Diurnal changes in the composition of gas from the intercellular spaces of maize leaves (*Zea mays* L.)

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The supply of oxygen presents no difficulty to land plants since their aerial parts, especially leaves, have unlimited possibilities of drawing it from the surrounding air. It could appear that the composition of the internal atmosphere of leaves differs little from that of air in view of the possibility for free exchange of gases with the surroundings through stomata. However, the liberation of oxygen into the atmosphere by the assimilating leaf and the liberation of carbon dioxide by the leaf in darkness is evidence for the existence of a partial pressure gradient for O_2 and CO_2 between the atmosphere of intercellular spaces and the surrounding air. Therefore, one should expect the composition of the internal atmosphere in the leaf to be different at any moment from that of air. The equilibrium between the concentration of gases dissolved in the liquid phase of the tissue (cytoplasm, cell sap and cell wall moisture), their pressure in intercellular spaces and in the surrounding atmosphere depends on the intensities of respiration and photosynthesis which cause the liberation or binding of oxygen and carbon dioxide in the cell.

However, little is known about the range of diurnal changes in the composition of gases within a leaf. On the other hand, changes of partial gas pressures in the intercellular spaces may substantially affect the biologically important composition of gases dissolved inside the cell itself. Direct measurement of the gas composition in the internal atmosphere of land plant tissues could, therefore, provide valuable information on the actual gaseous environment of these plants.

We have selected leaves as research material because of the functions attributed to green parts of plants as regards the supply of oxygen to other tissues which are either not in direct contact with atmospheric oxygen (e.g. submerged parts of aquatic plants — Gessner 1959) or periodically suffer from lack of oxygen in the soil (e.g. roots of crop

* The preliminary results of the present work were reported to the Congress of the Polish Botanical Society jointly with Mr Jerzy Czerski M.Sc. I take the opportunity to express my thanks to him for his valuable help in the experiments reported in this work, and for being given the chance to use the extraction apparatus of his design.

plants during protracted rainfalls, inundations etc.). Oxygen deficiency is believed to be the cause of many land plant diseases (Bergman 1959).

The interest in the composition of internal gases in various plant tissues is of long standing. The earliest survey of the subject is found in Berthelémy (1874). However most of the studies have been conducted on fleshy tissues such as fruits, tubers etc. or on lignified tissues like tree trunks and branches. Since these works are of little relevance for the study of the internal atmosphere in leaves, they are omitted in the present discussion, and those interested are referred to the studies of Stiles (1960) and Chase (1934).

The development of research on the gas composition in other, non fleshy, tissues is surveyed by Darlot (1951). This survey covers only a few contributions, all very old, and mainly concerned with aquatic plants. The most interesting seem to be the results obtained by that author himself who studied the diurnal changes in gas composition of intercellular spaces in submerged leaves of *Oenanthe phellandrium* Lamk., *Umbelliferae*. He established that on a cloudless day the proportion of oxygen in the gases (extracted in vacuum from a tissue immersed in mercury) rise towards a maximum in the afternoon (30.5 per cent) and then begins steadily to decline reaching a minimum just before sunrise (6.6 per cent).

The dependence of the composition of internal atmosphere in aquatic plants on light conditions was also established by Laing (1940) who had extracted gases from a plant tissue in Toricelli's vacuum. He observed considerable differences between the morning and afternoon in the oxygen content in *Nuphar advenum* petioles and rhizomes (leaves have not been examined).

Even earlier, Górski (1929) found that the oxygen concentration in gases evolving from a cross section of assimilating shoots of aquatic plants increased parallel with an increase in light intensity.

The available data concerning leaves of land plants are exceedingly slender. Aubert (1892) found in fleshy stems or leaves of several *Cactaceae* and *Crassulaceae* species, that is in tissues not typical for most land plants, examined in daylight, that there is 18.35 to 26.45 per cent O_2 and 0.22 to 1.5 per cent CO_2 in the gases extracted in vacuum from tissue immersed in water.

Fairly recently, an analysis of the composition of gases from sugar-beet leaves in a 24-hour cycle has been carried out by Rubin and Panasenko (1956). They have also used the vacuum extraction method, placing the tissue in a saturated NaCl solution. However, while in aquatic plants a clearly favourable effect of photosynthesis on the percentage content of oxygen is invariably observed, and not only in leaves but in other tissues as well, these authors ascertained an inverse

dependence. They found a lower oxygen content in sugar-beet leaves during the day than during the night.

This contradiction in the hitherto reported results is possibly due to differences between the plants studied and their ecological conditions, and possibly arising from various causes methodological in nature. It has provided an additional incentive for undertaking the present study.

EXTRACTION AND ANALYSIS OF GASES

Vacuum extraction makes it possible to remove free gases and presumably some of the dissolved ones within a relatively short period of time. None of the many apparatuses described seemed to us entirely satisfactory. The apparatus designed by Ulrich (1949) and used by Darlot (1951) and Binet and Darlot (1957) represents a certain progress in this field as compared with other methods, owing to the fact that by freezing the tissue in mercury before extraction, the possibility is reduced, in the opinion of these authors, of any major changes in the original composition of gases in the tissue. However, along with other devices, it has one drawback, namely it does not permit the determination in absolute values of the gaseous content of the tissue. In this method it is necessary to assume that the internal atmosphere of the tissue is homogeneous and that the gas sample obtained — big or small — is representative of the whole. Moreover, in order to collect a sufficient sample of gases (from 0.1 to 0.3 cm³ — Darlot does not specify the weight of leaves that these values correspond to) in Ulrich's apparatus, it is necessary to repeat the extraction several times, which considerably extends the duration of the analysis.

