LITERATURE CITED

BONNER, D. M., AND J. BONNER. 1940. On the influence of various growth factors on the growth of green plants. Amer. Jour. Bot. 27: 38–42.

BONNER, J. 1937. Vitamin B_1 a growth factor for higher plants. Science 85: 183–184.

———, AND G. AXTMAN. 1937. The growth of plant embryos in vitro. Preliminary experiments on the rôle of accessory substances. Proc. Nat. Acad. Sci. Wash. 23: 453–457.

———, AND P. S. DEVIRIAN. 1939. Growth factor requirements of four species of isolated roots. Amer. Jour. Bot. 26: 661–665.

———, AND J. ERICKSON. 1938. The *Phycomyces* assay for thiamin (Vitamin B_1): the method and its chemical specificity. Amer. Jour. Bot. 25: 685–692.

———, AND J. GREENE. 1938. Vitamin B_1 and the growth of green plants. Bot. Gaz. 100: 226–237.

———, AND ———. 1939. Further experiments on the relation of vitamin B_1 to the growth of green plants. Bot. Gaz. 101: 491–500.

BURKHOLDER, P. R., AND ILDA MCVEIGH. 1940. Growth of *Phycomyces Blakesleeanus* in relation to varied environmental conditions. Amer. Jour. Bot. 27: 634–640.

CHIBNALL, A. C. 1939. Protein metabolism in the plant. Yale Univ. Press.

HARRIS, L. J. 1938. Vitamins and vitamin deficiencies. 204 pp. Churchill, London.

HLAVATY, J. 1929. Sur la disparition de la vitamin B des grains germant dans un germois ou dans le sol. Compt. Rend. Soc. Biol. Paris 100: 587–589.

KÖGL, F., AND A. HAAGEN SMIT. 1936. Biotin und Aneurin als Phytohormone. Zeitsch. Physiol. Chemie 243: 209–226.

LEONIAN, L. H., AND V. G. LILLY. 1940. Studies on the nutrition of fungi. IV. Factors influencing the growth of some thiamin-requiring fungi. Amer. Jour. Bot. 27: 18–26.

PFÜTZER, G., C. PFAFF, AND H. ROTH. 1938. Der Einfluss des Stickstoffgehalts von Gerstenkörnern auf die Entwicklung und den Vitamingehalt der jungen Pflanzen. Biochem. Zeitsch. 297: 137–141.

ROBBINS, W. J. 1939. Growth substances and gametic reproduction by *Phycomyces*. Bot. Gaz. 101: 428–449.

———. 1939a. Growth substances in agar. Amer. Jour. Bot. 26: 772–777.

———, AND MARY BARTLEY. 1937. Vitamin B_1 and the growth of excised tomato roots. Science 85: 246–247.

———, AND K. C. HAMNER. 1940. Effect of potato extracts on growth of *Phycomyces*. Bot. Gaz. 101: 912–927.

———, AND F. KAVANAGH. 1938. Thiamin and growth of *Pythium Butleri*. Bull. Torrey Bot. Club 65: 453–461.

———, AND MARY BARTLEY SCHMIDT. 1939. Vitamin B_6 a growth substance for isolated roots. Proc. Nat. Acad. Sci. Wash. 25: 1–3.

RYTZ, W. 1939. Beitrag zum Aneurinstoffwechsel bei höheren Pflanzen. Ber. Schweiz. Bot. Gesell. 49: 339–399.

SCHOPFER, W. H. 1937. Recherches sur le métabolisme de l'azote d'un microorganisme acellulaire. Protoplasma 28: 381–434.

———, ET A. JUNG. 1937. Un test végétal pour l'aneurine. Méthode, critique, et resultats. Compt. Rend. V. Congrès International Technique et Chimique des Industries Agricoles. Schéveningue. 1: 22–34.

VON KUTHY, A. 1937. Über die Änderung des Gehaltes an Vitamin B_1 und C während der Keimung von Getreidesamen. Compt. Rend. V. Congrès International Technique et Chimique des Industries Agricoles. Schéveningue. 1: 119–126.

WILLIAMS, R. R., AND T. D. SPIES. 1938. Vitamin B_1 (thiamin) and its use in medicine. Macmillan, New York.

THE COMPOSITION OF THE INTERNAL ATMOSPHERE OF NUPHAR ADVENUM AND OTHER WATER PLANTS [1]

Harlow E. Laing

VARIOUS STUDIES have been made of the composition of the internal atmosphere of land plants, but few have been made with semi-submerged water plants. The older literature contains a number of papers, reviewed by Ursprung (1912) and Arber (1920), but the only recent study, other than the writer's, is that made by Colla (1931) who concluded that part of the oxygen of photosynthesis is used in respiration in *Nymphaea alba*. Since the gaseous content of the internal atmosphere of plants is undoubtedly related to the respiratory process, it was decided to determine the oxygen and carbon dioxide concentrations of the internal atmosphere of *Nuphar advenum* and certain other semi-submerged water plants (tables 1, 2 and 3), when the plants were under different conditions and at different times of the day and year.

