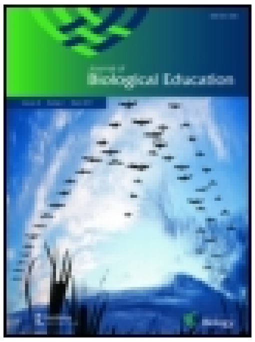

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Chlorophyll does not reflect green light – how to correct a misconception

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ABSTRACT
Plant leaves are green because they contain the green photosynthetic pigments, chlorophylls *a* and *b*. Popular science literature, and sometimes even textbooks, state that the greenness is caused by reflection of green light by chlorophyll. In the present study, we compared the reflectance spectra of green leaves to yellow or white leaves of the same species. Chlorophyll-deficient leaves reflected green light more efficiently than green leaves of the same species, which conclusively refutes the misconception. The data show that the green colour of leaves is caused by preferential absorption of blue and red light by chlorophyll, not by reflection of green light by chlorophyll. The data suggest that the cellulose of the cell walls is the main component that diffusely reflects visible light within plant leaves.

KEYWORDS
Chlorophyll; reflectance; green; green leaves; visible light; absorbance

Introduction

The colours of illuminated items are – with few exceptions like the blue colour of the sky – caused by wavelength-selective absorption of light. Wavelengths that are neither absorbed nor pass through, are (diffusively) reflected from the item, and the spectral distribution of the reflected light determines the colour. An opaque object either absorbs or reflects all incident light, and if the object is homogenous like a Lego brick, then the reflection spectrum of the material is essentially a mirror image of its absorption spectrum. In heterogeneous systems, one constituent may mostly reflect and another may absorb.

Chlorophylls *a* and *b* show strong absorption in the blue and red spectral regions but absorb poorly green light (500–560 nm) (Lichtenthaler and Buschmann 2001). Due to inhomogeneous broadening, the absorption spectra of both pigments are however wider *in vivo* than in organic solvents (Van Amerongen, Valkunas, and van Grondelle 2000), enabling wider absorption of photons throughout the illumination spectrum. Plants also contain carotenoids absorbing blue-green light (Lichtenthaler and Buschmann 2001). Nonetheless, due to the sheer number of pigment molecules, green light is overall absorbed only 20–30% less efficiently by leaves of land plants than red or blue light, and green light is also utilised in photosynthesis (Hershey 1995). Ability to utilise green light has been suggested to provide the leaves in lower layers of the canopy and chloroplasts in lower mesophyll layers with excitation energy when the topmost layers efficiently absorb blue and red light (Terashima et al. 2009).

The reflectance of light is widely used in remote sensing to estimate the chlorophyll content per surface area of the terrain. The estimation requires simultaneous recording of reflectance at two

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Supplemental data for this article can be accessed here.

wavelengths, from which one is strongly and the other poorly absorbed by chlorophyll. The ratio of reflectance in the red region (strongly absorbed) to reflectance in near-infrared (poorly absorbed) has been found to estimate chlorophyll content better than the red to green reflectance ratio (Le Maire, Francois, and Dufrêne 2004; Datt 1999).

Popularised science texts (see, e.g. Ortega 2020) and even biology textbooks (see, e.g. Raven, Evert, and Eichorn 2005) sometimes explain the green colour of plant leaves by stating that chlorophyll absorbs blue and red but reflects green light. The origins of this hypothesis are unclear, but it appears to be widespread. A Google search requiring the exact wording 'chlorophyll reflects green light' gave 4670 hits when tested on 4 October 2020, and Wikipedia repeats the misconception (Wikipedia article titled Chlorophyll; https://en.wikipedia.org/wiki/Chlorophyll, cited 27 April 2020).