For our purposes, it was essential to have an arrangement which would meet the following conditions: 1) completely remove the free gases from intercellular spaces of such delicate tissues as leaves, and 2) reduce to a minimum the changes in the primary gas composition. The first condition is fulfilled by an extractor designed by Czerski (1964) for quick determination of the size of intercellular spaces on the basis of the volume of gases extracted in vacuum from a plant tissue previously immersed in gas-free distilled water. As demonstrated by the author a one-minute extraction was sufficient for practically complete evacuation of free gases contained in the intercellular spaces of the leaf, even in the case of succulent plants. When using a vacuum of 17 mm of mercury we found that no more than about 2.5 per cent of the total amount of gases was left in intercellular spaces. No correction was introduced for this residual amount, identical in all readings. The exceptionally high efficiency of measurement as compared with other methods (up to 2 min. including the vacuum period) held out good prospects for meeting the second condition as well.

The analysis of the gases obtained was achieved by incorporating into the apparatus, between the calibrated capillary tube and the terminal taps of two Hempel's pipettes with absorbers, these being 30% KOH solution for CO₂ and alkaline pyrogallol solution for O₂. Instead of filling the apparatus with distilled water acidified saturated solution of NaCl was used.

This modification of the apparatus did not essentially change the measuring procedure described by Czerski (1964), it simply enabled analysis of the gas obtained without transferring it to another apparatus. The gas-measuring capillary tube, 50 cm in length and of 900 mm³ capacity was graded to read 2 mm³ which enabled measurement accurate to 0.5 mm³. The least amount of air that could be accurately analysed for oxygen content was 60 mm³.

The leaves of maize (*Zea mays* L.) var. Małopolanka, grown in sand cultures were used for the analysis. A large and relatively rigid leaf provided suitable material for measurement. In order to obtain as far as possible homogeneous material only the central part of the blade of fully developed leaves of identical age was analysed. In practice, counting from the bottom, the fourth leaf of a 5—6 week old plant was used. The leaves were being detached from the plant just prior to measurement. The volume of gases liberated from a leaf sample, weighing 1 to 2 gm varied between 200 and 400 mm³. Measurements were made six times in a 24 hour cycle, and repeated several times at one or two-day intervals. In measurements at night, the weighing and placing of leaves in the extractor, as well as the extraction itself, proceeded in light falling from an adjoining room.

CONDITIONS FOR PLANT GROWTH

The plants were grown in a light and temperature-controlled chamber. As the source of light 40W fluorescent lamps, providing 50% day-light and 50% white-light was used. An 11 hour photoperiod was employed. The 5—6 week old plants looked satisfactory, they were not etiolated, and the colour of leaves differed in no way from those grown in a greenhouse. The temperature for the light period was fixed at 22°C, and for the dark period, at 15°C. Relative air humidity in the chamber, although not controlled, remained fairly constant. It was about 70—80 per cent when the light was on, and increased to 100 per cent in the dark.

The small variation of relative air humidity in the chamber was reflected in the insignificant changes in the daily water content of the leaves. The leaves of the plants grown in controlled conditions had a higher and more constant water content (89.4—90.7 per cent) than those grown in the greenhouse (78.3—81.3 per cent). Prior to switching on the lights the plants guttated profusely.

Constant conditions in respect of light and temperature, and also largely in respect of relative air humidity permitted steady photosynthesis and respiration, undisturbed by such factors as sudden temperature variation, temporary cloud overcast or excessive dryness of the air, which have either a direct bearing on the two processes or indirectly affect them through transpiration rate and water content of the leaf.

DIURNAL CHANGES IN THE COMPOSITION OF GASES IN MAIZE LEAVES

In the first series of plants, grown under controlled conditions, with a photoperiod from 5.00 a.m. to 4.00 p.m., three day-time measurements were made in such a way as to have the first one some time after the commencement of photosynthesis (7.30 a.m.), the second, at midday, and the third, just before switching off the lights (before 4.00 p.m.). The three night-time measurements were distributed in a similar way.

Table 1

The composition of the internal atmosphere of maize leaves in twenty-four hours
A. Plants grown in controlled conditions

Time of measurements h	Number of measurements	mm ³ of gas per g fresh weight of leaf, average				%			
						experimentally obtained		with correction for respiration	
		CO ₂	O ₂	N ₂	total	CO ₂	O ₂	CO ₂	O ₂
5 00	10	9.5	48.7	199.8	258.0	3.7	18.8	1.1	20.1
7 30	15	7.5	50.5	191.0	249.0	3.0	20.3	0.0	23.7
11 30	13	6.8	50.5	197.0	252.0	2.7	20.0	0.0	23.5
16 00	11	6.3	52.7	189.0	248.0	2.5	21.2	0.0	24.7
18 30	18	6.0	48.0	176.0	230.0	2.6	20.8	0.0	22.3
22 00	10	7.6	52.8	207.6	268.0	2.8	19.7	0.4	20.9

Note: N₂ by difference.

