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Correlation of pH and Light Intensity on Flower Color in Potted *Eustoma grandiflorum* Grise.

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Abstract. The environment can affect the intensity of flower color in *Eustoma grandiflorum*. Low light and alkaline pH within the growing cell can lead to reduced color intensity. Two independent causes are responsible for the decrease in the intensity of flower color. 1) Older flowers were more alkaline than freshly opened flowers. A 7% **increase in pH was related with a 10% reduction in color intensity. 2) Flowers that open under low light were paler than those opening under high light intensity.** A 25% **decrease in light intensity was related to a 30% reduction in the concentration of anthocyanin and a 40% reduction in color intensity.**

Eustoma grandiflorum is a seed-propagated, cut-flower crop that has been extensively hybridized by the Japanese (Halevy and Kofranek, 1984; Roh and Lawson, 1984). Several dwarf pot-plant cultivars recently released include 'Blue Lisa' (Pan American Seed, West Chicago, Ill.), 'Little Belle Blue' (U.S. Dept. of Agr., Agr. Res. Serv., Beltsville, Md.), and 'Mermaid Blue' (Sakata Seed, Japan).

Preliminary postharvest observations by commercial growers showed that under low light conditions in the home, cut flowers often faded from dark purple (RHS color chart 78A) to light purple (RHS color chart 78D). This study was initiated to assess if the flowers of potted plants also fade and to determine environmental and biochemical causes for the fading.

In flowers, there is a pigment complex composed of several molecules of anthocyanin as well as numerous molecules of flavonoid copigments. It has been suggested (Goto et al., 1985) that this anthocyanin/copigment complex contains three subcomplexes ionically linked by a metal ion. Each subcomplex is composed of two copigment molecules and two anthocyanin molecules stacked on top of each other such that the electrons from each ring structure can interact. The electronic interaction of the rings produces the color we see. The pigments themselves express very little color under physiological conditions (Asen et al., 1972). The pH can affect the color of the anthocyanin/copigment complex by changing the electron interaction of the rings (Goto et al., 1985). As the pH increases, the color becomes bluer (Stewart et al., 1975).

In the present study, we examined the effects of pH, light, and the ratio of anthocyanin to copigment on *Eustoma* flower color.

Seedlings of the dwarf *E. grandiflorum* cultivars Blue Lisa, Little Belle Blue, and Mermaid Blue were grown using standard greenhouse practices established for *Eustoma* (Roh and Lawson, 1984). Five plants of each cultivar flowered in a greenhouse under natural spring photoperiod with a minimum of 18C and maximum of 25C at a maximum of 1050 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of sunlight. The flowering plants were then transferred to a laboratory at 23C where the remaining buds opened under 260 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of cool-white fluorescent light.

Flower petals were removed from either fresh flowers 1 day postanthesis or old flowers 6 days postanthesis. They were placed intact on microscope slides, covered with water, and coverslips placed over them.

The absorption of 7850-μm^2 sections of these petals was made using a Zeiss Photometer Microscope 03 equipped with a XBO 450W xenon arc lamp (Zeiss, New York). This light source has an emission spectrum similar to daylight. Absorption measurements were not made at a specific wavelength. Pale flowers had the same color or λ max as normal, darkly colored flowers. The microspectrophotomer is extremely sensitive. Measurements can be affected by fluctuations in electric current, humidity, temperature, etc. To reduce the effect of this sensitivity on absorption measurements, 50 sections from the middle of a single petal were measured. These measurements were combined for five flowers taken from the same plant and then averaged; thus, each reported mean was the result of 250 absorption readings for a plant. Five plants were used for each treatment.

