Leaf Senescence of Postproduction Poinsettias in Low-light Stress

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Abstract. Photosynthetic light harvesting was investigated under low-light stress conditions relevant to the problem of interior longevity of potted ornamental plants. Comparisons of leaf pigment levels and chlorophyll fluorescence excitation spectra were made for ‘Guthrie V-10 Amy’ poinsettia (Euphorbia pulcherrima Willd.), which has poor interior longevity, and ‘Eckespoint Lilo’ poinsettia, which has superior interior longevity. The results show that ‘Eckespoint Lilo’ had higher total chlorophyll content per leaf area and lower chlorophyll a : chlorophyll b ratio than ‘Guthrie V-10 Amy’. In low-light stress, ‘Eckespoint Lilo’ retained its chlorophyll or even accumulated higher levels than in high light, while ‘Guthrie V-10 Amy’ did not exhibit higher chlorophyll retention in low light. Both cultivars acclimated to low-light stress by decreasing the chlorophyll a : chlorophyll b ratio, and this acclimatization was evident sooner in younger, outer-canopy leaves above the pinch than in older leaves below the pinch. Both cultivars also increased the chlorophyll : carotenoid ratio in low light. These changes in pigment composition, which were essentially structural changes, were reflected in functional changes in light harvesting, as assessed by measurements of chlorophyll fluorescence excitation spectra.

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Interior longevity of potted ornamental plants is a point of practical concern for consumers and thus a point of economic concern for producers. Many environmental variables differ between a greenhouse, the typical production site, and the interiors of homes or offices, the typical consumption sites. Since maximum light levels in many homes and offices are no more than 5% of those in greenhouses, low-light stress is likely among the most important environmental factors influencing interior longevity. In the present project, the poinsettia was used as an economically important model system to investigate low-light stress effects on photosynthetic pigments and light harvesting.

Previous investigators have shown that low-light stress has deleterious effects on the postproduction performance of poinsettias. Most of these previous studies (Miller and Heins, 1986a, 1986b; Nell and Barrett, 1990; Scott et al., 1984a, 1984b; Shanks et al., 1970) focused on leaf and/or cyathia abscission and did not examine photosynthetic pigments or involved only visual estimates of loss of green from the leaves. In one of these studies (Nell and Barrett, 1990), four poinsettia cultivars were compared for leaf abscission under typical indoor conditions. ‘Eckespoint Lilo’ exhibited the lowest leaf drop of the four cultivars tested. In another experiment, leaf abscission in ‘Dark Red Annette Hegg’ and ‘Guthrie V-14 Glory’ increased as the interior light level decreased or as the interior temperature increased. No data regarding leaf photosynthetic pigments were reported.

Investigators in two studies (Nell and Barrett, 1986; Nell et al., 1990) recognized the importance of photosynthesis in the postharvest period and addressed this aspect of physiology by measuring the light compensation points and dark respiration rates in cultivars that differ in interior longevity. These two physiologi-
Materials and Methods

Plant material and growth conditions. Rooted cuttings of poinsettia (Euphorbia pulcherrima Willd.) cultivars Gutierrez V-10 Amy (‘Amy’) and Eckespoint Lilo (‘Lilo’) were supplied by the Paul Ecke Poinsettia Ranch (Encinitas, Calif.). ‘Amy’ is a poor interior performer, and ‘Lilo’ is a good interior performer (David Hartley, personal communication; Nell et al., 1990; Nell and Barrett, 1986, 1990). One cutting was transplanted per 15 cm in diameter plastic pot containing Sunshine mix #1 medium (Western Peat Moss Ltd., Vancouver, B.C.). A few days after transplanting, each pot was fertilized with 9 g Osmocote 12N–5.2P–12.5K (Sierra Chemical Company, Milpitas, Calif.). The potted plants were placed on a greenhouse bench under natural lighting that was typically 950 to 1300 µmol·m⁻²·s⁻¹ at canopy level at midday. For the first 4 weeks of establishment, however, an overhead shade cloth was used to attenuate the natural lighting by 66%. An automatic timer controlled irrigation with water through tubes that were positioned on the surface of the potting medium. Greenhouse thermostats were set for 20°C nights and 27°C days. Measured temperatures were 18 to 22°C nights and typically 23 to 29°C days, although midday temperatures in summers sometimes reached 35°C. In experiments run from October through March, supplemental light (100-W incandescent lamps located 1 m above canopy level at 1-m intervals along the length of the bench) was provided throughout nights to prevent premature flower initiation in young plants.

