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SPECTROPHOTOMETRY OF THE CHLOROPHYLL CONTENT IN THE LEAVES OF PLANTS

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It has been shown that the content of chlorophyll in a green leaf can reliably be determined using the colour coordinates. In this case the entire curve of spectral reflectivity is used, the shape of which is governed by the chlorophyll content in a green leaf. The conclusions drawn show that the more is the chlorophyll content in a leaf, the less is the light reflected by this green leaf (Kondratyev , Fedchenko, Barmina, 1982,pp.). However, from the point of view of reliable estimation of the chlorophyll in leaves from the light reflected by them, this conclusion seems to be too general, since this parameter does not always permits correct determination, which of the leaves contains more chlorophyll and which less.

Figure 1 exemplifies the curves of spectral reflectivities of various crops with different chlorophyll content.

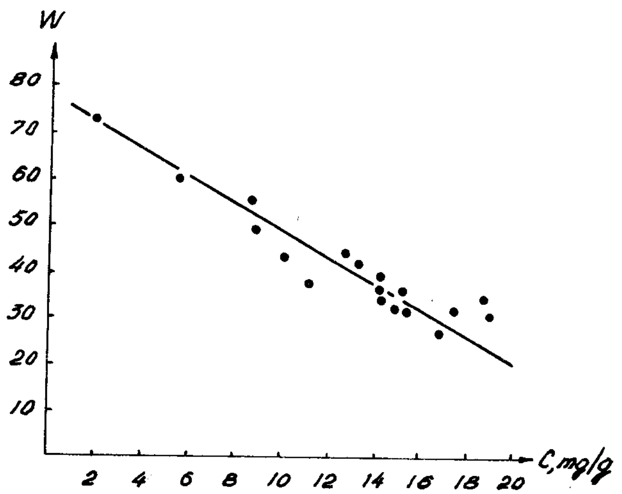

Figure 1. Spectral curves of reflection for leaves with different amounts of chlorophyll (mg/g). 1 - 4.32; 2 - 8.73; 3 - 7.34; 4 - 15.27; 5 - 12.03 .

It follows from this figure that not always the leaves with a greater (smaller) chlorophyll content reflect a smaller (greater) amount of light. This effect is particularly strong in the blue, green and red spectral regions. Naturally, if the colour coordinates are calculated using these spectral reflectivity curves and then the chlorophyll content is estimated from these colour coordinates, the accuracy will not be high. It will be shown below that spectral reflectivities of green leaves depend not only on their chlorophyll content, but also on the spectral reflectivity of a leaf itself, which can vary within wide limits.

Green leaves of buckwheat, potatoes, barley, sorge, and red clover were taken to the laboratory and photometered with the SF-18 spectrophotometer with spectral sensitivity from 400 to 750 nm. Then all these leaves were put in alcohol and kept there till complete extraction of chlorophyll, to be further photometered.

Table 1 presents the results of statistical processing of the spectral coefficients of reflection (SCR) for leaves of some crops without chlorophyll,and their 70%-confidence limits. An analysis of this table shows that the SCR's for the leaves of the above-mentioned crops negligibly differ. This points to the fact that the correlation between spectral reflectivities of leaves and their chlorophyll contents will be specific in every concrete case.

The following experiment was carried out to estimate the effect of the leaf's SCR on the accuracy of determination of its chlorophyll content.

Buckwheat plants were grown in special vessels. The leaves of these plants, with different contents of chlorophyll, were taken to laboratory, chopped, weighed and photometered with the SF-18 spectrophotometer. Simultaneously, the chlorophyll

Table 1. Spectral Coefficients of Reflection (%) for the Chlorophyll-Free Leaves of Some Crops* .

Crop	Wavelength, nm 400	450	500	550	600	650	700	750
Buckwheat	18.5±1.4	19.8±1.5	22.4±2.3	23.5±2.4	24.0±1.7	18.4±1.9	26.3±2.0	37.5±2.4
Red clover	22.1±1.8	25.1±3.5	26.2±3.7	28.4±3.5	29.4±3.1	25.1±4.1	29.1±2.8	43.4±3.1
Sorgo	19.1±1.7	19.7±1.7	22.4±1.5	26.4±2.0	27.5±2.1	23.1±1.9	27.4±2.0	35.1±2.7
Barley	18.1±1.3	18.5±1.5	21.4±1.8	22.9±2.1	23.7±1.9	17.5±1.6	25.6±1.6	35.4±1.7
Potatoes	15.0±0.9	17.0±1.0	19.1±1.6	23.1±2.0	24.1±1.9	20.1±1.7	25.1±2.4	36.0±2.5

* Data are averaged over 5 measurements.

content was determined chemically, using a conventional technique.

