1941

The absorption of radiant energy in plants

Jacob E. Dinger

Iowa State College

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THE ABSORPTION OF RADIANT ENERGY IN PLANTS

by

Jacob E. Dinger

A Thesis Submitted to the Graduate Faculty
for the Degree of
DOCTOR OF PHILOSOPHY
Major Subject Physics

Iowa State College
1941
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INTRODUCTION

The importance that radiation plays in plant development has long been known, and innumerable studies of the effect of various types and intensities of radiation upon plant growth have been made. Many studies of visible radiation in relation to plant growth have been made, but little attention has been given to the effects of the near infra-red region. Approximately one-half of the solar radiation energy reaching the earth is in the near infra-red region of the spectrum, and the question naturally arises as to the effect, if any, this radiation may have on plant growth. The studies of several workers indicate that the near infra-red does affect the plant growth.

Johnston (11) has made a study of the effect of the near infra-red out to a wavelength of approximately 1.4 μ on tomato plants. By means of the proper filters only visible radiation from a Mazda lamp was permitted to reach one group of plants, and the visible plus infra-red reached a second group. The light intensities were made equal in the visible portion of the spectrum as measured by a Weston photronic cell. The group of plants grown under visible plus infra-red radiation as compared with the group grown
under visible only showed an increase in total dry weight, height of plant and distance between internodes, a decrease in the total water requirement and a marked decrease in the amount of chlorophyll present in the leaves. In extreme cases a distinct yellowing and death resulted; so it appears that, if not actually destructive, this region of the infra-red spectrum is of little or no benefit in chlorophyll formation, but it does influence plant growth.

Arthur and Stewart (2) studied the growth of plants under Mazda, neon, sodium and mercury vapor lamps. The plants grown under the Mazda lamp in comparison to plants grown under the other lamps showed excessive stem growth and a pale green color. This difference was attributed to the excess infra-red in proportion to the visible output of the Mazda lamp. Arthur and Stewart also found (3) that stomata are completely closed under infra-red radiation.

At a temperature range of 73° - 78° F, the rate of water loss under a lamp without a filter was 2.5 times the loss under the same lamp with a filter that passed infra-red only. At the temperature range of 98° - 100° F, the visible rate of loss was only 1.3 times that of the infra-red which indicates that the infra-red produced a high rate of transpiration at the high temperature even if the stomata were apparently closed.
Review of Previous Studies

Brown and Escomb (4) made one of the first attempts to measure the energy absorbed by the plant leaf. In their work a balance sheet between the energy received by the leaf and the energy used by the leaf was set up. The fraction of the total incident energy which was transmitted by the leaf was determined by a Callendar radiometer. The remaining portion of the incident energy was assumed to be absorbed by the leaf, the reflected energy thus being entirely ignored. In this manner they found the fraction of absorbed energy to be between 70 and 80 percent for most of the leaves measured.

Pokrowski (15), Schull (16), and Hibben (9) all working with a spectrophotometer made measurements on transmission and also reflection throughout the visible range. The results of all these workers agreed as well as could be expected when variations in the leaves are considered. As a typical example of their results the following table is given from Pokrowski's work:
Seybold (17) used filters to isolate various portions of the spectrum and made measurements on the transmission of leaves. Using a Linke actinometer his results are somewhat different from those of the previously mentioned workers, perhaps because the degree of purity of spectrum was not nearly so great with filters as in the case of the spectrophotometer measurements. Seybold (18) also made reflection measurements by using an elliptical mirror to collect the scattered radiation, but again he used filters to select a certain portion of the spectrum.

Loomis (12) by using a Raske-Mulder color analyzer has made measurements on a large number of green leaves throughout the visible spectrum. Figure 1 shows the composite results of data taken from over 50 species of leaves.

In all the reflection and transmission measurements very little attention has been given to the region beyond .750\(\mu\). Seybold (18) gives an overall transmission of about 25 percent for the region from .770\(\mu\) to 3.00\(\mu\) for a num-

<table>
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<th>(\lambda) ((\mu))</th>
<th>480</th>
<th>500</th>
<th>550</th>
<th>600</th>
<th>620</th>
<th>650</th>
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<tr>
<td>Tilia parviflora</td>
<td>Reflection</td>
<td>.083</td>
<td>.101</td>
<td>.170</td>
<td>.135</td>
<td>.121</td>
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<tr>
<td></td>
<td>Transmission</td>
<td>.070</td>
<td>.283</td>
<td>.176</td>
<td>.148</td>
<td></td>
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<tr>
<td>Fraxinus excelsior</td>
<td>Reflection</td>
<td>.024</td>
<td>.041</td>
<td>.100</td>
<td>.070</td>
<td>.063</td>
</tr>
<tr>
<td></td>
<td>Transmission</td>
<td>.044</td>
<td>.062</td>
<td>.140</td>
<td>.096</td>
<td>.065</td>
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ber of leaves, but no attempt was made to measure the variation with wavelength.

