxidation of foods in living cells resulting in the release of energy." Other authors of current textbooks (Miller, 1938) and of monographs upon plant respiration are essentially in agreement with this interpretation as applied to plants. We have, therefore, emphasis placed upon a metabolic process, largely chemical in nature, whereby energy is released through the oxidation of foods within living cells. Such a definition is broad enough to include both aerobic (or oxygen) and anerobic forms of respiration.

Certain Factors and the Respiratory Gas Exchange.

Much of the early study of plant respiration was concerned with the influence of certain factors, chiefly external, upon plant respiration or upon related processes dependent on respiration. Since the exchange of gases is the outward sign of inward respiratory activity, the first step

* The writer wishes to thank the following individuals for reading the manuscript: Professors A. T. Guard, H. B. Knoll, C. L. Porter, and P. A. Tetrault

in building up a knowledge of plant respiration involved the discovery and demonstration of such an exchange. This discovery, in turn, waited upon the identification of the gases concerned in respiration. Therefore, although the Italian physician and plant anatomist Malpighi as far back as 1679 pointed out that seeds require air for their germination, progress in the subject was obliged to wait for the discovery of oxygen by Joseph Priestly in 1774. Three years later, the Swedish chemist and co-discoverer of oxygen, Scheele, was able to show that germinating seeds absorb and utilize oxygen and, at the same time, produce carbon dioxide. In 1779 the Dutch physician and plant experimenter, Ingenhousz, demonstrated that all living plants evolve carbon dioxide in darkness and that non-green plants also evolve carbon dioxide in the light as well.

A marked step in advance came with the introduction of quantitative methods of study by the Swiss plant physiologist and chemist, De Saussure, as illustrated by work published between 1797 and 1822. De Saussure measured the gas exchanges in germinating seeds and in darkened leaves, and compared the rates of oxygen consumption with those of carbon dioxide production. He found that frequently, but not always, these gases were exchanged in equal quantities. De Saussure also studied the production of water and of heat in plant respiration. Both of these he correlated with the measurable gas exchange.

Respiration is one of several physiological processes which are markedly influenced by temperature. Bonnier and Mangin in 1884 found that plant respiration, as measured by carbon-dioxide production, increases regularly with increased temperature until it finally reaches a maximum value where it remains at the same level until the death of the plant takes place at about 50°C. It was thus shown that plant respiration has no true optimum temperature.

Internal as well as external factors have also received study. Pal-ladin, at the turn of the century, published a report of the relation of carbohydrate supply to respiratory activity. He found that when starved, etiolated leaves were floated on sugar solutions their original low rate of respiration increased many fold. Sugar supply thus may limit respiration. The work of Spoehr and McGee (1923) concerned another and perhaps unexpected internal factor which may markedly influence respiration. These writers found that in leaf tissues wherein carbohydrate supply was adequate, increased respiration rates could be obtained by adding simple amino acids.

The contributions of the foregoing men, and of many others not mentioned, illustrate the slow gathering of knowledge concerning the general nature of the respiratory gas exchange and also the influence of various factors upon this process. As a result of these findings, speculation regarding the meaning of the observed phenomena began to develop. Some of the ideas put forward have been abandoned in the light of wider knowledge. Other concepts have been modified and retained as the result of further studies and still others are now in the process of formulation, testing, and modification.

Respiratory Concepts.

One concept which seems to have been widely held over a period of forty years is that of "double respiration." Following, and in spite of, the careful work and reasoning of De Saussure, the idea became widespread that plants carry on two distinct forms of respiration which alternate during the twenty-four hour day. Thus it was thought a "diurnal" respiration took place during the daylight hours and a "nocturnal" respiration took place during the hours of darkness. The diurnal respiration was characterized by the absorption of carbon dioxide and the evolution of oxygen. The nocturnal respiration, on the other hand, resulted in the reverse gas exchange—that is, the absorption of oxygen and the evolution of carbon dioxide. This misconception can be attributed to the use of the term "respiration" for both gas exchanges. Thus, as we now know, the actual daylight respiratory gas exchange was overlooked because it was masked by the greater magnitude of the reverse gas exchange of photosynthesis.

It remained for the German plant physiologist Sachs to sound the death knell for this double-respiration concept. Sachs in 1865 emphasized what had been shown previously by others—that two separate processes contribute to the carbon-dioxide-oxygen gas exchange, namely, photosynthesis and respiration. To quote Stiles and Leach (1936) in their monograph *Respiration in Plants*, "Sachs pointed out what he later called 'the scarcely conceivable thoughtlessness and obtuseness' in speaking of a double respiration of plants—of a so-called nocturnal respiration, by which was understood the evolution of carbon dioxide which occurs in true respiration."

