RESPIRATION OF THE LEAVES OF NUPHAR ADVENUM AND TYPHA LATIFOLIA [1]

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The leaves of *Nuphar advenum* and *Typha latifolia* are ordinarily submerged for part of their lifetime and many are aerial or semi-aerial for the remainder of the time. Therefore, the question as to the respiratory behavior of these leaves under different conditions of aeration is pertinent to an understanding of the adaptation of the plants to their habitat.

Material.—The species of plants used in these studies were *Nuphar advenum* Ait., and *Typha latifolia* L. An experiment on the leaves of *Pelargonium zonale* is included for the purpose of comparison.

The young leaf blade of *Nuphar advenum* is tightly rolled from both edges toward the mid-rib, the upper surface, which contains all the stomata, being innermost. As the petiole elongates, the blade very slowly uncoils until, by the time it has reached the surface of deep water, all of the folds and undulations have disappeared so that the blade frequently lies flat upon the surface of the water. In shallow water many of the blades reach the surface before they have become flat and, if the petioles are sufficiently stout, are held erect above the surface of the water. Thus, throughout the entire summer season, the typical plant of *Nuphar advenum* may have submerged, floating, and some aerial leaves. In the very early spring the first two or three leaves usually remain submerged, and their broad blades never become perfectly flat but remain very slightly undulant or wrinkled in appearance. The last unfolded leaves of autumn are small, purplish-pink, flat, and submerged, while the winter leaves consist only of the sharply-tipped, tightly-coiled blades that protrude often not more than half their length above the mud in the icy water.

In *Typha latifolia* the young leaves normally remain submerged until they attain a length of approximately six to ten inches, at which time the tips begin to extend above the surface of the water. New leaves appear in a continuous succession throughout the growing season until the frost of autumn reduces them to the familiar grayish-brown thicket of dead leaves, some of which surround decadent culms while others, borne from rhizomes that were too immature to produce a fruiting culm, simply droop from a point usually slightly elevated above the water. This slightly elevated point is the tip of the pointed spike of tightly compressed innermost young leaves that remain concealed under the dead outer leaves. These young leaves frequently remain alive throughout the winter and, when the breaking up of the ice and the action of wind and waves have torn the old leaves away at the end of winter, there may be seen the green tips of the tightly compressed young leaves protruding above the water.

Experimental results.—Mature leaves of *Nuphar advenum* were selected and weighed into six lots, care being taken to obtain as much uniformity as possible. Each lot was placed in a large museum jar serving as respiration chamber. The six jars were then closed by means of large rubber stoppers each fitted with two glass delivery tubes, one long and one short. The jars and contents were placed in a water bath kept at a constant temperature of 25°C. The delivery tubes were connected to an aerating apparatus described in a previous paper (Laing, 1940) in such a manner that the six lots were subjected to different oxygen concentrations, namely, air, 10, 3, 1.5, and 1 percent of oxygen, and purified nitrogen. The rate of respiration was determined by means of a modified Pettenkofer apparatus (Laing, 1940). The data are shown graphically in figure 1.

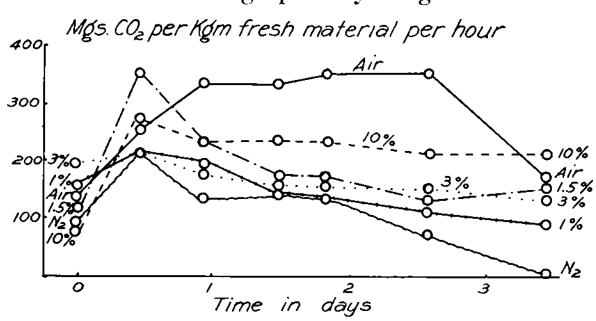

Fig. 1. Respiration of mature leaves of *Nuphar advenum* in air, nitrogen, and various concentrations of oxygen by volume.

The results show that the leaves evolved CO_2 rapidly during the first day following collection and preparation. This increase of CO_2-production, however, was not so great as in the case of rhizomes (Laing, 1940). The per cent decrease of respiration from the second day to the end of the experiment was very high in nitrogen and very low in 10 per cent of oxygen. The rate of decrease for the intermediate concentrations of oxygen was more or less related to the oxygen concentration. The per cent of decrease in air was intermediate between that in 3 and 1.5 per cent of oxygen.

[1] Received for publication January 19, 1940.
Paper from the Department of Botany of the University of Michigan, No. 701.
This investigation was conducted under the direction of Dr. F. G. Gustafson, whom the writer wishes to thank for various suggestions and encouragement. He also wishes to thank Dr. C. D. La Rue and Dr. C. C. Meloche for helpful information.
During part of the time that this research was conducted the writer was the holder of an F. C. and Susan Eastman Newcombe fellowship in Plant Physiology.

