ABSORPTION SPECTRA OF LEAVES. I. THE VISIBLE SPECTRUM[1]

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(WITH THIRTEEN FIGURES)

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Introduction

The many and varied responses of plants to light have stimulated an interest among plant physiologists in leaf absorption spectra. The discovery by Hagenbach (**4**) of the differences in the absorption spectra of leaf extracts and leaves has led to recognition of the fact that absorption spectra of leaves are not predictable from the spectra of their extracts. More recent work by Seybold and Weissweiler (**16, 17**) has further emphasized this fact by showing that leaves which differ widely in their pigment content may show similar absorption spectra.

The measurement of leaf absorption spectra is not a simple problem. The optical difficulties involved have been discussed by several workers (**11, 14, 15, 7, 18**) who have emphasized that the problem consists essentially in measuring the diffuse light reflected from and transmitted through the leaves. If the amounts of flux incident upon, transmitted and reflected by the leaf are known, the absorption may be readily calculated from the equation $A = I - (T + R)$, where A, I, T and R refer to the flux absorbed, incident, transmitted and reflected respectively. It should be emphasized that the reflected and transmitted light is scattered in all directions by the leaf surfaces and by the tissue interfaces. It is, therefore, necessary to obtain an integrated measure of this reflected and transmitted light; and, as several workers have pointed out, the integrating sphere is well adapted for this purpose.

A study of the extent to which the reflected and transmitted light conform to the cosine law for a perfect diffusing surface has been made by Dinger (**2**), and some of his unpublished data are presented here (figs. 1 and 2). It is evident that the reflection curve for these leaves conforms closely to the cosine law, and the transmission curve conforms less closely. Both curves indicate a large amount of scattering.

Probably the most extensive study of leaf absorption spectra is that of Seybold and Weissweiler (**16, 17**). These workers used the Hardy automatic recording spectrophotometer which, although limited to the visible spectrum, is nearly ideal for this type of work. With this instrument, both reflection and transmission can be accurately and quickly measured, with

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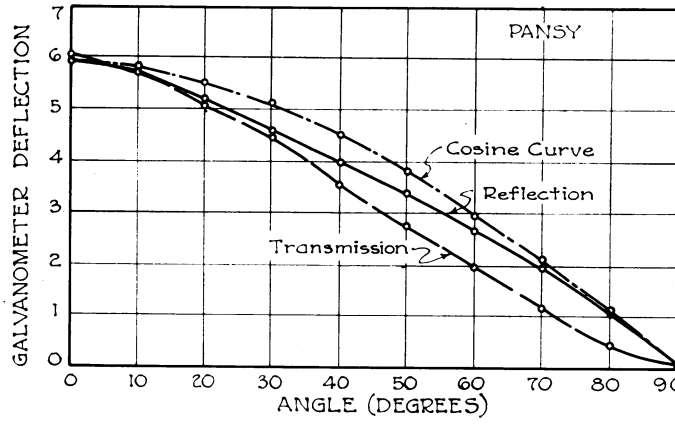
the integrating sphere principle. Absorption spectra were obtained for leaves of several species as well as for algal thalli. Normal leaves, water-infiltrated leaves and Chlorella suspensions showed an absorption maximum at about 680 mμ and a minimum at about 550 mμ. Dipping the leaves in boiling water or in ether for a few minutes shifted the absorption maximum to about 672 and 663 mμ respectively. A colloidal pigment preparation also



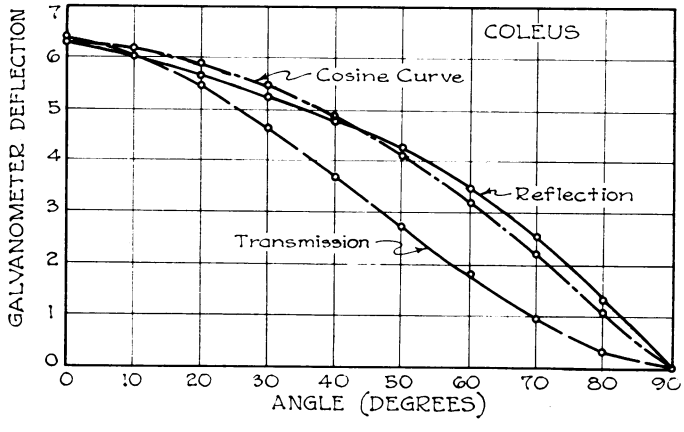


FIG. 1. (*Above*) Scattering of transmitted and reflected light by pansy leaves.

FIG. 2. (*Below*) Scattering of transmitted and reflected light by green Coleus leaves.

showed a slight shift in this absorption maximum. Comparisons between the absorption spectra of leaves grown in the shade and in the sun, and between normal and aurea leaves showed striking similarities.

More recently, RABIDEAU *et al.* (**12**) have employed the integrating sphere technique to make qualitative comparisons of absorption spectra of leaves, chloroplast suspensions and disintegrated chloroplast suspensions. With their apparatus they were able to extend the region investigated to

800 mμ. With one exception, the leaves showed an absorption maximum between 670 and 680 mμ and a minimum at 540 to 560 mμ. A leaf of Ananas gave a broad band in the red with an apparent maximum at 660 mμ. With most leaves, minor bands were evident at 600 to 620 and 640 to 660 mμ. The chloroplast and disintegrated chloroplast suspensions gave absorption spectra qualitatively similar to those of the leaves, but the bands were more sharply defined.

The present investigation was made with three purposes in mind. The first was to make a study of both the reflection and absorption characteristics of leaves and the effects of unusual leaf surfaces and of differences in leaf color. The second was to determine the effects on absorption spectra of dipping the leaves in boiling water and in ether, and of infiltrating the leaves with water. Some data relative to the effects of such treatments have already been reported by SEYBOLD and WEISSWEILER (**16, 17**) but have not as yet been confirmed by other investigators. The third purpose was to make a comparative study of the spectra of leaves, leaf extracts and chloroplast and disintegrated chloroplast suspensions. The extracts and suspensions were to be of such concentration that, when examined in a 1-cm. absorption cell, the quantity of pigment per unit cross-sectional area of light beam would be equal to that obtained with the leaf sample. In this manner the effect on absorption spectra of other variables than pigment quantity could be ascertained.

Materials and methods

The reflection data shown in figures 5 and 6 were obtained by means of a Razek-Mulder recording spectrophotometer. This instrument utilizes a diffuse light source, and the reflection normal to the leaf surface is measured by a phototube. The output from the phototube is fed to a galvanometer, and the galvanometer deflection is amplified optically and recorded photographically. The transmission data in figure 6 were obtained by clamping the leaf to a Weston photocell and exposing the leaf to the beam from a monochromator. The photocell output was fed to a galvanometer and the deflection read from a scale. Incident intensity was obtained by reading the deflection with the leaf removed from the front of the photocell. The absorption percentages were obtained by subtracting the sum of the reflection and transmission percentages from 100.

The data shown in the other figures were obtained with a spectrophotometer which was constructed specially for this work. The light source consisted of a projection lantern equipped with a 500 watt lamp. The monochromator was a single-prism instrument fitted with quartz optics. An integrating sphere, 25.5 cm. in diameter, was attached directly to the exit slit housing of the monochromator and held in place by a clamp and flange. The inner surface of the sphere was finished with a paint described by WALSH (**20**) which gives a white matt surface as required in sphere photometry.