Light and colour belong to elementary school science classes and to high-school physics. Studies of conceptions about light, colour and vision have revealed that upper elementary school children (grades 3–6) often have no idea about the roles of incident and reflected light in vision and that children tend to get confused when they find out that a brightly coloured item cannot be seen at all in complete darkness (Ward, Sadler, and Shapiro 2008). Various incorrect ('alternative') conceptions of colour, including the idea that colour is a permanent property of an object and independent of the colour of incident light, are common among upper elementary school students (Eaton et al. 1984; Valanides and Angeli 2008), and the misconceptions appear to be recalcitrant against traditional science teaching (Eaton et al. 1984). In Finnish high-school biology textbooks, the colour of plants is simply attributed to the presence of chlorophyll, without a further physical explanation (Happonen et al. 2018a, 2018b).

The misconception that chlorophyll reflects light may not belong to elementary school students' misconceptions because this misconception requires correct basic understanding on how the colour of an object is formed. Therefore, the misconception is expected to be one of the teachers and their educators. In the present study, we show that the reflectance of green light by plant leaves is not caused by chlorophyll, and plant leaves devoid of chlorophyll show higher, not lower, reflectance in the green region than green leaves. With these data, we seek out to falsify and correct the common misconception about chlorophyll reflecting green light. Furthermore, we provide simple tools for demonstrating the reflectance of light by leaves in a classroom.

Materials and methods

Plant material

Three different species of plants with different variations in pigment composition were used to measure reflectance of light. Green and yellow leaves of *Betula pendula* (silver birch) were collected from a local park in late autumn, *Euphorbia pulcherrima* (poinsettia), a variety with white upper leaves, was purchased from a local flower store and additional leaves were obtained as a gift from Ms. Eija Leino. Seeds of a variegated barley plant, *Hordeum vulgare* var. variegata ('cat grass') were bought from Moles Seeds, Colchester, UK, sown in a research greenhouse, where the germinated plants were grown at 21°C and in a 16 h light/8 h dark-light cycle with photosynthetic photon flux density of 100 μmol $m^{-2}s^{-1}$. From the two latter species, reflectance was measured from both white and green leaves of the specimens. Six-millimetre leaf disks were cut out from the selected plant leaves just before measuring.

Reflectance measurements

Spectra of specular reflectance between 400 and 800 nm were measured with an STS-VIS spectrometer equipped with an optical reflectance probe R600-7-UV-125 F, both from Ocean Optics, Dunedin, FL, USA. The spectrometer provides a spectrum from 370 to