MATERIAL.—The internal atmosphere of semi-submerged water plants is contained within cavities or canals that vary in size and extensiveness. The tissues of *Nuphar advenum* Ait. and *Peltandra virginica* (L.) Kunth. are soft, spongy, and very aeriferous throughout the entire plant, while those of *Pontederia cordata* L. are spongy in the petiole and rhizome but not in the root. The leaves and stolons of *Typha latifolia* L. and *Sparganium eurycarpum* Engelm. and the culms of *Scirpus validus* Vahl are

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spongy and aeriferous, while the rhizomes and roots are hard and contain a comparatively small internal atmosphere.

It has long been known that gases are able to pass readily from one part of the plant to another in the various species of water lilies (Dutrochet, 1831, 1837; Raffenau-Delile, 1841), but an important fact not brought out in previous studies is that gases are unable to pass so readily across the abscission zone of the very young leaf or into the very young root because of the compact mass of undifferentiated tissue that comprises these areas. As the petiole enlarges, the abscission zone becomes more narrow until finally only a few layers of cells intervene between the air cavities of the rhizome and those of the petiole. Similarly, as the root enlarges, the compact zone of cells becomes more narrow, and numerous air channels are opened up. A similar condition has been noticed in *Cladium mariscus,* one of the English twig rushes (Conway, 1937).

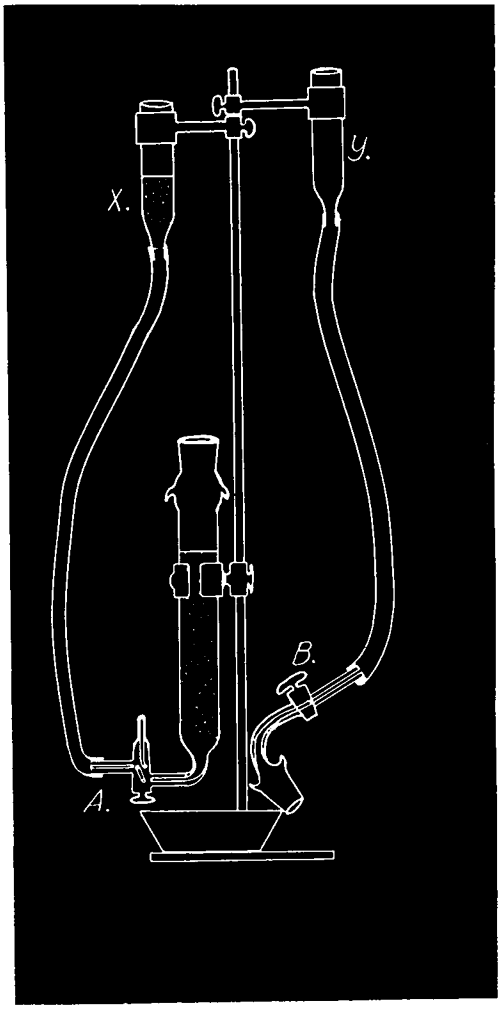

Fig. 1. Apparatus used for extracting gases.

Methods and apparatus.—Gases were extracted from bulky pieces of plant tissue by means of an apparatus (fig. 1) designed by Dr. F. G. Gustafson of the Department of Botany of the University of Michigan and modified by the author. The apparatus consists of a glass extraction tube 8 inches long and 1⅛ inches in diameter, to the lower end of which is fused at right angles an arm fitted with a ground glass tail-cock in an inverted position (fig. 1). The end of this arm is fitted with a piece of rubber tubing bearing at its other end an open glass tube (X) to facilitate the handling of the mercury. The upper end of the extraction tube is fitted with a hollow ground-glass stopper. The ground-glass stopper bears, at right angles, an arm fitted with an ordinary ground-glass stopcock (fig. 1, B). A rubber tube is used to connect this with an open glass tube (Y). The two little glass lugs on the ground-glass stopper and the two corresponding ones on the extraction-tube are for the purpose of holding the stopper in place by means of cotton twine or rubber bands. The method of operation is briefly as follows:

Beginning with the apparatus in position shown in figure 1, with stopcock A closed and the extraction tube about three-fourths full of mercury, a plug of plant tissue, cut from a bulky rhizome by means of a cork-borer, is inserted into the mercury by pushing with a wooden ram-rod in such a manner that no air is permitted to come in contact with the plug of plant tissue excepting at the ends where the tissue is protected by its own periderm. The plug of tissue is kept submersed in the mercury by means of a valve spring of a bass horn (tuba) while the large glass stopper is being inserted. The stopper is held in place by rubber bands or by twine wound back and forth over the lugs.

Mercury is then allowed to flow from tube X into the extraction tube and up into tube Y. Both stopcocks are then closed, tube X is removed, and the apparatus is inverted and arm A is attached to the gas sampler by means of a short rubber tube. The gas is then extracted under negative pressure by lowering tube Y. Then, by quickly raising tube Y, the gas is passed over into the sampler.