The results of the analyses are presented in Table 1. The composition of gases during a 24-hour period is characterized by a high degree of constancy both in absolute values and as percentages. However, the percentage oxygen content shows a clear upward trend during the light period. Already in the first daytime reading the oxygen content in the leaf was, at a level approximate to, but against expectations, never exceeding, that in the air. This level was maintained for some time after the lights had been turned off and photosynthesis had stopped (20.8 per

cent at 18.30 h). The lowest oxygen content was found to be in the last night-time reading, that is — before switching on the lights (18.9 per cent at 5.00 a.m.). The carbon dioxide content varied only slightly, increasing a little towards the end of the dark period (3.7 per cent).

Fig. 1 drawn from the values in Table 1, presents clearly the trends just discussed.

Similar results were obtained for plants of the same age, grown in the greenhouse. The analyses coincided with sunny and warm days in

Fig. 1. The composition of the internal atmosphere of maize leaves in twenty-four hours. Plants grown in controlled conditions.

1 — O_2 content obtained; 2 — O_2 content calculated (with correction for respiration); 3 — O_2 content in the air; 4 — CO_2 content obtained; 5 — CO_2 content calculated (with correction for respiration).

the latter part of July 1962. In this series, the first reading was being taken before sunrise, and the last, two hours after sunset. The measurement in the middle of the night was abandoned.

The results of these measurements are presented in table 2 and in Fig. 2. In spite of a general similarity of the changes in the composition

Table 2

The composition of the internal atmosphere of maize leaves in twenty-four hours
B. Plants grown in greenhouse

Time of measurements h	Number of measurements	Air temperature °C	Relative air humidity %	mm ³ of gas per g fresh weight of leaf, average				%			
								experimentally obtained		with correction for respiration	
				CO ₂	O ₂	N ₂	total	CO ₂	O ₂	CO ₂	O ₂
4 00	10	18	97	12.4	38.6	168	219	5.7	17.6	1.5	19.7
9 00	24	21	79	9.8	40.2	157	207	4.7	19.4	0.0	24.2
13 00	19	25	67	10.0	47.0	184	241	4.2	19.5	0.0	23.6
18 00	17	24	56	9.7	42.3	160	212	4.6	20.0	0.0	24.6
22 00	12	21	81	11.4	37.6	159	203	5.5	18.1	1.1	20.3

of gases during a 24-hour period, a lower oxygen content than in the first series was noticeable, even though the light conditions were undoubtedly better. Furthermore, the absolute and percentage value of CO_2 content was higher as compared with the plants grown under controlled conditions.

However, the composition of gases in intercellular spaces as determined experimentally is not identical with the actual composition in the leaf just prior to the analysis. This is due to the fact that during the measurement the leaf is isolated from the outer atmosphere for a short period of time. In these conditions, the respiring cells consume the oxygen present in the intercellular spaces and the carbon dioxide produced accumulates in the spaces instead of diffusing out of the leaf. Substantial amounts of it may also dissolve inside the cells themselves. Even if the period of time from immersion of the leaf in NaCl solution to the moment of gas removal is, as in the present work, scarcely two minutes including the vacuum period, the amount of carbon dioxide which will accumulate in intercellular spaces as a result of respiration, may cause a consider-

Fig. 2. The composition of the internal atmosphere of maize leaves in twenty-four hours. Plants grown in greenhouse. Descriptions as in Fig. 1.

able increase of the proportion of this gas. Similarly, the uptake of oxygen will bring about a decrease of the initial content of this gas in the intercellular spaces. This particular source of error has not been hitherto appreciated in most of the relevant works.

CORRECTION FOR RESPIRATION

The respiration rate of maize leaves grown in controlled conditions and in the greenhouse was examined to account for its effect in the course of measurement on the primary composition of gases in the intercellular spaces. Owing to the differences in temperature and light be-

tween day and night, measurement of the respiration rate was made twice, for halves of the same leaves, in a 24 hours cycle, before sunrise and at midday. The intensity was measured in Warburg respirometers at 22°C, that is at a temperature average for the premises in which the gas analyses were made. The results are presented in Table 3.

Table 3

Respiration rate of maize leaves ($\text{mm}^3 \text{O}_2/\text{g}$ fresh weight of leaf/h)
at $t = 22^\circ\text{C}$

No. of leaf	Plants grown in controlled conditions		Plants grown in greenhouse	
	4 ⁰⁰ a.m.	1 ⁰⁰ p.m.	4 ⁰⁰ a.m.	1 ⁰⁰ p.m.
1	246	307	260	282
2	192	271	270	311
3	170	204	292	296
4	198	246	284	300
Average	201	257	276	297
Average/min	3.3	4.3	4.6	5.0

In order to diminish any possible consequences of injury of the leaf, the rate of respiration of one half of it was measured alternately before sunrise and at midday, the other half being left undetached on the plant. It was found that the rate of respiration of the leaf at night was lower than that at midday. It was also established that the respiration rate for leaves in the greenhouse was higher than that in the growth chamber. Moreover, in both cases, considerable differences in the respiration rate of individual leaves of the same age were observed. The coefficient of respiration was 0.96.