Apex pruning and leaf tagging below the pinch. At 4 weeks after transplanting, the shade cloth was removed and the apex was pruned to leave eight fully expanded leaves below the pinch. On 55 plants of each cultivar in each experiment, a marker tag was attached to the petiole of the second leaf below the pinch, and these tagged leaves were later harvested for leaf pigment and chlorophyll fluorescence analyses.

Controlling photoperiod for flower initiation. Beginning at 1 week after plants were pinched and continuing for 7 weeks, the photoperiod was controlled to provide 14-h dark periods beginning daily at 1600 hr. This treatment to induce flowering and bract coloring was accomplished with an automated system that drew an opaque curtain over the greenhouse bench. An electric fan positioned under the bench and ducted through the curtain provided air exchange when the curtain was drawn over the bench.

Leaf tagging above the pinch and low-light treatment. After 7 weeks of photoperiod control and then 2 weeks of natural photoperiod, the plants were in full flower. Each plant then received a second marker tag which was placed on the petiole of a leaf above the pinch. A green leaf was selected on a flowering branch in the outer canopy, two leaves below the lowest red color. Five plants of each cultivar were set aside for the harvest representing time 0 of the shade treatment. Twenty-five plants of each cultivar were numbered and left on the open bench as high-light controls, and 25 plants were numbered and placed on the adjacent, low-light bench. This procedure provided five pre-identified plants of each cultivar in each treatment for the subsequent harvests at 7, 14, 21, 28, and 35 days.

Shade boxes provided the low-light environment on the adjacent greenhouse bench. Each shade box was 234 × 142 × 71 cm (l × w × h), and two boxes covered an entire bench. Two layers of fabric, with a separating gap of 5 cm, formed the ceiling and walls of each box. The fabric from Phifer Western (City of Industry, Calif.) was a 34 × 11 weave of almond-colored, vinyl-coated polyester threads. Light intensities inside the shade boxes were approximately 10 µmol·m⁻²·s⁻¹ at midday. The spectral transmission of the boxes was measured with a portable spectroradiometer (model LI-1800; LI-COR, Lincoln, Neb.). The shade boxes were especially effective in attenuating the shorter wavelengths (Fig. 1), thus simulating indoor incandescent lighting better than fluorescent lighting. Two 7 m³·min⁻¹ fans, located in diagonally opposite corners, blew air through attenuating diffusers into each box to exchange the air approximately two times per minute. This continuous air exchange insured that temperature, humidity, and gas concentrations in the box were similar to those experienced by the high-light control plants on the adjacent open bench.

Recording leaf abscission. At the beginning of the shade treatment (day 0) and on the five subsequent harvest dates, all of the main stem leaves remaining below the pinch on the five plants to be harvested that day were counted, and the percent abscission was determined.

Harvesting leaf disks. On day 0 of the shade treatment, disks 12 mm in diameter were punched midway between the center vein and the margin of the leaves on five plants of each cultivar. Two sets of disks per cultivar were harvested from the tagged leaves below the pinch, and two more sets from the tagged leaves above the pinch. Five disks per sample were combined, weighed, chopped into 2-mm² pieces, and frozen in test tubes on dry ice at the greenhouse bench. Similar dual harvests were made on each of the subsequent dates.