The following precautions must be observed in order to avoid error:

The possibility of a leak around the large glass stopper is forestalled by designing the apparatus so that it is necessary to invert it in order to extract the gasses and pass them over to the sampler. Thus leaks can be detected. Another precaution is the use of a rubber tube not exceeding 1.5 mm. in thickness to connect the sampler to the extractor. If the hose is very thick it is difficult to make sure that the ends of the glass tubes are close together. When these ends are not close together, air pockets are formed, thus introducing air into the gases to be analyzed. A similar tube is used to connect the sampler to the gas-analysis apparatus. All air pockets in the rubber hoses are dislodged by tapping lightly with the fingers while the mercury bottles are held as high as possible. Air pockets in horizontal capillary tubes are prevented by causing the mercury to pass slowly enough to fill all crevices. The tail-cock on the extraction apparatus and the similar one on the sampler are indispensable aids in avoiding error. The stopcocks and the large stopper are kept greased with regular stopcock grease of a quality known to hold well against pressure.

The gases were analyzed in a Henderson-Haldane apparatus by the method described by Novy *et al.* (1925) excepting that a hydrosulphite solution described by Fieser (1924) was used instead of the pyrogallate solution for absorbing oxygen.

Experimental results.—These studies are divided into two parts, namely, (1) the internal atmosphere of *Nuphar advenum,* (2) the internal atmospheres of other semi-submerged plants.

Part 1.—On January 9, a rhizome of *Nuphar advenum* situated about 6 inches deep in the mud under an additional 6 inches of water at the edge of a frozen lake, was dug up and immersed immediately in a pail of icewater and mud and kept in a refrigerator for 4½ hours. The internal atmosphere of the rhizome was then sampled by cutting a plug of tissue about 10 inches from the apical end by means of a cork-borer, and the gases were extracted and analyzed as previously explained. The internal atmosphere of this rhizome at the time of sampling contained 2.2 per cent of oxygen and 5.45 per cent of carbon dioxide. If there were an error in this experiment, the effect would be to increase the amount of oxygen and decrease the amount of carbon dioxide because of the difficulties involved in protecting the rhizome from the influence of atmospheric oxygen and in preventing the outward diffusion of the carbon dioxide while transporting and storing the material. Therefore it is safe to conclude that the oxygen content of the rhizome of *Nuphar advenum* in the winter time is very low, probably not over 2 per cent.

An experiment was performed to determine the rate of the utilization of oxygen in the interior of the rhizomes of *Nuphar advenum* at ordinary room temperature. Duplicate plugs were taken side by side from a vigorous rhizome that had been lying on the laboratory table covered with a moist towel. A sample of the internal atmosphere was taken immediately from one of these plugs and was found to contain 19.6 per cent of oxygen and 1.2 per cent of CO_2. The other plug was put into an extractor and submerged in mercury and allowed to respire without any additional air for 5.6 hours. The internal atmosphere was then sampled and was found to contain 0.07 per cent of oxygen and 20.9 per cent of CO_2. Therefore it is apparent that the rhizome of *Nuphar advenum* lying in moist air contains an atmosphere relatively high in oxygen content and that the rhizome uses this oxygen rapidly in respiration at room temperature.

In order to determine the influence of light and the position of the leaf in respect to the surrounding water upon the internal atmosphere of the petioles and rhizomes of *Nuphar advenum,* several experiments were performed in December upon plants growing in a tank in the greenhouse. Samples of gas were taken from rhizomes and petioles in mid-forenoon soon after photosynthesis had commenced and in mid-afternoon while photosynthesis was still rapid. The petioles, each about 10 inches long, were classified according to whether the blade was aerial, floating, or submerged. The petiole was cut into three equal parts and the atmosphere of each part was

Table 1. *Oxygen and carbon dioxide content of the internal atmosphere of rhizomes and petioles of Nuphar advenum grown in the greenhouse in December.*

Direction of sunlight	Position of blade	Part of the petiole	Per cent by volume: O_2 A.M.[a]	O_2 P.M.[b]	CO_2 A.M.	CO_2 P.M.
Direct	Aer.[c]	Aer. upper	12.1	18.3	3.6	0.5
Direct	Aer.	Subm. middle	4.8	14.3	6.5	1.7
Direct	Aer.	Subm. basal	3.2	9.6	7.4	1.9
Direct	Subm.[d]	Subm. upper	1.7	16.9	3.6	0.8
Direct	Subm.	Subm. middle	0.5	17.7	7.1	0.6
Direct	Subm.	Subm. basal	0.9	16.1	7.5	1.9
Oblique	Fltg.[e]	Subm. upper	13.0	12.3	4.2	4.9
Oblique	Fltg.	Subm. middle	9.4	9.9	5.7	5.7
Oblique	Fltg.	Subm. basal	9.1	9.2	6.8	6.3
Mirrored	Fltg.	Subm. upper	..	16.5	..	2.6
Mirrored	Fltg.	Subm. middle	..	15.0	..	2.8
Mirrored	Fltg.	Subm. basal	..	14.1	..	4.6
		Part of the rhizome				
Direct	Varied	Subm. apical	10.5	14.8	10.8	5.2
Direct	Varied	Subm. basal	2.2	13.7	18.1	7.7

[a] A.M. = mid-forenoon.
[b] P.M. = mid-afternoon.
[c] Aer. = aerial.
[d] Subm. = submerged.
[e] Fltg. = floating.

analyzed separately (fig. 2). The data are given in table 1.