Without going any deeper into these differences, the following conclusions were derived from the data obtained. The amount of carbon dioxide accumulating in intercellular spaces as a result of respiration in the course of the analysis itself is higher in daytime than at night, and slightly higher for the greenhouse plants than for those from the growth chamber. Furthermore, it may considerably vary in the readings for individual leaves. Similarly, corresponding losses are incurred in the original oxygen content of the intercellular spaces.

However, even when the total amount of gases contained in the intercellular spaces of the leaf is known as well as the respiration rate and time of analysis, it is not an easy proposition to make a correction for respiration.

It follows from the differences in respiration rate during day and night (Table 3) that a different correction must be applied to daytime

readings and a different one to the night readings. Additional differences are caused by changing oxygen conditions for respiration in the course of measurement. In the first stage of analysis, the leaf is being immersed in the liquid, and thus becomes isolated from the surrounding air (for about 1 min.), however respiration proceeds normally since the oxygen contained in the intercellular spaces is still available and the pressure is equal to atmospheric. In the second stage, during the extraction proper, already in the first few seconds, air is expelled from the intercellular spaces and accumulates above the liquid in which the leaf is immersed. At this stage the leaf respire in conditions practically devoid of oxygen. Anaerobic carbon dioxide can readily evolve from the cell. Also during the day, though not at night, the cell is presumably saturated with dissolved photosynthetic oxygen, which may be consumed by respiration in the vacuum, and will exude by that much less, along with the free gases.

In order to bring in the correction it is also necessary to assume: a) that the leaf surrounded by the liquid in the extraction vessel, in the period prior to vacuum formation (i.e. for one minute) respire with an intensity equal to that measured in the Warburg respirometer, since the intercellular spaces contain a sufficient amount of oxygen, and, the infiltration and plasmolysis of the leaf takes place only after the removal of gases from the intercellular spaces; b) that the respiration rate of the leaf in vacuum (one minute) is equal to that prior to vacuum formation; and c) that the respiration coefficient is equal to one.

Taking into account all these assumptions, the volume of carbon dioxide obtained experimentally has to be reduced in all the readings taken in the 24-hour period by the amount of gas that may accumulate during two minutes of respiration of the isolated leaf. The volume of oxygen obtained should be correspondingly increased by the amount that may have been used up in the two minutes in question during the daytime analyses. For the night analyses only half that amount should be corrected for since the tissue while in vacuum had neither free nor dissolved oxygen available for respiration.

The diurnal changes in carbon dioxide and oxygen content in the intercellular spaces of maize leaves, after considering the correction just discussed, are presented in the last columns of Tables 1 and 2, and in Figs 1, 2 as broken lines. The primary oxygen content in the intercellular spaces at daytime turns out to be considerably higher than the oxygen content in the air, and almost identical for greenhouse and growth chamber plants. At night, oxygen stays at a level equal to that in the air, slightly diminishing to a minimum just before sunrise (aver. 19.9 per cent). The high average oxygen content, recorded at 6.30 p.m. for leaves taken out in the darkness (Table 1) was attributed by the author to the possibility of photosynthesis proceeding in the extraction

vessel. During this measurement, the sun was still high in the sky and the generally dark room, with windows facing North-West, had the best illumination at that time of the day, the effect of which was initially not appreciated.

The percentage share of carbon dioxide in the intercellular spaces during "day" measurements was immensely in excess of that in the air, but in absolute figures, proved to be even lower than the calculated amounts of this gas derived from respiration. Presumably the correction made was insufficiently accurate, though possibly some of the gas managed to diffuse into the gas-free liquid surrounding the leaf due to the high solubility of carbon dioxide, even in saturated NaCl solution.

The above method of making an allowance for respiration during the measurement of gas in leaves, although not free from simplifications, represents an effort to arrive at an account of gaseous exchange in the intercellular spaces with the view to a more critical appraisal of the data on their percentage composition obtained by this as well as other authors.

CHANGES IN THE GAS COMPOSITION IN ISOLATED OAT LIVES IN LIGHT AND IN DARKNESS

Owing to the difficulty encountered, due to the uncertain nature of many of the assumptions in making a correction for respiration in the amount of gases measured experimentally, the primary content of oxygen and carbon dioxide in intercellular spaces of leaves under light or darkness was calculated in another way.

With this in view, oat leaves (*Avena sativa* L.) taken from plants exposed for a few hours to diffused daylight, after weighing (about 0.5 g), were immersed in the liquid filling the extraction vessel and timed. The extraction vessel was immediately covered with black cloth and the whole apparatus filled up according to the usual method but without employing the vacuum pump. Only after 1, 6, 11, or 16 minutes of isolation of the leaf in the liquid, the extractor was connected to the vacuum pump and the gases extracted for one minute. Altogether, including the vacuum period, the leaves respired in isolation for 2, 7, 12, or 17 minutes meanwhile consuming the oxygen originally present in the intercellular spaces.

Likewise the gas content in another lot of leaves of the same series was determined after different periods of isolation in the extraction vessel, but with the extractor illuminated with a 100 W electric bulb placed at a distance of 15 cm. In these conditions, the preponderance of photosynthesis over respiration, presumably not very big in view of the poor and uneven illumination of the leaf, brought about an increase in the amount of oxygen over the unknown initial content of this gas in the intercellular spaces prior to immersion of the leaf.

