FLORAL COLOURS AND THE PHYSIOLOGY OF VISION

Part II. The Green Colour of Leaves

By Sir C. V. Raman

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Nearly all vegetation—from the blades of grass on the ground to the tops of lofty trees in the forest—has green as its characteristic and dominant colour. In this part of the memoir, we shall concern ourself with the question, what is the origin of this colour? In other words, what is the spectral character of the radiation that excites this sensation in our visual organs? The answer to the query that emerges from the investigation is a surprising one. We shall in the course of our enquiry have much to say also about other questions, particularly the following. What is the relationship between the perceived colour and the absorption spectra of the pigments responsible for it? Why do different species of plants, shrubs and trees exhibit different shades of green in their foliage? What is the origin of the progressive change of colour following its first appearance of a leaf during its further development to full size and maturity?

A spectacular display of colour is that provided by a rice-field grown under irrigation, when after the seedlings have been transplanted, they multiply and cover the whole area. In bright sunshine, the field appears as a brilliant sheet of green, the exact shade of the colour depending on the state of growth. Viewing such a field through a direct-vision pocket spectroscope and comparing the spectrum then seen with that of the sunlit sky above the field, it is found that the violet and blue parts of the spectrum are absent in the light of the rice-field, while, on the other hand, the green and red sectors are both conspicuous and are of comparable strength. The superposition of the green and the red should have resulted in the field appearing of a golden-yellow colour instead of the bright green which it actually exhibits. Indeed, when sky-light is viewed through a glass filter of a golden-yellow hue, its spectrum also covers the green and red sectors and resembles that of the rice-field. Why then does the rice-field with a similar spectrum appear green instead of golden-yellow? The answer to this question emerges when the spectral composition of its colour is critically examined. We may usefully begin with a few remarks on the techniques of observation.

Even the thinnest of leaves when held up in sunlight does not permit of the sun's disk being seen through it. In other words, the incident light is completely diffused or scattered besides suffering absorption within the material. It is very commonly the case that the upper and lower surfaces of a leaf present a very different appearance. The former exhibit in diffuse daylight a deeper colour and are also smoother. In consequence, when held at the proper angle to a beam of light incident on it, there is an observable reflection or glitter at the surface. On the other hand, the colour of the lower surface appears diluted by admixture with white light and by reason of its roughness, the regular reflection by the surface is weakened and may indeed not be noticeable at all.

In the circumstances stated above, we have two distinct and alternative methods of studying the colour of green leaves. The first is to observe the *upper* surface of the leaf holding it towards the light in such position and viewing it at such an angle that the surface reflection or glitter is unobservable and only the light emerging from the interior of the leaf is seen. The second method is to observe the light which emerges *through* the leaf when it is held up against the source of light. In the latter case, the slit of the observing spectroscope may be brought up close to the leaf. It then makes no difference which side of the leaf faces the source of light. In either case, it is desirable to use a powerful source of illumination. Indeed, direct sunlight is most suitable, though useful observations may also be made by holding up the leaf against the sky or alternatively using a powerful artificial source such as a tungsten-filament lamp of high candle power. Quite simple methods suffice for making the observations, a direct-vision pocket spectroscope being that all is needed. It should be provided with a wavelength scale capable of being focused independently and also capable of being adjusted laterally so that its readings can be checked against those of the lines in the solar spectrum.

The colour of leaves depends a good deal on the species under study. The colour also exhibits very significant changes during the progress of a leaf to maturity after its first appearance. Further significant changes appear when it has passed maturity and turns yellow before finally dropping off. These changes in colour, so far from being a source of embarrassment in the investigation, are actually helpful since they enable us to correlate the observed variations of colour with the changes in its spectral composition and thereby assist us in understanding the nature of the relationship between them.

We may at this stage turn our attention to the colouring matters whose presence in green leaves has been proved by extraction with suitable solvents and their chemical identity established after their separation from each other by appropriate methods. These colouring matters have been found to be of two kinds, *viz.*, the carotenoids and the chlorophylls respectively. The carotenoids present in green leaves are of two kinds, *viz.*, β-carotene and dihydroxy-α-carotene or xanthophyll. The chlorophylls are also two in number and have been designated as chlorophyll (*a*) and chlorophyll (*b*) respectively. The absorption spectra of the carotenoids in a state of solution have been thoroughly investigated. β-carotene and xanthophyll exhibit somewhat similar absorption spectra which appear predominantly in the wavelength region between 350 mμ and 500 mμ. At wavelengths greater than 520 mμ the absorption by these carotenoids is quite negligible. Chlorophyll (*a*) and chlorophyll (*b*) behave a little differently from each other. Chlorophyll (*a*) exhibits two pronounced maxima of absorption, one near the red end of the spectrum at about 660 mμ and the other in the violet at about 420 mμ; between 640 mμ and 450 mμ, its absorption is very weak, but there is a perceptible increase in the wavelength range between 600 mμ and 630 mμ with a distinct maximum at 615 mμ. The principal absorption maxima of chlorophyll (*b*) appear respectively at 640 mμ and 460 mμ, while between 620 mμ and 480 mμ its absorption by it is very weak, though a small rise appears around 590 mμ.

