

Light-induced, dark-reversible colour shifts in petals of *Phlox*

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Flowers of some *Phlox* (*Phlox x paniculata* L.) varieties undergo daily colour shifts, being blue in the early morning, turning red during the day, and returning to blue in the evening. The colour shift, which occurs only in the upper (adaxial) petal surfaces, is due to the daily changes in ambient light. In the laboratory, colour shifts could be induced by 2.5 h of ultraviolet, visible or far-red light and recorded by reflectance spectrophotometry.

There are indications that irradiations with different kinds of light cause qualitatively different colour shifts, and that thus more than one photoreceptor pigment and more than one primary light reaction may be involved. The presence of phytochrome was demonstrated in petals of white *Phlox* flowers by in vivo transmission spectrophotometry. It is therefore possible that colour shifts in coloured *Phlox* flowers are mediated by phytochrome. Possibly the movement of ions (e.g. hydrogen ions) into or out of the vacuole (where the visible pigments are located) is affected by light absorption in a pigment in the tonoplast.

Additional key words – Anthocyanin, far-red, *Phlox x paniculata*, phytochrome, ultraviolet radiation.

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Introduction

It was noticed that flowers of a variety of *Phlox* growing in a garden changed colour during the day. When an inflorescence was covered with aluminium foil, it was more blue than its neighbours when the foil was removed at noon, and it was concluded that the colour shifts were, at least in part, due to the diurnal variations in ambient light. We decided to investigate this phenomenon more closely, as it might be of general significance.

Although phytochrome regulation of anthocyanin synthesis is well established (review by Mancinelli 1983), and naturally occurring, irreversible changes in petal colours have been described (Blank 1958, Ram and Mathur 1984, Paige and Whitham 1985), reversible diurnal changes, to our knowledge, have hitherto not been reported. Steiner (1972) described a wavelength dependence of the relative amounts of anthocyanin in

isolated petals, suggesting differential light effects on synthesis and breakdown of various anthocyanidins, but this, too, seems unrelated to our findings, so a closer investigation seemed motivated.

Abbreviation – UV-A, ultraviolet radiation in the region 320-400 nm.

Materials and methods

Inflorescences of a violet variety of *Phlox x paniculata* growing outdoors in Lund, Sweden were picked during late August and early September 1984 and dark-adapted for at least 1 h. Flowers were then removed. One petal from each flower was removed and its reflectance spectrum recorded as described below. The rest of the flower was placed with the adaxial surface down in a plastic Petri dish and covered with the lid,

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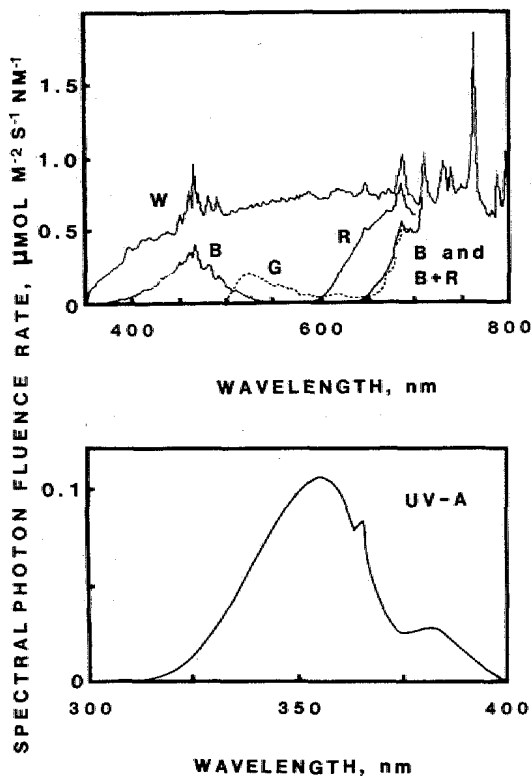


Fig. 1. Spectra of the lights used. Upper panel, xenon lamp without (W) or with various Plexiglas filters: blue (B), green (G), red (R) and a combination of blue and red (B + R, only far-red light transmitted). Lower panel, "black light" (UV-A).

which was lined with moistened filter paper to prevent water loss. The Petri dish was irradiated from below, so that the adaxial petal surfaces were exposed to the light. Irradiation was carried out in an apparatus with a xenon lamp as described previously (Björn et al. 1963) at a temperature of ca 26°C. Either white light (filtered only through glass and the plastic Petri dish) was used, or 3 mm Plexiglas filters (Röhm & Hass) no. 627 (blue), 705 (green) or 501 (red) were placed under the Petri dishes. Petals were removed at intervals, their reflectance measured, and comparisons made with measurements taken on petals from the same flower before irradiation. The spectral composition of the lights is shown in Fig. 1.

