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PITCH PERFECT

grass, football pitch, photosynthesis, light-dependent reaction, wavelength, absorption spectrum, redox indicator, chlorophyll, chloroplast

biology

16–18 years

1 | SUMMARY

In this project, students use different coloured lights to investigate the effect of the wavelength of light on the rate of photosynthesis and growth of grass. After evaluating the experimental evidence, they will be able to recommend which colour of light should be used in lighting rigs to best support the growth and recovery of grass on football pitches between matches.

2 | CONCEPTUAL INTRODUCTION

In temperate regions, natural daylight is limited during much of the football season, particularly during the short days of the winter months. Lighting rigs are used to speed up grass growth on the parts of a pitch that are shaded and to encourage the rapid recovery of grass damaged by wear and tear during a football match **(FIG. 1)**.

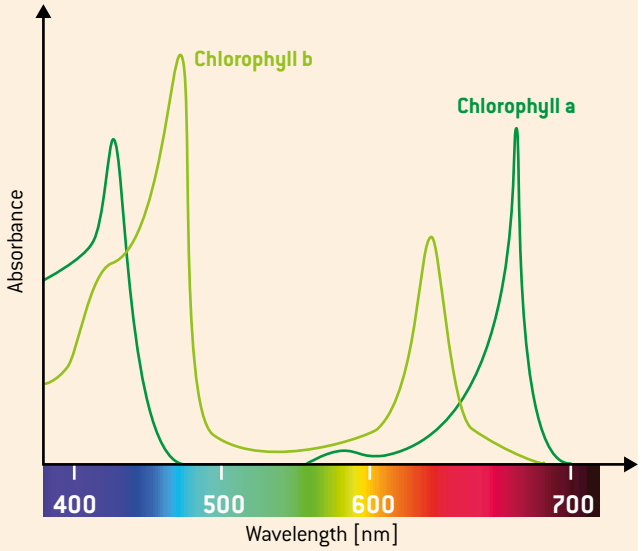
FIG. 1 Lighting rigs to speed up grass growth

FIG. 2 The visible spectrum [1]

V: violet, B: blue, G: green, Y: yellow, O: orange, R: red

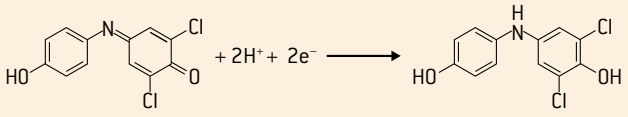
The visible spectrum is made up of a range of different wavelengths of light, i.e. different colours **(FIG. 2)**. The most common photosynthetic pigment, chlorophyll, is actually a mixture of two pigments (chlorophyll a and chlorophyll b) that absorb some wavelengths of light more than others, showing maximum absorption of red and blue light and minimum absorption of green light **(FIG. 3)**.

FIG. 3 Absorption by chlorophyll depending on wavelength of light [2]



The energy absorbed by chlorophyll is used in the light-dependent reactions of photosynthesis to excite its electrons to higher energy levels. The energy gained by these electrons is subsequently used in redox reactions to release energy, which is used to make ATP. This product, alongside another product of the light-dependent reactions (reduced NADP), is used by the plant in the Calvin cycle to make glucose. The plant uses glucose as an energy source and a raw material for the synthesis of a wide range of organic materials required for healthy plant growth.

FIG. 4 DCPIP: 2,6-Dichlorophenolindophenol



Oxidised DCPIP (blue)

Reduced DCPIP (colourless)

The rate of photosynthesis can be investigated using a redox indicator called DCPIP, which is blue when oxidised and colourless when reduced **(FIG. 4)**. When DCPIP is added to chloroplasts freshly extracted from plants, it is reduced by the electrons (and protons) produced during the light-dependent reactions of photosynthesis when the chloroplasts are illuminated. The faster these reactions occur, the faster the rate at which DCPIP is reduced. In one investigation, the students determine the rate at which DCPIP is reduced (decolourised) under different coloured lights in order to determine the effect of the wavelength of light on the rate of photosynthesis. In a second investigation, the students illuminate trays of grass for one week using different coloured lights and then harvest the grass to find its fresh mass as a measure of how much the grass has grown. The stu-

dents then evaluate the results of both experiments in order to recommend which colour of light should be used in lighting rigs to most effectively support the growth and recovery of the grass on a football pitch.