It is obvious that there is a diurnally changing gradient of oxygen and carbon dioxide in the petiole and the rhizome. In the morning, before much photosynthesis has taken place, the remote or basal end of the rhizome is very low in oxygen and high in CO_2, but a few hours of photosynthesis changes this situation. The amount of CO_2 decreases greatly and the increase of oxygen is also very great. Thus, the oxygen concentration is high during the daytime but, as the previous experiment has shown, the rhizome uses this oxygen rapidly so that the concentration is low in the morning. The reverse is true of the CO_2.

Fig. 2. Cut petiole of *Nuphar advenum.*

Also, there is quite a difference in the oxygen and CO_2 concentration in the upper and lower portion of the petiole. The position of the blade whether aerial, floating, or submerged, and the angle at which the sun's rays strike the blade, were found to be important factors influencing the gaseous content of the petiole. It was possible to increase the oxygen content of the petioles of floating leaves by using a mirror to reflect the oblique December sunlight more directly onto the leaf.

These experiments demonstrate clearly the ready gaseous interchange between the rhizome and the leaf and its relation to photosynthesis.

During the month of June, similar experiments were performed in the field. The extraction apparatus was mounted upon a table set in the lake in the midst of a luxuriant growth of pond lilies so that the extraction could be accomplished with the minimum hazard of experimental error. The depth of the water was approximately 20 inches. Because of the short nights and long days, it was decided to darken the plants for part of the time in order to observe the contrast between the composition of the internal atmosphere of darkened plants and those that were allowed to photosynthesize. An inverted empty oil barrel, perforated so as to admit air but excluding light, was used to darken the plants. Care was taken to darken only isolated plants so that there was no underground connection with the rhizomes of neighboring plants. The procedure of analysis was the same as that already described excepting that (a) the gases were extracted only from the upper and lower portions of the petiole and not from the midportion, since the fact of the gradient in petioles had already been established, and (b) analyses were made of gases extracted from roots and peduncles as well as from rhizomes and petioles. The data are given in table 2.

These data confirm those obtained with plants in the greenhouse. In all comparable cases, the oxygen content of the various parts of the plant in bright sunlight in the field in June was slightly higher than that in the greenhouse in December. That was undoubtedly caused by better illumination and the longer duration of photosynthesis during June. The longer period of darkness in the field, due to the use of the barrel, however, caused the percentage of oxygen to become very low and the carbon dioxide content to rise rather high in the petioles, thus proving without a doubt that the origin of a large proportion of the oxygen of the internal atmosphere of *Nuphar advenum* is photosynthetic, particularly when the oxygen content rises much above 2 or 3 per cent, since respiration tends to keep the oxygen content of the petioles and rhizomes low when the leaves are not illuminated. It should be mentioned again that the aerial leaves under the barrel were well aerated.

These field data also indicate that the gradient of gases previously noticed in the petioles and rhizomes, extends into the roots, and that the relative concentration of oxygen in any particular part of the underground portion of the plant is largely dependent upon the distance of that part from a photosynthetic source of oxygen, whether the part in question be a root or a remote portion of the rhizome. The data also show the correlative fact that when condi-

Table 2. *Oxygen and carbon dioxide content of the internal atmosphere of petioles, peduncles, rhizomes, and roots of Nuphar advenum plants growing in their natural habitat in June.*

		Per cent by volume			
		O_2		CO_2	
Part of the petiole used	Position of blade	Dark	Sunny	Dark	Sunny
Subm. upper	Subm.[b]	1.5	18.9	11.0	4.9
Subm. basal	Subm.	1.1	15.4	10.9	4.2
Aer. upper	Aer.[a]	2.3	19.5	12.3	2.3
Subm. basal	Aer.	1.6	18.0	10.2	2.4
Part of the peduncle used	Position of flower				
Subm. upper	Subm.	...	16.7	...	2.5
Subm. basal	Subm.	...	19.2	...	0.9
Aer. upper	Aer.	9.3	...	6.3	...
Subm. basal	Aer.	3.7	...	10.0	...
Part used	Location				
Root	Attached 9 to 12 inches from apex of rhizome	1.6	7.9	10.1	9.8
Rhizome	15 inches from apex of same rhizome	0.6	7.0	17.7	4.9
Rhizome	Near apex of same rhizome	3.9	11.7	19.8	9.7
(Some leaves were aerial and some submerged)					

[a] Aer. = aerial.
[b] Subm. = submerged.

tions are unfavorable for photosynthesis, the oxygen content becomes very low and hence that respiration must be at least partially anaerobic.

