RESPIRATION OF THE RHIZOMES OF NUPHAR ADVENUM AND OTHER WATER PLANTS [1]

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NUMEROUS STUDIES have been made on the respiration of land plants and some on water plants, but very few such studies have been made on semi-submerged water plants (Samantarai, 1938). Yet, from the standpoint of the environment, semi-submerged plants offer very interesting material because of the belief that they were once terrestrial and have subsequently invaded the water, and because it is difficult to understand how the rhizomes of plants with terrestrial form are able to endure the condition of low aeration that exists in the mud at the bottom of a pond (Cole, 1932).[2] That this ability is not due to particular structural features may be deduced from the fact that rhizomes of very diverse structures are found imbedded in the submerged mud. For example, there are the thick spongy rhizomes of *Nuphar advenum, Nymphaea tuberosa, Pontederia cordata,* and *Peltandra virginica* in contrast to the small, tough, firm rhizomes of *Typha latifolia, Sparganium eurycarpum, Scirpus validus,* and *Acorus Calamus,* the firm corms of *Sagittaria latifolia,* and the small, tough-rooted crowns of *Asclepias incarnata.*

Furthermore, the ability to endure the nearly anaerobic conditions of the submerged mud is not associated with a particular habit of growth and fruiting, because all of the species of thick spongy rhizomes mentioned above as well as one of the toughest and firmest of rhizomes to be found anywhere, namely, that of *Scirpus validus,* grow progressively through the mud, branching only occasionally, sending up fruiting culms at irregular intervals, and always dying and becoming decayed at the rear; while the hard young rhizomes of *Typha latifolia* and *Sparganium eurycarpum* and the corms of *Sagittaria latifolia* are formed one at the terminal end of each of the slender spongy stolons that radiate from a parent plant which dies at the end of its fruiting season.

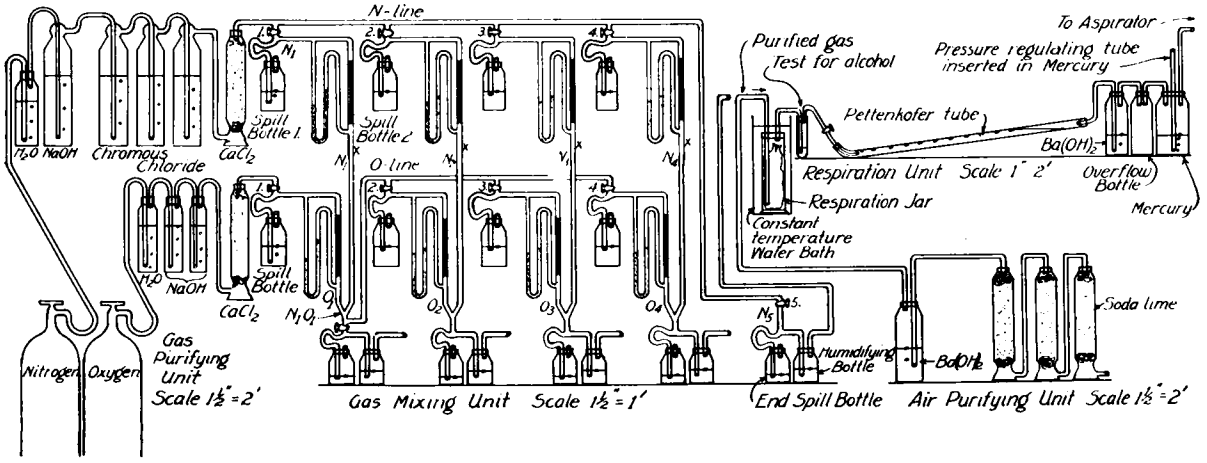

Fig. 1. Apparatus composed of gas purifying, gas mixing, and respiration units.

Also, as will be explained more fully in a later paper, the ability of these rhizomes to endure practically anaerobic conditions is not exclusively dependent upon the fact that they possess an internal atmosphere that contains, at times, appreciable quantities of oxygen; because, at other times, this internal atmosphere contains very little and sometimes practically no perceptible amount of oxygen.

From the foregoing statements, it is apparent that a study of the respiration of these plants should contribute much toward a better understanding of their mode of life.

METHODS AND APPARATUS.—Because of the bulkiness of the material, the method used was that of absorbing the carbon dioxide produced by the material in a standardized solution of barium hydroxide, titrating the remaining solution against a 0.1 N solution of oxalic acid using phenolphthalein as indicator, and calculating the amount of CO_2 absorbed per hour per unit weight of material.