It is necessary here to remark that the absorptive behaviour of the pigments as exhibited in the green leaves is not necessarily identical with that of the same materials separately in a state of solution. Nevertheless, the results quoted are useful as aids in the interpretation of the observations made with green leaves. Holding up a green leaf against a brightly lit sky and examining the light transmitted through it with a pocket spectroscope, it is found that the spectrum seen lies between the limits 520 mμ and 645 mμ. The position of these limits is not found to differ sensibly as between leaves showing a light green and a dark green colour respectively. Nor do these limits alter appreciably when we examine leaves in different stages of growth. Using bright sunlight, however, a feeble extension of the spectrum beyond 645 mμ to greater wavelengths can be observed. The dark absorption bands evidently due to chlorophyll lying in that region can then be seen. Likewise, using sunlight, the short wavelength limit is pushed down to about 500 mμ.

The cut-off apparent at 500 mμ and complete at 520 mμ is clearly ascribable to the absorption by the carotenoid pigments. This is indicated by the fact that leaves which have turned yellow also exhibit a cut-off at 520 mμ. Likewise, as we shall see later on, the petals of flowers with a golden-yellow hue ascribable to the presence of carotenoid pigments also exhibit a cut-off at the same wavelength. We thus arrive at the conclusion that the wavelength limits between which the light transmitted by green leaves has a sensible intensity, *viz.*, 520 mμ and 645 mμ are set respectively by the absorption due to the carotenoid pigments and the chlorophylls.

From the remarks and observations set forth above, it is clear that the green colour of leaves which we perceive is *not* a consequence of the powerful absorption by chlorophyll at the red end of the spectrum. Indeed, this region is of such low luminosity that its removal can have no sensible effect on the observed colour. *Actually, the operative factor is the absorption by the pigments present in the leaf of the yellow region of the spectrum.* This absorption is not strong. It nevertheless suffices profoundly to alter the character of the visual sensation excited by the light which has traversed the material containing these pigments. Indeed, on a careful examination of the spectrum of the light emerging through a green leaf, it is evident that the yellow sector in the wavelength range between 570 mμ and 590 mμ is weakened relatively to the regions on either side of it. Such weakening is appreciable even with the thinner and immature leaves and is conspicuous with the thicker and maturer leaves. The yellow sector is completely absent in the case of leaves which are dark green in colour. Thus, the differences in the observable colour of the leaves in these different cases are clearly ascribable to the variations in the extinction of the yellow part of the spectrum relatively to the green and to the orange and red sectors on either side of it.

As the yellow sector of the spectrum is a part of which the luminous efficiency is high, it is only to be expected that its extinction would have a readily observable effect on the perceived colour of the emerging light. Even so, it remains to be explained why the part of the spectrum in the wavelength range between 590 mμ and 645 mμ which is observable even with the thickest and darkest leaves and whose visual brightness is certainly not negligible in comparison with that of the spectral region between 520 mμ and 570 mμ seems to exercise little or no influence on the perceived colour. While it is undoubtedly the case that the red is weakened relatively to the green in the light which penetrates the leaf, the fact that it has no visual effect indicates that in the absence of the yellow sector which is the connecting link between the green and the red, the green sector masks the effect of the red, in other words, prevents its coming within the range of perception.

As visual observations of the spectral transmission by green leaves present no difficulty, any observer can satisfy himself regarding the statements made above. It appeared, however, desirable to exhibit the facts objectively with the aid of photographically recorded spectra. The non-uniform sensitivity of panchromatic films in the spectral region under consideration makes it rather difficult to accomplish this in a satisfactory manner. That the spectral ranges of transmission by a green leaf and by a yellow flower are not very different is, however, easy enough to exhibit in spectrograms. Figure 1 and Fig. 2 reproduced in Plate I are intended to do this. Figure 1 exhibits the recorded spectra of a green leaf and of a yellow flower side by side and Fig. 2 those of the yellow flower alone. The red end of the spectrum appears at the extreme left in each case.

Postscript dated the 9*th August* 1963: Since the foregoing paragraph was written, it has been found possible using an "Agfa Raman Plate" to obtain satisfactory spectrograms demonstrating the effects described above.

SUMMARY

The light emerging with sensible intensity after passage through the material of a green leaf is observable in the spectral regions of green and red extending from 520 mμ to 645 mμ. These limits are set by the carotenoid and the chlorophyll pigments in the leaf which exercises powerful absorptions at smaller and at greater wavelengths respectively. The characteristic absorption bands of the chlorophylls can be seen under strong illumination at the extreme red end of the spectrum. But they do not sensibly influence the observed colour of the leaf, since the extreme red is of very low luminous efficiency. Actually, the operative cause which determines the observed colour of the leaf is the absorption by the leaf pigments manifested in the yellow region of the spectrum between 570 mμ and 590 mμ. The colour of the leaf appears a deeper green with increasing strength of that absorption.