Another interesting fact brought out by the field experiments (table 2) is that the concentration of oxygen in the internal atmosphere of the basal portion of the peduncle of a submerged flower on a sunshiny day is higher than that of the upper portion thus suggesting that respiration is more rapid than photosynthesis in the submerged flower of *Nuphar advenum* in bright sunlight. This suggestion is further substantiated by the fact that when the plant was darkened the concentration of oxygen in the internal atmosphere of the aerial upper portion of the peduncle of an aerial flower was found to be 9.3 per cent, while that in the submerged basal portion was 3.7 per cent. Under similar circumstances, the oxygen content of the internal atmosphere of the upper portion of the petiole of a darkened aerial leaf was 2.3 per cent, while that in the basal portion was 1.6 per cent. The concentrations of carbon dioxide in the bases of the peduncle and petiole were about the same, i.e., 10.0 and 10.2 per cent respectively; but there was about twice as much carbon dioxide in the upper portion of the petiole as there was in the upper portion of the peduncle, the amounts being 12.3 and 6.3 respectively. Apparently respiration must have proceeded more rapidly in the darkened aerial leaf than in the darkened aerial flower, or else the gaseous exchange must have been more rapid between the flower and its medium than between the leaf and its medium. It is true that the floral parts, excepting possibly the outermost husks, were thinner and more delicate than the leaf and hence gaseous exchange may have been facilitated thereby. The concentration of oxygen and carbon dioxide in the internal atmosphere of a darkened submerged leaf on the same plant were nearly the same as those of the aerial leaf (table 2).

Part 2.—In table 3 are given the data for the other species of plants studied. These results show that the conclusions drawn from the study of *Nuphar advenum* are applicable, with minor variations, to the various other species of semi-submerged water plants. In *Pontederia cordata, Typha latifolia, Sparganium eurycarpum,* and *Scirpus validus,* the influence of photosynthesis upon the oxygen content of the internal atmosphere is much less noticeable than in *Nuphar advenum;* but, nevertheless, a similar gradient of gases is present, the effect of atmospheric oxygen being much more noticeable.

In *Peltandra virginica,* a greater contrast is shown between the composition of the internal atmosphere of the darkened and that of the well-illuminated plant than exists in *Pontederia cordata et al.,* but less than in *Nuphar advenum.* This corresponds with the fact that the relative size of the leaf blade of *Peltandra virginica* is intermediate between that of *Nuphar advenum* and that of the other species mentioned. Another correlative attribute is the fact that the extensiveness of the spongy aeriferous tissues of *Peltandra virginica* is intermediate, as previously mentioned.

TABLE 3. *Oxygen and carbon dioxide content of the internal atmosphere of petioles, leaves, culms, rhizomes and stolons of semi-submerged plants growing in their natural habitat.*

	Per cent by volume			
	O_2		CO_2	
Part of the plant used	Dark	Sunny	Dark	Sunny
Pontederia cordata L.				
Aer.[a] upper part of petiole	18.6	19.4	2.1	1.3
Subm.[b] basal part of petiole	10.3	13.1	7.9	7.2
Subm. rhizome	4.6	8.8	15.7	13.5
Peltandra virginica (L.) Kunth				
Aer. upper part of petiole	10.0	19.2	9.1	1.0
Subm. basal part of petiole	3.2	11.7	14.8	10.8
Subm. rhizome	0.4	6.1	21.6	17.8
Typha latifolia L.				
Aer. parts of leaves	17.3	17.8	2.4	2.6
Subm. basal parts of leaves	10.6	15.7	10.8	4.5
Subm. soft stolon of same plant	8.7	9.7	13.8	13.8
Subm. young rhizome at end of stolon 10 inches from parent plant	...	2.1	...	27.5
Subm. young rhizome at end of stolon 20 inches from parent plant	...	2.5	...	38.0
Aer. upper part of culm	17.6	8.3	9.3	7.0
Subm. basal part of culm	9.8	13.0	6.3	8.1
Sparganium eurycarpum Engelm.				
Aer. part of leaves	15.7	18.0	4.1	3.1
Subm. basal part of leaves	11.0	13.3	7.5	8.8
Subm. soft stolon of same plant	...	7.2	...	7.5
Subm. young rhizome of same plant	0.9	...	11.5	...
Scirpus validus Vahl				
Aer. upper part of culm	17.5	19.6	1.6	1.2
Subm. basal part of culm	7.0	18.7	7.2	2.3
Subm. apical part of rhizome	...	9.7	...	9.5
Subm. remote part of rhizome	...	4.3	...	11.9
Subm. apical part of rhizome (not near culm)	0.4	...	15.2	...
Subm. remote part of rhizome (near attachment of culm)	2.7	...	8.2	...

[a] Aer. = aerial.
[b] Subm. = submerged.

In *Typha latifolia,* darkening the plant had little effect upon the composition of the internal atmosphere of the submerged stolon, but did cause a slight lowering of the oxygen content of the leaves. Comparing this observation with that of the species previously described, it may be stated that there appeared to be a direct relationship between the relative area of the blade of the leaf and the extent to which the gradients of oxygen and carbon dioxide change from periods of illumination to periods of darkness, and *vice versa;* the leaf area and extent of change of gradient being greatest in *Nuphar advenum,* less in *Peltandra virginica,* and least in *Pontederia cordata, Typha latifolia, Sparganium eurycarpum,* and *Scirpus validus.*

Another interesting fact brought out by the data is the extreme fall of gradient of gases between the relatively soft aeriferous stolons and the comparatively firm young rhizomes of *Typha latifolia.* The fact that this fall is greater, the greater the distance from the parent plant, indicates that part of the oxygen is probably obtained from the parent plant, but that the young rhizomes sometimes must endure conditions of extremely low aeration even in the summer time when respiration is at its maximum due to warm temperature.