The apparatus used was a modification of the apparatus described by Pettenkofer (1862) and sub-

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[2] Cole (1932) found that the water at a depth of ten inches in the mud of a pond contained 0.35 cc. of oxygen per liter while the water at the surface of the mud contained 2.36 cc. when the depth of the water above the mud was eight feet. These conditions are equivalent to those of moist gaseous mixtures containing only 0.035 and 0.236 per cent of oxygen, respectively.

sequently modified by Pfeffer (1885), and Palladin (1923). This apparatus ordinarily consists of two units, namely, a gas purifying unit and a respiration unit (fig. 1). However, it was necessary to insert a gas mixing unit in order to obtain the various concentrations of oxygen desired. Traces of carbon dioxide and oxygen were removed from the nitrogen gas by passing it through sodium hydroxide and chromous chloride solutions, respectively. A tube of phosphorus was inserted occasionally to verify the absence of oxygen. The oxygen gas was passed through a solution of sodium hydroxide to remove carbon dioxide. Air was passed through granular soda lime to remove carbon dioxide and then through a solution of barium hydroxide as an extra precaution. In order to insure complete absorption of carbon dioxide, the Pettenkofer tubes used were much longer and more slender than those usually employed; a tube designed to contain 100 cc. of barium hydroxide solution was approximately six feet in length.

The gas mixing unit (fig. 1) consisted of a series of capillary tubes attached to manometers and governed by adjustable "spill-bottles." Dibutyl phthalate was used as the manometric fluid because of its low vapor pressure. Distilled water was used in the spill bottles where evaporation could easily be remedied. The eight capillary tubes used were selected from a number of tubes previously calibrated. Tubes of large diameter through which air passed quite rapidly were calibrated by measuring the amount of air collected over water in an inverted burette within a given period of time and at a given manometric pressure. Tubes of very small diameter were calibrated by counting the number of bubbles released from a chosen standard delivery tube at a certain depth in water, within a given period of time, at a given manometric pressure, and within a very narrow range of temperature. Since the rate of flow of a gas through a capillary tube is inversely proportional to the viscosity of the gas when other factors are constant, it was possible to convert the readings obtained with air to those of the desired gas by means of a table of viscosities. It was possible then to calculate the percentage of oxygen that would be present in a mixture of oxygen and nitrogen when the gases, each flowing from a separate capillary tube, were finally mixed by being passed into a single Y tube. Thus, a primary mixture of oxygen and nitrogen was made at point N_1O_1 (fig. 1) by means of the first set of two capillaries, and this primary mixture was then diluted with different amounts of nitrogen to form three secondary mixtures at points O_2, O_3, and O_4, respectively. The resulting mixtures were analyzed in a Henderson-Haldane apparatus and found to be very accurate. In fact, it was found to be possible to produce mixtures by this method with satisfactory assurance of their probable accuracy even when the mixtures contained oxygen in quantities too minute to be determined by ordinary methods of analysis. Since any moisture present is likely to condense in capillary tubes and thus introduce an error, all moisture was removed from the purified gases by passing them over granular calcium chloride before introducing them into the capillary system. This necessitated the humidifying of the gases before they entered the respiration chamber. The method of conducting the dry gases under positive pressure to the humidifying bottles is illustrated at point N_5 (fig. 1), where the accompanying end spill bottle was used to regulate the pressure. The gases then were drawn from the humidifying bottles under negative pressure provided by the aspirator, through the respiration jars, thence through the Pettenkofer tubes where the carbon dioxide was absorbed, and finally through the testing solution of barium hydroxide to insure that all of the carbon dioxide had been absorbed. A mercury pressure regulator inserted just before the aspirator was used to insure a constant pressure. The respiration chambers were immersed in a constant temperature water bath, which was kept at 25°C.

PROCEDURE.—Fresh rhizomes were prepared by removing all of the leaves and roots, care being taken not to rupture the periderm needlessly. The terminal bud of each rhizome was removed in some experiments and left intact in others, as will be pointed out later. In *Nuphar advenum,* when the terminal bud was left intact, the petioles of a few of the large inner leaves were cut in such a way as to leave about 3 cm. of the petioles surrounding the embryonic leaves, thus insuring that no injury was done to the bud. Although rhizomes are characteristically irregular in size and shape, an effort was made to choose comparable material and to secure as much uniformity as possible in all respects. The prepared rhizomes were put into large-mouthed cylindrical museum jars or large test tubes which were then closed by means of large rubber stoppers, through each of which there extended one long and one short glass tube. In some experiments each jar contained but one rhizome, while in other experiments there was more than one rhizome in each jar. The jars were placed in a water bath kept at a temperature of 25°C., and were connected to the aerating system as shown in figure 1.