Coblentz (5) while making an extensive study of the reflecting power of various substances included measurements on a few green leaves. His results for a red oak leaf showed a reflectivity of 18 percent at \( \lambda_{0.8} \) with an almost uniform decrease to 11 percent at \( \lambda_{2.8} \). Chestnut oak showed a decrease from 12 percent at \( \lambda_{0.8} \) to 5 percent at \( \lambda_{2.8} \).

Zscheile (21) has determined the absorption curves for carefully purified chlorophylls a and b. To determine the absorption coefficients more accurately at the points of maximum absorption in the blue and red he used a concentration of \( 0.0007 \) gm./liter; whereas, in the region of lesser absorption he used a concentration of \( 0.0150 \) gm./liter. The agreement of the resulting absorption coefficients for the overlapping regions indicates that Beer's Law is valid for these concentrations.

Zscheile (21) described a method for making a quantitative spectral analysis of chlorophyll which not only enables a calculation of the total concentration of chlorophylls a and b but also the ratio of the amount of chlorophyll a to b. This method is valid only if Beer's Law holds, and his results show this method to give accurate results at the concentrations of \( 0.0010 \) and \( 0.003 \) gm./liter.
Figure 1
Review of Methods and Techniques

Photographic materials and photoelectric equipment which are generally used for detecting radiation throughout the visible and ultra-violet region of the spectrum are not sensitive to radiation having a wavelength greater than 1.2\(\mu\). A thermopile is therefore used for detecting the radiation in the infra-red region. The output energy of a thermopile, however, cannot be directly amplified by a vacuum tube circuit because the low impedance of the thermopile cannot be matched by the high impedance of a vacuum tube. The usual method is to connect the thermopile to a sensitive galvanometer and amplify the galvanometer deflection by some means. There is a tendency for the thermopile to cause a zero drift of the galvanometer which makes it necessary to design the method of amplification in a manner such that the drift will be eliminated or reduced to a minimum.

Hardy (8) described the resonance radiometer for amplifying the thermopile output and also for eliminating drifts. By his method the radiation falling on the thermopile was periodically interrupted with a frequency corresponding to the natural period of the galvanometer. A beam of light which had passed through a grating was reflected from the mirror of the galvanometer through a
second grating and focused by means of a split convex lens on the junctions of a compensated thermopile which was connected to a secondary galvanometer. The spacings in the two gratings were arranged so that as the primary galvanometer was periodically deflected the reflected beam of light illuminated first one junction then the other of the compensated thermopile which resulted in a greatly amplified periodic swing of the secondary galvanometer. The amplitude of this swing was proportional to the energy striking the primary thermopile and the zero drift of the primary galvanometer was eliminated.

Firestone (6) has described a somewhat modified form of Hardy's resonance radiometer. The reflected beam of light from the primary galvanometer fell upon a phototube, and the optical system was so arranged that as the primary galvanometer was deflected the light intensity reaching the phototube varied linearly with the deflection of the galvanometer. The output of this phototube was amplified by a specially designed circuit for reducing drifts to a minimum, and the output of the amplifier caused the deflection of a secondary galvanometer.

Taylor (19) has described a method for amplifying galvanometer currents in which the reflected beam of light from the primary galvanometer was split by two concave mirrors and focused on two phototubes connected in opposi-
tion to one another. An unequal illumination of the two phototubes resulted in a greatly amplified deflection of the secondary galvanometer in the phototube circuit. A method described by Moss (14) for amplifying galvanometer deflections is very similar to that of Taylor's except the phototubes were replaced by a split photronic cell.

Amdur and Pearlman (1) have described a null method for measuring a small e.m.f. The unknown e.m.f. was connected in series with a galvanometer and a low resistance. By means of an auxiliary circuit a current could be passed through the small resistance in such a direction that the resulting IR drop opposed the unknown e.m.f. and could be made equal to this e.m.f. as indicated by a zero deflection of the galvanometer. The current through this small resistance also passed through a larger resistance across which the fall in potential was measured by a Type K potentiometer. The potentiometer measurement was thus proportional to the unknown e.m.f. and a knowledge of the ratio of the two resistances gave the absolute value of the measured e.m.f.
STATEMENT OF PROBLEM

Radiant energy must be absorbed by some component of the plant before it can have any effect upon the plant. In this investigation a study of this absorption has been made throughout the spectral range from .35\(\mu\) to 2.6\(\mu\). The problem has divided itself into the following parts:

1. A determination was made of the angular distribution of the radiation scattered by a leaf upon transmission and reflection. A knowledge of this distribution was necessary in a study of the transmitted and reflected radiation.