Irradiation was also made with UV-A (320–400 nm), and in this case radiation came from above from 4 Philips 20 W/08 "black light" fluorescent tubes. The Petri dishes were turned upside down, so that the radiation impinged upon the adaxial petal surfaces.

Radiation was measured with an Optronics Model 742 spectroradiometer interfaced with a Hewlett-Packard HP-85 microcomputer.

Measurements of colour changes were first attempted

using ordinary transmission spectrophotometry with an Aminco DW-2a spectrophotometer. However, since only the cells in the adaxial epidermis changed colour, this proved unsuitable, and instead we improvised a method for reflection spectrophotometry. One "arm" of a 1 mm thick Y-shaped fiberoptic light conductor (Optrode 3070, Oriel Scientific Corp., 250 Long Beach Blvd., Stratford, CT, USA) was fitted to the entrance slit of the Optronics spectroradiometer and the other "arm" to a small lamp compartment containing a 7 V, 0.4 A incandescent lamp fed from a transformer. The "base" of the Y was fitted with a light-tight rubber cup which was pressed against the petal to be measured in such a way that the end of the light conductor was about 3 mm from the petal. Light from the lamp was conducted to the petal, and part of the reflected light conducted to the spectroradiometer. We scanned the 570–650 nm region at 5 nm intervals. Using the HP-85 computer, data were stored on tape until processed. Measurements from 5 or 10 flowers were averaged, and the "absorbance change", $\log [(reflectance \text{ before irradiation}) / (reflectance \text{ after irradiation})]$ plotted as a function of wavelength. It should be noted that values above the zero line in the diagrams (Fig. 2) mean a decrease of reflectance. Measurements were concluded within a few minutes after the termination of the treatments stated.

It was not feasible to measure phytochrome in the coloured variety of *Phlox*, and phytochrome was therefore assayed in petals of a white variety. Measurements were done with the dual wavelength method and an Aminco DW-2a spectrophotometer as described by Björn and Björn (1976). The sample (a standard 10 × 10 mm cuvette packed with petals) was irradiated alternately with 660 and 730 nm actinic light (20 nm half bandwidth, almost saturating) at right angles to the measuring beams (10 nm bandwidth) and the reversible change in absorbance at various wavelengths relative to 800 nm recorded after each irradiation.

Results and discussion

When violet *Phlox* flowers were exposed to acetic acid fumes they turned bright red. When exposed to ammonia gas they turned first blue, then yellow. With extracts buffered to various pH values, the main colour change from red to blue took place between pH 6.5 and 7.0. Irradiations with white light as well as different coloured (blue, green, red) light all resulted in reflectance changes in the upper petal surfaces (Fig. 2). With white light an appreciable reflectance change took place already in 1 h (not shown). Since the plastic colour filters used all transmitted far-red light, we tested also a combination of blue plus red plexiglas, transmitting only above 650 nm. Also such irradiation resulted in a reflectance change of the upper petal surface (Fig. 2). UV-A and far-red radiation decreased the short wavelength reflectance (increased the absorbance) without changing the red reflectance, while white light first in-