Three different types of experiment were made. In the first group of experiments, the rhizomes were kept in water through which either air or nitrogen was bubbled. The experimental conditions were, therefore, essentially anaerobic and semi-anaerobic, thus resembling somewhat the conditions of the habitat.

The second group of experiments was similar to the first in all respects excepting that the medium consisted of moist air part of the time and moist nitrogen gas part of the time, hence causing the experimental conditions to be either completely aerobic or completely anaerobic, the only moisture present being in the form of vapor.

In the third group of experiments, various mixtures of oxygen and nitrogen were used. The essential significance of these experiments is that they explore the realm of respiratory behavior that inter-

TABLE 1. *Respiration of fresh rhizomes in aerated water and in water through which nitrogen was bubbled.*

Species	Conditions	Mgms. CO_2 per Kgm. material per hr.			
		1st day	3rd day	7th day	12th day
Nuphar advenum	Air	61.5	59.7	44.5	...
	N_2	63.2	45.1	25.9	...
Nymphaea tuberosa	Air	50.6	48.4	46.0	16.0
	N_2	37.4	13.2	23.0	17.1
Peltandra virginica	Air	79.7	79.2	78.1	58.3
	N_2	74.8	73.3	72.6	49.5
Pontederia cordata	Air	37.4	39.6	39.6	...
	N_2	35.2	48.4	13.2	...
Typha latifolia	Air	154.0	105.6	105.6	...
	N_2	105.6	114.4	70.4	...

venes between conditions of complete anaerobiosis and complete aerobiosis. As it is impossible to include all of the experiments performed, only a few typical ones will be described.

RESULTS.—*Rhizomes submerged in water.*—In this group of experiments, bulky portions of representative rhizomes of five different species (table 1) were kept entirely submerged in water in separate respiration jars, two jars being used for each species. Through each of the two jars used for each of the three species, *Nuphar advenum, Nymphaea tuberosa,* and *Peltandra virginica,* air from which the carbon dioxide had been removed and nitrogen from which both the carbon dioxide and oxygen had been removed were bubbled during alternate periods of time consisting of several days each. Through one of each of the two jars containing the rhizomes of *Pontederia cordata* and *Typha latifolia* respectively, purified nitrogen was bubbled continuously, and purified air was bubbled continuously through the other jar. Numerous readings were taken of the production of carbon dioxide.

Immediately after the rhizomes of *Nuphar advenum* had been removed from the respiration jars at the end of the eighteenth day of the experiment, a test was made of the rate of evolution of CO_2 by the microorganisms contained in the water remaining in the jars. This test showed that the proportion of the CO_2 that was due to the activity of microorganisms was probably not greater than 20 per cent of the total amount evolved during the latter days of the experiment. A similar percentage was estimated for *Nymphaea tuberosa,* but for *Peltandra virginica* the amount was probably not over 10 per cent. There appeared to be little, if any, microorganic activity in the jars containing *Pontederia cordata* and *Typha latifolia,* probably because these experiments lasted only seven days. While the presence of microorganisms is an objectionable feature, such experiments are not without value for the purpose of comparison, even though the data lack the degree of quantitative precision that it is possible to obtain by the methods used in later experiments where the rhizomes were in a medium of moist gas instead of water.

At the end of each of the five experiments, it was noticed that the rhizomes were still quite firm, there being only a few areas of dead tissue on the surfaces of *Nuphar advenum* and *Nymphaea tuberosa,* somewhat less on *Peltandra virginica,* and none at all on *Pontederia cordata* and *Typha latifolia.* Vigorous growth of shoots had occurred on all the rhizomes of *Pontederia cordata* in nitrogen as well as in air, but none in the rhizomes of *Typha latifolia,* either in nitrogen or in air. The fact that noticeable growth did not occur in the rhizomes of *Typha latifolia* in spite of its higher respiratory rate shows that growth and respiration do not necessarily go together. However, it is a fact that the rhizomes of *Typha latifolia* contained numerous buds, which, as will be pointed out later, are able to grow when conditions are favorable. Since the buds had previously been removed from the rhizomes of *Nuphar advenum, Nymphaea tuberosa,* and *Peltandra virginica,* there was no opportunity to observe the effect of the experimental conditions on growth.

The evolution of CO_2 on the first, third, seventh, and (for two species) twelfth days of continuous experimental conditions are given in table 1. Since the graphs of these experiments are essentially similar to those of the second group, they are omitted for the sake of brevity.