2. Measurements were made to determine the percentage of the incident energy transmitted by the leaf.

3. Measurements were made to determine the percentage of the incident energy reflected by the leaf.

4. A study was made of the absorption spectra of acetone-ether extracts, solution of the yellow pigments in ether, acetone-petroleum ether extracts, and a solution of saponified chlorophyll in water. The difference between the absorption coefficients of the acetone-ether
and the yellow pigments in ether gives the absorption coefficients of the chlorophyll, if one assumes the pigment molecules to be in the same state of association and chemical combination in the two solutions. By varying the concentration of the solutions the concentration which permitted the use of Beer's Law was determined.
Lambert's Cosine Law defines a perfectly diffuse reflector as one which redistributes the radiation it receives in such a manner that, whatever be the directional distribution of the incident radiation, the intensity of the reflected radiation from each element of the surface in any given direction is proportional to the cosine of the angle which that direction makes with the normal to the surface. On the other hand, in the case of specular reflection the direction of the reflected radiation is dependent upon the direction of the incident radiation. As leaf surfaces range all the way from those with extremely glossy cuticles to those with a fine tomentose surface, the distribution of scattered radiation from leaves of different species may be expected to vary. All reflection does not take place at the surface but much radiation is internally reflected within the cells of the leaf; therefore, even in the case of very glossy leaves some scattering will occur.

Lambert's Law defines a perfectly diffuse transmitter as one which scatters the transmitted radiation such that
the intensity of the radiation in any direction is proportional to the cosine of the angle between that direction and the normal to the surface. Scattering upon transmission is produced by multiple internal reflections within the cells of the leaf; therefore, the degree of scattering is dependent upon the thickness and density of the leaf.

Reflection and Transmission by Leaves

In a discussion of absorption of radiation by leaves Mestre (12) has divided the incident radiation into four parts such that

\[ P_o = P'_s + P'_r + P + P_a \]

where \( P_o \) is incident radiation, \( P'_r \) is radiation reflected at the surface, \( P'_s \) is scattered radiation emerging through the surface of incidence, \( P \) is the transmitted radiation and \( P_a \) is the radiation which is absorbed. A measurement of \( P_o, P'_s + P'_r \), and \( P \) will permit a calculation of \( P_a \).

It is obvious that the radiations \( P'_s \) and \( P'_r \) cannot be separated but must be measured together. As \( P'_s \) is returned through the surface of incidence after multiple internal reflections within the leaf, there has been an opportunity for selective absorption by various components of the leaf. Therefore, it is expected that the reflected
radiation will be similar in quality to the transmitted radiation P.

The length of the mean light path in the leaf for the P and P' radiation depends upon the number of internal reflections within the leaf. If the leaf is compact, there will be in effect fewer internal surfaces at which reflections may take place and thus there will result a decrease in the mean light path. If, however, the leaf is spongy and the intercellular spaces are filled with air, more internal reflections will occur and the mean light path will be lengthened. As the chances for absorption of the P and P' radiations are lessened by a decrease in the mean light path, it is expected that a thin compact leaf will show greater transmission than will a leaf which contains more air spaces, other things being equal.

Absorption by Solutions

The exponential law of absorption of radiation by a solution is given by the expression

\[ I = I_0 e^{-\alpha d} \]  

where \( I_0 \) is the intensity of the incident radiation, \( I \) is the intensity of transmitted radiation, \( \alpha \) is the specific absorption coefficient and \( d \) is the thickness of the cell. From expression (1) it is clear that a knowledge of the
Intensity of the incident and transmitted radiation and the thickness of the cell permits the calculation of the specific absorption coefficient $\alpha$.

Let us consider the case when radiation is passed through two solutions of equal thickness in series. Let the specific absorption coefficients of the solutions be $\alpha'$ and $\alpha''$ respectively. Expression (1) for the first solution becomes

$$I' = I_0 e^{-\alpha' d}$$

and for the second solution

$$I'' = I_0'' e^{-\alpha'' d}$$

but for the second solution $I_0''$ is equal to $I'_0$, the intensity of radiation transmitted by the first solution; therefore, expressions (2) and (3) combine to give

$$I'' = I_0'' e^{-\alpha' d} e^{-\alpha'' d} = I_0 e^{-(\alpha' + \alpha'')} d$$

hence

$$I = I_0 e^{-\alpha d}$$

where $\alpha$ is now the specific absorption coefficient of the combination of solutions.

We see that the specific absorption coefficients are additive, so the total specific absorption for a solution is the sum of the absorption coefficients of the component parts. It follows, therefore, that the specific absorption coefficient for any one component of a solution can be ob-
tained by taking the difference between the specific ab-
sorption coefficient of the solution as a whole and the
specific absorption coefficient for the remaining compo-
nent parts, providing the concentration of all components
remains unchanged.

