Photosynthesis in Submerged Land Plants.

By Harry V. Heimburger.

Detmer,[1] in his "Practical Plant Physiology," describes an experiment to show the evolution of oxygen in photosynthesis. He uses for this purpose a water plant which is placed under a glass funnel in a vessel of water and the evolved gas collected in a test tube. The same experiment is described in a number of text-books, mention usually being made that water plants must be used. *Elodea*, *Myriophyllum*, *Ceratophyllum*, *Hippuris Potamogeton*, *Chara* and *Spirogyra* are suggested by various writers. It is to be inferred from most of the texts that no especial difficulty is met in performing this experiment, though a few of the authors consulted mention some difficulties and suggest that the experiment is not always entirely satisfactory. One writer says, "It is better to allow the apparatus to stand several days in the sunlight in order to catch a full tube of the gas."[2] Another says, "After two or three days of hot sun, enough of the gas can be obtained to make the oxygen test."[3] Again we are told, "The glowing of the splinter shows that the gas is oxygen"[4] intimating that a very pronounced test is not to be expected; though others say that the spark bursts at once into flame.

In one text[5] the use of watercress in the experiment is recommended. A few of the texts examined, do not say definitely what sort of plants to use, but usually it is implied that water plants are required. In only one text, of those we have been able to examine, is it even intimated that land plants may be used. Doctor Coulter[6] says, "If an active leaf or a water plant be submerged in a glass vessel, and exposed to the light, bubbles may be seen coming from the leaf surface and rising through the water." The illustration accompanying this text shows what appears to be the leaf of some tree or shrub. In several texts it is expressly emphasized that land plants will not serve for this purpose. Atkinson says,[7] "Land plants.

[1] Detmer-Moor, Practical Plant Physiology, pp. 35-37.
[2] Atkinson, College Botany, p 62.
[3] Hunter, Essentials of Biology, p. 126.
[4] Atkinson, Elementary Botany, p. 51.
[5] Reynolds Green, Vegetable Physiology, p. 164.
[6] Coulter, Plant Relations, pp. 29, 30.
[7] Atkinson, Elementary Botany, p. 51.

however, will not do this when they are immersed in water, but it is necessary to set up rather complicated apparatus."

Doctor Ganong, who may be regarded as an authority in plant physiology, in commenting on this experiment criticises severely the statement that land plants may be used. He says, "An erroneous experiment, given in several text-books, accompanied by a false illustration, is that one in which leaves of land plants placed under water are represented as giving off bubbles of oxygen which rise through the water. It is true that leaves which are enveloped in a film of air do carry on some photosynthesis under water, but the amount is so small that it is doubtful if any visible bubbles of oxygen are released, the tiny quantities being taken directly into solution."[8]

It is the purpose of this paper to show that some land plants do carry on photosynthesis, when submerged in water, and that for purposes of the experiment described above, are even better than the water plants ordinarily used.

In September of 1911 my attention was called to the fact that *Melilotus alba*, when submerged in water, could carry on photosynthesis, with considerable evolution of gas, and that the gas is particularly rich in oxygen. At the suggestion of Professor Howard J. Banker a number of simple preliminary experiments were carried on to test the power both in Melilotus and in several other land plants. It was intended that more careful experiments should be performed later to determine the percentage of oxygen in the gas and the rate of the evolution of the oxygen under varying conditions. While this quantitative work has not been done, the results so far obtained are so striking as to appear of interest and worthy of note.

In the experiments as performed, a few leaves of the plant under observation, were placed in the usual manner in a glass funnel which was inverted in a large glass jar full of water. A test tube of 30cc. capacity was used to collect the evolved gas. Carbon dioxide was generated by treating ordinary limestone with hydrochloric acid, and a stream of this gas was kept bubbling through the water outside the funnel. The whole apparatus was exposed to sunlight in a south window.

With *Melilotus alba*, using three or four vigorously growing shoots, having approximately one hundred leaves, from 60 to 80cc. of gas was

[8] Ganong, A Laboratory Course in Plant Physiology, p. 103.

obtained in four hours. This gas was so rich in oxygen that a glowing splinter thrust into the tube burst into flame with an explosive snap. The same leaves were left in the apparatus and on the second day yielded more than 30cc. of gas, giving a good spark test. On the third day, however, the water in the jar showed green and the plants were becoming macerated. Only a very little gas was evolved during this day, the quantity being insufficient for the spark test. With none of the plants used was any gas obtained after the second day, and sometimes only a little on the second day.

Similar tests were made with *Melilotus officinalis, Trifolium pratense, Trifolium repens, Lactuca canadensis, Arctium minus* and *Nepeta cataria*. With all these plants a good evolution of gas was obtained, at least during the first day. *Lactuca* and *Arctium* evolved very little gas during the second day and both showed considerable maceration after sixteen to twenty hours in the apparatus. With *Nepeta* the evolution of gas appeared to be more rapid than in *Melilotus*, though this might have been due to difference in light intensity or because of a greater leaf surface being exposed. No accurate measurements of leaf surface were made in any of the tests, but approximately the same leaf area was used in each case. In all the plants used, except *Nepeta*, there was less gas evolved than with *Melilotus*, but in every case there was a free evolution of the gas and not at all the tiny quantities that Doctor Ganong suggests. In all cases, too, the gas gave a very good spark test, showing it to be very rich in oxygen.

It will be noted that all the plants named above, have either a hairy or waxy covering to the leaves so that they do not become wet when immersed in water but are really enclosed in a thin film of air. When exposed to bright sun this film grows thicker and thicker until it becomes a bubble of considerable size. After a time a portion of the bubble breaks off and rises through the water into the test tube, this process continuing while the plant is in bright sunlight.

In the case of *Nepeta* and *Arctium* there are a number of minute bubbles formed on the surface of the leaf, these being more numerous and forming more rapidly on the under side. These bubbles increase in size, merging together to form large bubbles until they become so large that portions break off and escape into the tube. With *Melilotus*, *Trifolium* and *Lactuca* the film of air seems continuous and becomes a large bubble which practically encloses the leaf. The bubbles which rise into the test tube are large, there being no streams of tiny bubbles as in the case of the water plants.

The rate of the evolution of the gas depends on the brightness of the light and the supply of carbon dioxide. If a part of the light is shut off the rate becomes slower and the evolution of gas ceases when the light becomes dim. The rate becomes slower if the bubbling of carbon dioxide through the water is stopped. Enough carbon dioxide diffuses into the water from the air for the evolution of gas to go on slowly, but a rapid evolution is not obtained unless the water is kept charged with the carbon dioxide.

In one experiment leaves of *Melilotus* were dipped for an instant into 50% alcohol and then immediately immersed in water. These leaves became wet, no film of air was present and no evolution of gas occurred though the plants were exposed to the sunlight for more than six hours.

These experiments were performed in May of 1912, after which time nothing further was done except that in October *Melilotus* and *Nepeta* were used in demonstration experiments before a class. A good quantity of gas was obtained. The rate of evolution was slower than in May, probably due in part to difference in light and part to difference in the condition of the plants. The plants used in May were young vigorous shoots, while only old plants could be found in October.

As a plant to use in demonstrating the evolution of oxygen in photosynthesis, I am quite sure that either *Melilotus* or *Nepeta* will prove entirely satisfactory. They are not only much easier to obtain than the aquatic plants, which are said to be necessary for this experiment, but the results are more quickly attained and are more striking than is usually the case with the conventional aquatics used for this purpose.

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