In summarizing the results of these five experiments, it should be pointed out that the rhizomes in the jars through which nitrogen was bubbled produced less CO_2 than the rhizomes in the jars through which air was drawn. Nevertheless it is remarkable how much CO_2 these rhizomes under anaerobic and nearly anaerobic conditions produced, and how well this rate was sustained throughout the experiments. When the measurement of CO_2 was begun soon after the collection and preparation of the samples, an initial outburst of CO_2 during the first two days was noticed in both aerated and non-aerated water. This was not observed when the collection of CO_2 was postponed until the third or fourth day. A decrease in the CO_2 output was noticed when nitrogen was introduced following a period of respiration in aerated water, and usually an increase of CO_2 was produced

TABLE 2. *Respiration of fresh rhizomes in moist air and in moist nitrogen gas. Average of the readings taken on the second and fourth days.*

Species	Conditions	Mgms. CO_2 per Kgm. material per hr.			
		1st day	3rd day	6th day	10th day
Nuphar advenum	Air	59.2	51.0	23.6	12.4
	N_2	50.7	33.8	12.4	7.8
Typha latifolia	Air	48.4	33.0	44.0	...
	N_2	41.8	24.2	37.4	...
Scirpus validus	Air	55.0	46.2	59.4	...
	N_2	66.0	26.4	30.8	...
Asclepias incarnata	Air	72.6	85.8	91.3	...
	N_2	83.6	46.2	79.2	...
Sagittaria latifolia	Air	81.4	60.5[a]	70.4	...
	N_2	61.6	26.4[a]	15.4	...
Sparganium eurycarpum	Air	44.0	33.0[a]	28.6	...
	N_2	30.8	25.3[a]	26.4	...
Acorus Calamus	Air	72.6	73.8[a]	52.8	...
	N_2	72.6	48.4[a]	59.4	...

when air was admitted to the previously non-aerated medium, but this increase was not lasting. The significance of the results of this group of experiments is that they furnish experimental evidence under controlled conditions that the rhizomes of water plants can respire either in aerated or non-aerated water and that the rhizomes of at least some species can endure prolonged exposure to anaerobic conditions remarkably well.

Rhizomes in air.—In this group of experiments, fresh rhizomes of each of seven different species (table 2) were prepared as usual and placed in a medium of moist air instead of water. Moist nitrogen was introduced into one jar of *Nuphar advenum* during the fifth hour after the collection of carbon dioxide was begun, and into the other jar on the eleventh day, at which time air was reintroduced into the first jar. In all of the other six species, nitrogen was introduced into one of the jars during the twenty-seventh hour. In the experiments with *Typha latifolia, Scirpus validus,* and *Asclepias incarnata,* air replaced nitrogen and nitrogen replaced air on the eighth day, and the experiment was then continued until the fourteenth day. The experiments with the other species lasted only seven days and no changes were made after the twenty-seventh hour.

The experiment with *Nuphar advenum* was halted for inspection on the twentieth day. The rhizomes were taken from their jars, inspected for a few minutes, and, after having been found to be in excellent condition, were returned to their respective jars; and the experiment was continued for 10 more days. Upon inspection of the material at the end of the experiment, it was discovered that the rhizomes, while not so fresh in appearance as on the day when they were dug, nevertheless were still quite firm with only a few small softened areas. Fortunately there had been no abrasion on the periderms of these rhizomes, and all of the leaf scars had been well healed. Consequently molds were present only to a very slight extent. Similar conditions of endurance were noticed in the other species.

Graphs typical of the respiratory behavior of these species are presented in figures 2, 3, and 4, and a condensed summary of a few essential points of the data are given in table 2.

The results of the experiments of this group show that the rhizomes of semi-submerged water plants are able to respire both aerobically and anaerobically. Furthermore, there is a marked similarity between the different species. In all cases, the introduction of nitrogen was followed by a decrease in the production of CO_2; and the introduction of air, following a period of anaerobiosis, resulted in an increased production of CO_2 (fig. 2 and 3). Excepting in the experiments with *Asclepias incarnata* and *Sagittaria latifolia,* there was a general tendency for respiration to decrease with time both in air and in nitrogen. Since the collection of CO_2 did not begin until the third day after collecting and preparing the samples, any initial outburst of CO_2 that may have occurred previous to that time was not recorded. It is probable, however, that the rapid decrease in production of CO_2 by *Sagittaria latifolia* represents the return of the rate of respiration to normal, following the initial outburst. The same may be true to a lesser extent of the other species. Although the rate of CO_2 production was higher aerobically than anaerobically in all of the species, nevertheless, in most of the species, the lower rate in absence of air was maintained throughout the experiments nearly as well as in air. The results also indicated that the rhizomes and buds of these water plants were able to endure anaerobic conditions for a considerable period of time, depending on the species. It might be interesting to add that the results of another experiment not reported in this paper show that the rate of respiration in the rhizome of *Nuphar advenum* decreases with distance from the terminal bud.