It follows also that if the concentration of a solu-
tion is increased by some factor the specific absorption
coefficient of the solution is increased by the same fac-
tor. Therefore, if we let
\[ \alpha = c \beta \]  \hspace{1cm} (6)
where \( \beta \) is the absorption coefficient for a solution of
unit concentration and thickness and \( c \) is the concentra-
tion, expression (1) becomes
\[ I = I_0 e^{-c\beta \lambda} \]  \hspace{1cm} (7)
which is known as Beer's Law.

The distinction between \( \alpha \) and \( \beta \) is given by equation
(6). \( \alpha \) is the specific absorption coefficient for a given
solution of unit thickness, whereas, \( \beta \) is the absorption
coefficient for a solution of unit concentration and unit
thickness.

From the above definition of \( \beta \) it follows that, if \( \beta \)
is measured for various values of the concentration, a
graph of \( \beta \) plotted against the concentration should result
in a straight line of zero slope. If the line does not
have a zero slope, Beer's Law is not valid for the solution
at the concentration in question. A deviation from Beer's Law probably results from a chemical reaction between solute and solvent or dissociation of the absorbing molecules.
EXPERIMENTAL

Apparatus

General description

Figure 2 illustrates the arrangement of the apparatus. Lens L, focused the light source S, on the spectrometer slit. The auxiliary circuit provided a null method for obtaining a reading proportional to the energy reaching the thermopile. For a very accurate setting of the zero position of the galvanometer G the deflection was amplified by means of the amplifier circuit. This arrangement provided a quick method of taking and rechecking the readings so that the error which would result from a zero drift over a period of time was eliminated.

The component parts of the apparatus will be described in detail.

Light source

The light source was a tungsten filament 110 volt 100 watt light bulb designed for use in a small projection lantern. The size and shape of the filament made it possible to focus a long narrow beam of light on the spec-
troemer slit. A 110 volt battery supplied the current for the source.

Spectrometer

The spectrometer, figure 3, which was used to obtain a monochromatic light beam was a quartz instrument manufactured by the Gaertner Scientific Corporation with special calibration ranging from 0.180 μ to 3.0 μ. The instrument made use of a Cornu prism. Dispersion tables furnished by the manufacturers provided the necessary data to determine the slit widths which would give the desired purity of spectrum.

Thermopile

The thermopile was a Coblentz vacuum thermopile with twelve junctions. The twelve hot junctions were placed so that the total receiver area was about 0.15 cm. wide and 1.5 cm. long. The metal case enclosing the thermo-elements was continuously evacuated by means of a Cenco Hyvac pump. The thermopile is shown in place on the spectrometer in figure 3.

Galvanometer

The galvanometer was a low resistance, high sensitivity Leeds and Northrup instrument. The thermopile resistance was such as to make the galvanometer critically damped. It
Figure 2

AUXILIARY CIRCUIT

SPECTROMETER

AMPLIFIER CIRCUIT
Figure 3. Spectrometer.
was necessary to mount the galvanometer on a sponge rubber shock absorbing shelf to eliminate the vibration of the suspension by earth tremors.

**Auxiliary circuit**

The auxiliary circuit was a modified form of the Amdur and Pearlman null method for measuring a very small e.m.f. Closing the switch SW, figure 2, simultaneously opened the light shutter SH and closed the branch A of the circuit thus setting up a current in such a direction so that the fall in potential across $R$, opposed the e.m.f. developed by the thermopile TH. Resistances $R_1$, $R_2$, and $R_3$ made it possible to adjust this current so that the IR drop across $R$, was exactly equal to the thermopile e.m.f. as indicated by a zero reading of the galvanometer $G$. The current as measured by the milliammeter AM, range 50 milliamperes, was proportional to the energy reaching the thermopile. The resistance $R_1$ consisted of a large copper bus bar with a number of terminals which made it possible to select a value of $R$, which would result in a convenient deflection of the milliammeter.

The variable resistances $R_5$ and $R_6$ of branch B provided the means of adjusting the zero setting of the galvanometer.
Galvanometer deflection amplifier

The galvanometer deflection was amplified by means of the circuit shown in figure 2. A beam of light from the source $S_1$ was reflected by the galvanometer upon the right angle mirror $M$ which consequently reflected light on the phototubes $P_1$ or $P_2$ or both depending upon the position of the galvanometer mirror. When the light intensities falling on the two tubes were equal the amplified output of the tubes caused the two halves of the double target electric eye tube $E$ to appear the same. A very slight deflection of the galvanometer would cause a relative change in the intensity of light striking the phototubes and the relative change in the amplified output of the phototubes would cause a relative change in the two halves of the electric eye tube.