Identical measurement series, only reduced to 12 min., were carried out with oat leaves taken from plants previously kept in darkness for a few hours.

By tracing the average percentage oxygen content from successive readings as a function of the isolation time of the leaf, when wither

Fig. 3. Percent oxygen content of intercellular spaces of oat leaves isolated in light (a) or in dark (b) in relation to isolation period. A, B — oxygen content prior to isolation in leaves of plants previously kept in light (A) or in dark (B).

Fig. 4. Oxygen content (in absolute values) in intercellular spaces of isolated oat leaves in relation to isolation period. Descriptions as in Fig. 3

in light (Fig. 3a) or in darkness (Fig. 3b) it is possible to read directly off the graph, taking the point of intersection of the curves with the y-axis, the percentage content of oxygen in the leaf at time $t=0$, that

is — at the moment just before isolation. Oxygen content thus determined was equal to 22.8 per cent for leaves kept in light prior to detaching and 20.4 per cent, for leaves previously kept in the dark.

The same figures will be obtained if absolute values of the average amounts of oxygen in the extracted gases, in light or in darkness (Fig. 4), are taken as a function of the period of isolation. Through extrapolation of curve *b* it is possible to determine with a large degree of accuracy the initial absolute value of oxygen, and knowing the average sum total of the gases, to calculate also the percentage content of this gas in intercellular spaces just before immersion of the leaf in the liquid.

It follows from Fig. 4 that the intensity of oxygen consumption during the first minutes of isolation is nearly constant in both series, being 6.2 and 6.6 mm³ O₂/g. min., that is — 372 and 396 mm³/g. per hour. Respiration rate of oat leaves verified by the Warburg method was roughly the same, 390 mm³/g. per hour. This confirmed the correctness of the assumption that a short immersion of the leaf in saturated NaCl solution causes no changes in the physiological state of the leaf until the salt has infiltrated the tissue. Therefore, the initial content of oxygen in intercellular spaces was presumably 94 mm³ per g of leaf tissue for previously illuminated plants, that is — taking the average sum total of the gases as equal to 412 mm³/g (compare Table 4), it accounted for 22.8 per cent. For plants kept in dark, the initial oxygen content of 82 mm³/g corresponded to 20.4 per cent of the average total amount of gases (402 mm³/g).

The complete quantitative data for this series are presented in Table 4. The total amount of gases obtained after two minutes of isolation of the leaf in light differed little from that obtained when the leaf was kept in isolation for a longer period of time. On the other hand in darkness, the sum total of the extracted gases decreased as the period of isolation increased. Particularly the carbon dioxide content both in absolute values and as percentage increased out of proportion to the amount of oxygen consumed. Presumably, a certain amount of the gases, mainly CO₂, managed to diffuse to the gas-free NaCl solution and therefore could not be extracted from the dissolved state during a minute-long vacuum. This seems feasible considering the great solubility of this gas even in a saturated salt solution, especially if its partial pressure in the intercellular spaces considerably increased in the dark. It also could be experimentally demonstrated that small amounts of gas having a high percentage of carbon dioxide are released from the liquid after a lengthy (half-hour) extraction in vacuum extended beyond the actual leaf analysis, with the leaf examined being already removed from the apparatus. The composition of gases obtained in vacuum from freshly

prepared salt solution was similar to that in water and contained negligible amount of carbon dioxide. Reference may be also made to the experiments of Binet and Darlot (1958) on *Oenanthe* and *Elodea* shoots kept for several hours or even for several days in darkness and in unaerated water. They never found more than 8 per cent carbon dioxide in gases subsequently extracted from intercellular spaces of these plants. However, they did discover by a chemical method considerable amounts of this gas in the water surrounding the plant.

Table 4

The gas content of oat leaves isolated from the air and kept in light or in dark as affected by the time of isolation

Isolation period min	Light conditions	mm ³ of gas per g fresh weight of leaf				%		
		total	CO ₂	O ₂	N ₂	CO ₂	O ₂	N ₂
A. Plants previously kept in light								
17	in light	420	0	116	304	0.0	27.6	72.4
12	in light	429	0	113	316	0.0	26.4	73.6
7	in light	417	0	107	310	0.0	25.7	74.3
2	in light	409	0	97	312	0.0	23.7	76.3
2	in dark	416	9	82	325	2.2	19.7	78.1
7	in dark	402	23	51	328	5.7	12.7	81.6
12	in dark	374	23	23	328	6.2	6.2	87.6
17	in dark	325	25	7	293	7.7	2.1	90.2
B. Plants previously kept in dark								
12	in light	413	0	98	315	0.0	23.7	76.3
7	in light	420	3	93	324	0.7	22.1	77.2
2	in light	407	12	85	310	3.0	20.9	76.1
2	in dark	396	14	69	313	3.5	17.5	79.0
7	in dark	374	37	36	301	9.9	9.6	80.5
12	in dark	336	29	8	299	8.8	2.4	88.8

Owing to the difficulty in accounting for the amount of carbon dioxide resulting from the above-mentioned loss, an accurate determination of its content in the gases from intercellular spaces both in absolute and relative values, is hard to accomplish. However, this does not apply to oxygen since owing to the large quantities of it in the intercellular spaces and its low solubility coefficient, it is possible to determine its content by the above method.