In vitro model pigment systems were constructed as previously reported (Asen et al., 1986). Stock solutions of the anthocyanin or flavonol copigment containing twice the required normality were prepared in citrate-phosphate buffer at pH 2.40 and 0.28 N NaOH, respectively. An equal volume of anthocyanin was then mixed with an equal volume of copigment. The pH of the mixture was varied by changing the pH of either the anthocyanin or copigment stock solution. Individual flavonoids (anthocyanins and copigments) of 'Little Belle Blue' were detected by high-performance liquid chromatography (Asen et al., 1986). Anthocyanins were separated on a C_{18} column using a 30-min elution program of 0% of 10% acetonitrile in 1.5% phosphoric acid and 15% acetic acid, followed by a 10-min linear increase to 20% and held at 20% for 10 min. Flow rate was 1 $ml \cdot min^{-1}$ and the elutant was monitored at 540 mm. Flavonols were separated on a C_{18} column using a 9-min elution program of 8% acetonitrile in 3% triethylamine at pH 3.0, followed by an 8% to 12% linear increase over 0.1 min and a 12% to 30% linear increase over 50 min. The flow rate was 3.5 $rnl \cdot min^{-1}$ and the elutant was monitored at 340 nm.

There was no difference in the intensity of color of fresh flowers 1 day postanthesis between 'Blue Lisa' and 'Mermaid Blue' grown under high (1050 $\mu mol \cdot m^{-2} \cdot s^{-1}$) or low light (260 $\mu mol \cdot m^{-2} \cdot s^{-1}$) (Table 1). The color of fresh 'Little Belle Blue' flowers was less intense than that of the other two cultivars when grown under high light and was the same intensity when grown under low light.

Under high light intensity, the flower color of every cultivar became less intense with time. After 6 days postanthesis, flowers of 'Little Belle Blue' had lost 10% of their initial absorption, while the flowers of 'Blue Lisa' lost 35% and those of 'Mermaid Blue' 40% (Table 1).

Fresh flowers of 'Little Belle Blue' that opened under low light had 40% less absorption than flowers that opened under high light; the flowers of 'Blue Lisa' and 'Mermaid Blue' that opened under low light had 63% less absorption than those flowers that opened under high light (Table 1). The pale

Table 1. Relative absorption of fresh and old flower petals from *Eustoma* cultivars that opened in high or low light (1050 and 260 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Fresh flowers were measured at 1 day postanthesis, old flowers at 6 days postanthesis.

	Relative absorption[z]		
	High light		Low light
Cultivar	Fresh	Old	Fresh
Blue Lisa	14.2 ± 2.6	9.3 ± 2.7	5.2 ± 1.0
Little Belle Blue	9.9 ± 1.3	8.9 ± 1.6	5.9 ± 1.5
Mermaid Blue	13.8 ± 3.5	8.4 ± 0.9	5.0 ± 0.3

[z]Values in relative absorption units are reported as the mean ± $t_{95\%}$ × SD of five plants per treatment (n = 5).

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Table 2. Relative concentration of the major flavonoids from *Eustoma* 'Little Belle Blue' found in pale flowers formed under low light (260 μmol·m^{-2}·s^{-1}) or dark flowers formed under high light (1050 μmol·m^{-2}·s^{-1}).[z]

Intensity		Flavonols[y]			Anthocyanin[y]		
Light	Color	Km	acKm	Total	De-1	De-2	Total
Low	Pale	27.5 ± 1.4	59.2 ± 1.2	125 ± 50	5.8 ± 0.9	88.1 ± 0.9	33 ± 18
High	Dark	22.7 ± 1.5	48.9 ± 1.1	125 ± 40	8.8 ± 0.9	78.5 ± 1.6	112 ± 61

[z]Values in relative percent are reported as the mean ± $t_{95\%}$ × SD. For example, in the flowers formed under low light, Km represents 27.5% of the total flavonols present. Total concentrations are reported as micrograms per milligrams of dry weight. Each value is the result of averaging data obtained from five plants.
[y]Km = kaempferol-3 rhamnosylgalactoside-7-rhamnoside, acKm = kaempferol-3-p-coumaroylrhamnosylgalactoside-7-rhamnoside, De-1 = delphinidin-3p-coumaroylgalactoside-5-glucoside, De-2 = delphinidin-3-p-coumaroylrhamnosylgalactoside-5-glucoside.

Table 3. The absorption at 575 nm of a solution of delphinidin-3,5-diglucose (De) and kaempferol-3-rhamnosylgalactoside-7-rhamnoside (Km).

