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Photosynthesis measurements based on the determination of carbon increase in plants

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With the exception of the Sachs method (1884), which is now obsolete as it is inconvenient and innacurate, other methods of measurement of the photosynthesis applied by Blackman (1905), Lundegårdh (1922), Boysen-Jensen (1928), Kostytchev (1928) and Maximow (1928) are based on the determination of the decrease of CO_2 in the air surrounding the plant.

Those methods are most suitable for solving many problems connected with the assimilation of CO_2, but they seem not to be adequate for obtaining accurate data concerning the intensity and course of assimilation in natural conditions, as the measurements last a short time and usually are applied only to a part of the plant or even a single leaf owing to the limited size of the apparatus.

Results obtained by those methods cannot be generalised to the whole plant or to longer periods, as this might lead to wrong conclusions. E. g. Singh and Lal (1935) have found by measuring once a week the photosynthesis in wheat by Blackman's method, that the assimilation is most intense during the blooming period. This does not seem to agree with the increase in dry weight. In a six year study of pot-cultures of oats, barley, wheat and rye I have stated many times that for these plants the increase in the weight is most intense in the initial period of growth up to the time when the heads begin to form, then it decreases rather rapidly and in the blooming period it is much smaller than during the preliminary growth period.

Thus it seems that for the determination of the real intensity of photosynthesis as it takes place in natural conditions measurements should be made lasting over longer periods and not on the decrease of CO_2

in air but on the increase of carbon in the plant itself, growing in open air in natural conditions. Therefore, I have undertaken the determination of the carbon increase in plants by means of three methods descibed farther.

Methods and results

Experiments were performed in 1937 and 1939 on young (10—20 days old) oat plants for which I have found from sand cultures in 1934, 1935, 1936 and 1939 that their weight should increase about 20% in 24 hours calculating the rate of increase in compound interest according to V. H. Blackman.

Such an intense increase must be caused by an equally intense photosynthesis, hence the increase in carbon should also attain about 20% and mights be easily measured even by not very accurate methods.

In order to avoid complications connected with the translocation of assimilation products in the plant I have analysed the whole plant. For this reason the plants were not grown in soil from which it is difficult to extract the roots but in water cultures.

The technique of water cultures is so well developed, that plants grow as well as on soil. Besides, in a water culture we may easily make qualitative and quantitative changes in the culture solution, and study the effect of particular chemical elements on photosynthesis.

The seeds remained for 24 hours in water germinated on damp filtering paper for 48 hours and were then placed on a paraffined net over the culture solution, the roots being immersed in the liquid. The age of the plants was counted from the time of putting the seeds into water once with swelling begins the activity of enzymes, respiration and growth. Plants used for the experiments were selected for uniform development. I have analysed plants in sets of 10 to 20 together in order to eliminate casual individual differences. The carbon content in the plants were determined by Messinger's method, modifyed by Küster and Stallberg (1893), plants being burnt in a mixture of concentrated sulphuric acid and potassium bichromate and the oxydation of produced gases being completed in a biebig's oven. From the quantity of CO_2 absorbed in a weighed container with KOH I calculated the quantity of carbon in a given plant sample. Water and chlorine produced by burning the plants were absorbed in traps with concetrated sulphuric acid and KJ placed between the incineration flask and Liebig's oven.

1. Measurement of the photosynthesis by the absolute increase of carbon (in mg) in 1 average plant — orientation method (1937).

If we take two sets of plants from among uniformly developed plants and analyse one before and the other after photosynthesis, we may, in spite of experimental errors due to inequalities of weight between individual plants, find the increases in carbon which we shall express in per cent. (Table 1).

Table 1

	Average C content per plant		Increase in carbon
	in mg	in relative numbers	
20.VI.1937 Age of plants 19 days Number of plants in a set — 10			
At 6.30 a. m. (before photosynthesis)	18.3	100	
At 8 p. m. (after photosynthesis)			
	a) 23.8 b) 22.0		
	mean 22.9	125	25%
23.VI.1937 Age of plants 10 days Number of plants in a set — 20			
At 5.30 a. m. before photosynthesis)			
	a) 8.3 b) 9.3		
	mean 8.8	100	
At 8 p. m. after photosynthesis)			
	a) 9.8 b) 10.7		
	mean 10.25	117	17%

The intensity of photosynthesis was found to be 17% or 25% per day which is in agreement with the rate of increase in dry weight of oats at this stage of growth. (Table 2).