DISCUSSION

Of the works concerned with the composition of gases in plant tissues only a few are applicable for discussion here in the light of results obtained in the present work. This is due to the differences in plant material used for investigation, in the extraction procedure itself, and especially in its duration which has an essential bearing upon the composition of the gases obtained. However, the most important difference lies in the fact that none of the workers operated with absolute values in the gas analyses.

Regarding the diurnal changes in the percentage composition of gases in maize leaves, our results are fully comparable in respect of the character of the curve with the data of Darlot (1951) for submerged leaves of an aquatic plant (*Oenanthe phellandrium* Lamk.). However while the daily maximum oxygen content in the afternoon hours ranged from 22 to 30.5 per cent for that plant, in our experiments, is accounted, after a correction for respiration, for no more than 24.7 per cent. This is explained by the rapid exchange of gases between the atmosphere of intercellular spaces and the surrounding air, unrestricted by water in the case of leaves of land plants. The submerged leaves of aquatic plants owing to a considerably slower diffusion of gases from intercellular spaces to the water surrounding them are in a position to accumulate considerable amounts of oxygen in conditions favouring assimilation. For the very same reasons, the oxygen content of underwater leaves of *Oenanthe phellandrium* used to drop at night to 8—10 per cent whilst in the intercellular spaces of maize and oat leaves it kept at a level close to oxygen content in the air.

These results throw some light on the role of stomata in the gas exchange of leaves. Apparently the closing of stomata, limiting, as we know, the transpiration and photosynthesis, presents no particular obstacle to the exchange of gases necessary for respiration. This is evident from the absence of any marked increase in carbon dioxide concentration in the intercellular spaces at night, and especially the absence of any substantial drop in oxygen concentration that would necessarily take place if the internal atmosphere of the leaf was completely isolated from the surroundings as the result of stomata closing. During experimental isolation of the leaf from the surrounding air, consumption of oxygen from the intercellular spaces, in spite of its considerable volume, was substantial already after twelve minutes.

There is no convincing data on the effect of stomata opening on respiration available. Pisek and Winkler (1956), having found no relationship between respiration and the opening of stomata suggested the possibility of an intensified diffusion of carbon dioxide and oxygen through the cuticle as a result of increased concentration gradient of

these gases. In fact, Dugger (1952) reports that at an increased concentration of carbon dioxide in the surroundings to 1 per cent the uptake of this gas through the non-stomate leaf epidermis is as effective as through stomata at a normal concentration of this gas in the air. The small difference between oxygen concentration inside the leaf and in the air, as established in this work, would suggest that also the exchange of this gas is not particularly hindered at night. In fact, the gradient of oxygen concentration between the air and the surface of cells bordering on intercellular spaces is much bigger, than would be borne out from the average value of the percentage oxygen content in the intercellular spaces, and possible adequate for an efficient diffusion of this gas though the epidermis when the stomata are closed.

If however the leaves of a land plant be isolated from the air surrounding them, that is, be placed in conditions specific for submerged leaves of aquatic plants, as was done in the present work, when oat leaves were immersed for a few minutes in the extraction liquid, they will readily accumulate oxygen and consume carbon dioxide when exposed to light, behaving in a similar way as the leaves of an aquatic plant. In these conditions, after 17 minutes of isolation in poor light of an oat leaf, on the average 27.6 per cent oxygen was obtained that is — even more than Darlot obtained for an aquatic plant under natural conditions. At the same time, no carbon dioxide was detected.

In darkness, on the other hand, oat leaves out of touch with the surrounding air rapidly consumed oxygen from the intercellular spaces and accumulated carbon dioxide. The amount of oxygen consumed in terms of leaf weight and isolation period conformed to the respiration rate of leaves of the same plants, as determined in Warburg respirometers. The absolute figure for the accumulated carbon dioxide was however incongruously small, assuming R.Q. to be 1.0.

Also Rubin and Panasenکو (1956) having isolated undetached sugar-beet leaves exposed to light by covering them with a layer of vaseline, found a certain increase of oxygen content after a few hours in the isolated leaves (13.4 per cent) as compared with control leaves (10. per cent). Conversely, at night, the control leaves contained more oxygen (17.4 per cent) than the isolated ones (16.5 per cent). However, in disagreement with these results which point to a clear effect of light, through photosynthesis, upon the increase of oxygen in the gas content of leaf, those authors found an inverse daily rhythm of oxygen content in unisolated leaves to that established by the present investigations, and even at night when the peak oxygen content was reached, they found no more than 17 per cent of this gas. Likewise, carbon dioxide increased from 0.9 per cent at night to 7.3 per cent in the day. This pattern of oxygen and carbon dioxide content fluctuation in leaves

during night and day, inverse to the anticipated one, they attribute to the effect of respiration which they had ascertained, is considerably higher during the day. However, they omitted to correct for oxygen consumption in the course of the analysis itself, (they also make no mention of its duration), and hence, it seems, they obtained values for oxygen not only below the real ones but possibly a semblance of an inversed daily rhythm.

In fact, a higher rate of respiration of leaves during the day related to increased temperature may lead to a reduction of the rate of apparent photosynthesis (Pisek and Winkler 1956; Decker 1958) and a possible drop in oxygen concentration in the intercellular spaces of the leaf. However, it is hard to explain why the maximum oxygen content in sugar-beet leaves in the experiments of those authors had occurred at night in the absence of photosynthesis, and not, say, in the morning hours in conditions favouring maximum apparent photosynthesis.