The double throw switch $SW_1$ made it possible to use either one or two stages of amplification for the output of the phototubes. Tubes $T_1$ and $T_2$ as well as $T_3$ and $T_4$ were in the same envelope, the tube being a double triode R.C.A. 6SC7. The phototubes $P_1$ and $P_2$ were R.C.A. 929 tubes and the electric eye tube $E$ was an R.C.A. 6AF6G. A six volt battery provided the current for the filament heaters. To obtain a clear cut pattern on the electric eye tube it was necessary to keep the amplifier free of all A. C. currents.
The auxiliary circuit and the amplifier circuit were enclosed in metal boxes to provide the proper shielding. Figure 4 is a photograph of the auxiliary circuit and the galvanometer deflection amplifier.

**Sensitivity of apparatus**

A change of 1 milliamperc in the resistance $R$, produced a sufficient change in the fall of potential across $R$, so that the resulting galvanometer deflection was detected by the galvanometer deflection amplifier. A calculation of $R$, from a knowledge of its resistivity and dimensions gives a value of $5 \times 10^{-6}$ ohms. The product of current and resistance gives a value of $5 \times 10^{-5}$ volts as the change in voltage which was detected by the apparatus.

Ising (10) has developed an expression which places a natural limit on the useful amplification of galvanometer deflections. If the galvanometer deflections are amplified to a high enough degree, the Brownian fluctuations of the suspended system will become apparent and therefore further amplification is useless. The response of the suspended system to the Brownian movements is dependent upon the restoring torque of the coil suspension, the moment of inertia of the coil, the damping of the system and the temperature of the coil. Taking into considera-
Figure 4. Auxiliary circuit and galvanometer deflection amplifier.
tion these factors, Ising has developed the following expressions for the smallest changes in current strength and potential difference that can be recognizable by a galvanometer at 18°C, with one single observation:

\[(d_i)_{\text{min}} = 4.48 \times 10^{-10} \frac{1}{\sqrt{T_0 R}} \text{ amperes (1)}\]

\[(d\nu)_{\text{min}} = 4.48 \times 10^{-7} \sqrt{\frac{R}{T_0}} \text{ volts (2)}\]

where \(R\) is the resistance of the galvanometer circuit and \(T_0\) is the undamped period of the suspension.

The galvanometer circuit of figure 2 had a resistance of 15 ohms, and the galvanometer had a period of 3 seconds. The application of equation (2) shows that the smallest voltage change that can be detected by this galvanometer is 10^-7 volts. The smallest changes in voltage which were detected with the galvanometer and deflection amplifier differ by a factor of 5 from the natural limit placed on this galvanometer by the Brownian movements.

Further amplification of the deflection could have been achieved by increasing the intensity of the light beam which was reflected from the galvanometer mirror and thus increasing the relative change in the light striking the two phototubes for a given deflection.

The sensitivity of the galvanometer is 2 \(\times\) 10^-7 volts/mm. when a light beam is reflected on a scale at a distance of
50 cm. from the galvanometer mirror. The voltage change of $5 \times 10^{-7}$ volts thus produced a deflection equivalent to 0.025 mm. on a scale placed 50 cm. from the mirror.
Procedure

Scattering by leaves

A collimated beam of light was made to strike the leaf normally. An R.C.A. 929 phototube was mounted on an arm which made it possible to rotate the phototube in a circular path about the leaf at the center, and in this way the phototube could be placed at any desired angle with the normal to the leaf. The phototube with the necessary B supply was connected to a galvanometer. The galvanometer deflection plotted against the angle which the position of the phototube made with the normal to the leaf gave the scattering curve of the leaf. For transmission measurements the leaf was placed directly over an opening in the lamp housing with the phototube at a distance of 13 cm. from the illuminated portion of the leaf. For reflection measurements the leaf was mounted at a distance of 13 cm. from the lamp housing which permitted the use of a 12.5 cm. radius for rotating the phototube about the leaf.

Transmission by leaves

The radiation transmitted by a leaf is diffusely scattered by the leaf, the degree of scattering depending
upon the thickness and structure of the leaf. In measuring the transmitted energy it is necessary to measure all or a known portion of this scattered energy. The method employed in this investigation in determining the total transmitted energy by the leaf was to place the leaf between the exit slit of the spectrometer and the thermopile and obtain the resulting reading. The leaf was then replaced by a material, in this instance paper, whose scattering properties were the same as those of the leaf, but the percentage of transmitted energy was known. In both cases the fraction of the total transmitted energy reaching the thermopile element was the same; therefore, the ratio of the two readings multiplied by the percentage transmission of the second material gave the percentage transmission of the leaf for the particular wavelength in question. A measurement of the scattering properties of different grades of paper have shown that it is possible to select a grade of paper which has scattering properties very similar to the leaf in question. Observations made in the laboratory of the University of Michigan have shown that bond paper has no absorption band throughout the portion of the spectrum which was used in this investigation.