Extracting and quantitating pigments directly from leaf tissue. Chopped leaf samples were held on dry ice in the dark for no more than 24 h before leaf pigments were extracted and quantitated. One of the dual sets of five chopped leaf disks was ground in 5 ml acetone for 1 min with a Biohomogenizer (Biospec Products, Bartlesville, Okla.). Three ml acetone, used to rinse the homogenizer probe, and 2 ml water were pooled with the ground leaf tissue to total 10 ml. The extract was centrifuged at 13,000g for 10 min, and the supernatant was filtered through a polytetrafluoroethylene membrane having 0.2-µm pores (Acrodisc 13CR PTFE from Gelman Sciences, Ann Arbor, Mich.). Absorbances of the filtrate were measured at 460, 645, and 663 nm against a blank of 80% (by volume) acetone in water. Concentrations of chlorophyll a, chlorophyll b, and total carotenoids were calculated from the

Fig. 1. Transmission spectrum of shade box used to expose poinsettias to low-light stress. The average of 30 scans of light intensity at 10 locations inside the box was divided by the average of 30 scans of light intensity at 10 locations just outside the box.
absorbances (Embry and Nothnagel, 1988).

Preparin g a thylakoid membrane fraction. The second set of five chopped leaf disks was processed to obtain a thylakoid membrane fraction. The method of Percival and Baker (1985), used by us to prepare millet thylakoids (Embry and Nothnagel, 1988), was slightly modified to accommodate the large amount of milky latex present in aqueous homogenates of poinsettia leaf tissue. The filtrate obtained by passing the homogenate through a nylon net (Embry and Nothnagel, 1988) was centrifuged at 13,000×g for 90 sec, instead of the 3000×g for 90 sec used for millet. The pellet thus obtained from five chopped leaf disks was resuspended in 0.5 ml resuspension buffer consisting of 100 mM sorbitol, 5 mM NaCl, 5 mM MgCl2, and 10 mM N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid at pH 7.0. This thylakoid suspension was supplemented with glycerol to 15% (by volume) and then stored frozen in the dark at liquid nitrogen temperature until analyzed.

Analyzing pigment ratios and chlorophyll fluorescence in thylakoids. While chlorophyll fluorescence can be measured directly from intact leaf tissue, the chlorophyll concentration in leaves is so high that much of the emitted fluorescence is reabsorbed by other chlorophyll molecules before it can escape the leaf. Because this reabsorption is wavelength dependent, chlorophyll fluorescence spectra obtained from intact leaves are strongly distorted (Percival and Baker, 1985). This artifact was avoided in the present study by using thylakoid membrane suspensions at concentrations sufficiently dilute that reabsorption was negligible.

Thylakoid membrane preparations in vials were removed from the liquid nitrogen freezer and quickly thawed by immersion in warm water. The suspension was removed from the vial, diluted by adding at least nine volumes of resuspension buffer, centrifuged at 13,000×g for 90 sec. and resuspended in 0.5 ml resuspension buffer. Pigment concentrations in this thylakoid suspension were determined by drawing a 25-µl aliquot, extracting it with 80% (by volume) acetone, passing the extract through a filter (Acrodisc 13CR PTFE, German Sciences, Ann Arbor, Mich.), and measuring absorbances as described above for pigments extracted directly from leaf tissue. A further aliquot from the thylakoid suspension was mixed with appropriate amounts of resuspension buffer and 3-(3,4-dichlorophenyl)-1,1-dimethyleurea (10 mM stock in ethanol) to obtain a suspension with total chlorophyll at 2 µg·ml⁻¹ and 3-(3,4-dichlorophenyl)-1,1-dimethyleurea at 20 µM.

Fluorescence excitation spectra were measured at 25°C with a spectrofluorometer (model 112A Fluorolog 2; Spex Industries, Edison, N.J.). Slit widths of 2.50 mm were used and resulted in 9.2 nm bandpass resolution in the single excitation spectrometer and 4.5 nm bandpass resolution in the double emission spectrometer. Excitation spectra were corrected for variation in excitation energy and differences in optical paths by use of 1,1’,3,3’,3’,3’-hexamethyldi-indocarbocyanine perchlorate as a quantum counter dye (Nothnagel, 1987). A colored glass filter (model KG-3; Fisher-Schuman Corp., New Rochelle, N.Y.) was used in the excitation path to attenuate stray light from the very strong near-infrared emission lines of the Xe (model XBO 150 W/1; Osram Corp., Montgomery, N.Y.) arc lamp (Nothnagel, 1987). Stray and scattered light were further reduced by a 700-nm short-wave pass interference filter (model 700FL07-50; Andover Corp., Salem, N.H.) that was used in the excitation path, and a 730-nm long-wave pass colored glass filter (model RG-9; Schott Optical Glass, Duryea, Pa.) that was used in the emission path.