In *Sparganium eurycarpum* the gradients of oxygen and carbon dioxide were found to be practically similar to those in *Typha latifolia* under comparable conditions, and therefore need no special discussion.

In the illuminated plant of *Scirpus validus,* the submerged parts contain approximately twice as much oxygen as is contained in the corresponding parts of a darkened plant, whereas the difference is only slight in the aerial parts. In this species, the aerial parts are photosynthesizing culms that arise vertically from various points on a submerged horizontal rhizome. The low figures for oxygen in the darkened plant indicate that respiration surely must be largely anaerobic at least part of the time. The substantial amount of oxygen in the rhizome of an illuminated plant shows that conditions favoring aerobic respiration also are frequent.

Discussion and conclusions.—This study shows that there is a gradient of oxygen and carbon dioxide in the rhizomes and petioles of semi-submerged plants. The changing of the gradient with the amount of illumination in warm weather suggests that the oxygen is largely the result of photosynthesis. The circumstantial evidence would warrant the general conclusion that part of the oxygen is utilized in respiration and part of the carbon dioxide is probably utilized in photosynthesis. The direct evidence shows that the oxygen of the internal atmosphere of *Nuphar advenum* actually is used up in respiration. Undoubtedly there is some loss of both gases by diffusion into the medium, the carbon dioxide diffusing the more readily. Also, it is probable that some oxygen and some carbon dioxide diffuse from the water into the plant. This should be more noticeable in the winter time because it should constitute the major cause of the exchange of gases near freezing temperature. In these semi-submerged water plants the inter-cellular cavity containing the internal atmosphere is somewhat like a leaky reservoir. While it serves to contain what is put in, still it cannot hold it against the inevitable losses due to the photosynthetic use of the carbon dioxide, the respiratory use of the oxygen, and the general losses due to diffusion.

As mentioned in the anatomical discussion in the introduction, there is not an easy pathway for the movement of the gases of the internal atmosphere into the petiole of the very young leaf of *Nuphar advenum.* As the leaf grows, however, the intervening partition becomes thinner, permitting of a more ready movement of the gases. The internal atmosphere of the rhizome while in connection with the internal atmosphere of the photosynthesizing older leaves, probably does not easily influence the almost anaerobic conditions surrounding the young leaves. Consequently the young leaves which continue to grow forth from the bud throughout the season are, at first, practically entirely dependent upon the surrounding water for their supply of oxygen. Although the young leaves are able to endure prolonged periods of anaerobiosis, nevertheless when they have attained maturity, it is quite essential, due to adaptations that they have developed, that more oxygen should be available (Laing, 1940b). Consequently, it is important that leaves that have once become aerial or floating should not thereafter become submerged. Submergence is brought about by violent wave action, or by a rise in the water level. Rickett (1924) found that 100 per cent of the plants of *Nuphar advenum* of Green Lake, Wisconsin, were in a southwest corner protected from winds in water not over one meter in depth. It is a matter of almost universal observation that water-lilies and pond lilies inhabit areas where violent wave action and radical changes in the water level are infrequent or practically non-existent. Mild wave action, however, is tolerated, and the lily pads literally ride the waves. Down deep in the rhizome the oxygen of photosynthesis is being utilized in respiration. The oxygen is not stored but is utilized so rapidly in respiration that an internal atmosphere, containing as high as 19.6 per cent of oxygen, loses all of its oxygen through respiration within 5.6 hours at room temperature. The temperature in the shallow water of a pond-lily bed on a summer day is often not lower than 23°C. and occasionally higher. As the weather becomes cooler, the water itself contains more oxygen, the leaves photosynthesize less, and the rhizome respires less. There probably is never a time, however, when aerobic respiration is exclusive throughout the rhizome. Always in some part and often throughout the entire rhizome, anaerobic processes are undoubtedly taking place. It has been observed that the odor of a freshly dug rhizome of *Nuphar advenum* is similar to that of one which has respired for a week in nitrogen, while the odor of a rhizome that has respired in air has changed from the pungent, ester-like, vinegary fresh odor to one more subdued and potato-like; and, also, freshly dug rhizomes of *Nuphar advenum* have been found to contain alcohol (Laing, 1940a), and were able to respire anaerobically continuously for several weeks.