Slit widths which gave a purity of spectrum of .020 μ were used for transmission measurements. However, wider slits were necessary for the reflection measurements so
the data presented later were taken with slits giving a purity of spectrum of $\lambda /\mu$ or less thus making it possible to compare the reflection and transmission measurements. Observations were taken at $\lambda /\mu$ intervals from $0.7\mu$ to $2.6\mu$.

**Reflection by leaves**

The method employed in measuring the percentage of energy reflected by the leaf consisted of a comparison of the reflection of the leaf with a material having similar scattering properties and a known reflectivity.

An optical system was arranged at the exit slit of the spectrometer which made it possible to project the radiation upon the leaf normally. The receiving element of the thermopile was placed at an angle of about 10 degrees with the normal to the leaf on the side of the leaf receiving the incident radiation. A reading of the thermopile response to the reflected radiation with a leaf in this position was taken. The leaf was then replaced by a material, in this instance bond paper, whose scattering properties were the same as that of the leaf and having a known reflectivity. The ratio of the first reading to the second multiplied by the percentage reflection of the second material gave the percentage reflection of the leaf for the particular wavelength under consideration.
Slit widths giving a purity of spectrum of .1\(\mu\) or less were used and measurements were taken at .1\(\mu\) intervals from .7\(\mu\) to 2.6\(\mu\).

**Absorption by solutions**

The absorption cells containing the liquids under consideration were placed in front of the entrance slit to the spectrometer. Two cells of equal thickness were used, the one containing only the solvent and the other the solution. Means were provided for interchanging the two cells at each observation. The ratio of the two readings, taking into account the difference in reflectivity of the two cells, gave the fraction of the incident light not absorbed by the solute.

Slits widths giving a purity of spectrum of .020\(\mu\) were used and readings were taken at intervals of .025\(\mu\).

Data were taken with different concentrations of the solute. These concentrations have been designated as 4X, 2X, 1X, \(\frac{1}{2}X\), \(\frac{1}{4}X\) and \(\frac{1}{8}X\), a concentration of 1X meaning that the solute from one square centimeter of leaf area was dissolved in 1 cubic centimeter of the solvent. The other solutions had the indicated multiple concentration of the 1X concentration. As an absorption cell of 1 cm. thickness was used a concentration of 1X presented the same amount of the extracted solute per unit area as had been present in the leaf.
PRESENTATION AND DISCUSSION OF RESULTS

Scattering by Leaves

Figures 5, 6, 7 and 8 show the transmission and reflection scattering curves for Ficus elastica, maize, pansy and coleus in comparison with a cosine curve. The galvanometer deflections were multiplied by the proper factor to bring all curves to the same scale.

The top surface of the Ficus elastica leaf has a glossy surface and hence exhibited an appreciable amount of specular reflection, but the bottom has a matt surface and the scattering curve approximated the cosine curve. Because the leaf is thick and heavy the transmission scattering approached the cosine law of scattering.

The reflection from the top and bottom surfaces of the maize, pansy and coleus leaves approached the cosine law of scattering. The scattered transmitted radiation from these leaves does not conform to the cosine law as well as does the reflected radiation. It was observed that in general the bottom surface was a more diffuse reflector than the top surface.
Transmission and Reflection by Leaves

Figures 9 and 10 compare the percentage transmission of the leaf, acetone-ether extract, yellow pigments in ether and a saponified solution of chlorophyll in water for potato and corn plants. In comparing the absorption of the leaf with extracts from the leaf in solution, the optics of the leaf cannot be ignored. If the absorption spectra of the pigments were not changed by chemical reactions and molecular dissociations upon extraction, it would be expected that the leaf would show greater absorption because of the multiple internal reflections which take place in the leaf resulting in a greatly increased probability of absorption by the pigments. The wide difference between the absorption curves for pigments \textit{in vivo} and in solution indicated that the absorption spectra of the pigments were altered upon extraction. It is pointed out by Mestre (12) that the pigments are probably altered chemically upon extraction and the form of the absorption curves is a complex function of the polarity and dielectric constant of the solvent and of the electronic configuration of the pigment molecules.

Figure 11 compares the percentage transmission of a dark green, light green and yellow hibiscus leaf. The yellow leaf does not show the chlorophyll absorption band in
the red end of the spectrum.

Figure 12 compares the transmission of the white portion of a variegated coleus leaf with the green portion of the same leaf. There was no selective absorption by the white portion and the total transmission was much higher. It is reasonable to assume the percentage reflected to be comparable to the percentage transmitted so it may be concluded that very little radiation was absorbed by the white leaf in the visible region of the spectrum.

Figures 13, 14, and 15 show the reflection and transmission by Ficus elastica, maize, and potato leaves in the near infra-red.