However the weighing of 660 plants, investigation of which I do not describe here in detail, has convinced me that individual differences in weight and therefore in carbon contents were so great (up to 24%) that in spite of careful selection and operating with bigger sets (10—20 specimens) it is not possible to compensate individual differences and therefore this method is not accurate and may serve rather for demonstration purpose than for research work.

Table 2

Rate of increase of dry weight of oats in sand cultures

Year	Period (age of plants)	increase in dry weight in compound interest per 24 hours
1934	17 — 25 days	17%
1935	10 — 14	18,5
	14 — 18	22
	18 — 22	13
1936	24 — 30	19
	15 — 20	15
	20 — 25	14

2. Measurement of photosynthesis in plants by the increase of their carbon percentage by constant weight.

We may determine the increase of carbon in plants independently of casual differences in plant weight namely by the percentage of carbon in the plants mass.

A plant begins the assimilation process with a certain provision of carbon which corresponds to a certain per cent of its total fresh weight. If the total mass of the plant would not change in the course of the assimilation then the percentage of carbon would grow at a rate corresponding to the intensity of assimilation. In fact the mass of plants is usually subject to changes during the day, we may however take into account the carbon content after the photosynthesis together with the weight found before the photosynthesis, for calculating the percentage of carbon as if by constant weight. We then divide the percentage of carbon by constant weight found after the photosynthesis by the percentage found in plants before and expressing the surplus in percents we obtain the real increase in carbon during photosynthesis. (Table 3).

3. Measurement of photosynthesis by the increase of the ratio C/N in plants to which the supply of nitrogen was interrupted during photosynthesis.

The increase of the carbon content in plants may be measured independently of any determination of mass if we base exlusively on the chemical constitution — namely on the ratio of carbon to some other elements f. i. nitrogen, phosphorus or potassium. If we determine the initial ratio C/X in the plants and place them in a culture solution lacking the element X, so as to avoid any increase in X during photosynthesis

Table 3

23.VI.1937. Age of plants — 10 days. Number of plants in a set — 20

	Fresh weight in g.		C content		Increase in C(%)	C percentage			Increase in C(%)
	morning (before phot.)	evening (after phot.)	in mg	in relative numbers		real in time of harvest	in reference to morning weight	in relative numbers	
Plants weighed and analysed at 5.30 a. m. (before photosynthesis)									
	a) 3,2	—	165,5			5,15	—		
	b) 3,5	—	187			5,34	—		
mean			176	100		5,25	—	100	
Plants weighed morning and evening and analysed at 8 p. m. after photosynthesis									
	a) 3,2	3,5	196			5,60	6,13		
	b) 3,5	3,62	214			5.92	6,11		
mean			205	117	17%		6,12	117	17%

then by analysing the plants after photosynthesis we should find the ratio C/X of so many $^0/_0$ higher as the $^0/_0$ of carbon has increased in the plants.

The experiments proved that culutre solutions without phosphorus or without potassium caused an abnotmal rise of the ratio C/P or C/K which can ba explained only by exosmosis of phosphorus respectively potassium from the plants to the nutrient solution lacking of the given element. Potassium appears in plant cells almost exclusively in the form of ions K' and phosphorus in great part in the form of ions H_2PO_4', HPO''_4 and PO'''_4, especially in young plants. Both those elements may be subject to exosmosis. On account of these difficulties with potassium and phosphorus only the ratio C/N proved to be a reliable index of carbon increase in plants. Besides I have found a marked decrease of the ratio C/N in plants dried for 2 hours at 105^0C in comparison to plants analysed in fresh state, which proves that carbon is lost during the drying. Therefore, it seems reasonable that analysis of plants in fresh state is the only correct method. (Table 4).

Table 4

22.VIII.1939 Age of plants 15 dyas	Number of plants in a set	Fresh weight g.	C content (mg)	N content (mg)	C/N ratio	Relative C/N ratio	C increase
At 5 a. m. (before photosynth.	8	5,1	238	23	10,3	100	
At 6 p. m. (after photosynth.	6	4,05	194	15,7	12,4	120	20%

Among other results (obtained with dried plants) it may be interesting to note, that the exclusion of potassium from the culture solution for less than 20 hours caused a retardation of the photosynthesis even though before the experiment the plants had been sufficiently supplied with potassium, growing on a complete culture solution. (Table 5.)