A high carbon dioxide content — many times as high as that in the air, obtained also in this study during daytime measurements, when good conditions for assimilation had prevailed prior to the analysis, could be fully explained by the formation of this gas as a result of respiration in the course of the analysis itself. The amount of carbon dioxide found, taken in absolute figures, is even lower in daytime than the amount that presumably should accumulate from respiration, possibly because of a partial diffusion of carbon dioxide from intercellular spaces to the liquid surrounding the leaf. Therefore, it should be assumed that originally the atmosphere of the leaf in daytime contains no more carbon dioxide than is present in the air. At night the amount of carbon dioxide is clearly bigger than that which could be accumulated as a result of respiration in the course of the analysis. It is also presumed that even a greater amount of carbon dioxide managed to diffuse to the salt solution than during day measurements. After a correction for respiration its content in the intercellular spaces declines to 0.4 — 1.5 per cent. Perhaps these values, too, are either too high or too low since it is probable that, on the one hand, the percentage composition of gases removed from the intercellular spaces is affected to some extent by carbon dioxide dissolved in the liquid phase of the leaf tissue and expelled along with the free gases in vacuum, and on the other, that a part of the accumulated carbon dioxide originating from respiration might diffuse to the liquid surrounding the leaf, thereby resulting in a decreased percentage of this gas. Therefore, great caution should be exercised with regard to the reliability of the figures obtained in view of the small amounts of carbon dioxide derived from direct measurement (from 0 to about 20 mm³). However, clear daily fluctuations of carbon dioxide concentration were observed to be in an inverse relation to those of oxygen.

Darlot, using a different method, also found considerable amounts of carbon dioxide (up to 1.5 per cent) in the leaf of an aquatic plant, in daytime, this together with a high percentage of oxygen pointing to intensive photosynthesis. He assumed that the excess CO_2 comes from that dissolved in the cell fluids and liberated along with the free gases. However, that author disregards the fact that the green parts of plants exposed to light are the place of intensive carbon dioxide consumption. It seems that in spite of freezing the tissue in mercury to -55°C for the purpose to slowing down the metabolism during the analysis, applied in his experiments, an unavoidable derangement of the initial composition of gases takes place as a result of respiration; it occurs precisely when the leaf is immersed in mercury but still before its solidification. In this connection, the data for oxygen obtained by that author are presumably also somewhat below the real figures and require a correction for respiration.

The omission of this correction is the reason why various authors find considerable amounts of carbon dioxide in intercellular spaces of tissues (compare the specification given by Binet and Darlot 1957) presumably in fleshy tissues as well, and correspondingly lower values for oxygen. Only Burton (1950), determining by a vacuum method the oxygen content of potato tuber kept at different temperatures, took into account oxygen used up during the analysis. He established experimentally that this amount of oxygen is not so small since it comes to about 18 per cent of that obtained after a half-hour extraction.

The failure to consider the effect of respiration of tissue during extraction on the composition of the gases obtained led, to unwarranted far-reaching conclusions, especially on the part of Pontonowicz (1954) who investigated gas relations and respiration rate of poppyheads during ripening. Having established considerable differences in the contents of oxygen and carbon dioxide in the chamber of the head and in the intercellular spaces of separately analysed poppyhead walls, including placentae, he assumed the existence of hindered gas exchange between the chamber and the intercellular spaces of the placenta. It seems however, that in this case, too, the high respiration rate of placenta during the measurement itself is responsible for the high percentage of carbon dioxide and low of oxygen in gases extracted from the placenta tissue, as compared with the composition of gases in the air chambers of the poppyhead instantaneously expelled by squashing it while immersed in saturated sodium chloride solution.

A direct determination of the actual composition of gases in the intercellular spaces of the tissue without resorting to a correction for respiration is hard to accomplish with the existing methods. This prompted the author to compute, on the basis of gas contents determined

in isolated oat leaves kept in light or dark after different periods of isolation, the amount of oxygen at time $t=0$ from which farther changes in the gas content proceeded. The oxygen content in oat leaves thus calculated is 22.8, when plants were previously kept in light, and 20.4, when in dark. These data agree with those for maize leaves obtained after correcting for respiration, thereby confirming the accuracy of the correction.

A more detailed comparison of the present results with those of others is rendered impossible by the fact that none of the latter specifies absolute values for the amount of gases obtained.

The extractor used in the present work, initially constructed for a gasometric determination of intercellular spaces in plant tissues, makes it practically possible to completely remove the free gases together with a certain amount of gases dissolved in the liquid phase of the tissues. The proportion of dissolved gases in the total amount contained by tissues with developed intercellular spaces is however not very big. Assuming that the solubility of gases in the liquid phase (moist cell-walls, protoplasm, cell sap) when in equilibrium with the air is equal to 87 per cent of the solubility of these gases in water (Intern. Critical Tables, 1920, for oxygen, and Burton and Spragg, 1950, for nitrogen), it may be calculated that 1 g of fresh weight of leaf, with the volume of intercellular spaces being 200 mm^3 , and the water content equal to 80 per cent, will contain 200 mm^3 of free gases at 20°C and only about 13 mm^3 of dissolved gases. Of the dissolved gases, only an insignificant amount, not affecting the gasometric determination of the volume of intercellular spaces, will be liberated during a one-minute extraction (Czerski 1964). Owing to the high nitrogen and oxygen content in intercellular spaces, the share of dissolved nitrogen and oxygen will also have no essential effect on the percentage composition of the free gases.