[The Journal for July (27: 449–582) was issued July 30, 1940]

This experiment was repeated three times with younger leaves to test the accuracy of the conclusions, care being observed during the second and third repetition to prevent overlapping of the leaves by adjusting the leaves on wire frames. Also, during the second and third repetitions only four gaseous mixtures were used; air, 10 and 3 per cent of oxygen, and purified nitrogen. The respiratory behavior was found to be similar to that of mature leaves.

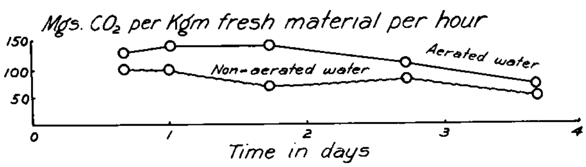

Fig. 2. Respiration of young leaves of *Nuphar advenum* in aerated and non-aerated water.

A test for alcohol production by the leaves in the various concentrations of oxygen and nitrogen indicated that reducing substances such as alcohol were produced in nitrogen and in the lower concentrations of oxygen. Here, as well as in the respiration of rhizomes, previously described (Laing, 1940), there is evidence that aerobic and anaerobic respiration occur simultaneously when the oxygen concentration of the medium is low. This same phenomenon was observed by Gustafson (1936) while studying the respiration of tomato fruits and by Laing (1940) in rhizomes of *Nuphar advenum.*

At the end of the experiments it was observed that the different mixtures of oxygen and nitrogen had affected the leaves differently. It was found that the mature leaves of *Nuphar advenum* had endured 1.5 per cent of oxygen best, 3 per cent of oxygen next best and 1 per cent of oxygen third best; but had deteriorated rapidly in moist air and quite rapidly in 10 per cent of oxygen and in nitrogen. In contrast to these results, it was found that the younger leaves had endured pure nitrogen or a very low concentration of oxygen best and were injured most by the 20 per cent of oxygen in the air, the injury being greatest at the margin and least near the mid-rib. Mature leaves that had been injured by aerobic conditions had become yellow and russet-colored like leaves normally are in autumn, showing a break-down of chlorophyll. Those injured by too little oxygen had developed areas of a dead buff color that appeared watersoaked. The break-down of the mature leaves in nitrogen was probably associated with the extreme decrease in respiration.

In another experiment with *Nuphar advenum,* 275 grams of petioles cut into 10-inch lengths were put into each of the six large respiration jars. These were connected in the usual way to the following gaseous mixtures: air, 10, 3, 1.5 and 1 per cent oxygen, and nitrogen.

The per cent decrease in respiration did not show any relationship with the oxygen concentration. The results did show, however, that petioles respire more nearly like rhizomes than like leaf blades, because on the third day of typical experiments with each, the rhizomes had averaged about 30 mg. of CO_2 per Kgm. of fresh material, the petioles about 33 mg., and the leaf blades about 180 mg. Presumably some of this rate of CO_2 production was caused by the difference in the rapidity of gaseous exchange due to the difference in bulkiness, but most of it was probably due to a difference in the number of active cells.

For purposes of comparison an experiment was conducted on the respiration of young leaves of *Nuphar advenum* in water. Since pond-lily leaves are very buoyant, the wire frames were inverted before being put into the large jars, so that the leaves would not become loosened and buoyed up to form a packed mass at the surface of the water. Two sets were prepared and put into two large jars, which were then filled with water high enough to cover the leaves. Air was bubbled through one jar and nitrogen through the other.

The leaves in the aerated water (fig. 2) respired more vigorously than those in the nitrogenated water, but there was a gradual decrease in both lots. Alcohol was formed in the watery medium of both lots, as was determined by the blue color obtained by warming a sample of the water into which a mixture of 5 drops of 5 per cent potassium dichromate and 5 cc. of 30 per cent nitric acid had been put. Enough alcohol was formed by the leaves in nitrogenated water so that some passed off during the experiment and was collected in a tube containing some of the mixture of nitric acid and potassium dichromate, changing the color after a collection period of fifty-seven hours. No color change was noted in a tube of indicator solution connected to the aerated material, thus showing that less alcohol was formed by the leaves in aerated water than by the leaves in the water through which nitrogen was passed.

In order to compare the respiratory behavior of the leaves of *Nuphar advenum* with those of some familiar land plant, the leaves of *Pelargonium zonale* were used. The gaseous mixtures used were air, 10 and 3 per cent oxygen, and nitrogen.