Table 5

22.VIII.1939 Age of plants 15 days.	Number of plants	Absolute dry weight gr	Percent of C, N and P in dry weight			Ratio		Ratio in relative numbers	
			C	N	P	C/N	C/P	C/N	C/P
Morning at 5 a. m. (before photosynthes.)	111	5.81	43,6	5,21	1,40	8,37	31,2	100	100
Evening at 6 p. m. (after photosynthes.)									
Complete culture solution	89	5,49	44,9	4,83	1,29	9,30	34,8	111	111.5
N—free	91	5,45	44,3	4,26	1,28	10,40	34,6	124	111
P—free	65	4.00	44,5	4,86	1,08	9,15	41,2	109	132
K—free	71	4.59	44,2	5,00	1,32	8,84	33,5	105,5	107.7

On a complete solution and on a solution free of phosphorus the ratio C/N was 111 and 109, whereas on a potassium-free solution it was only 105,5. On a complete solution and on a nitrogen-free solution the ratio C/P was 111,5 and 111, whereas on a potassium-free solution it was only 107,5. The agreement of the rations C/N and C/P in solutions containing potassium is striking: 111 — 109 — 111,5 — 111; it is a good test of the correctness of the method. The effect of potassium on the photosynthesis had been observed a long time ago. Nobbe (1870) noticed that plants unsufficiently supplied with potassium are unable to produce starch which was later confirmed by many others. Briggs (1922) was first to prove experimentally that by exclusion of potassium from the culture solution the photosynthesis is retarded and Pirson (1937) discovered an inverse effect — that by supplying potassium to plants suffering from scarcity of this element the photosynthesis is immediately accelerated. My experiments lead to the conlusion that the intensity of the photosynthesis depends more on the absorption of potassium than of nitrogen or phosphorus though the latter are the basic components of proteins and demand for them in the period of rapid growth is great. Potassium is adjusting the chemical and physical processes in the cells. Potassium increases the swelling of biocolloids affecting the physical condition of the nucleus, cytoplasm and cell membrane, which may have an important influence on cell division and on growth.

It should also be noticed that potassium is the only radioactive element among those indenspensable to plants. The potassium isotope K^{40} appearing in very small quantities (0,012%) produces β and γ radiation. Its half-life period is 10^9 years (Riezler 1942). Considering that 1 g. of fresh plant weight contains about 5 mg K (0,5%) we find that about 9 atoms K^{40} desintegrate per minute per gramm fresh weight of plant what corresponds to $1,6 \times 10^{-13}$ cal. approximately. The same plant during the same time by respiration yields some 10^{17} molecules CO_2 and gains thereby $1,5 \times 10^{-2}$ cal./min. Thus the amount of energy from the radiation of potassium is 10^{11} times smaller than that coming from respiration and cannot play any part in the plants energy — balance. However a single K^{40} atom when desintegrating produces 100.000 times more energy than a C atom when oxydized to CO_2, what may have a certain physiological importance as a stimulus. Besides the β radiation is accompanied by a weak γ radiation. We know that intense radiations kill living tissues, but the weak potassium radiation may have favourable stimulating effect. In this respect sodium which is not radioactive cannot be substituted by potassium in spite of their physical and chemical similarity.

The younger and the more active are the cells the more potassium they contain. Potassium constantly wandering from older to younger parts of the plant.

The concentration of potassium in the tissue is closely linked to its activity. The part of potassium in the plant differs completely from that played by other elements which serve mainly as building materials for the plant organism.

The measurement of photosynthesis in conjunction of absorption of mineral compounds by plants lead to most interesting problems connected with their nutrition and metabolism.

Conclusions

The photosynthesis was measured by the determination of carbon increase in the plants themselves instead of measuring the decrease of CO_2 in the surrounding air as it was usually done in other methods.

1. The increase of carbon in plants may be found from:
    a) the absolute increase in carbon (in mg) in a average plant,
    b) the incease in carbon percentage „at constant weight“,
    c) the increase of the ratio C/N in plants grown on a nitrogen — free culture solution.
2. Results obtained by those methods are in agreement with the real rate of growth increase.
3. The measurement of absorption of mineral compounds by plants together with the measurement of photosynthesis makes it possible to make a step toward the better understanding of the general metabolism of plants.
4. Using method (c) I could state the influence of potassium on the rate of photosynhtesis: elimination of K from the culture solution for less than 20 hours has a retarding effect on carbon assimilation.

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