The leaf specimen was held in the center of the sphere by a rectangular holder which was pivoted to permit swinging the leaf out of the light beam

when desired. The sample was trimmed to fit the holder and always included the midrib or a primary vein which fitted into a slot at one side of the holder and projected into a small cup of water. By this means the tendency of the sample to dry out while the measurements were being taken was greatly decreased.

An RCA type 1P-22 photomultiplier tube was used as the detector. It was mounted in the bottom of the sphere in such a manner that only reflected light from the sphere walls could strike the photocathode. The voltage supply for the tube consisted of a stabilized electronic power supply which provided a potential of from 900 to 1000 volts DC between the cathode and ninth dynode, and a separate 45 volt battery for the last stage between the ninth dynode and the anode. A complete description of this apparatus has been given by Moss (**9**). The output was read directly by means of a microammeter.

The present investigation was confined to the visible spectrum. The slits were adjusted to a calculated band width of 10 mμ throughout the spectrum, and readings were made every 10 mμ. Duplicate or triplicate determinations were made for each of the species, treatments or preparations, and either a mean value or a representative curve was chosen to be presented here. Three measurements were necessary at each wavelength setting to obtain the leaf absorption spectra. A reading with the sample in the sphere but out of the light beam gave a measure of the incident flux. A reading with the sample in the light beam gave a measure of the combined transmitted and reflected fluxes. The amount of flux reflected was measured by taking a reading with the sample in the light beam but with a metal clip directly behind it. This clip was painted a dull black on the side facing the leaf and white on the other side. Absorption was calculated from the equation $A = I - (T + R)$, where A, I, T and R refer to the flux absorbed, incident, transmitted and reflected respectively.

The absorption spectra of the extracts were measured in the same apparatus by placing first the solvent, then the extract, in a 1 cm. Beckman absorption cell which was mounted between the exit slit of the monochromator and the sphere. The spectrum was scanned three times with the solvent in the cell, then three times with the extract in the cell. Readings were taken every 10 mμ, and mean values computed. The absorption was calculated by taking the difference between the transmission of extract and solvent and expressing this difference as a percentage of the transmission shown by the solvent. This method involves the customary assumption that the refractive indices of solvent and extract are essentially the same. A similar procedure was followed in measuring the absorption spectra of the chloroplast and disintegrated chloroplast suspensions, except that the cell containing the suspending medium or suspension was mounted in the leaf holder in the center of the sphere. A correction for reflection from the cell and suspended chloroplasts was made by blocking off the transmitted light with a piece of dull black paper. Several tests were made to determine the accuracy which could be expected from the apparatus. All indicated that

the amount of scattered light was negligible, and that instrumental errors were within the limits required. Transmission curves obtained with the apparatus described above and with a Beckman spectrophotometer checked within 2%, except in steep portions of the curves where the discrepancy was attributable to band width differences.

Most of the plant material used was grown in the greenhouse. Care was taken to avoid drying or deterioration of the material prior to the determinations. Unless otherwise stated, the sample was mounted in the sphere in such a manner that the light was incident on the upper surface of the leaf. The leaf extracts were prepared by essentially the same method as that used by SEYBOLD and WEISSWEILER (**16, 17**). Twenty-five square centimeters of leaf tissue were extracted with methanol and the extract made up to a volume of 25 ml. The extracts were stored in the dark at zero degrees centigrade, and analyzed as soon after the extraction as feasible.

The chloroplast suspensions were prepared by a modification of the method reported by GORHAM and CLENDENNING (**3**). The filtered brei was centrifuged for 10 minutes at 300 to 350 g. to eliminate the large particles, and the chloroplasts were thrown down by centrifuging 10 minutes at 2000 g. and resuspended in 0.5 M sucrose. The disintegrated chloroplast suspensions were prepared by grinding the leaf laminae for 10 minutes in a mixture of ice and distilled water with the Waring Blendor. The material thrown down by centrifuging 10 minutes at 4000 g. was discarded, and that which was thrown down at 20,000 g. was resuspended in distilled water and kept for analysis. The suspensions were made up to a volume such that they would have the same quantity of pigments per unit volume as the extracts. This was done by centrifuging the solid material from an aliquot of the suspensions, extracting this material with methanol, and comparing the extract with the equal-area leaf extract using a Duboscq colorimeter. From this comparison, the proper dilution factor for the suspension was calculated. As a result, the leaves, leaf extracts, chloroplast suspensions and disintegrated chloroplast suspensions were all comparable as to quantity of pigment per unit cross-sectional area of light beam.

Experimental results

Absorption and reflection spectra for several species were obtained; but, because of their similarity, not all of them are presented here. The results obtained with six species are shown in figure 3. Bean, spinach, Swiss chard and tobacco were chosen as representative of species having relatively thin leaves with no unusual surface characteristics. A considerable uniformity in their absorption and reflection spectra will be noted, with the exception of the high reflection and low absorption shown by tobacco in the neighborhood of 550 mμ. The absorption minimum at 550 mμ, the points of inflection at about 600 and 630 mμ, the maximum in the neighborhood of 680 mμ and the sharp drop beyond 680 mμ are typical of most of the species examined. Much less detail is shown in the absorption spectra at the shorter wave-

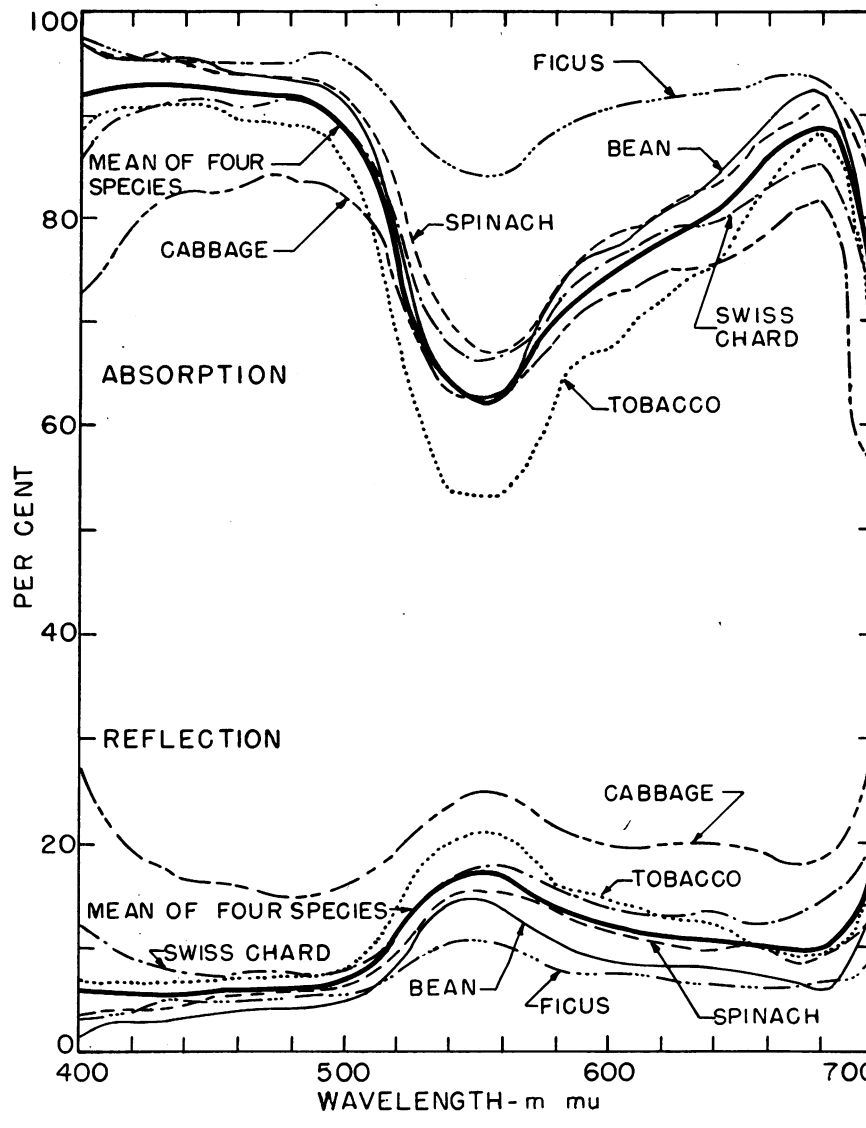


FIG. 3. Absorption and reflection of light by leaves of six species, together with smoothed average curves for bean, spinach, Swiss chard, and tobacco. Reflection of green light (550 mμ) averaged 17% and absorption 62%.