The data indicated that there was a 10 per cent decrease of respiration in air, a 25 per cent increase in 10 per cent oxygen, 18.2 per cent decrease in 3 per cent oxygen, and an 89.1 per cent decrease in nitrogen, from the second to the fifth day of the experiment. The great decrease in respiration in nitrogen was probably associated with the death of the leaves. Tests showed that alcohol was produced by the leaves in nitrogen, but none by the other leaves. The different concentrations of oxygen had different effects on the leaves of *Pelargonium zonale.* The leaves that had been in nitrogen were all dead and had assumed a dark lusterless color. They were the first ones to become dry. Those that had been in 3 per cent oxygen were changed least in color at the end of the experiment, but the true condition of injury became more apparent as they were taken out of the jars and permitted to dry. They turned a dull ashy gray color and were the second to become dry. Those that had been in air were the most yellow but were still glossy. They were the third to become dry. Those in 10 per cent oxygen appeared to be in the best condition at the end of the experiment and they were the last to become dry, some of the petioles remaining plump even on the eighth day of drying.

Six samples, each composed of 55 gm. of fresh green leaves of *Typha latifolia,* were prepared by cutting the leaves into lengths of about 15 to 30 cm. These leaves were placed in large test tubes which were connected to the following gaseous mixtures in the usual way: air, 10, 3, 1.5 and 1 per cent of oxygen, and nitrogen. Care was observed to avoid including any diseased or discolored specimens. Most of the leaves appeared to be about middle-aged, physiologically speaking, i.e., neither young nor old.

In this experiment the per cent decrease of respiration was greatest in 1 per cent and least in 1.5 per cent of oxygen, and there was no consistent correlation between the decrease in the rate of respiration and the per cent of oxygen. The decrease of CO_2 production during the first three days of the experiment was greater in nitrogen, 1 per cent, and 1.5 per cent of oxygen than it was in 3 per cent of oxygen. During that time there was no permanent decrease of respiration in 10 per cent of oxygen, and there was an actual increase of respiration in 20 per cent of oxygen or air. During the fourth day there was some decrease of respiration in all the lots excepting the one in 1.5 per cent of oxygen, which had been generally low throughout the experiment.

At the conclusion of the experiment the leaves that had been in moist air were found to be in an advanced stage of yellowing. Yellowing had also begun in those leaves that had been in 10 per cent of oxygen, but the condition was much less advanced. There was no noticeable yellowing in the other lots. A similar condition was noticed at the end of the respiration experiment with leaves of *Pelargonium zonale* described above, and again in the culms of *Scirpus validus* which were included in a supplementary experiment not reported here. A comparison of the yellowing of the leaves of *Pelargonium zonale, Typha latifolia,* and the culms of *Scirpus validus* indicated that most of the yellowing was associated with the higher concentration of oxygen, as was previously noticed in the experiment with the mature leaves of *Nuphar advenum.*

Discussion.—The rate of decrease of CO_2 evolved by excised leaves of different species under standardized conditions may be taken as an indication of the relative effect of the conditions upon respiration. After four days, the per cent of decrease of evolution of CO_2 by the excised leaves of the following species, in air and in nitrogen respectively, were: *Nuphar advenum* (young leaves), 25 and 50; *Typha latifolia,* 20 and 47.8; *Nuphar advenum* (mature leaves), 40 and 94.1; and *Pelargonium zonale,* 10 and 89. These data show that the young leaves of *Nuphar advenum* are able to continue anaerobic respiration and to endure anaerobic conditions remarkably well but that this ability is lost when the leaves become mature and float on the water or extend into the air above the water. The ability of the mature leaves to endure anaerobiosis is approximately equal to that of the relatively rugged leaves of *Pelargonium zonale,* but is still greater than that of such leaves as those of *Encelia farinosa* and *Verbena ciliata,* reported by Gustafson (1932). The leaves of *Encelia farinosa,* after fifty hours in nitrogen, were respiring only 17 per cent as rapidly as they had respired at the beginning of the experiment, while a similar lot in air still respired 77 per cent as much as at the beginning. The leaves of *Verbena ciliata* showed even a greater difference between those in nitrogen and those in air.

Although the leaves of *Typha latifolia* are thicker and perhaps more rugged than those of *Nuphar advenum,* nevertheless it is interesting to note how well they can endure anaerobic conditions and continue to evolve CO_2 after four days in nitrogen.

The significant facts brought out by these studies are that there are some differences in the respiratory behavior of the leaves of the different species of plants studied, that there is a difference between the behavior of young and mature leaves of *Nuphar advenum,* and that the submerged leaves of such water plants as *Nuphar advenum* and the leaves of *Typha latifolia* are able to endure anaerobic conditions much better than the aerial leaves of the familiar species studied.

SUMMARY

Respiratory studies were made of the leaves of the semi-submerged water plants *Nuphar advenum* and *Typha latifolia,* and of the land plant *Pelargonium zonale.* A modified Pettenkofer method was used. Observations were made on the effect of different mixtures of oxygen and nitrogen upon the respiration and endurance of these leaves.