Similarly, the rhizomes and stolons of the other species studied have been found able to endure long periods of anaerobic conditions (Laing, 1940a). When oxygen is available, it undoubtedly is used in respiration. When oxygen is unavailable, respiration is anaerobic. It is probable that anaerobic conditions prevail practically continuously somewhere in each plant, and that aerobic and anaerobic respiration proceed simultaneously much of the time. Certainly in the firm young rhizome of the illuminated plant of *Typha latifolia,* buried in the mud beneath six or eight inches of water, respiration must be chiefly anaerobic, because the internal atmosphere was found to contain as low as 2.1 per cent of oxygen and as much as 38 per cent of carbon dioxide, while at the same time in the soft stolon to which the young rhizome was attached the respiration must have been largely aerobic since the internal atmosphere of the stolon contained 9.7 per cent of oxygen and 13.8 per cent of carbon dioxide (table 3). Similarly, in *Scirpus validus* and *Sparganium eurycarpum,* anaerobic conditions were found to prevail in the young rhizomes of darkened plants in mid-summer; while in the photosynthesizing plants of these as well as all other species studied, excepting *Typha latifolia,* the oxygen content was well above 3 per cent, which has been found (Laing, 1940a) to be sufficient for aerobic respiration at 25°C. inasmuch as no easily detectable amounts of alcohol or other reducing substances indicative of anaerobic respiration were formed at that concentration.

What has been said concerning the significance of the constantly changing gradient of oxygen and carbon dioxide in the several species of water plants studied is undoubtedly true of many other comparable species of semi-submerged plants.

SUMMARY

Samples of internal atmosphere were extracted from the leaf petioles, culms, and rhizomes of the following species under different conditions and at different times of the year by means of an apparatus described in the text, and analyzed for O_2 and CO_2 content: *Nuphar advenum, Peltandra virginica, Pontederia cordata, Typha latifolia, Sparganium eurycarpum,* and *Scirpus validus.*

It was found that the oxygen concentration is very low in the internal atmosphere of the rhizomes of *Nuphar advenum* in mid-winter (2.2 per cent or less), while the carbon dioxide concentration is higher (5.4 per cent or more). This indicates that at least some anaerobic respiration takes place in the rhizome during the winter.

In summer, the oxygen content of remote portions of the rhizome falls very low (0.6 per cent) after the plant has been darkened, thus indicating that some anaerobic respiration occurs during warm weather; while at mid-afternoon of a sunny day the oxygen content of the remote portions of the rhizome may rise as high as 7 per cent. Therefore, respiration is aerobic part of the time, anaerobic part of the time, and probably there are times when aerobic and anaerobic respiration occur simultaneously. The same is true of the other semi-submerged species studied.

In all of these plants, the presence of an oxygen gradient from the leaf to the rhizome with the highest point in the leaf on a sunny day definitely shows that the oxygen produced in photosynthesis diffuses into the rhizome, and the carbon dioxide, which also has a gradient, but in the reverse order, diffuses into the leaf and is there used in photosynthesis. When the plant is not photosynthesizing, the oxygen in the rhizome is quickly used up, and carbon dioxide accumulates. In a previous study (Laing, 1940), it was found that these plants were able to grow and respire without injury in very low concentrations of oxygen. These two facts explain how semi-submerged plants are able to grow in the mud at the bottom of ponds and streams.

It was found that a direct relationship seems to exist between the relative area of the blade of the leaf and the extent to which the gradients of oxygen and carbon dioxide change from periods of illumination to periods of darkness, and *vice versa;* the leaf area and extent of change of gradient being greatest in *Nuphar advenum,* less in *Peltandra virginica,* and least in such species as *Pontederia cordata, Typha latifolia, Sparganium eurycarpum,* and *Scirpus validus.*

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LITERATURE CITED

Arber, Agnes. 1920. "Water Plants." Cambridge Univ. Press.

Colla, Silvia. 1931. Sulle variazione del contenuto in O_2 e in CO_2 negli organi de *Nymphaea alba* L. Boll. Soc. di Biol. Sperm. 6: 823–826.

Conway, Verona M. 1937. Studies in the Autecology of *Cladium mariscus* R. Br. New Phytol. 36: 64–96, part 3.

Dutrochet, M. 1831. Memoire sur les organes aérifères des végétaux, et sur l'usage de l'air que contiennent ces organes. Ann. d. Sci. Nat. 1 ser. T 25, 1832, p. 242–259.

———. 1837. Memoirs pour servir à l'histoire anatomique et physiologique des végétaux et des animaux. T 1, p. 336.

Fieser, L. F. 1924. A new absorbent for oxygen in gas analysis. Jour. Amer. Chem. Soc. 46: 2639.

Laing, H. E. 1940a. Respiration of the rhizomes of *Nuphar advenum* and other water plants. Amer. Jour. Bot. 27: 574–581.

———. 1940b. Respiration of the leaves of *Nuphar advenum* and *Typha latifolia.* Amer. Jour. Bot. 27: 583–586.

Novy, F. G., F. G. Novy, Jr., H. R. Roehm, and M. H. Soule. 1925. Microbic respiration. Jour. of Infectious Diseases 36: No. 1, 109–167.

Raffenau-Delile. 1841. Evidence du mode respiratoire des feuilles de *Nelumbium*. Ann. des Sci. Nat. 2 ser. Bot. 16: 328–332.