lengths. The heavy lines represent the mean values of the absorption and reflection spectra of the four species. It should be noted that the curves of reflection and absorption are roughly complementary, although less detail is shown by the reflection curve.

Ficus was chosen to represent the thick, heavily pigmented type of leaf. As compared with the more normal type, it showed high absorption across the entire visible spectrum, with the absorption bands less clearly defined

and with less difference between the absorption at 550 and 680 mμ. The absorption peak in the red was spread out between 660 and 680 mμ. The reflection was correspondingly low and showed relatively little variation.

The effect on absorption and reflection spectra produced by the presence of a highly reflecting material on the leaf surface is shown by the curves for cabbage. Reflection was 8 to 28% higher than the mean curve for the four normal species. Absorption was low, though not so low as tobacco in the region of 500 to 630 mμ. Part of this difference can be attributed to the fact that the reflection curve for tobacco showed greater variation in this region.

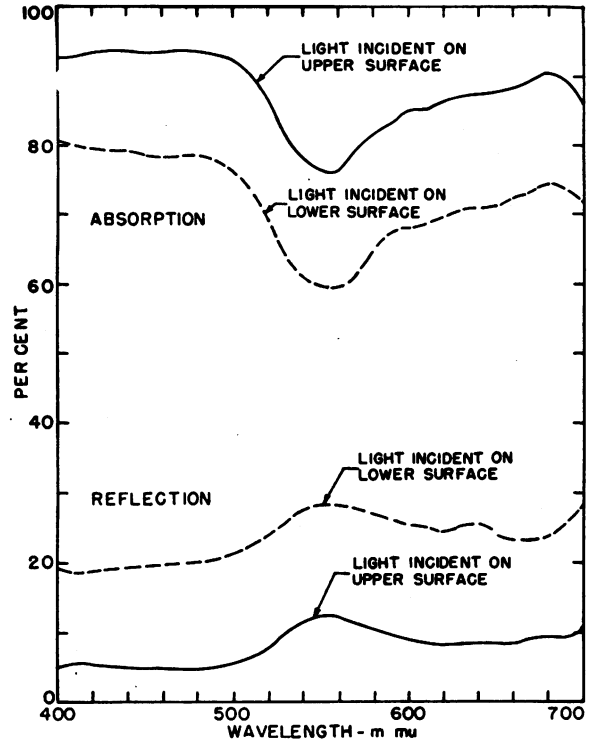


FIG. 4. Absorption and reflection of light incident on the upper, dark green, or lower, white, surface of white poplar leaves.

The effect of surface character is shown even more strikingly by the curves for white poplar in figure 4. These were obtained with the light incident first upon the upper, then the lower surface of the leaf. The difference in the absorption curves can be accounted for almost quantitatively by the difference in reflection. It should be noted that the absorption curve for the light incident upon the upper surface follows the general pattern set by the curves in figure 3, but is intermediate between that of such species as spinach and that of Ficus.

The relationship between leaf color and reflection spectra is shown in figure 5. It is interesting that yellow and orange leaves show greater reflection in the green than does the green leaf. However, the high reflection

throughout the yellow and red accounts for their characteristic colors. Reflection in the blue is nearly the same for leaves differing greatly in their apparent color. It should be remembered that reflection from leaves is made up of two components. One is the reflection occurring at the first air-cuticle interface, which probably is fairly uniform throughout the spectrum. The other is the reflection occurring at interfaces within the leaf. This second component of reflection is subjected to selective absorption by the materials through which the light must pass, and accounts largely for the spectral character of the reflection curves. This point is supported by the

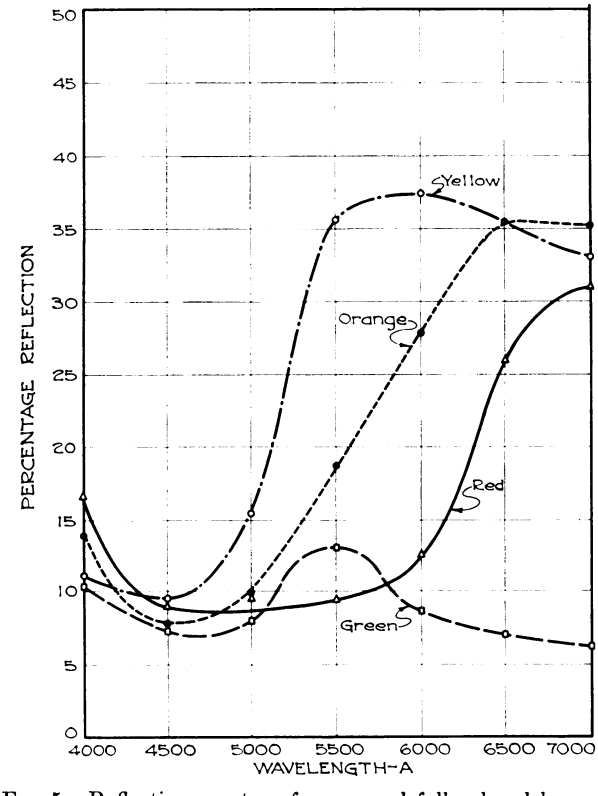


FIG. 5. Reflection spectra of green and fall colored leaves.

curves for infiltrated leaves in figures 10 and 11, which show that infiltration reduces the internal reflection and thereby tends to flatten the curve.

Figure 6 shows the reflection, transmission and absorption for yellow leaves. It will be noted that the transmission and reflection curves are similar and of the same order of magnitude. Both are complementary to the absorption curve.

ABSORPTION AND REFLECTION SPECTRA OF TREATED LEAVES

The effect of boiling upon the absorption spectra of leaves is shown in figures 7 and 8 for bean and Kalanchoe. The spectrum of the crude methanol extract is included in figure 7 for comparison. The relatively high

concentration of acids in Kalanchoe leaves makes the preparation of pigment extracts difficult. The effect of boiling the bean leaf was principally to decrease its absorption in the region beyond 480 mμ and to shift the position of the absorption peaks toward the blue. There was a significant decrease in reflection beyond 520 mμ. The decrease in absorption and reflection can be attributed to a partial filling of the air spaces in the leaf by water. The shift in the spectrum was most noticeable in the far red, and caused the peak to move to a position intermediate between that for the normal leaf and the extract.