The thylakoid suspensions were held in 1×1×4.5-cm polystyrene cuvettes during the fluorescence measurements and were magnetically stirred to prevent settling. When the 2.50-mm excitation slits were used, the excitation spectrometer illuminated a 3.3×8.0-mm area on the front face of the cuvette. As the excitation spectrometer was scanned from 400 to 700 nm, the incident light intensity in this illuminated area varied between 60 and 250 µmol·m⁻²·s⁻¹, with the integrated average being 160 µmol·m⁻²·s⁻¹. Front-face collection of emitted light, i.e., collection at an angle of 22.5° relative to the incident beam, was used to further minimize reabsorption artifacts. Chlorophyll fluorescence emission was measured at 734 nm in the band arising from vibrational sublevels in the main chlorophyll transition (Goedheer, 1972).

The thylakoid suspensions, which had been held in the dark, were pre-illuminated in the spectrofluorometer for 40 sec before initiating the excitation scan. Control experiments involving fluorescence measurements at fixed excitation and emission wavelengths showed that specimens containing 3-(3,4-dichlorophenyl)-1,1-dimethyleurea and preilluminated as described exhibited no time-dependent fluorescence induction kinetics over a time period equal to that required to complete an excitation scan.

Before summing for average, excitation spectra were normalized to unit area intensity integrated between 400 and 700 nm. Differences between cultivars or between plants at different times and conditions were highlighted by expressing the excitation spectra as difference spectra. For these calculations, the excitation spectrum for thylakoids from leaves below the pinch of ‘Lilo’ at day 0 was scaled to relative intensity of 100 at the long wavelength peak that occurred in the 660 to 680 nm range. The area intensity of this spectrum integrated between 660 and 680 nm was then used as the standard. Before subtracting to obtain difference spectra, all other spectra were scaled so their area intensities integrated between 660 and 680 nm matched that of the standard.

Combining results from separate experiments. Data reported for leaves below the pinch were the combined results from three experiments conducted in separate years (cutting transplant dates 15 Nov. 1989, 17 July 1990, and 29 Jan. 1991). Data for leaves above the pinch were not recorded in the first experiment, so the reported data were the combined results from two experiments. Data on the time course of changes in leaf abscission and pigment levels involved discrete measurements that were amenable to statistical analysis. Analysis of variance and the F test of significance were performed with the InStat program (GraphPad Software, San Diego, Calif.) for personal computers. Fisher’s LSD (Dowdy and Weardon, 1983) was subsequently calculated at P = 0.05.

Results

The two poinsettia cultivars selected for this study had been previously judged as inferior (‘Amy’) and superior (‘Lilo’) performers in interior conditions where several environmental variables differ from a greenhouse. When abscission of main stem leaves below the pinch was examined in a comparison where light was the only (within the limits of our experimental abilities) environmental variable, ‘Amy’ was more susceptible to low-light stress than ‘Lilo’ (Fig. 2). After several weeks of shade treatment, abscission in ‘Amy’ was much greater in low light than in high light while abscission in ‘Lilo’ was not significantly different between the treatments.

Total chlorophyll levels in leaves below (Fig. 3A) and above (Fig. 3D) the pinch were higher in ‘Lilo’ than in ‘Amy’ during the postproduction period. ‘Lilo’ responded to the low-light environment by maintaining (below the pinch) or even slightly increasing (above the pinch) the chlorophyll content. In contrast, ‘Amy’ in low light did not exhibit enhanced chlorophyll levels either below or above the pinch.