The Ficus elastica leaf showed much greater reflection than transmission. Because the leaf is very thick there is more opportunity for a larger portion of the radiation to be returned by internal reflections through the incident surface resulting in a high reflection. The fact that the water absorption bands were so pronounced in the reflected radiation indicates that a large portion of the total reflected radiation was internally reflected.

In contrast to the Ficus elastica leaf the relatively thin corn leaf showed a larger percentage of the incident radiation transmitted than reflected. The reflected radiation, however, again showed strong absorption in the water bands indicating that, even in the case of a thin leaf, much
of the reflection was internal.

For the potato leaf, which is of medium thickness, the transmission and reflection were about the same.

The transmission and reflection curves for the leaves show that approximately 15 percent of the incident radiation was absorbed in the region from .8μ to 1.3μ. The water bands at 1.5μ and 2.0μ absorbed about 55 and 85 percent respectively. Extracted solutions of the pigments in the leaves showed no absorption between .7μ and 2.6μ in the near infra-red so the absorption in the water band regions was probably due entirely to the water.

It is shown by Fowle (6) that the solar energy in the regions of the water bands at 1.5μ and 2.0μ is completely absorbed by the water vapor present in humid air; therefore, plants which are grown in sunlight may not have the opportunity to absorb this infra-red radiation. However, plants grown under Mazda lamps receive much infra-red radiation which can be absorbed by the water present in the leaves. This absorption results in heating of the leaf and it is very probable that the effect of infra-red radiation on plants which has been observed by various workers is due to this heating effect.
Absorption by Solutions

Figures 16 and 17 show the absorption spectra of an acetone-ether extract from fresh hibiscus and potato leaves. The absorption coefficient $\alpha$ is plotted against the wavelength. Because the concentration of the solution was successively increased by a factor of two, Beer's Law would predict that the ordinates of each curve should be just one-half the corresponding ordinates of the curve immediately above. However, it is evident that marked deviation from this law occurred at the higher concentrations, particularly at the shorter wavelengths. Concentrations of $\frac{1}{2}$ and less are seen to have obeyed Beer's Law. Figure 18 illustrates the deviation at various wavelengths from Beer's Law for the acetone-ether extract of hibiscus leaves. Beer's Law does not hold for those concentrations and wavelengths for which the curves are not parallel to the axis.

Figures 20 and 21 give the absorption spectra of the yellow pigments in ether from corn and potato leaves. Again there was a deviation from Beer's Law at the higher concentrations, but the deviation was not as great as in the case of the acetone-ether extract. The deviations at various wavelengths for extracts from potato and hibiscus leaves are shown in figures 19 and 22.

The acetone-ether extract contained both chlorophyll
and the yellow pigments, but was washed to remove water soluble pigments. The difference between the absorption coefficients of the acetone-ether extract and the absorption coefficients of the solution containing only the yellow pigments should give the absorption coefficients of the chlorophyll alone. Figures 23, 24 and 25 show the absorption curves obtained in this manner for chlorophyll from corn, hibiscus and potato leaves. In obtaining the correct absorption curves for chlorophyll in this manner it is necessary that the yellow pigment molecules retain the same degree of association in the solution containing only the yellow pigments as do the molecules in the acetone-ether extract. If both the acetone-ether extract and the yellow pigments in ether solution obey Beer's Law upon dilution of the solutions, then one may assume that no further dissociation and chemical reaction of the pigments is taking place. The concentrations of 1X and less for both the acetone-ether extract and the yellow pigments in ether solution obeyed Beer's Law which indicates the pigments were in the same state of association; therefore, the chlorophyll by difference curves can be considered correct for these concentrations. For the concentrations of 1X and higher the molecules may or may not be in the same state of association in the yellow pigment solution as in the acetone-ether extract, and the chlorophyll by difference curves at
these concentrations may or may not represent the absorption of chlorophyll in ether at these concentrations.

The absorption spectra of saponified potassium chlorophyllide taken from corn, hibiscus and potato leaves are shown in figures 26, 28 and 30. A marked deviation from Beer's Law occurred only in the region below .425μ as illustrated by figures 27, 29 and 31. A comparison of figures 16 and 17 with figures 26, 28 and 30 shows that the maximum absorption in the red for the acetone-ether extract did not appear at the same wavelength as the absorption in the red by the saponified potassium chlorophyllide in water. The acetone-ether extract had a maximum at about .660μ and the chlorophyllide in water at about .640μ.

Figure 32 gives the absorption spectra for acetone-petroleum ether extract from corn leaves. The curves are very similar to those of the crude ether extract, and they also show a pronounced deviation from Beer's Law at the blue end of the spectrum for concentrations greater than 1/4X.

The concentration of the solutions of 1/4X as determined by colorimetric means is dependent upon the validity of Beer's Law; such methods should not be used for crude ether extracts with a concentration much greater than this value. Methods such as the one described by Zscheile (20) for making a quantitative spectral analysis of chlorophyll to determine the relative amounts of chlorophylls a and b should
not be attempted with the concentrations for which Beer's Law does not hold.