The data indicate that the young leaves of *Nuphar advenum* and the leaves of *Typha latifolia* were able to respire anaerobically for some time, the rate of decrease of respiration being about 50 per cent in four days. Respiration of the mature leaves of *Nuphar advenum* in nitrogen, however, decreased 94 per cent in four days, showing that they had lost the ability to sustain anaerobic respiration. A comparison was made with the mature leaves of *Pelargonium zonale* in which the rate of anaerobic respiration decreased 87 per cent in four days.

On the basis of fresh weight the rate of respiration of the leaf blades of *Nuphar advenum* was greater than that of the petioles, and that of the petioles was somewhat greater than that of the rhizomes.

The leaves of *Nuphar advenum* and *Pelargonium zonale* produced alcohol in 3 per cent and less of oxygen.

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INTERMITTENTLY OPERATED IRRIGATOR CONES AS INDICATORS OF SOIL-MOISTURE CONDITION IN DRYING CYLINDER CULTURES OF WHITE LUPINE [1]

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THE MOISTURE condition of a soil mass may be considered either with regard to its physical characteristics or with regard to its capacity to maintain an adequate supply of water for plant roots. It is thus necessary to distinguish between physical soil-moisture condition and physiological soil-moisture condition. As a wet aerated soil dries out, its physical moisture condition changes gradually and continuously till absolute dryness is approached, but its physiological condition remains healthful till drought effects in the plants begin to appear, after which this condition changes rapidly for the worse. For such a drying soil mass, physical moisture condition may be measured or estimated from time to time in several ways, as in terms of average moisture content, soil-moisture potential or water-supplying power. On the other hand, physiological moisture condition can be estimated only by means of plant indicators, which show the approach and progress of drought injury in terms of growth retardation, turgor lowering, wilting and withering. The onset of drought injury, which is of prime interest from the viewpoint of physiology and ecology, corresponds to a fairly definite value of initial water-supplying power, a value essentially constant for a very wide range of kinds of soil and soil porosities. But the critical average soil-moisture content at the onset of drought injury varies widely with the kind of soil considered. Therefore moisture-content values cannot be interpreted with reference to plant health unless the kind of soil is specifically taken into account.

Root absorption is confined to the soil within very short distances of the absorbing surfaces. Although the moisture of that restricted portion of the soil mass may remain unmeasured, estimates of soil moisture that represent the average condition within the region of growing roots may be taken as approximate measures of the current subterranean moisture environment. Thus the average volumetric moisture content of the soil mass within the region occupied by a root system represents the amount of water within reach of the exploring roots. This average is an approximate measure of the soil's moisture reserve. It is expressed as grams of water present in 100 ml. of soil.

The average moisture content of a mass of soil is generally expressed gravimetrically as grams of water per 100 gm. of dry soil. That form of expression is valuable for comparisons so long as all soils considered are characterized by similar dry weights per unit of volume, but it cannot be directly interpreted with reference to plant health, for plants are not directly influenced by the specific weight of the soil about their roots. Because of these considerations and because volumetric percentages seemed to be worthy of further study, they are employed in this account.

The use of dry soil-point cones (Livingston and Koketsu, 1920; Livingston and Norem, 1937; Marshall, 1931; Wilson, 1927; Wilson and Livingston, 1932) as water absorbers in the study of initial water-supplying power led Livingston and Norem (1937) to the suggestion that water-saturated porous-porcelain pieces might be somewhat similarly employed to secure estimates of the *initial water-absorbing power* of the soil. Those authors described an arrangement by which a hollow porous-porcelain cone permanently buried in the soil to be tested was filled with water and allowed to give off water to the adjacent soil for a standard initial period (one hour), after which the cone was emptied by the observer and left without water supply for many days, until the time of the next test. For that instrument a reading was just the volume of water that moved from cone to soil within the one-hour period.

The results of an extensive series of greenhouse experiments with flower-pot cultures, in which their intermittently operated porous-porcelain irrigator cones were tested with drying soil masses at the onset of serious drought injury to the plants, led the authors just mentioned to the thought that an arrangement of this type might prove useful for appraising soil-moisture condition in the vicinity of plant roots at the onset of permanent wilting (Briggs and Shantz, 1912; Miller, p. 190, 1938; Veihmeyer and Hendrickson, 1928), at least for a

[1] Received for publication February 26, 1940.

Botanical contribution from the Johns Hopkins University, no. 153.

In the interpretation of the results secured and in the preparation of this paper, much greatly appreciated help was received from Professor Burton E. Livingston, of the Laboratory of Plant Physiology, the Johns Hopkins University.