3 | WHAT THE STUDENTS DO

3 | 1 Safety advice

The chemicals used in this investigation are low-hazard, but students need to be aware of the general risks in using electrical equipment (lamps, blender and electronic balance) and must wear safety goggles as part of good laboratory practice.

3 | 2 Preparations

A complete list of all necessary materials can be downloaded on the Science on Stage website.[3]

1. Sow ryegrass seeds in seven small trays (8 cm × 16 cm × 5 cm depth). Each tray must contain the same mass of potting compost and must be seeded evenly with the same mass of grass seed (sufficient to cover the surface of the compost). Place the seed trays on a sunny windowsill and grow for five weeks. Water regularly as required to keep the compost moist, using distilled water and adding the same volume of water to each tray. It is not possible to control environmental factors such as humidity and temperature, but as all of the trays are kept in the same location, each tray of grass is subject to the same environment fluctuations.
2. After five weeks, harvest the grass using scissors, leaving a sward height of 3 cm. Use the harvested grass for the "rate of photosynthesis" investigation (steps 3–12) and the seven trays of grass for the "rate of growth" investigation (*3.4*). Both investigations require seven bench lamps, each fitted with a RGB 3W B22 LED Global Bulb Light (these bulbs are available at low cost from common online stores). Each bulb is supplied with a remote control that can be used to set the colour to either red, orange, yellow, green, blue, violet or white **(FIG. 5)**. The same seven lamps and bulbs can be used for both investigations in order to cut costs.

3 | 3 Effect of the wavelength of light on the rate of photosynthesis

3. Add approximately 30 g of fresh grass leaves (harvested in step 2) to 250 cm^3 of cold sucrose/pH 7.5 buffer solution. This is prepared by dissolving 2.7 g hydrated disodium hydrogen phosphate, 1.0 g anhydrous potassium dihydrogen phosphate, 33 g sucrose and 0.25 g potassium chloride in 250 cm^3 distilled water.
4. Blend for 60 seconds to break open the cells and release the chloroplasts. Filter using a muslin cloth to remove cell debris. Store the filtrate on ice.
5. Dip one end of a capillary tube into the chloroplast extract so that the extract is drawn up into it. Remove the capillary tube and use a tissue to dry off the outside of the capillary tube. This tube is your colour reference tube (it is coloured green).
6. Use a Pasteur pipette to add 1.0 % DCPIP solution to the rest of the chloroplast extract, one drop at a time, shaking the bottle gently to mix it. The DCPIP solution is prepared by dissolving 0.1 g DCIP and 0.4 g potassium chloride in 100 cm^3 distilled water. It must be freshly prepared.
7. Add enough DCPIP until the extract changes colour permanently from green to blue-green, then wrap the whole bottle in aluminium foil as quickly as you can to keep the chloroplast + DCPIP extract in the dark.
8. Position a bench lamp with a violet bulb 8 cm above a white tile (do not switch it on yet). Place the coloured reference tube from step 6 on the tile. Now dip three capillary tubes into the chloroplast + DCPIP extract, dry them off as before and place them beneath the violet lamp next to the colour reference tube. Do this as quickly as possible. These are your experimental tubes **(FIG. 6)**.
9. Switch the lamp on and start the stopwatch.
10. Record the time required for the colour of each experimental tube to match the colour of the reference tube (t) in a suitable table (sample data is given in **FIG. 7**). Because the colour of the tube contents is very difficult to see under dif-

FIG. 5 The lamps were fitted with RGB 3W B22 LED Global Bulb Lights, which are supplied with a remote control to set the light colour to either red, orange, yellow, green, blue, violet or white.