Rhizomes in different oxygen concentrations.— In this group, the rhizomes were subjected to a medium of moist gaseous mixtures of oxygen and nitrogen which were prepared as explained in the section on methods and apparatus. In some of the experiments the gaseous mixtures used were air, 4.6, 1.5, 0.4, and 0.1 per cent of oxygen, and purified nitrogen, while in the rest of the experiments the mixtures were air, 10, 3, 1.5 and 1 per cent of oxygen, and nitrogen. Since the collection of CO_2 was begun soon after the samples were prepared, a more rapid evolution of CO_2 was observed early in each experiment.

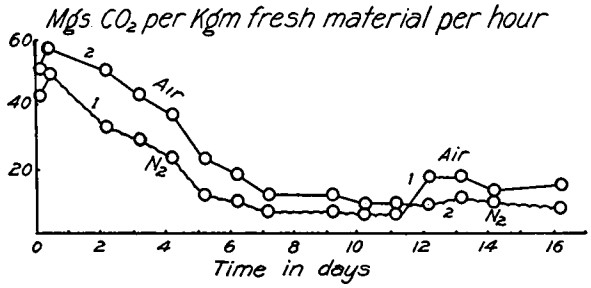

Fig. 2. Respiration of rhizomes of *Nuphar advenum* in moist air and in moist nitrogen gas.

In summarizing this group of experiments (fig. 5, 6, 7, 8, and 9), it may be said that the rate of respiration was usually highest in air, lowest in nitrogen, and intermediate in the various other concentrations of oxygen. There was usually a decrease in the rate of respiration in all media during the course of an experiment, the rate of decrease being only slightly greater in nitrogen and the lower concentrations of oxygen than in air. By dividing the difference between the initial amount of CO_2 produced and that at the end of an experiment by the initial amount, one obtains the per cent of decrease in respiration for the duration of the experiment. In one typical experiment with *Nuphar advenum* (fig. 5) the per cent of decrease of respiration from the second day to the end of the experiment was 28.4 in air, 65.7 in 4.6 per cent O_2, 67.7 in 1.5 per cent O_2, 67 in 0.4 per cent O_2, 42.1 in 0.1 per cent O_2, and 73 in nitrogen. The decrease was thus not much greater in nitrogen than in some of the other gaseous mixtures containing appreciable amounts of oxygen. This shows the ability of these plants to sustain the rate of CO_2 production quite well even under anaerobic conditions.

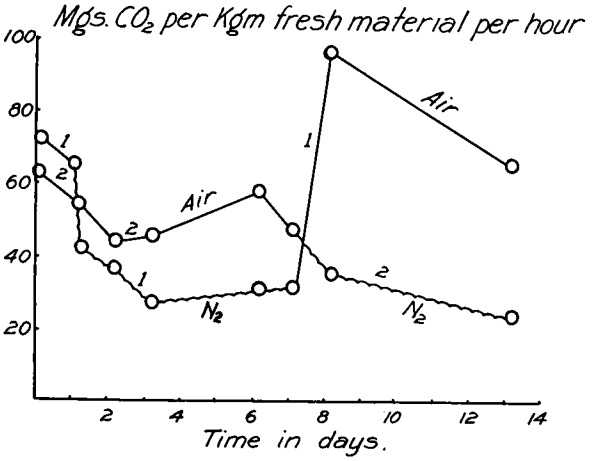

Fig. 3. Respiration of rhizomes of *Scirpus validus* in moist air and in moist nitrogen gas.

In another experiment with the rhizomes of *Nuphar advenum* (fig. 6), the plant material in air (solid line) shows that there was a more or less steady increase in the respiratory rate from the beginning. The rate in 10 per cent of oxygen at first increased more than that in air, but later came to the same level. This more rapid rate of respiration in 10 per cent of oxygen than in air is assumed to be due to some physiological difference in the rhizomes, because usually the respiration in air was greatest. The increased output of CO_2 that is known to be associated with the simultaneous occurrence of respiration and fermentation may account for the comparatively high rate in 1.5 and 3 per cent of oxygen. In the experiment with *Pontederia cordata* (fig. 7) the relatively high respiratory rate in 10 per cent of oxygen was associated with the relatively greater number of growing points (buds and shoots) and the relatively larger surface area of the material in that container.