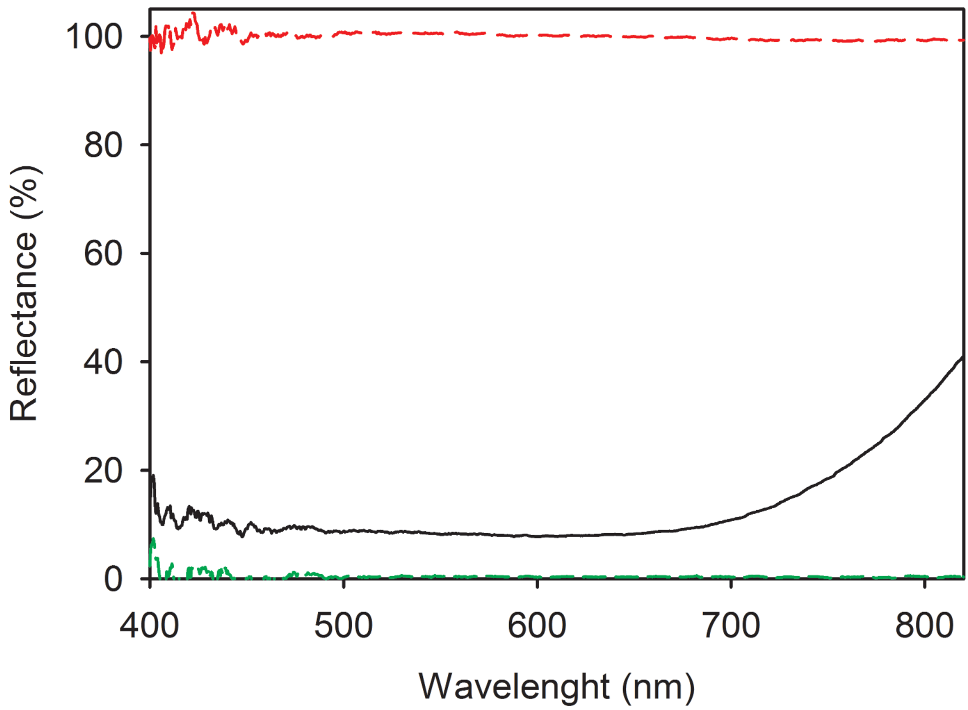

Figure 1. Reflectance spectra of the matt black paper used under the leaf samples (black, solid line), 100% reflection standard (red, long-dashed line) and a Vantablack surface (green, dashed line). Specular reflectance was measured with an STS-VIS spectrometer, using a 250 W halogen lamp as a light source. For the measurement, the probe was placed 7 mm above the surface and aligned with the surface normal. Each curve represents an average of 10 technical replicates, and the data have been smoothened with a moving median.

800 nm with 1024 data points but data below 400 nm were not used because of low signal quality. The reflectance probe was aligned with the normal of the sample surface and placed 5 mm above it. A 250 W halogen lamp, driven by a stable voltage source, was attached to one end of the bifurcated reflectance probe. A reflectance standard from Labsphere, Inc. (North Sutton, NH, USA) was used to define full reflectance. For calibration of the spectrometer, reflectance was also probed from a Vantablack-VIS (Surrey Nanosystems, Surrey, UK) surface to ensure that the instrument zero truly indicates zero reflectance (Figure 1). Reflectance spectra of leaf discs were measured on top of a matt black cardboard or on white office paper, as indicated. Interference by external light was blocked by doing the measurements in a darkroom and covering the setup with a black matt foil (Edmund Optics, York, UK).

Pigment quantification

Pigments from parallel samples of *Hordeum* and *Euphorbia* were extracted with N, N-dimethylformamide (DMF) from 6-mm diameter leaf disks. Each disk was incubated in 1 ml of DMF overnight (white poinsettia for a few hours), and absorbance was measured at 480, 646.8, 663.8 and 750 nm; the very small 750 nm absorbance was subtracted from other absorbance values to compensate for light scattering. *Betula* leaf disks were incubated only shortly to obtain the carotenoid to chlorophyll ratio. Pigments were quantified according to Wellburn (1994).

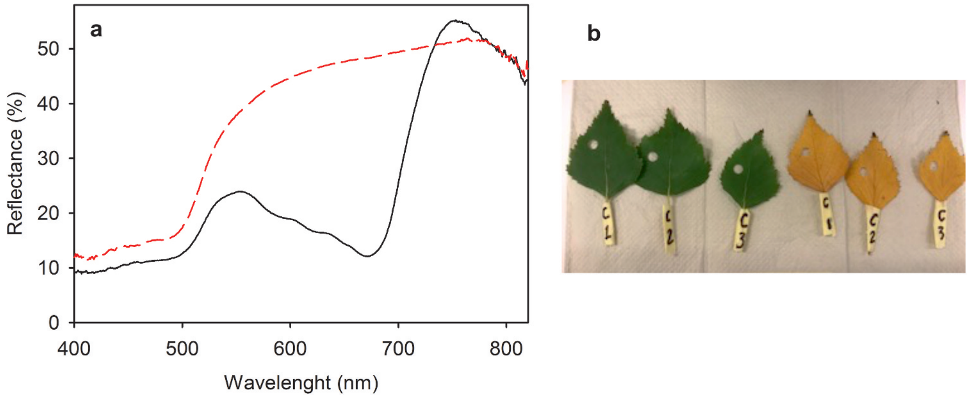

Figure 2. Reflectance spectra of green (black, solid line) and yellow (red, dashed line) leaves of *B. pendula* (a) and examples of the leaves (b). Specular reflectance was measured with an STS-VIS spectrometer, using a 250 W halogen lamp as a light source. For the measurement, a leaf disk was placed on a matt black cardboard at a 5 mm distance from the probe, and the probe was aligned with the surface normal. Each curve represents an average of 6 independent biological replicates, and the data have been smoothened with a moving median using a window of 9 data points.