Rickett, H. W. 1924. A quantitative study of the larger aquatic plants of Green Lake, Wisconsin. Trans. Wisc. Acad. Sci. 21: 381.

Ursprung, A. 1912. Zur Kenntniss der Gasdiffusion in Pflanzen. Flora N. F. Bd. 4 (G. R. Bd. 104) 129–156.

NEW MARINE ALGAE FROM SOUTHERN CALIFORNIA. I.[1]

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The excellent work of Setchell and Gardner has done much to make known the nature and extent of the very interesting marine flora of the Pacific Coast of North America, but a considerable amount of work will yet be necessary before the marine algae of this region may be said to be fairly well known. The smaller forms are of course less well known than the larger. During the past few years of collecting, many of these smaller forms have been studied by the writer, a number of which are of considerable interest. The following account of a study of three of these small plants, which seem to be new to science, will indicate the nature of the interesting results to be expected from further study.

Methods.—Morphological and cytological studies of these three plants were made in the usual manner. The fixing solution was composed of 1 gm. of chromic acid and 1 cc. of glacial acetic acid per 100 cc. of sea water. N-butyl alcohol was employed in dehydration and embedding and serial paraffin sections were made and stained with Heidenhain's iron-alum-haematoxylin.

Dermocorynus **occidentalis** *sp. nov.*—Plants forming thin, brownish red, horizontally expanded thalli, 1–3 cm. diam., bearing numerous erect simple fruiting branches 1–2 mm. high; basal thallus 50–100–(170) μ thick, composed of erect filaments of cells mostly 4–6 μ diam. and sometimes grading into a more or less evident hypothallus of several layers of larger horizontally elongate cells; fruiting branches usually simple, narrowed at the base, and composed of longitudinal branching medullary filaments and forking anticlinal cortical filaments; tetrasporic branches somewhat flattened; cystocarpic branches cylindrical or somewhat flattened; tetrasporangia 12–16 × 22–26 μ, scattered over the entire surface of the fruiting branches, cruciately divided; antheridia covering the more or less cylindrical branches; cystocarps one to several, 90–130 μ diam., embedded in the erect branches, often bulging, but without pore or perithecium; carpospores 10–15 μ diam., in a dense globular mass.

Plantae ex thallis crustaceis tenuibus fusco-rubris compositae, horizontaliter expansae et 1–3 cm. latae, ramos erectos simplices numerosos fructiferos 1–2 mm. altos sustinentes; partibus prostratis 50–100–(170) μ crassis, ex filis cellularum erectarum 4–6 μ crassis compositis, et interdum cum hypothallis plus minusve distinctis et ex cellulis majoribus et horizontaliter elongatis compositis; ramis fructiferis plerumque indivisis, ad basim constrictis, ex filis medullaribus tenuibus furcatis et ex filis corticalibus anticlinalibus furcatis compositis; ramis tetrasporangiiferis plerumque paulum compressis; ramis cystocarpiiferentes plerumque cylindratis; tetrasporangiis 12–16 × 22–26 μ crassis, in tota superficie externa ramorum dispersis, cruciatim divisis; antheridiis ramos cylindratos circumvestientibus; cystocarpiis plerumque pluribus, 90–130 μ crassis, in ramis erectis sitis, saepe tumentibus, sine carpostomiis aut peritheciis distinctis; carposporis 10–15 μ crassis, in massis globosis densis aggregatis.

Dermocorynus occidentalis has been found by the writer at a number of places along the coast of southern California, chiefly in Los Angeles County, where it seems to be more or less common, although it is easily overlooked because of its small size. It is probably equally common farther south along the coast of California, since it has been collected by the writer as far south as Punta Banda, Lower California, Mexico. A fruitless search was made for it in the Monterey region of central California during the summer of 1939. The plants are generally found in the lower tidal zone along rocky shores. They seem to be strictly saxicolous in habit. Tetrasporic, cystocarpic, and antheridial plants have been collected, and they are all very similar in general aspect, consisting of numerous erect fruiting branches, more or less lanceolate in shape, arising from a thin brownish-red crust several centimeters in diameter (fig. 1, 2) and irregular in outline. Two types of fruiting branch never appear intermixed on the same basal stratum, although asexual plants, for example, may be found growing adjacent to sexual plants. The fruiting branches of cystocarpic plants are commonly cylindrical, whereas those of tetrasporic plants are distinctly flattened, being 2–3 times as wide as thick. They may be somewhat flattened in male and cystocarpic plants also. The fruiting branches are usually entire, but a few instances of forked fruiting branches were found in the case of cystocarpic plants (fig. 1). Numbers 650 (tetrasporic) and 2408 (cystocarpic) in the herbarium of the writer have been selected as type specimens. The former was collected from rocks at low tide level, Emerald Bay, Laguna Beach, Orange County, February 2, 1935. The latter was collected at low tide level at Laguna Beach December 8, 1938.

Dermocorynus Montagnei Crouan is the type and only previously known species of this genus. There seems to be no record of further collections of *D. Montagnei* since it was first described over seventy years ago by the Crouan brothers (1867) from material dredged in the vicinity of Brest on the west coast

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