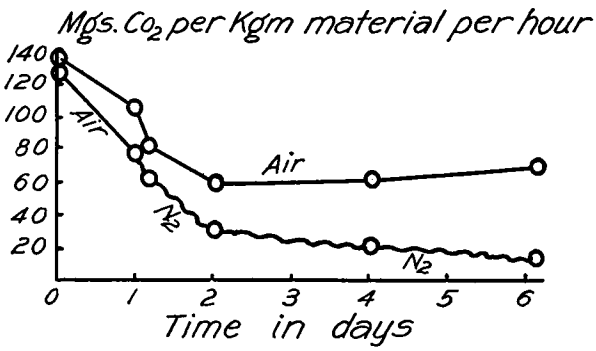

Fig. 4. Respiration of young corms of *Sagittaria latifolia* in moist air and in moist nitrogen gas.

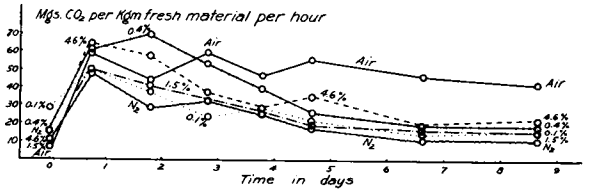

Fig. 5. Respiration of rhizomes of *Nuphar advenum* in air, nitrogen, and 4.6, 1.5, 0.4 and 0.1 per cent of oxygen by volume.

In the experiment with *Peltandra virginica* (fig. 8) the low rate of respiration of the material in 1.5 per cent of oxygen is accounted for by the fact that this material consisted of a single large rhizome having a relatively small surface area and few growing

points. The three small rhizomes that comprised the material in one per cent of oxygen possessed a greater relative area and more growing points than any other lot of material in the experiment.

In the experiment with *Typha latifolia* (fig. 9) the relatively higher respiratory rate in 4.6 per cent of oxygen was associated with a very rapid growth of new shoots from the lateral buds of mature rhizomes. As will be pointed out in a later paper, the optimum range of concentration of oxygen for maximum rate of growth of the shoots of *Typha latifolia* is very narrow, being approximately 4.6 per cent of oxygen. This is more narrow than that for most of

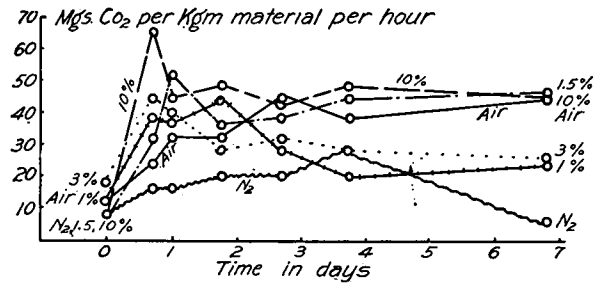

Fig. 6. Respiration of rhizomes of *Nuphar advenum* in air, nitrogen, and 10, 3, 1.5, and 1.0 per cent of oxygen by volume.

the other species studied. For that reason it was in the study of *Typha latifolia* that the most noticeable relationship between respiration and growth was observed.

In some experiments, tests were made for the production of alcohol by the rhizomes in the different concentrations of oxygen. The test was made by passing the gas coming from the respiration jars through test tubes containing a solution composed

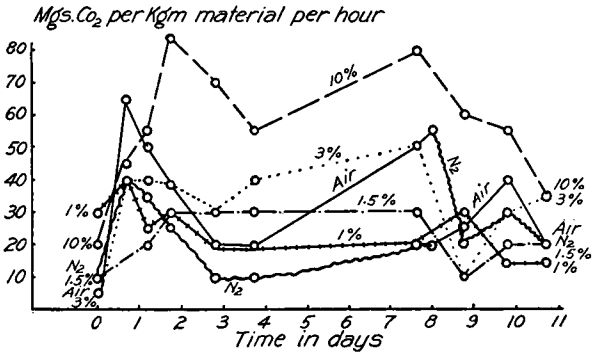

Fig. 7. Respiration of rhizomes of *Pontederia cordata* in air, nitrogen, and various concentrations of oxygen.

of 5 cc. of 30 per cent HNO_3 and 5 drops of 5 per cent $K_2Cr_2O_7$ solution. This solution, which is yellow at first, gradually changes through a series of green color shades to a clear blue in the presence of reducing substances such as alcohol, and the time required to cause a color change may be taken as a measure of the relative rate of the production of reducing substances. The production of alcohol by the rhizomes of *Nuphar advenum* in nitrogen and those in 1 per cent of oxygen caused a complete color change to blue within 17 hours, and those in 1.5 per cent of oxygen within 111 hours. In 3 per cent of