Another nineteenth-century concept advanced in 1878 by the German botanist Pfeffer, and later abandoned by him, was the belief of a direct connection between fermentation and aerobic, or oxygen, respiration. This concept has been called the "Theory of Connection." It was borrowed from the animal physiologist Pfüger, and applied to plants by Pfeffer. Pfeffer's idea was that normal plant respiration consisted of two steps. The first step could take place under anaerobic conditions and yielded both alcohol and carbon dioxide. It was therefore considered to be identical with yeast fermentation. The second step was aerobic in character and oxidized the alcohol produced in step one completely to carbon dioxide and water. The fermentation process, therefore, was looked upon as the natural first step in the normal oxygen respiration of seed plants. (Fig. 1.)

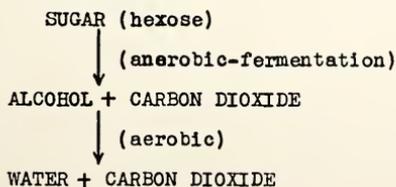


Fig. 1 Pfeffer's Scheme

In line with this concept, it was supposed that one-third of the carbon contained in the original sugar appeared as carbon dioxide in the fermentation step. This would represent the maximum yield of carbon dioxide under anaerobic conditions, as in an atmosphere of pure hydrogen, for example. The second step, which was dependent upon the presence of free oxygen, converted the carbon of the alcohol product to carbon dioxide so that all of the carbon of the original sugar would appear as carbon dioxide. Therefore, the ratio of carbon dioxide respired by plants in hydrogen to that produced in air should be one to three. This ratio could not be verified experimentally, perhaps, because of the toxic effects of the anaerobically accumulated alcohol. Also, it was shown that alcohol is less easily oxidized by plants than sugar. For these and other reasons Pfeffer virtually abandoned the view that alcoholic fermentation constitutes a part of normal seed-plant respiration. He said in 1897 as quoted in Kostyshev's (1927) *Plant Respiration*: "These primary causes, which in normal respiration bring about the oxidizing action of oxygen, in the absence of free oxygen . . . effect reactions which *did not take place* wholly or in part, reactions out of which arise carbonic acid as well as other products of intramolecular respiration."

In spite of the fact that Pfeffer himself gave up the theory of connection, the fundamental idea back of it was not dead. In 1910 and the following years, the Russian physiologist Kostyshev advocated a modification of Pfeffer's concept. (Fig. 2.) Sugar (hexose) is again

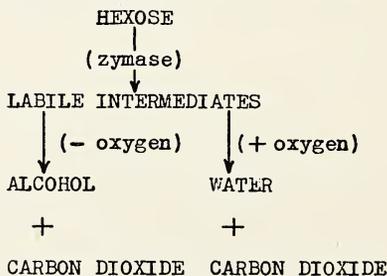


Fig. 2 Kostyshev's Schema

the starting point of his theory and under the action of zymase, demonstrable in plant tissues, produces certain labile compounds intermediate in the process of fermentation. Such compounds as acetaldehyde and pyruvic acid have been suggested as probable intermediates.

Following this first step, which is anaerobic in nature, the intermediate compounds in the presence of oxygen become oxidized to carbon dioxide and water. In the absence of free oxygen, however, some of these intermediates undergo oxidation and some undergo reduction yielding the typical fermentation products of carbon dioxide and ethyl alcohol.

Kostyshev's modification overcame at least some of the objections to the original concept. For example, the difficulty of securing a rapid oxidation of alcohol by seed plants is obviated by assigning the actual

production of alcohol to anaerobic conditions only. The main path of normal respiration thus turns aside before the final alcohol product is reached. Instead, the labile intermediate compounds are completely oxidized to the end products of oxygen respiration.

Blackman in 1928 suggested a schema which is a further modification and expansion of that of Kostyshev. (Fig. 3.) In the first place,

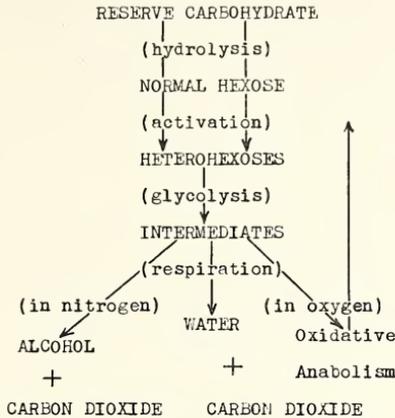


Fig. 3 Blackman's Schema

carbohydrate reserves such as starch or sucrose form the initial substrate. This necessitates a preliminary hydrolytic step to form hexose sugar. Since recent studies on the structure of sugars had described an active gamma-form of hexose, Blackman suggested that an activation step should precede the actual chemical breakdown of the hexose sugar. This cleavage step he labelled "glycolysis." It is anaerobic in nature and corresponds generally to the first step in Kostyshev's schema. Also following Kostyshev's concept, Blackman's schema gives ethyl alcohol and carbon dioxide as the final products of seed-plant respiration in nitrogen, and carbon dioxide and water as final products in oxygen (air).