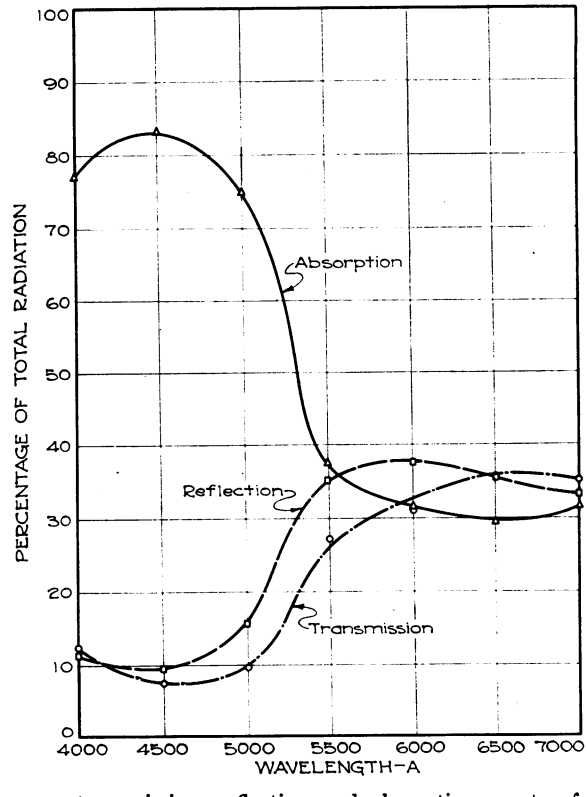


FIG. 6. Average transmission, reflection and absorption spectra for a number of yellow leaves.

The spectrum of the boiled Kalanchoe leaf showed a different pattern, although the blue shift in the peak at 680 mμ was evident. In the absorption curve for bean there were suggestions of absorption bands at 500, 580 and 620 mμ. In the curve for Kalanchoe there was no suggestion of a band at 480 mμ; a new band was found at 540 mμ, and one at 620 mμ. Even more striking were the shift in the absorption minimum from 550 to 585 mμ and the increased reflection in the red. It would be difficult to arrive at an adequate explanation for these differences without careful chemical and spectrophotometric work. A probable explanation may be found in the high acid content which leaves of succulents often contain. With heating, this

acid could lead to pigment degradation, as shown by the development of brown coloring in heated leaves.

The effects of dipping the leaves in ether as well as those resulting from dipping in boiling water are shown in figures 9, 10 and 11. The effect of the boiling was much the same for spinach and tobacco as for bean, resulting principally in an overall reduction in reflection and absorption at the longer wavelengths, and a shift which was particularly noticeable in the

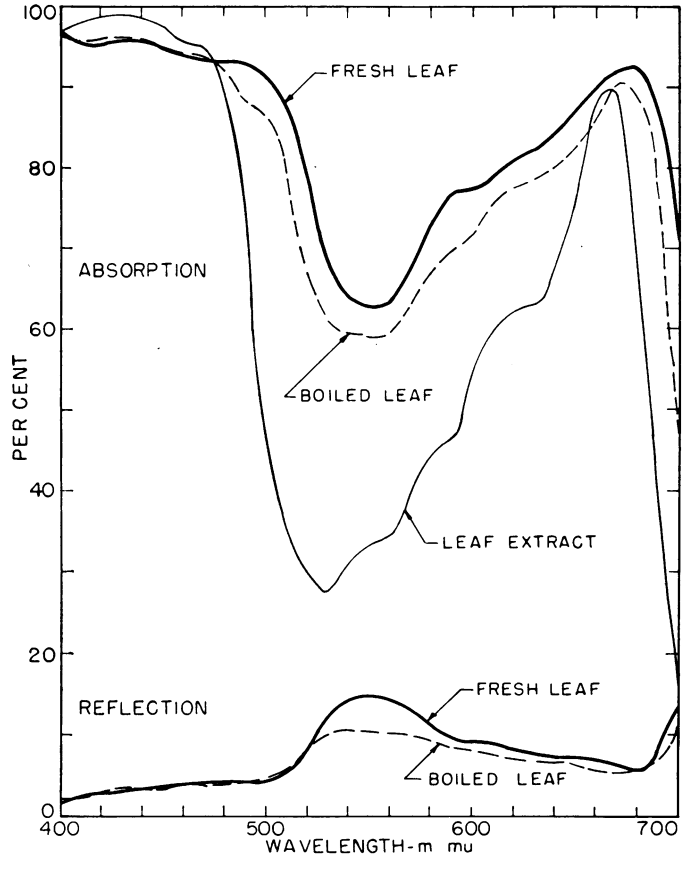


FIG. 7. Transmission and reflection spectra for bean leaves and a methanol extract containing an equal quantity of pigments.

position of the absorption peak in the red. The curves obtained for a Swiss chard leaf dipped in boiling water resemble those obtained for Kalanchoe. The same absorption peaks at 540 and 620 mμ and the shift in the absorption minimum may be found.

There was also a lack of uniformity in the results obtained by dipping the leaves in ether. With spinach, both the absorption and reflection curves were shifted toward shorter wavelengths. The shift in the position of the red absorption maximum was greater than was obtained by dipping the leaf in boiling water, but the peak was not shifted so far as that of the ex-

tract. Although the amount of reflection was only slightly altered, the absorption was markedly decreased. The curves for Swiss chard and tobacco present a different aspect. With Swiss chard, the reflection maximum, which was at about 550 mμ in the fresh leaf, had been nearly eliminated by the ether dipping, and with tobacco the maximum had been lowered greatly. This reduction was reflected partly in the increase in absorption throughout the region, but it should be noted that the increase in absorption was approximately double the reduction in reflection. With both species, the posi-

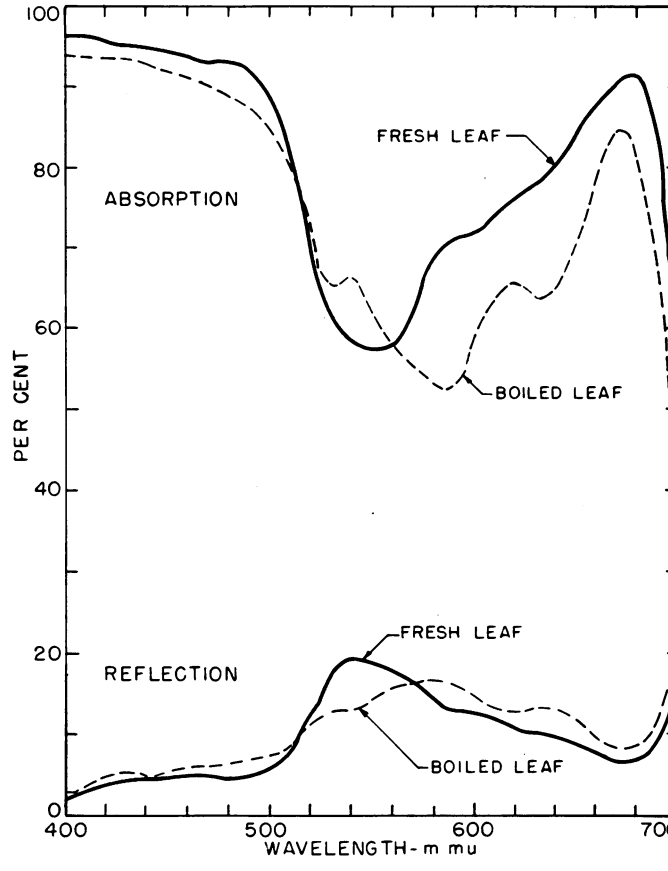


FIG. 8. Spectra of live and freshly boiled leaves of Kalanchoe.

tion of the red absorption maximum coincided with that obtained by dipping the leaves in boiling water. A possible explanation for these differences may be found by considering the relative thickness and permeability of the leaves and the length of time they were immersed in the ether, since these factors would affect the extent to which the pigments were extracted from the chloroplasts and the extract diffused throughout the leaf tissue. In no case was there any observable removal of pigment from the leaf.