The colour coordinates and their sums were calculated from the obtained spectral curves of reflection (Kondratyev, Fedchenko, Barmina, 1982, pp.   ). Then the sum of the colour coordinates was plotted as a function of the chlorophyll content in the buckwheat leaves shown in Fig.2.

Next, a few green leaves of various crops (buckwheat, potatoes, barley, sorgo, red clover) were photometered in laboratory, and the colour coordinates and their sums were calculated, from which (Fig.2) the amount of chlorophyll contained in these leaves was determined. These values are listed in Table 2 (column 2). Then these leaves were put into special vessel with alcohol and kept there till the total

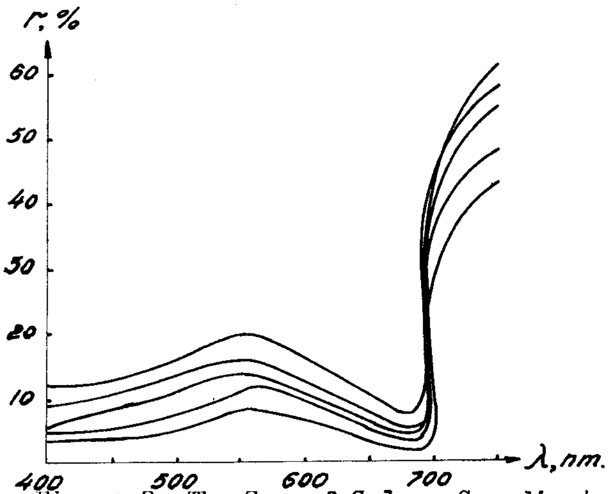

Figure 2. The Sum of Colour Coordinates vs. the chlorophyll amount in the leaves of buckwheat.

Table 2. The Chlorophyll Content (mg/g) Estimated Using the Colour Coordinates (CC) and the Conventional Technique (CT).

The number of sample, crop	CC	CT	Deviation	The sum of CC
1. Buckwheat	10.4	10.1	0.3	74.5
2. Buckwheat	4.3	4.4	-0.1	76.0
3. Buckwheat	16.0	15.9	0.1	77.1
4. Buckwheat	7.2	7.6	-0.4	77.4
5. Buckwheat	7.3	7.6	-0.3	77.6
6. Buckwheat	10.0	9.7	0.3	74.8
7. Buckwheat	15.2	11.5	3.7	68.5
8. Buckwheat	19.5	17.3	2.2	72.3
9. Sorgo	2.5	5.4	-2.9	81.5
10. Sorgo	3.7	7.8	-4.1	80.0
11. Sorgo	9.5	11.1	-1.6	78.9
12. Sorgo	10.1	9.7	0.4	73.8
13. Barley	2.0	5.6	-3.6	83.1
14. Barley	5.9	6.4	-0.5	77.1
15. Barley	3.5	3.7	-0.2	76.8
16. Barley	7.8	8.4	-0.6	77.1
17. Red clover	15.4	13.1	2.3	75.2
18. Red clover	17.5	14.1	3.4	69.3
19. Red clover	14.3	10.6	3.7	70.4
20. Potatoes	12.3	8.5	3.8	66.9
21. Potatoes	9.4	6.7	2.7	67.6
22. Potatoes	10.2	7.9	2.3	69.1

extraction of chlorophyll. The amount of extracted chlorophyll estimated using the conventional technique is given in Table 2 (column 3) (Baslavskaya, Trubetskova, 1967). Column 4 presents the colour coordinates sums for chlorophyll-free leaves.

Examination of this table shows that spectral reflectivities of the chlorophyll containing leaves expressed in terms of colour coordinates depend not only on the amount of chlorophyll in them, but also on the spectral reflectivity of the leaf itself. This points to the fact that the accuracy of estimating the amount of chlorophyll in leaves from the colour coordinates depends also on the spectral coefficient of reflection of the leaf itself. A comparative analysis of the results of estimation of the amount of chlorophyll using the colour coordinates and the technique described by Baslavskaya and Trubetskova (1967) verifies the conclusion drawn.

Upon extrapolation of the relationship $W = f(C)$ to the ordinate axis, we see that W , at the zero amount of chlorophyll, corresponds to $W_o=76.5$. It is seen from Table 2 that the values of the amount of chlorophyll estimated with the two techniques coincide only when W_o of the chlorophyll-free leaves (column 4) is close to 76.5 . In all the other cases they are quite different.

Thus, practical realization of the techniques suggested for the estimation of chlorophyll in leaves is as follows. First, a green leaf has to be photometered and then the colour coordinates are calculated from the resulting curve of reflection. This done, the leaf is put into a vessel with alcohol and kept there till the total extraction of chlorophyll. The leaf without chlorophyll is again photometered, and W_o is calculated. Then, using the colour coordinates for the green leaf, the amount of chlorophyll in this leaf is estimated from the graph for the given W_o.

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