Variable	Absorption
De:Km[z]	
10:1	0.952
1:1	0.723
0.5:1	0.478
0.1:1	0.079
pH[y]	
5.0	0.781
5.5	0.702
6.0	0.629

[y]1 × 10^{-3} M Km and 1 × 10^{-3} M De.
[z]pH = 5.6, Km = 1 × 10^{-3} M.

flowers had flavonoid (anthocyanin and copigment) profiles similar to those of dark flowers that opened under high light (Table 2). The total amount of flavonols present was identical in the pale and dark flowers; however, the pale flowers contained 70% less total anthocyanin (Table 2). The ratio of anthocyanin to copigment decreased 25% in those flowers opening in low light. This was related to a 40% reduction in absorption. In the in vitro model system, a 20% reduction in the anthocyanin : copigment ratio resulted in a 34% decrease in absorption (Table 3).

The reduction in the concentration of anthocyanin could occur either through decreased biosynthesis or increased degradation. Several anthocyanase enzymes have been reported (Sakamura and Obata, 1961). It is unlikely that the paler color of the newly opened flowers was due to increased degradation of anthocyanin, since most instances of degradation are the result of advanced senescence and are not influenced by light intensity (Sakamura and Obata, 1961).

Newly opened flowers of 'Little Belle Blue' had a vacuolar pH of 5.1, while the pH of 6-day-old flowers was 5.5. This 7% increase in pH was related to 10% less absorption. Light intensity had no affect on this change in pH. Several investigators have previously reported the effects of pH on the intensity of flower color. In 'Black Prince' fuchsia *(Fuchsia speciosa* Hort.) freshly opened calyxes had a pH of 3.6 and an absorption of 2.8, while old flowers had a pH of 4.2 and an absorption of 1.7 (Stewart et al., 1975). A 14% increase in pH was related to 39% less absorption. In 'Better Times' rose (Rosa complex hybrid) fresh flowers had a pH of 3.9 and an absorption of 0.80, while old flowers had a pH of 4.5 and an absorption of 0.37 (Asen et al., 1971). A 13% increase in pH was related to 54% less absorption.

As the pH increases, the color of the flowers becomes more blue (Stewart et al., 1975). In both rose and fuchsia, the older flowers, besides being lighter in color, were also bluer. In *Eustoma* we did not detect a change in color as the flowers aged and became more alkaline. There may not have been a change in color because freshly opened *Eustoma* flowers are already quite blue and only slightly acidic. The largest changes in color occur with red flowers at the more acidic pHs (Stewart et al., 1975).

In vitro model systems have been used to test pH effects on the absorption of anthocyanin/copigment complexes. A 1:1 molar ratio of a solution of 5 × 10^{-3} M cyanidin-3,5-diglucoside and quercetin had 19% less absorption when the pH was raised 18% from 4.2 to 5.1 (Asen et al., 1972). In this study, the absorption of a 1:1 molar ratio solution of 1 × 10^{-3} M delphinidin-3,5-diglucoside (De) and kaempferol-3-rhamnosyl-galactoside-7-rhamnoside (Km) closely resembled the absorption for the intact *Eustoma* flower. In the flower, a pH increase from 5.1 to 5.5 was related to 10% less absorption, while the same increase in pH of the in vitro solution also resulted in 10% less absorption (Table 3).

There appear to be two reasons for the reduced intensity of flower color. The first is related to changes in the pH and results in a reduction in color intensity as open flowers age. The second reason is related to changes in light intensity and results in flowers that open under low light intensity being less intensely colored than flowers opening under high light intensity.

Through selection and breeding it could be possible to genetically improve the characteristic reduction in the intensity of flower color as *Eustoma* flowers age. The flowers of many older cultivars and species change in pH as they age while the modem cultivars do not. In the older 'Crimson Carefree' geranium (Pelargonium complex hybrid), the pH increased from 2.9 to 5.4 as the flower aged (Stewart et al., 1975), while in the new 'Crimson Fire', the pH did not change as the flower aged (Asen and Griesbach, 1983).

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