Results

Silver birch leaves were collected in late autumn, when both green and yellow leaves are available. Green silver birch leaves, collected in late autumn, contain 40–50 µg chlorophyll cm^{-2}, and the chlorophyll concentration drops rapidly to less than 10 µg cm^{-2} before the leaf falls (Mattila et al. 2017). The reflectance spectra of green birch leaves showed the familiar spectral shape of a plant leaf, with 9–15% reflectance in blue (400–490 nm) and red (640–700 nm), 24% reflectance in green (500–570 nm), and strong reflection of far-red light (>700 nm) (Figure 2). The maximum of the reflectance of visible light was recorded at 550 nm (Figure 2). Yellow leaves reflected all colours of visible light more strongly than green leaves, and their reflectance in the green region was roughly twice as high as that of the green leaves (Figure 2). However, below 490 nm, the reflectance of the yellow leaves was only slightly higher than that of the green leaves, and above 770 nm, the reflectance values from green and yellow leaves were identical.

Variegated barley produces both green and white leaves. The chlorophyll concentrations of the green leaves vary, whereas white leaves contain no chlorophyll (Table 1). Reflectance measurements from both leaf types showed that the white leaves reflected more light throughout the whole spectrum than the green leaves. In the green region, white leaves reflected approximately 30%, whereas green leaves reflected less than 10% of light (Figure 3). In the red region (640–700 nm), the reflectance of the white leaves was similar as in the green region, but green leaves reflected less red than green light. Both types of leaves reflected less blue than green light but the white leaves still reflected more than 20%, whilst the green leaves reflected only 4–6% (Figure 3).

The Poinsettia variety used for the study has dark green lower leaves and white upper leaves containing no chlorophyll (Table 1). The white leaves are somewhat transparent, which prompted

Table 1. Pigment composition of leaves of *H. vulgare* and *E. pulcherrima*. Pigments were measured from samples similar to those used for the reflectance measurements and quantified spectrophotometrically according to Wellburn (1994). The dash indicates that no quantitative extraction was done.

	Chlorophyll ($a + b$), µg/cm^2	Carotenoids, µg/cm^2	Carotenoid to chlorophyll ratio µg/µg
B. pendula, green leaves	-	-	0.29
B. pendula, yellow leaves	-	-	1.23
H. vulgare, green leaves	22.38 ± 3.62	4.04 ± 0.59	0.18
H. vulgare, white leaves	0.00	0.23 ± 0.06	
E. pulcherrima, green leaves	58.75 ± 10.80	10.05 ± 1.78	0.17
E. pulcherrima, white leaves	0.0	0.0	