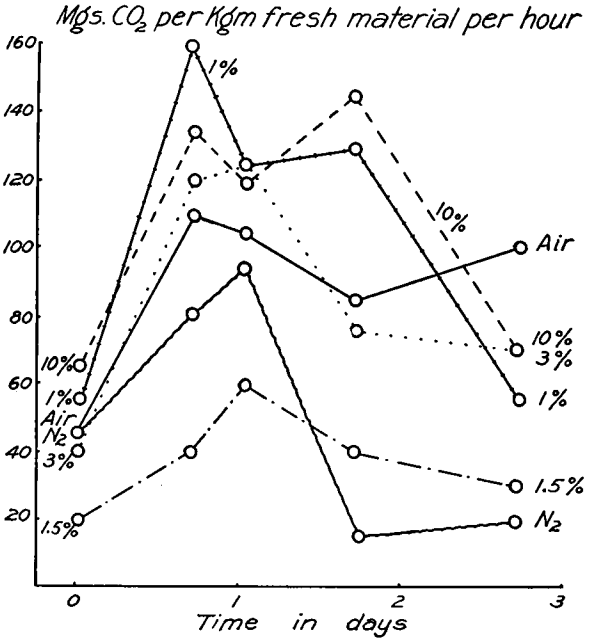

Fig. 8. Respiration of rhizomes of *Peltandra virginica* in air, nitrogen, and various concentrations of oxygen.

oxygen, the color change had advanced only to the green color stage within 135 hours. There was no color change in 10 per cent of oxygen nor in air. Thus it was shown that there is a quantitative relationship between the amount of reducing substances produced and the degree to which oxygen is lacking in the medium.

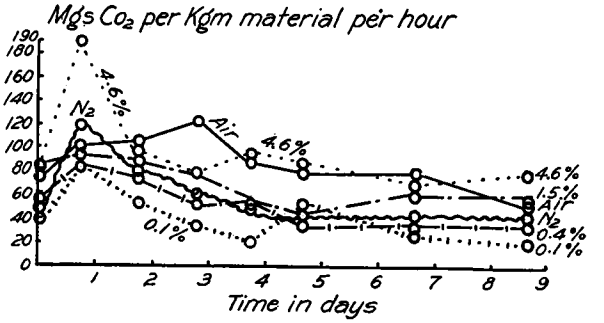

Fig. 9. Respiration of rhizomes of *Typha latifolia* in air, nitrogen, and various concentrations of oxygen.

At the close of each experiment with *Nuphar advenum* it was noticed that the rhizomes that had been in nitrogen or one of the low concentrations of oxygen had an aromatic ester-like odor characteristic of freshly dug rhizomes, while those that had been in air had a much less intense odor. Therefore a test was made to determine whether or not these plants produce alcohol while growing in their natural habi-

tat. Alcohol was distilled in the summer time from freshly dug rhizomes of *Nuphar advenum* in quantities large enough to be detected easily by means of the above color test. Aerial leaf blades of *Nuphar advenum,* however, failed to show any traces of alcohol. The presence of alcohol in the rhizome but not in the aerial leaf shows that anaerobic respiration or fermentation had undoubtedly occurred in the submerged portion of the plant. Rhizomes of *Nuphar advenum* dug from under the ice of a frozen lake in mid-winter possessed the usual terminal cluster of coiled young leaves commonly noticed in very early spring, thus showing that growth is practically continuous excepting during extremely low temperatures. These rhizomes contained an abundance of food reserves, as evidenced by the starch-filled cells of the uniseriate reticulum that makes up the bulk of the tissue, and they were practically comparable to those dug in midsummer insofar as their respiration was concerned. This abundance of food and the ability to tolerate the by-products of fermentation help to explain how these plants can endure the anaerobic conditions of their habitat.

Discussion and conclusions.—In these experiments it has been found that the rhizomes of all of the water plants studied and particularly those of *Nuphar advenum* were able to continue anaerobic respiration for long periods of time without appreciable injury. The fact that these rhizomes respired at a lower level anaerobically but that respiration was maintained over a long period of time is evidence that they are adjusted to a condition of low oxygen concentration. On the other hand, the fact that they respired aerobically without noticeable injury indicates that they are also adjusted to a plentiful supply of oxygen. It has been mentioned previously that the oxygen concentration of the water of the muddy habitat is quite low. In a later paper, data will be presented showing that the oxygen concentration of the internal atmosphere of the rhizomes in their native habitat is also frequently very low, although at other times oxygen may be present in concentrations as high as 10 per cent or more of the internal atmosphere, by volume. It is therefore probable that these rhizomes in their native habitat respire aerobically at times and anaerobically at other times according to the amount of oxygen present in the internal atmosphere. In fact, aerobic and anaerobic respiration probably proceed simultaneously much of the time.