The curves obtained from leaves infiltrated with water are typical of those obtained with several species. The effect was to reduce the internal

reflection at interfaces within the leaf tissue and thus lower both the reflection and absorption. The position of the principal absorption bands remained unchanged.

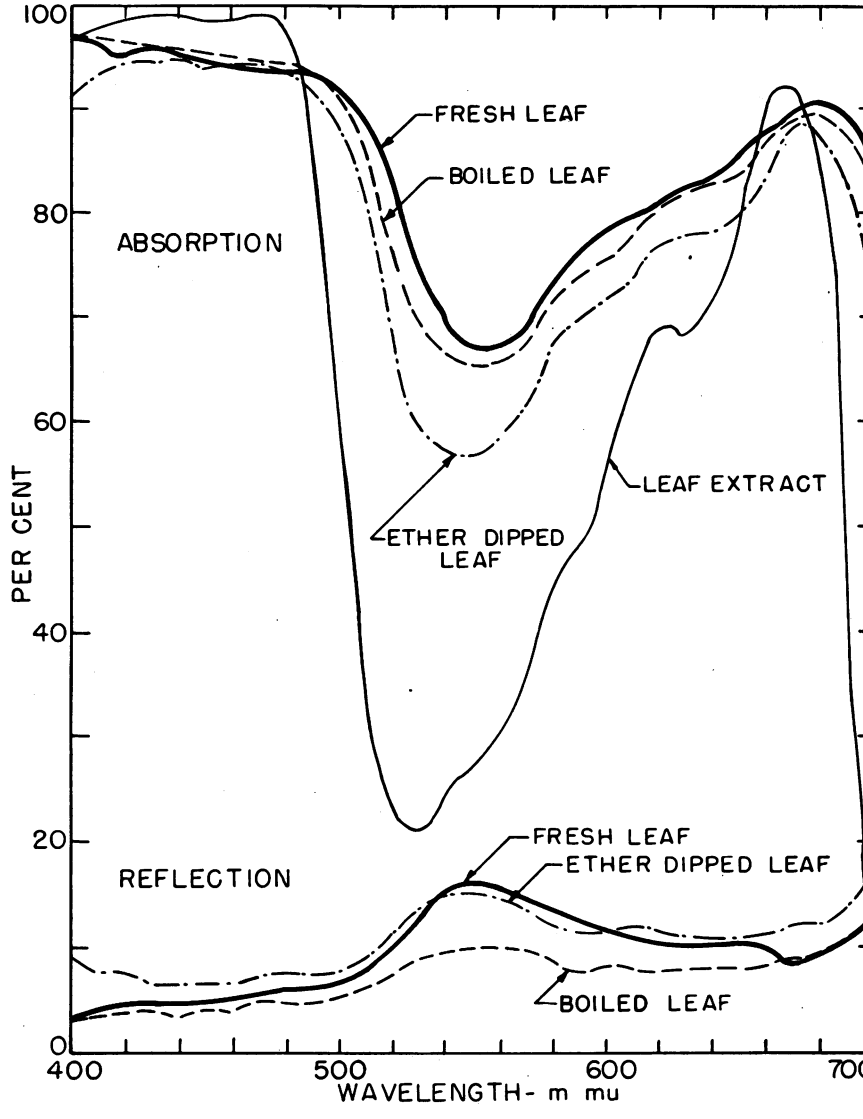


FIG. 9. Leaf and extract spectra for spinach.

ABSORPTION SPECTRA OF CHLOROPLAST AND DISINTEGRATED CHLOROPLAST SUSPENSIONS

The results of a series of experiments designed to compare the absorption spectra of fresh leaves with those of chloroplast and distintegrated chloroplast suspensions of such concentration that quantity of pigment would not be a factor are summarized in figures 12 and 13. There was a progressive

narrowing of the principal absorption bands from the fresh leaf to the chloroplast and distintegrated chloroplast suspensions, but the position of the maxima and minima remained essentially unchanged. In the curves for spinach the absorption in the blue and in the neighborhood of 680 mμ was

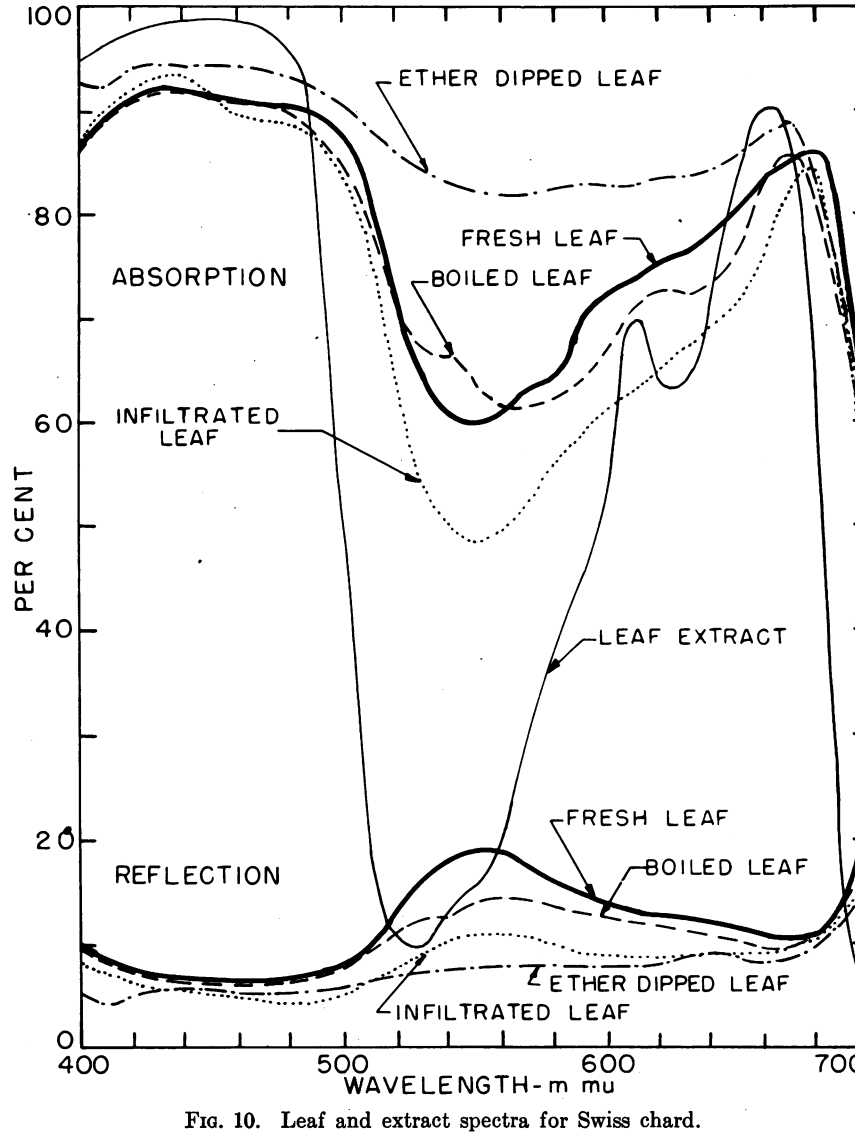


FIG. 10. Leaf and extract spectra for Swiss chard.

higher for the distintegrated chloroplast than for the chloroplast suspension, and the reverse was true in the curves for Swiss chard. A small error in the dilution factor for one or more of the suspensions could account for this difference.