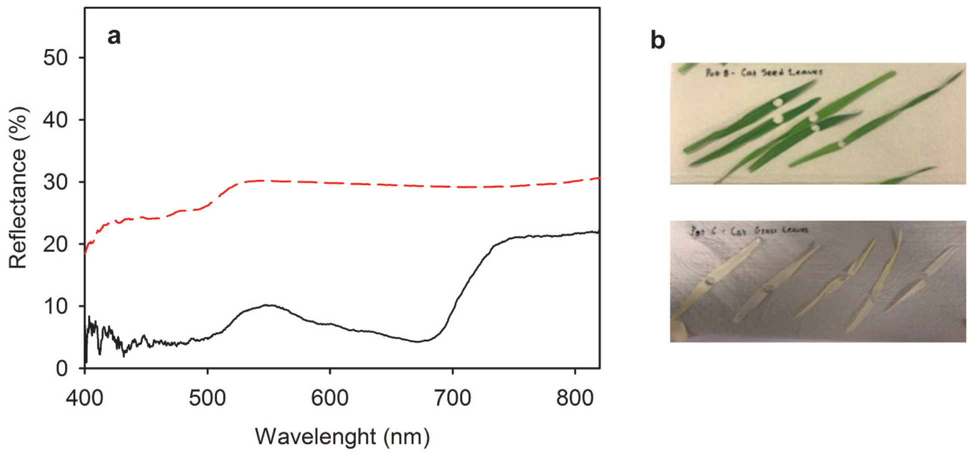

Figure 3. Reflectance spectra of green (black, solid line) and white (red, dashed line) of *Hordeum vulgare* cv. variegata leaves (a) and examples of the measured leaves (b). Specular reflectance was measured with an STS-VIS spectrometer, using a 250 W halogen lamp as a light source. For the measurement, a leaf disk was placed on a matt black cardboard at a 5 mm distance from the probe, and the probe was aligned with the surface normal. Each curve represents an average of 10 independent biological replicates, and the data have been smoothened with a moving median using a window of 9 data points.

us to measure their reflectance on both black and white background. In both backgrounds, white poinsettia leaves reflected much more light than green leaves (Figure 4a). When measured on a black background, the difference was smaller (Figure 4b) but still clear.

Discussion

White and yellow leaves with very low chlorophyll concentrations (Table 1) show significantly higher reflectance of green light than the green leaves of the same species (Figure 2 –Figure 4), thus immediately falsifying the hypothesis that chlorophyll reflects green light. Our results are in agreement with results of Gitelson and Merzlyak (1996) who showed that increasing chlorophyll *a* concentration is inversely, rather than directly proportional to the reflectance of green light.

Specular reflectance appears to depend on the surface quality and on the chlorophyll content of the leaf. The glossy birch and barley leaves reflect light more strongly than the matt, high-chlorophyll *Euphorbia* leaves throughout the measured spectrum. The differences in chlorophyll content (Table 1) partially explain the differences in reflectance below 700 nm but above that chlorophylls *a* and *b* absorb light very poorly. The present data also show that transmission of light from the background through the leaf can be significant. This was demonstrated by the *Euphorbia* spectra that changed dramatically when the background was changed, indicating strong dependence of reflectance on the reflectivity of the background. Dependence of leaf reflectance on the background may be important for the interpretation of remote sensing data.

While chlorophylls *a* and *b* absorb throughout the visible spectrum, carotenoids only absorb blue-green light (Zur et al. 2000). In green leaves, the contribution of carotenoid absorption to the reflectance is difficult to distinguish from the contribution of chlorophyll *b*, as both absorb roughly at the same range of wavelengths. However, the spectra of the chlorophyll-deficient leaves show that the decrease in reflectance in all pigmented leaves when approaching 500 nm from the long-wavelength side is largely caused by carotenoids. This conclusion is based on the finding that the decrease in reflectance around 500 nm is much larger in the yellow, more carotenoid-rich birch leaves (Figure 2, Table 1) than in the white leaves of variegated barley and *Euphorbia* with very low carotenoid content (Figures 3 and Figures 4).