Although the amount of CO_2 produced aerobically was greater than that produced anaerobically, nevertheless it was seldom three times as much. If respiration in terms of the amount of carbon dioxide produced is taken as the criterion, then respiration in air is greater than that in the absence of oxygen, but if the amount of respirable material used is the criterion, then it is probable that respiration is greater in the absence of oxygen. This is according to the equation for aerobic and anaerobic respiration which states that only one-third as much carbon dioxide is produced anaerobically as aerobically from one molecule of glucose assuming that 2 molecules of alcohol are formed for each 2 molecules of CO_2. It is therefore evident that because of the lack of oxygen and consequent anaerobic respiration in these plants, more food material is utilized in respiration than would be utilized if the plants had access to more oxygen. Hence, while life in the water has some advantages such as more uniform temperature and freedom from drouth, nevertheless growth may be reduced due to excessive utilization of food in anaerobic respiration.

Alcohol was formed by rhizomes while in a medium containing as high as three per cent of oxygen. The lower the content of oxygen in the medium, the more alcohol was formed. Alcohol was not detected when the medium contained 10 and 20 per cent of oxygen. Therefore it is concluded that the presence of alcohol indicates an insufficient amount of oxygen for complete aerobic respiration, and that aerobic and anaerobic respiration occur simultaneously in low oxygen concentrations. It should also be pointed out again that in nature these plants produce alcohol.

A relationship was noticed between the respiratory rate and the number of growing points or buds, whether or not growth actually occurred. Also the rate of respiration was closely associated with the amount of active protoplasm as illustrated by the rhizomes of *Nuphar advenum* in which the rate of respiration was greater in the apical portions than in the more remote parts.

It was also noticed that the smaller rhizomes usually produced more CO_2 per hour, weight for weight, than did the larger rhizomes. It is assumed that this is due to the facilitation of the exchange of gases by the relatively larger surface exposed by the smaller rhizomes.

The more rapid production of CO_2 that usually occurred during the first few days following the preparation of the samples has been noticed by other investigators. The cause has been attributed by Richards (1897) at least partly to wound stimulation, by Johnstone (1925) to mechanical facilitation of the exchange of gases, by Hopkins (1927) to the increased sugar content of the cells, and by Andus (1936) to the stimulating effect of merely handling the material.

When nitrogen was admitted in order to produce anaerobic conditions following a period of aerobic respiration, the production of CO_2 began at once to decrease. This same effect was found in the respiration of the cactus *Echinocereus fendleri* by Gustafson (1932), and in the respiration of seeds by Leach and Dent (1934) and Leach (1936). However, it is at variance with the results of an experiment, not reported here, on the respiration of plums, and also with the results obtained by Blackman (1928) with apples, and by Gustafson (1930) with tomato fruits. In the case of fruits, the sudden introduction of nitrogen in the place of air, causes a sharp increase in the output of CO_2, which is followed soon afterwards by a decrease to an amount somewhat less

than that which was produced during aerobic respiration.

SUMMARY

Respiratory studies have been made with the rhizomes and corms of *Nuphar advenum, Typha latifolia, Nymphaea tuberosa, Acorus Calamus, Sagittaria latifolia, Sparganium eurycarpum, Asclepias incarnata, Scirpus validus, Peltandra virginica,* and *Pontederia cordata.* A modified Pettenkofer method was used. In part of the experiments the plant material was immersed in water through which either air or nitrogen free of oxygen was bubbled. In other experiments the material was surrounded by gas mixtures of air, 10, 4.6, 3.0, 1.5, 1.0, 0.4, and 0.1 per cent of oxygen, or purified nitrogen.

It was found that these rhizomes and corms were able to respire anaerobically for long periods of time without any noticeable injury. The amount of CO_2 released was somewhat lower under anaerobic conditions than under aerobic conditions, but the percentage of decrease during an experiment was not much greater in the absence of oxygen or in low concentrations of oxygen than in air.

It was noticed that small rhizomes having more surface exposed and those having a greater proportion of active growing points respired more rapidly than large rhizomes or those having a smaller proportion of active cells, even when in a less favorable concentration of oxygen.

It was also noticed that the rate of evolution of alcohol was inversely related to the oxygen concentration up to and including three per cent of oxygen.

When rhizomes of *Nuphar advenum* were kept in low concentrations of oxygen, they gave off the strong aromatic odor characteristic of freshly dug rhizomes. However, after a few hours of exposure to air, the intensity of the odor was very greatly diminished. This fact, together with the fact that freshly dug rhizomes were found to contain alcohol, tends to show that these rhizomes in their normal habitat respire at least partially anaerobically.

A method of mixing gases is discussed.

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