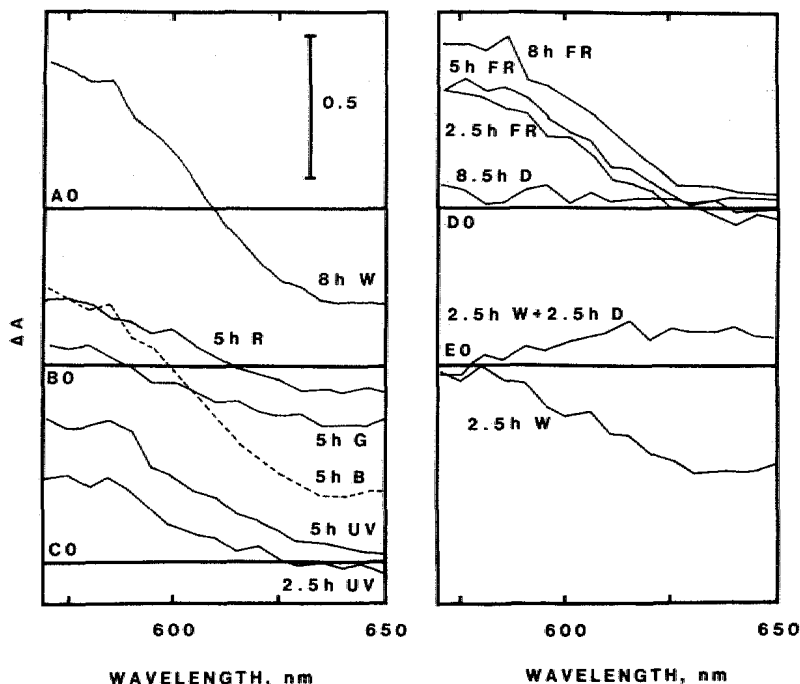


Fig. 2. Reflectance changes in coloured *Phlox* petals. The horizontal lines AO, BO etc. are the base lines (no reflectance change) for the different samples. Along the vertical axis is plotted $\Delta A = \log [(light\ reflected\ from\ petal\ before\ irradiation)/(light\ reflected\ from\ the\ same\ petal\ after\ irradiation)]$. Thus a trace above the base line means decreased reflectance. The light treatments were: With base line AO – 8 h white (W). With base line BO – 5 h red (R), 5 h green (G), 5 h blue (B). With base line CO – 2.5 h UV-A, 5 h UV-A. With base line DO – 2.5 h far-red (FR), 5 h far-red (FR), 8 h far-red (FR), 8.5 h dark (D). With base line EO – 2.5 h white (W), 2.5 h white followed by 2.5 h dark. Each curve represents an average from at least five experiments with different flowers. Vertical bar shows scale for all traces except for those belonging to base line CO which are 1.2 times expanded.

creased red reflectance and, with prolonged treatment, decreased reflectance at shorter wavelengths. The effect of coloured light was similar to that of white. The light-induced change was reversed during subsequent darkness.

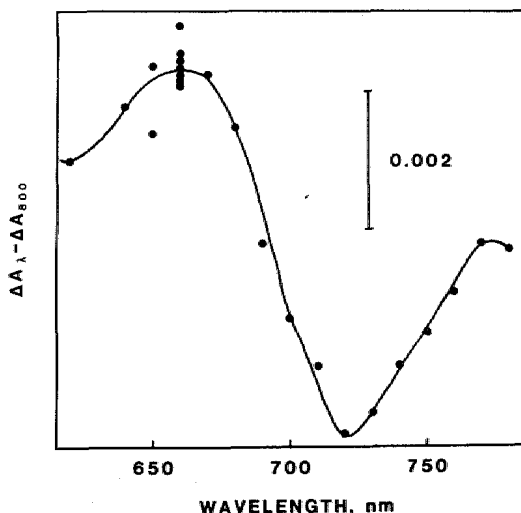


Fig. 3. Absorption difference spectrum (730 nm irradiated minus 660 nm irradiated) for white *Phlox* petals packed in a cuvette.

Petals have no detectable chlorophyll. Even if direct light effects on flavonoids have been reported (Miquel 1961, Sperling et al. 1966, Timberlake and Bridle 1965, see below), it is possible that the colour changes are mediated by one or more pigments regulating ion transport into or out of the vacuoles.

The observation of reversible absorbance changes in white *Phlox* petals (Fig. 3) brought about by red and far-red light makes probable the presence of phytochrome also in coloured petals, but we do not know if the phytochrome, or some of it, is associated with the tonoplast.

The pigment of *Phlox x paniculata* flowers is probably one or several anthocyanins present in the vacuoles. In *Phlox drummondii* flowers Harborne and Smith (1978) have identified delphinidin 3,5-diglucoside, cyanidin 3,5-diglucoside, and pelargonidin 3,5-diglucoside. No colour differences could be seen between extracts of irradiated and darkened flowers. There was no indication of a photochemical change in the pigment. Sperling et al. (1966) have observed light-dependent reversible absorbance changes in solutions of different flavylum compounds structurally related to pelargonidin, but the authors point out that this phenomenon was not seen with pelargonidin. Light absorption by anthocyanins is known to be affected not only by pH, but also by various metal ions (Harborne 1967, Metzner 1973, Luckner 1984). The reflectance increase in the long wavelength region (the decrease of reflectance in the short wave-

length region, respectively) may be caused by acidification of the cell sap, or by movement of metal ions. Changes in membrane permeability as a result of phytochrome phototransformation have been observed by several authors (cf. Kendrick 1983). Electrical potential measurements (Jaffe 1968, 1970) indicate that red light stimulates a H^+ efflux through the plasmalemma.

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