Because of the many indications that the pigments in the chloroplasts may be in a colloidal state, the absorption curve for a colloidal suspension

of the crude pigment extract from Swiss chard is presented in figure 13. Several differences between this curve and that obtained for the fresh leaf will be noted. There was a shift of nearly 40 mμ in the position of the absorption minimum for the colloidal suspension, and a shift of at least 5 mμ

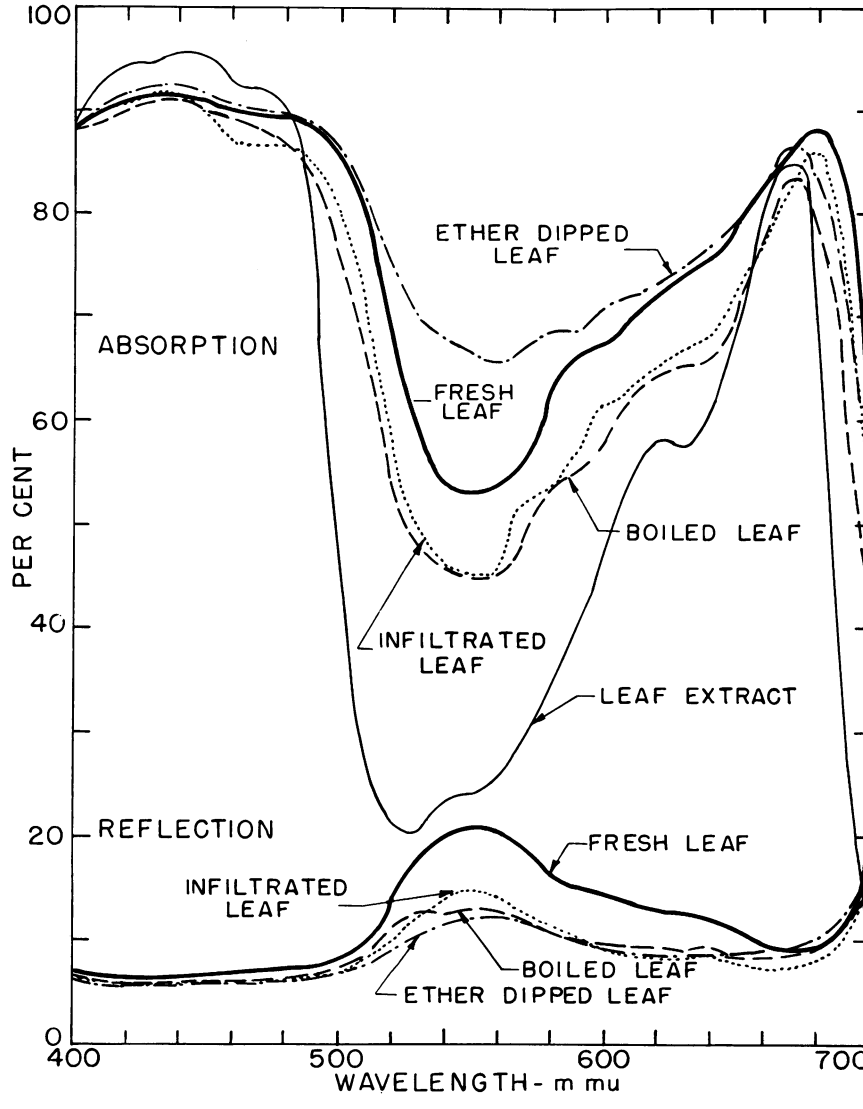


FIG. 11. Leaf and extract spectra for tobacco.

in the position of the red maximum. The latter shift has been noted by many other authors.

Discussion

The success of any spectrophotometric study is dependent to a large degree upon the accuracy of the apparatus used. The instrumental problem

is complicated by the optical nature of the material to be investigated. Leaves contain a variety of pigments whose physical state in the living leaf remains unknown. A considerable portion of the incident light is reflected

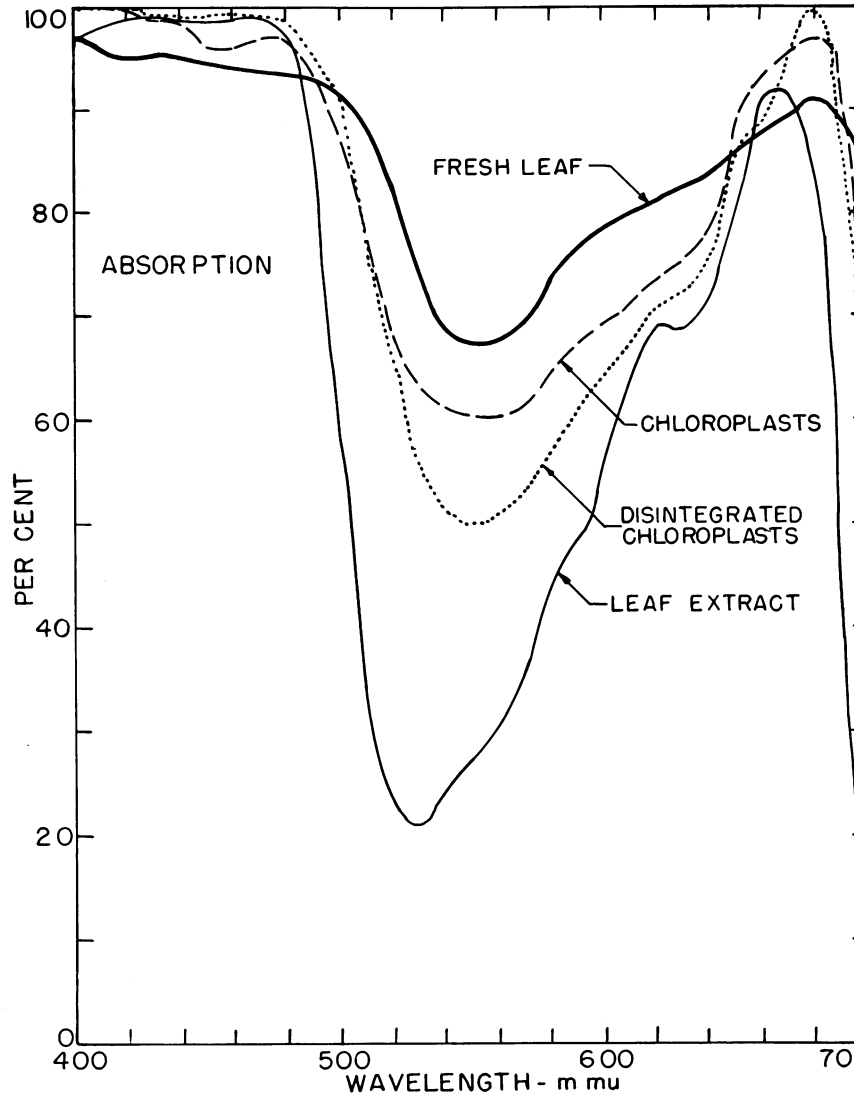


FIG. 12. Absorption spectra of equivalent quantities of pigments from spinach leaves in four physical states. Note that the high absorption of green light and the red band at 680 mμ are characteristic of all the curves except that for the methanol extract.