As regards carbon dioxide, even small absolute amounts of dissolved gas liberated during extraction may substantially change the initial content of this gas in the intercellular spaces owing to its considerable solubility, especially at night, at an increased molecular pressure of the gas in the intercellular spaces.

Small diurnal variation in the absolute values of free gases (and therefore of the volume of intercellular spaces as well) in maize leaves, may be due to inadequate uniformity of the plant material under investigation. A bigger volume of intercellular spaces (per 1 g of tissue) in the leaves of maize grown in artificial light is presumably due to a more "shade bearing" anatomical structure as compared with the plants grown under natural light (Sifton 1945).

If, as ascertained in the present work, the content of gases in the intercellular spaces of leaf, and especially that of oxygen, is subject to

a definite diurnal variation, then it would be worthwhile to verify experimentally whether in land plants, the underground parts of which may experience at least periodically an oxygen deficit, the changes in the percentage composition of gases in the leaf are transmitted to underground parts, and if so — what is the mode of transmission.

SUMMARY AND CONCLUSIONS

The percentage composition and absolute content of gases in intercellular spaces of maize leaves were determined periodically within a 24-hour cycle, using a vacuum extraction method.

The volume of gases, which at the same time was a measure of the volume of intercellular spaces, was found to be av. 251 mm^3 for plants grown in artificial light, and about 217 mm^3 for those in natural light, calculated per 1 g of leaf tissue.

The main processes affecting the composition of the internal atmosphere of leaves are photosynthesis and respiration. Accordingly, having considered a correction for respiration of leaves during the analysis itself, a source of error not appreciated in previous investigations, it was established that the atmosphere of the intercellular spaces was during daytime much richer in oxygen than the surrounding air (up to 24.7 per cent) and carbon dioxide was undetectable; during darkness the oxygen content in the leaf used to fall just below that in the air to reach a minimum before sunrise (approx. 19.9 per cent). The carbon dioxide content at night was highest before sunrise, and accounted for about 1.5 per cent. Due to the low amounts of carbon dioxide in leaf gases the figure for its concentration should not be regarded as absolutely reliable. But they clearly show that the daily variation of carbon dioxide content in the leaf is inversely related to the changes of oxygen content.

In short term experiments with oat leaves, isolated from the air through immersion in the liquid filling the extractor and exposed to light or kept in darkness for varying periods of time, the gas content in the intercellular spaces was determined. Thus, without making a correction for respiration the absolute and percentage content of oxygen was found by extrapolation to time zero. In oat leaves exposed to light for several hours prior to the experiment, the oxygen content in leaves was 22.8 per cent; for plants kept in darkness, 20.4 per cent.

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Zmiany w składzie gazów w przestworach międzykomórkowych liści kukurydzy (Zea mays L.) w ciągu doby

Streszczenie

Oznaczono skład i zawartość bezwzględną gazów w przestworach międzykomórkowych liści kukurydzy w cyklu dobowym, metodą usuwania gazów w próżni.

Ilość znalezionych gazów, będąca jednocześnie miarą objętości przestworów międzykomórkowych, wynosi średnio 251 mm^3 dla liści roślin hodowanych w świetle sztucznym i ok. 217 mm^3 dla liści roślin ze światła naturalnego — w przeliczeniu na 1 g blaszki liścia.

Głównymi procesami wpływającymi na skład wewnętrznej atmosfery liści są fotosynteza i oddychanie. Zgodnie z tym, po uwzględnieniu poprawki na oddychanie liścia podczas samej analizy — źródła błędu nie docenianego w dotychczasowych pracach — znaleziono, iż w dzień atmosfera przestworów jest znacznie bogatsza w tlen od otaczającego powietrza (do 24,7%) i praktycznie nie zawiera CO_2 ; w nocy zawartość O_2 w przestworach spada nieco poniżej zawartości tego gazu w powietrzu i jest minimalna przed świtem (ok. 19,9%). Zawartość CO_2 w nocy jest najwyższa przed świtem (ok. 1,5%). Z uwagi na małe ilości CO_2 w gazach liścia, wartości liczbowe stężenia tego gazu w przestworach należy uważać za względne. Świadczą one jednak o wyraźnych wahaniami dobowych w stężeniu CO_2 , odwrotnych do zmian w stężeniu O_2 , w atmosferze liści.

W krótkotrwałych doświadczeniach oznaczono skład atmosfery wewnętrznej liści owsa po różnym czasie izolowania ich od otaczającego powietrza i ekspozycji na światło lub utrzymania w ciemności (do 17 min). Metodą ekstrapolacji, bez konieczności wprowadzenia poprawki na oddychanie, wyznaczono zawartość bezwzględną i procentową tlenu w przestworach międzykomórkowych liści w momencie poprzedzającym analizę. Dla roślin owsa, znajdujących się przed doświadczeniem parę godzin na świetle, zawartość O_2 w liściach wynosiła 22,8%, dla roślin przebywających w ciemności — 20,4%.