The spectrograms of figure 33 show qualitatively the absorption bands of the various solutions. An iron arc was used as source thus making it possible to observe the absorption in the near ultra-violet. The acetone-ether extract containing chlorophyll absorbed strongly in the ultra-violet, but the yellow pigments in ether showed very little absorption in the ultra-violet out to the wavelength \( .3\mu \) beyond which the solvents became absorbing; this would indicate that the chlorophyll molecule absorbs ultra-violet radiation between \( .3\mu \) and \( .4\mu \). An incandescent source was also used to show more clearly the absorption band of chlorophyll in the red at \( .660\mu \).
Figure 7

GALVANOMETER DEFLECTION

ANGLE (DEGREES)

PANSY

Cosine Curve
Reflection
Transmission

Figure 8

GALVANOMETER DEFLECTION

ANGLE (DEGREES)

COLEUS

Cosine Curve
Reflection
Transmission
Figure 11

Figure 12
Figure 14

MAIZE LEAF

TRANSMISSION

REFLECTION - BOTTOM

REFLECTION - TOP

PERCENTAGE

WAVELENGTH - MICRONS

Figure 14
Figure 15

POTATO LEAF

WAVELENGTH (MICRONS)

PERCENTAGE

Transmission

Reflection - Bottom

Reflection - Top
Figure 17

CRUDE ETHER EXTRACT
POTATO LEAF

Graph showing various extract concentrations.
Figure 18

Figure 19
YELLOW PIGMENTS IN ETHER-CORN LEAF

Figure 20
Figure 21: Yellow pigments in ether-potato leaf.

Figure 22: Yellow pigments in ether-potato leaf.
CHLOROPHYLL BY DIFFERENCE
HYBISCUS LEAF

Figure 24
CHLOROPHYLL BY DIFFERENCE
POTATO LEAF IN ETHER

Figure 25
Figure 26

Figure 27
POTASSIUM CHLOROPHYLLIDE IN WATER-HIBISCUS LEAF

Figure 28

POTASSIUM CHLOROPHYLLIDE IN WATER-HIBISCUS LEAF

Figure 29
Figure 30

POTASSIUM CHLOROPHYLLIDE IN WATER - POTATO LEAF

Figure 31

POTASSIUM CHLOROPHYLLIDE IN WATER - POTATO LEAF

CONCENTRATION
Figure 32

CRUDE PETROLEUM
ETHER EXTRACT
CORN LEAF
Corn

Iron arc  
Blank  
Yellow Pigments in ether  
Acetone-ether extract

Incandescent  
Blank  
Yellow pigments in ether  
Acetone-ether extract

Iron arc  
Blank  
Saponified chlorophyll in water

Incandescent  
Blank  
Saponified chlorophyll in water

Hibiscus

Iron arc  
Blank  
Yellow pigments in ether  
Acetone-ether extract

Incandescent  
Blank  
Yellow pigments in ether  
Acetone-ether extract

Iron arc  
Water  
Saponified chlorophyll in water

Incandescent  
Water  
Saponified chlorophyll in water

Figure 33. Absorption spectrograms of extracts from corn and hibiscus leaves.
SUMMARY

The observations of this investigation may be summarized briefly as follows:

1. The distribution of reflected and transmitted radiation by a leaf, in general, approached the Lambert Cosine Law of diffuse scattering. Pronounced deviation from this law for the reflected radiation was observed when the leaf surface was glossy. The transmitted scattering by thin leaves did not conform to the cosine law, but showed a component of specular transmission.

2. The absorption curves for solutions of extracted pigments differed from the absorption curves of the leaf. In the process of extraction chemical reactions and dissociations of the pigment molecules may take place which change the absorption spectra of the pigments. The cellular structure of the leaf probably has an effect upon the absorption curves of the pigments in vivo.

3. A comparatively small percentage of the incident radiation in the region from .8μ to 1.3μ was absorbed by the leaf. Thick leaves reflected a large percentage of this radiation and transmitted a somewhat smaller per-
-63-

percentage. Thin leaves transmitted a larger percentage than was reflected in this region.

4. The absorption bands of water at 1.5\(\mu\) and 2.0\(\mu\) were apparent in both the transmitted and reflected radiation of leaves. The fact that the water bands appeared in the reflected radiation indicates that much of the reflected radiation was internally reflected.

5. Acetone-ether extract of fresh leaves, yellow pigments in ether, potassium chlorophyllide in water, and acetone-petroleum ether extracts from a number of leaves showed a marked deviation from Beer's Law at concentrations of 0.06 gms./liter and above particularly in the short wavelength portion of the visible spectra.
LITERATURE CITED


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