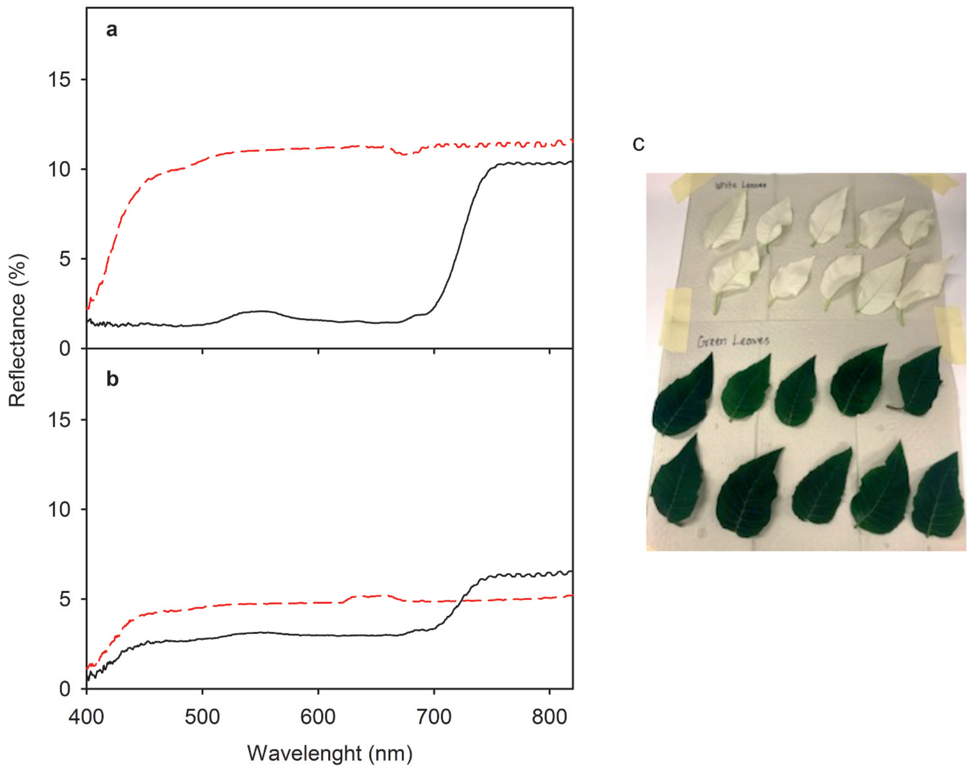

Figure 4. Reflectance spectra of green (black, solid lines) and white (red, dashed lines) of *E. pulcherrima* leaves placed on white (a) or black (b) background; the measured samples are also shown (c). Specular reflectance was measured with an STS-VIS spectrometer, using a 250 W halogen lamp as a light source. For the measurement, a leaf disk was placed on white copy paper (a) or on a matt black cardboard (b) at a 5 mm distance from the probe, and the probe was aligned with the surface normal. Each curve represents an average of 10 independent biological replicates, and the data have been smoothened with a moving median using a window of 9 data points.

If chlorophyll does not reflect green and near-infrared light, what then causes the reflectivity of leaves in these wavelength regions? Our data do not pinpoint a specific biomolecule among the constituents of the leaves, but cellulose of the cell walls is an obvious candidate, as white paper, composed of cellulose, is highly reflective in the whole visible range (see Figure 4). The large amount of cell walls in a plant leaf supports the suggestion that they are mainly responsible for the reflectance of leaves.

The sensitivity curve of the human eye reflected in the 1931 CIE luminous efficiency function (see Solomon and Lennie 2007 for a review of the human visual system) peaks in green, and therefore green light has a higher visible impact than other visible wavelength ranges. The spectral sensitivity of the human eye deepens our perception that plant leaves are definitely green, even if green light might be only slightly enriched in the light reflected from a leaf (Figure 4).

We have prepared two tools for the demonstration of why plant leaves are green. Appendix 1 is a short slide show demonstrating the function of chlorophyll in a leaf. This slide show can also help students to understand colour and vision in a more general sense. Appendix 2 demonstrates that in heterogeneous materials, one component often absorbs light while another is responsible of the diffuse reflection. A stroke of marker pen on white paper is an extreme example, as it appears colourful because the marker pen ink blocks the diffuse reflection of some wavelengths from the underlying paper. Such pigments do not leave any coloured mark on black paper. Crayons functioning with this principle are common, although many crayons, as well as watercolours, also contain reflective ingredients. Appendix 2 contains a video demonstration with white and black paper and a few colours, including marker pens, crayons and a plant extract in which the main pigments are chlorophylls *a* and *b*. The demonstration can either be shown as a video or reproduced in a classroom, when the teacher has made sure that the students understand the role of reflected light in the perception of colour.

In conclusion, plant leaves are green because green light is less efficiently absorbed by chlorophylls *a* and *b* than red or blue light, and therefore green light has a higher probability to become diffusely reflected from cell walls than red or blue light. Chlorophylls do not reflect light.

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Disclosure statement

No potential conflicts of interest were reported by any of the authors.

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