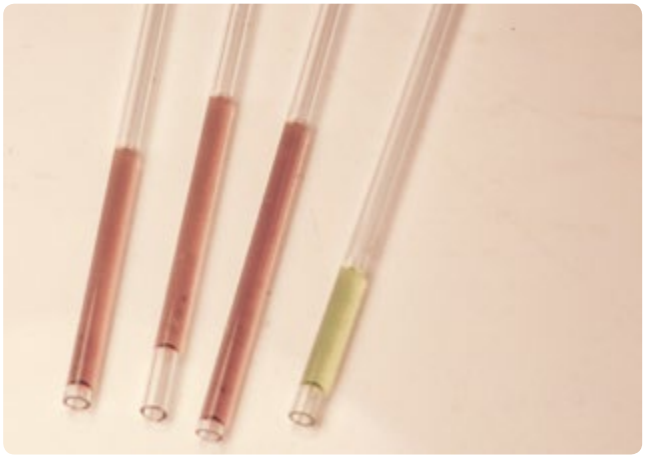


FIG. 6 Comparison of the colour of the experimental tubes (containing chloroplast extract + DCPIP) before illumination compared to a colour reference tube (containing chloroplast extract without DCPIP).

FIG. 7 Sample data on the effect of wavelength on rate of reduction of DCPIP (as a measure of the rate of photosynthesis)

Bulb colour	Wavelength of light [nm]	Time taken for experimental tube to match colour reference tube [s]				Mean rate of DCPIP reduction $= \frac{1,000}{t}$ $\left[\frac{1}{s}\right]$
		Tube 1	Tube 2	Tube 3	Mean	
Violet	420	660	660	640	653	1.53
Blue	450	520	520	520	520	1.92
Green	520	>900	>900	>900	>900	0.00
Yellow	570	680	740	760	727	1.38
Orange	620	520	520	560	533	1.88
Red	680	440	420	400	420	2.38
White	/	500	520	540	520	1.92

ferent coloured lights, the remote is used to switch the coloured bulb to "white" for one second every 20 seconds to check the colour matching.

11. Repeat steps 9 and 10 for the other five bulb colours and for a bulb emitting white light **(FIG. 8)**.
12. Calculate the mean reduction time and record the mean rate of colour change (1000/*t*). If there is no colour change after 15 minutes, record "no change" and record the rate of colour change as "0".

FIG. 8 The experimental and colour reference tubes were illuminated with different colours of light, recording the time for colour matching as an indication of the rate of decolourisation of DCPIP and thus the rate of photosynthesis.

3 | 4 Effect of the wavelength of light on growth rate

Place the seven trays from step 2 in a darkened room, illuminating each tray with a bench lamp fitted with a RGB 3W B22 LED Global Bulb Light. For each tray, use the remote control provided to set the colour to either red, orange, yellow, green, blue, violet or white. Leave the trays fully illuminated for six days and water periodically as required **(FIG. 9)**.

After six days, harvest the grass from each tray using scissors (cut the grass down to the base of the stem) and use an electronic balance to find the fresh mass of the grass harvested from each tray. Record the data in a suitable table (see sample data in **FIG. 10**).

FIG. 9 Trays of grass were illuminated using different coloured lights for six days before harvesting the grass to measure fresh mass as an indication of growth rate.

FIG. 10 Sample data of the effect of the wavelength of light on the fresh mass of grass harvested after six days of illumination (as a measure of the rate of grass growth)

Bulb colour	Wavelength of light [nm]	Fresh mass of grass harvested after 6 days illumination [g]
Violet	420	4.15
Blue	450	6.02
Green	520	3.66
Yellow	570	4.09
Orange	620	5.54
Red	680	6.23
White	/	5.43

4 | CONCLUSION

The students who participated in this project gained a better understanding of the light-dependent and light-independent reactions (Calvin cycle) of photosynthesis, in particular how the products of the light-dependent reactions are used in the Calvin cycle and how this affects plant growth rate. Students benefited from discussing the importance of controlling as many variables as possible during the germination and growth of the grass

seedlings (e.g. depth of compost, watering regimes, distance of coloured lamps from trays of grass) and also during the investigation of the rate of photosynthesis (e.g. distance of the coloured lamps from the chloroplast-containing extract). These discussions gave students a better understanding of the importance of valid experimental design in investigations.

After evaluating the results of both experiments, the students concluded that there was a correlation between the rate of photosynthesis and the rate of grass growth for different coloured lights and that the rate of photosynthesis and growth was highest in red light and lowest in green light. These results were as expected, given the absorption spectrum of chlorophyll **(FIG. 3)**.