There is, however, a final additional feature which Blackman has labelled "oxidative anabolism." In this step some of the carbon which might be expected to be released under aerobic conditions as carbon dioxide fails to appear. It is argued that this carbon must therefore be built back into one of the substances preceding, or intermediate in, glycolysis. This addition to the schema was included in an effort to explain the higher rates of carbon-dioxide production obtained with apple fruits in nitrogen compared to those obtained in air.

A different point of view of the respiratory process is emphasized by certain other writers. Let us look at the schema suggested by the Russian physiologist Palladin in 1908. Palladin made boiling water extractions of tissues from many different plants and found that a large proportion of the species studied contained substances which would color red or violet when treated with hydrogen peroxide and peroxidase. These extracted substances he termed "chromogens" and the oxidation products,

"respiration pigments." Palladin considered that these substances play a part in plant respiration as follows: (Fig. 4.) The chromogens could

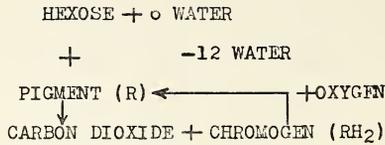


Fig. 4 Palladin's Schema

be oxidized by means of the action of oxidase and free oxygen to form respiratory pigments and water. These respiratory pigments could then bring about the oxidation of the substrates of respiration by removing hydrogen from them anaerobically. The pigments are thus reduced to form the chromogens which can be used again after oxidation in the air.

Here we have emphasis placed upon the anaerobic oxidation of the substrates of respiration. The use of free oxygen is confined to the restoration of the "hydrogen acceptor," as it is called, which in this case is the respiratory pigment. This general viewpoint is in accord with that held today concerning certain phases of cellular oxidations.

Within the past ten years several schemata which involve a series of organic acids and enzyme systems have been suggested for explaining oxidative changes taking place in living cells. One of the best known of these suggestions is that of the citric-acid cycle of Krebs as discussed by Barron (1943). This cycle, in condensed form (Fig. 5), shows

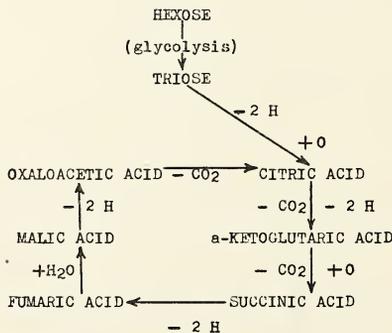


Fig. 5 Krebs Citric Acid Cycle

the oxidation of the glycolytic products of hexose sugar involving the formation of pyruvic acid which is combined with oxalacetic acid and oxidized to citric acid at the beginning of the cycle. Carbon dioxide is split off in the formation of the citric acid and in the two following steps of the condensed cycle.

The remainder of the citric-acid cycle is concerned with the regeneration of the oxalacetic acid to combine with more pyruvic acid formed by the partial oxidation of triose. Coupled with the series of acids which

make up the cycle is a system containing an oxygen activating mechanism including cytochrome and oxidase, plus appropriate dehydrogenase enzymes.

In spite of the fact that the Krebs cycle was based upon pigeon-muscle experiments, efforts have been made by Machlis (1944) and by Henderson and Stauffer (1944) to explain plant respiration upon a similar basis. Excised roots of barley and tomato were treated with oxidase and dehydrogenase inhibitors as well as with respiratory intermediates including fumarate, malate, succinate, and citrate. Studies made upon the responses in respiration to such treatments led these writers to the conclusion that some modified form of the Krebs cycle may function in the respiration of these roots.

Conclusion:

In conclusion it should be pointed out that from simple beginnings in the discovery of facts and the postulation of theories, progress in the study of plant respiration has led to an ever increasing accumulation of facts and to concepts of increasing complexity. When, and if, a complete picture of this fundamental process is eventually gained, we shall doubtless be amazed at the consummate chemist which the minute plant cell is proved to be.

References

- Barron, E. S. G., 1943. Mechanisms of carbohydrate metabolism. An essay on comparative biochemistry. *Advances in Enzymology* 3:149-189.
- Henderson, James H. M. and John F. Stauffer, 1944. The influence of some respiratory inhibitors and intermediates on growth and respiration of excised tomato roots. *Am. J. Bot.*, 31:528-535.
- Kostyshev, S., 1927. *Plant Respiration*. Translation by C. J. Lyon, P. Blakiston's Son and Company, Philadelphia.
- Machlis, Leonard, 1944. The influence of some respiratory inhibitors and intermediates on respiration and salt accumulation of excised barley roots. *Am. J. Bot.*, 31:182-192.
- Meyer, Bernard S. and Donald B. Anderson, 1939. *Plant Physiology*. C. Van Nostrand Company, Inc., New York.
- Miller, Edwin C., 1938. *Plant Physiology*. Second Edition. McGraw-Hill Book Company, Inc., New York.
- Spoehr, H. A. and J. M. McGee, 1923. Studies in plant respiration and photosynthesis. Carnegie Inst. Wash. Publ. No. 325. Washington
- Stiles, Walter, and William Leach, 1936. *Respiration in Plants*. Second Edition, Revised. Methuen and Co., Ltd., London.