FIG. 1

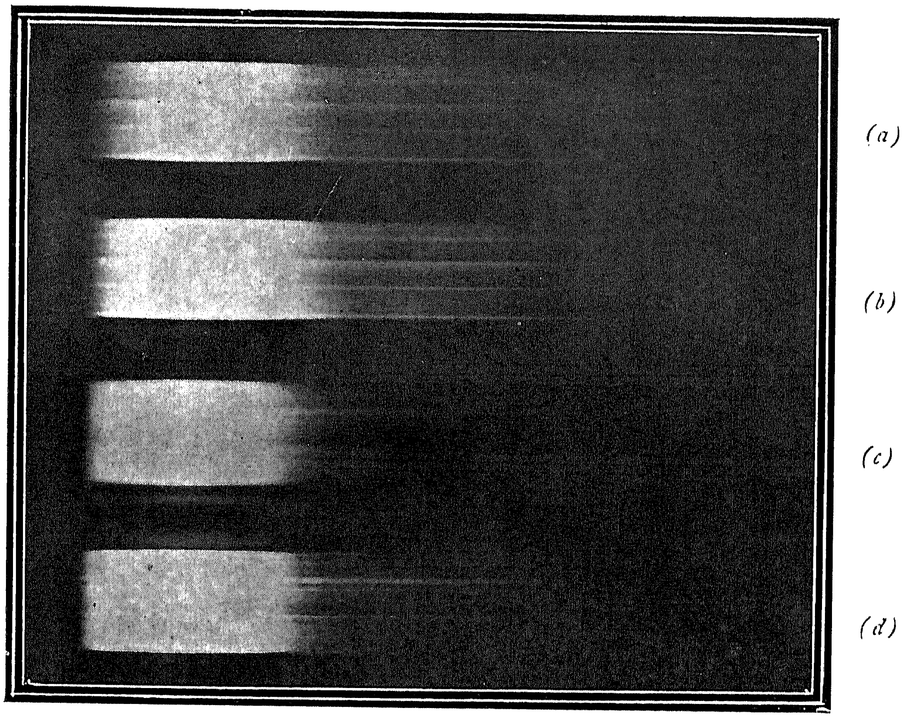

FIG. 2

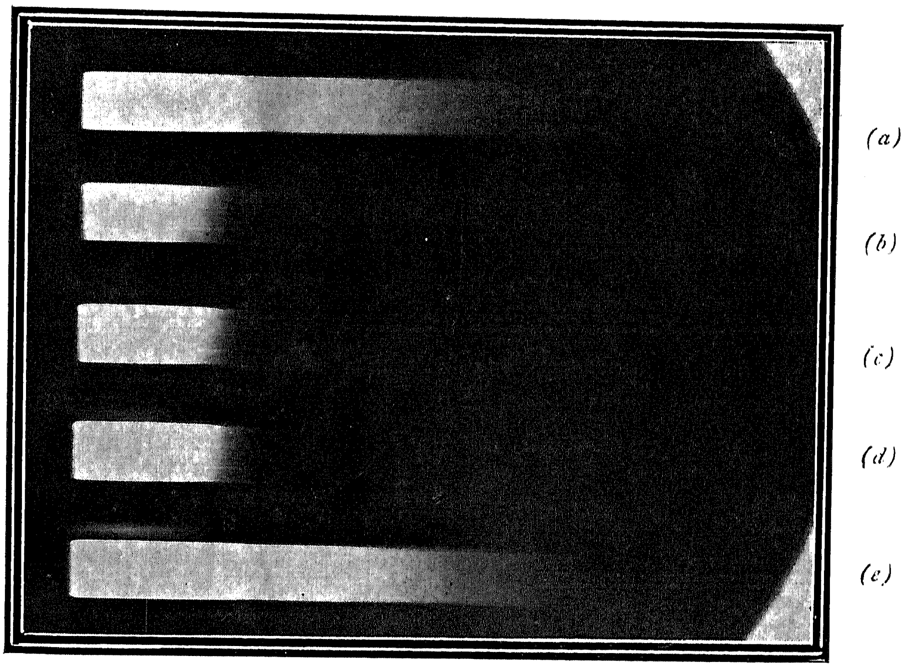

FIG. 1 (*a*) and (*b*). Spectrum of Green Leaf. (*c*) and (*d*) Spectrum of Yellow Flower.
FIG. 2 (*a*) and (*e*). Spectrum of Light Source. (*b*), (*c*) and (*d*). Spectrum of *Bignonia grassilis*