The results for blue light were not as high as expected, and this generated an interesting discussion on why this might be. Students suggested that this could be related to the different proportions of chlorophyll a and chlorophyll b in the chloroplasts (since chlorophyll a absorbs less blue light than chlorophyll b). Even so, blue light has more energy than red light and therefore in theory should excite more electrons than red light, leading to a faster rate of photosynthesis and a faster rate of growth. Further research revealed a possible explanation: chloroplasts contain another group of photosynthetic pigments called carotenoids, which include orange pigments (carotenes) and yellow pigments (xanthophylls). These pigments show maximum absorption of blue light and, like chlorophyll b, they transfer the energy they absorb to chlorophyll a to bring about the excitation of electrons in the light-dependent reaction. However, the transfer of energy is inefficient. Although this dissipation of energy may seem wasteful, it may be necessary to protect the plant from the potentially damaging effects of the high energy of blue light.

In making their final recommendations, students suggested that lighting rigs could lead to more efficient grass growth and recovery if they used red light, but football grounds use high-pressure sodium (HPS) lights. The inventor of mobile lighting rigs (Kolbjørn Saether—personal communication) explained that his company had been involved in several research programmes together with the Norwegian Crop Research Institute to find out about the effect of artificial lighting on grass growth. They investigated several parameters, such as light intensity, light amount per day, temperature and nutrition. However, they did not investigate the effect of the wavelength of the light, and they are very interested in the outcomes of our investigation.

Personal experience

During chloroplast extraction, blending releases enzymes that damage the chloroplasts and slow down the rate of photosynthesis (the activity of these enzymes is reduced by using a cold extraction buffer and keeping the chloroplast extract on ice). During the investigation, students became aware of the fact that chloroplast extracts lose activity over time. To overcome this problem and to make valid comparisons, students set up the rate of photosynthesis experiments as quickly as possible, staggering the experiments and using different bulbs in the shortest time possible so that all of the extracts used were as fresh as possible.

It was impossible to compare the colour of the chloroplast extracts in the experimental tubes with the colour reference tube under different lighting regimes. This was one of the benefits of using bulbs that could be controlled using a remote to periodically switch the bulb to "white" to check for colour matching. Another benefit of these bulbs is that they did not heat up, as any increase in temperature would have affected both the growth rate of the grass and the rate of decolourisation of DCPIP. This also enabled students to leave the lamps on continuously and safely for six days.

The figures recorded in **FIG. 7** and **FIG. 10** for the wavelength of light of different colours must be considered approximate, since each colour is made up of a range of wavelengths in a continuous spectrum.

5 | COOPERATION OPTIONS

Students from different schools and colleges could compare their results for both investigations, their improvements of the experimental design, and their investigations of the effects of the wavelength of light on the rate of photosynthesis in other plant species.

REFERENCES

[1] https://commons.wikimedia.org/wiki/File:Linear_visible_spectrum.svg (08/03/2016)
[2] Chlorophyll_ab_spectra2.PNG: Aushulz derivative work: MOtty [CC BY-SA 3.0 (http://creativecommons.org/licenses/by-sa/3.0) or GFDL (http://www.gnu.org/copyleft/fdl.html)], via Wikimedia Commons (08/03/2016)
[3] www.science-on-stage.de/iStage3_materials

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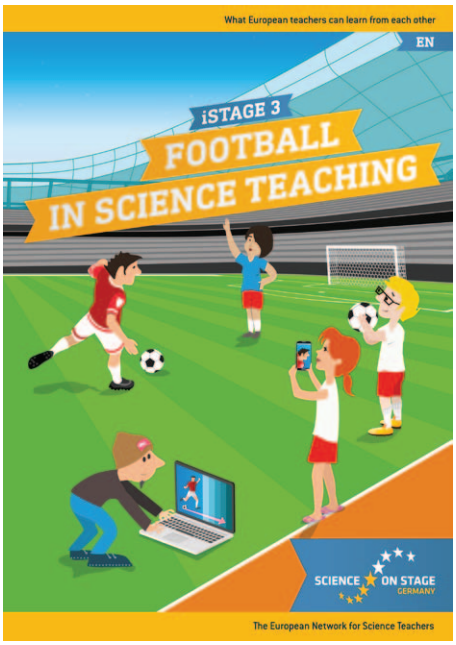

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