from the external surfaces, and another portion which is internally reflected is modified by the selective action of the pigments through which it passes. This reflected light is neither perfectly spectral nor diffuse in character, and the degree to which it approaches the cosine law for diffuse reflectance varies

between species and, to some degree, within a single species. The transmitted light is scattered also. The numerous air spaces and interfaces in the leaf tissue cause internal reflection which lengthens the effective light path within the leaf. As a result of all these factors, the absorption spec-

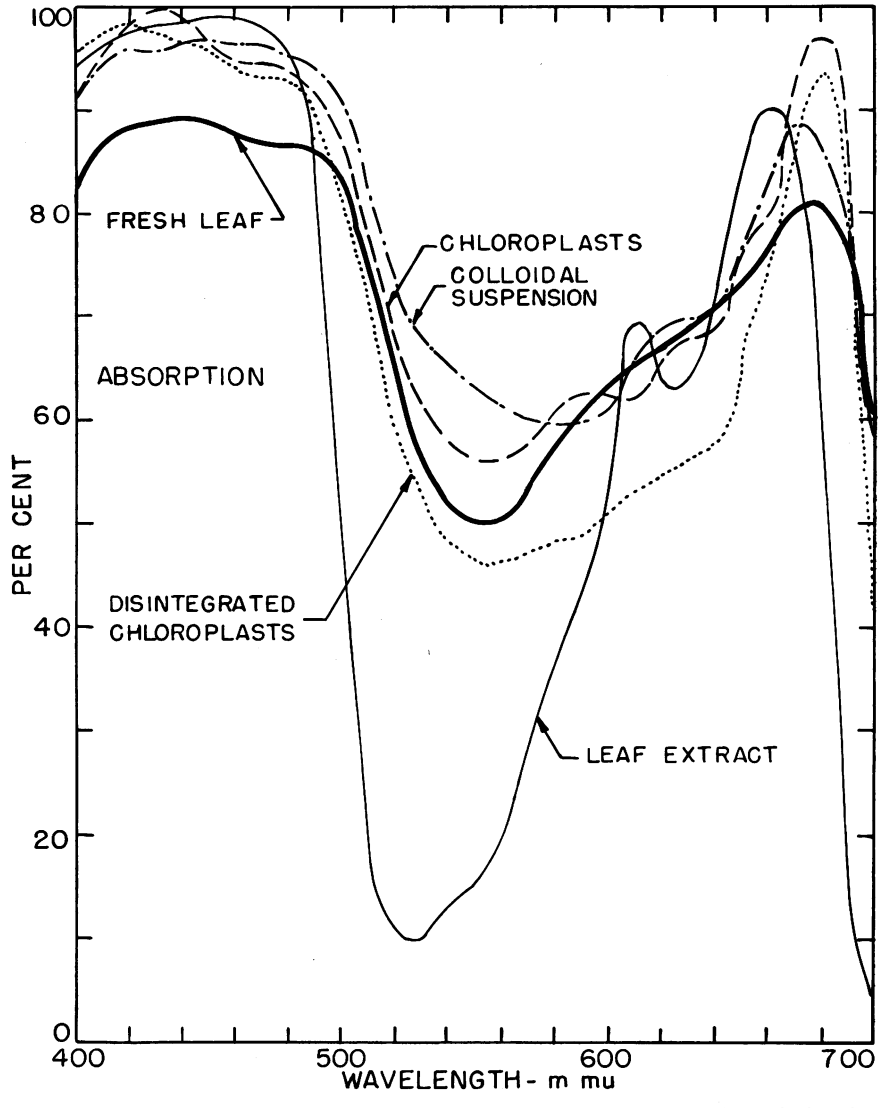


FIG. 13. Absorption spectra of equivalent quantities of pigments from Swiss chard leaves in five physical states.

trum of the leaf as a whole can be obtained only by an integrated measurement of the scattered light reflected from and transmitted through the leaf. The absorption spectrum of the pigments within the leaf is still more difficult to obtain, and will be discussed later. The selection of the com-

ponent parts of the apparatus used in the present work was influenced largely by their availability. The major choice was in the type of detector and its accompanying circuit. The selection of a photomultiplier tube proved to be a wise one from the standpoint of simplicity, economy and linearity of response. It is believed that the apparatus used was entirely adequate for the purpose.

Figure 3 shows that the absorption curves for most of the species have a maximum at or close to 680 mμ. The corresponding maximum for Ficus was not clearly defined but was between 660 and 680 mμ. The absence of a distinct absorption maximum, or an apparent shift in its position toward shorter wavelengths, is typical of thick or heavily pigmented leaves with a glossy surface. If the percentage transmission is computed for a dark, glossy leaf such as that of Ficus in the region 650 to 690 mμ, values of 0.5 to 3.0% are obtained. The shape of the absorption curve is then clearly dependent upon the reflection curve and does not correspond exactly to the absorption characteristics of the leaf. If such a leaf is infiltrated with water, the internal reflection will be reduced and a more pronounced absorption peak at or near 680 mμ will be found. This relationship between the reflection and absorption curves must be kept in mind when interpreting leaf absorption spectra.

Several investigators have attempted to study the absorption spectrum of the pigments in the leaf but no critical analysis of the problem has yet appeared. BROWN and ESCOMBE (**1**), SEYBOLD (**15**) and others have attempted to measure light absorption by white and green portions of varigated leaves. However, it was pointed out by WILLSTÄTTER and STOLL (**21**) and again by SCHANDERL and KAEMPFERT (**13**) that such a procedure is invalid because it neglects the effect of internal reflection and implies that all the pigment is concentrated in a layer along the lower surface of the leaf. Other investigators have used water-infiltrated leaves and have compared the absorption spectra before and after extracting the pigments with alcohol. This procedure is objectionable in other respects. A partial solution to the problem can be obtained by using the white and green portions of a varigated leaf and infiltrating both portions with water to reduce the internal reflection. Unfortunately, it is difficult to find a pigment-free portion of leaf tissue large enough to fill the light beam of the instrument employed.

SEYBOLD and WEISSWEILER (**17**) have attached considerable importance to the relative absorption in the three broad regions 400 to 500, 500 to 600 and 600 to 700 mμ. The absorption percentages obtained by these workers for *Sambucus niger* for these regions were 90, 74 and 83, and the ratios of absorption were respectively 108 : 89 : 100. An estimate of the absorption percentages for these three regions was made from the mean absorption curve for four species in figure 3. The resulting percentages were 92, 71 and 84; and the ratios were 110 : 85 : 100. The average absorption for the entire visible spectrum was estimated to be 82% and the reflection 10%.

Seybold and Weissweiler also obtained curves comparing the absorption and reflection by white poplar leaves when the light was incident on the

upper and on the lower surfaces. Their data show a difference of about 30% in both reflection and absorption. Figure 4 shows that the difference obtained in the present investigation was about 15%. This discrepancy can probably be attributed to the use of a young leaf in the present work on which the white hairs characteristic of the lower surface were not fully developed.

The discovery by HAGENBACH (**4**) of the shift in the absorption curve of the extracted pigments, and SORBY's discovery (**19**) of the effect on the absorption curves of leaves produced by dipping them in boiling water find confirmation in the data presented here. The effect produced by dipping the leaves in ether was investigated by SEYBOLD and WEISSWEILER (**16**), and their results are to a large degree substantiated here. The curves for spinach (fig. 9) contain a strong suggestion that the spectra of the boiled leaf and ether-dipped leaf are intermediate between the spectrum shown by the fresh leaf and that shown by the methanol extract. That dipping a leaf in ether should result in an absorption spectrum similar to that of an extract is not surprising, for the effect seems to be a partial extraction with the ether extract diffused throughout the leaf tissue. The same bands which appear in the spectrum of the extract were found in the spectra of the ether-dipped and boiled leaves, and suggested in the spectrum of the fresh leaf.

The absorption curves obtained with treated leaves of other species do not conform so well to the progressive transition found for the treated leaves of spinach. The absorption curve for the boiled Kalanchoe and Swiss chard leaves (figs. 8 and 10) were clearly out of line with this suggestion. They are most easily explained as resulting from degradative changes caused by leaf acids. In both species the boiled leaves had the brown color associated with phaeophytin. The effect of dipping the leaves in ether appeared to vary with the length of treatment. With prolonged treatment there was a marked reduction in reflection and a corresponding increase in absorption. With this increase in absorption, the bands became indistinct and a more uniform curve resulted, as shown by the curves for Swiss chard in figure 10 and to a less extent for tobacco in figure 11. The leaves of these species were given a much longer treatment than that given the spinach leaf. The absorption curve for the boiled tobacco leaf was similar to that for the spinach. However, the curve was intermediate in position between that of the fresh leaf and that of the extract.

SORBY (**19**) explained the boiling effect on leaf spectra by stating that the pigments were initially present in the free state, and, on heating, became dissolved in melted fatty material. WILLSTÄTTER and STOLL (**21**) arrived at essentially the same conclusion on the basis of the observation that the spectrum of the boiled leaf was like that of a solution of chlorophyll in phytol. It may be argued that boiling is an extremely harsh treatment for so labile a system as that of the plant pigments in the living leaf. However, NOACK (**10**) showed that distinct changes could be produced by temperatures much lower than that of boiling water. He prepared a colloidal dispersion of leaf material and showed that the natural fluorescence was mark-

edly decreased by heating to 70° for a few seconds. Longer heating caused a reappearance of the fluorescence. These reactions were interpreted as indicating that the chlorophylls are initially bound to protein and that this bond was broken by heating, leaving the chlorophylls in a colloidal state. Further heating caused them to become dissolved in the melted lipoids. MESTRE (**6**) investigated the effect of heating leaves and found a time-temperature relationship for the shift in the absorption spectrum which closely paralleled that for albumin coagulation. A similar effect was obtained for chloroplast suspensions by HAGENE and GOAS (**5**). Both of these latter investigations tend to support the conclusions of Noack.

The data presented here are consistent with Noack's hypothesis that the initial effect of heating is the denaturation of protein with rupture of the chlorophyll-protein bond, followed by formation of a solution of chlorophyll in melted lipoids. However, the data do not exclude other possible explanations, and the existence of an intermediate colloidal state, as proposed by Noack, remains unproved.

An attempt to obtain some data relative to the problem of determining the amount of absorption which can be attributed to the pigments in a leaf as distinct from the leaf as a whole has been made by determining the absorption spectra of chloroplast and disintegrated chloroplast suspensions. These suspensions were of such a concentration that the amount of pigment contained in an aliquot of suspension placed in a 1 cm. absorption cell was the same as that in a portion of leaf of equivalent area. The results are presented in figures 12 and 13, with the absorption curves for fresh leaves and leaf extracts included for comparison. Some discrepancy is found between the curves in the two figures which is probably due to errors in computing the proper dilution factors. However, the data indicate that as progressively smaller particle sizes were used there was a progressive reduction in absorption throughout the central portion of the visible spectrum and a sharpening of the absorption maxima and minima. Reflection from the disintegrated chloroplast particles was detectable only in the region of 500 to 600 mμ. Reflection from whole chloroplasts was appreciable throughout a broader region. The effect of reducing the particle size was partly a reduction of reflection which, in turn, tended to raise the absorption maxima and lower the minima. The same absorption bands were found in the suspensions as in the fresh leaf, and no shift in their position had occurred.

MILNER *et al.* (**8**) have recently described the preparation of very fine suspensions of chloroplast material. These suspensions, when activated by the addition of neutral salts in the presence of methanol, show an appreciable amount of the photochemical activity associated with the original chloroplasts as measured by the Hill reaction. Furthermore, successive precipitation and resuspension produces photochemically active material with an essentially constant chlorophyll/protein ratio. Absorption spectra for these suspensions were not given. Since the activity was found to be dependent upon the degree of aggregation, it would be interesting to know

what effect, if any, the state of aggregation had upon the absorption spectra. A correlation between the effect of various treatments on the photochemical activity and on the absorption spectra would also be of interest. From their work it appears that the fundamental photochemical unit must involve an association of both lipoid material and protein with the pigments.

Summary

Absorption and reflection spectra for leaves of four dicot species possessed certain features in common. There was an absorption maximum in the red at 680 mμ and a minimum in the green at 550 mμ, with points of inflection suggestive of minor absorption bands at about 600 and 640 mμ. Absorption was high throughout the blue but no distinct peak was present.

Mean absorption and reflection curves were obtained from the data for these four species. From this mean absorption curve, average values of absorption for the three regions 400 to 500, 500 to 600 and 600 to 700 mμ of 92, 71 and 84% respectively were obtained. For the region 400 to 700 mμ, the absorption was 82% and the reflection 10%.

Curves obtained for Ficus show the reduced reflection, increased absorption and general flattening characteristic of the absorption and reflection spectra of thick leaves. The white, tomentose, lower surface of a white poplar leaf resulted in an increase in reflection of about 15% and a corresponding decrease in absorption throughout the spectrum when the light was incident on the lower surface. Yellow, orange and red leaves showed about the same reflection in the blue but a much higher reflection in the red, as compared with a green leaf. The yellow and orange leaves reflected much more of the green and yellow light than did the green leaf. Transmission and reflection spectra for yellow leaves were similar, and both were complementary to the absorption spectrum.

Dipping leaves in boiling water or in ether for a few minutes shifted their absorption spectra toward the blue and sharpened the absorption bands. The change was more pronounced when the leaves were dipped in ether than when they were boiled. The boiled leaves showed a reduced absorption throughout the central portion of the spectrum. Those dipped in ether showed either an increased or decreased absorption throughout this region, depending primarily on the length of treatment. Leaves infiltrated with water showed no shift in their absorption spectra, but rather a decrease in absorption throughout most of the visible region and a sharpening of the band at 680 mμ.

Some data relative, to the absorption spectrum of the pigment complex freed from interference by the leaf structure were obtained by comparing the absorption spectra of leaves and leaf extracts with those of chloroplast and disintegrated chloroplast suspensions. These suspensions and extracts were so prepared that, when placed in a 1 cm. absorption cell, the quantity of pigment per unit cross-sectional area of light beam was the same as that obtained when using a sample of leaf tissue. The maxima and minima for

the suspensions agreed in position with those for the fresh leaf, but were more sharply defined. The effect of progressively smaller particle size in reducing the multiple reflection and thereby the absorption, particularly in the green, was also evident.

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