At the start of the postproduction period, the chlorophyll a :
chlorophyll b ratios in thylakoids from leaves below and above the pinch were slightly lower in ‘Lilo’ than in ‘Amy’ (0 days in Fig. 3B and E). As the shade treatment progressed, both ‘Lilo’ and ‘Amy’ had lower chlorophyll a : chlorophyll b ratios and higher chlorophyll : carotenoid ratios in low light than in high light. The chlorophyll fluorescence excitation spectrum for thylakoids from ‘Lilo’ leaves showed strong bands in the violet to blue wavelengths of 400 to 500 nm and in the red wavelengths of 600 to 700 nm (Fig. 4A). These bands arise as the sum of light-harvesting contributions from each of the effective photosynthetic pigments in the thylakoid membranes. Chlorophyll a absorbance in thylakoid membranes is most prominent in Soret bands peaking in the wavelength regions of 430 to 440 nm and in first excited state bands peaking at 670 to 680 nm. Chlorophyll b absorbance is similarly most prominent in a band peaking from 460 to 480 nm and in a weaker band peaking ≈ 650 nm. The shorter wavelength bands of the chlorophylls are strongly overlapping with the absorbance by carotenoids, which peaks from 420 to 500 nm and is relatively prominent around 487 nm in thylakoid membranes (Sauer and Calvin, 1962; Siefermann-Harms, 1985, 1987).

These characteristic wavelengths for each type of pigment were used to assign the origins of the peaks and valleys appearing in fluorescence excitation difference spectra. The difference spectrum of ‘Amy’ minus ‘Lilo’ at the start of the postproduction period (Fig. 4B) exhibited a valley near 650 nm, a wavelength of peak absorbance by chlorophyll b. This valley indicated relatively less light harvesting by chlorophyll b in ‘Amy’, a result consistent with the lower chlorophyll a : chlorophyll b content ratio in ‘Lilo’ than in ‘Amy’ at this time (0 days in Fig. 3B).

Fig. 3. Time course of effect of light level on pigment contents of leaves below (A–C) and above (D–F) the pinch of postproduction poinsettias. Total chlorophyll (A and D) was determined by extraction directly from leaf tissue. Pigment ratios (B, C, E, and F) expressed on weight/weight basis were determined by extraction from thylakoids to enable closest comparison with chlorophyll fluorescence excitation (Figs. 4–6). The LSD values were calculated at $P = 0.05$. Fig. 2. Time course of effect of light level on abscission of main stem leaves below the pinch of postproduction poinsettias. At 0 days on the abscissa, the low-light plants were moved into the shade boxes while the high-light plants remained on an open bench. As necessitated by the destructive sampling for pigment quantitation and fluorescence measurements, separate lots of plants were used for each time point. Thus, apparent declines in abscission with time, as for ‘Lilo’ low-light plants between 21 and 35 days, were due to variations between the lots rather than to an impossible reversal of abscission. The LSD was calculated at $P = 0.05$. 
the changes with time were due to changes in the low-light plants high-light difference spectra alone, however, did not reveal whether leaves below the pinch of 'Amy' and 'Lilo'. These low-light minus differences appeared sooner in leaves above the pinch than in 35 days (Fig. 5, E–H) of shade treatment showed that larger Comparison of the difference spectra at 7 days (Fig. 5, A–D) and 455- to 500-nm region of chlorophyll b and carotenoid absorbance. content ratios in high light than in low light (Fig. 3 B and E). The result consistent with the higher chlorophyll a : chlorophyll b ratio because while light-harvesting complex II binds chlorophyll b ratio in low light (Lichtenthaler, 1987). The relative increase in light-harvesting complex II may also be responsible for the commonly observed increase in the total chlorophyll : carotenoid ratio because while light-harvesting complex II binds chlorophyll and carotenoid pigments, its chlorophyll a : β-carotene content ratio is very high compared to the ratios for reaction center complexes (Lichtenthaler, 1987).

Regarding the comparison of ‘Amy’ and ‘Lilo’, the generally higher chlorophyll content of ‘Lilo’ (Fig. 3 A and D) and the resulting greater absorbance of light may contribute to the superior performance of ‘Lilo’ in interior environments. It is likewise
relevant that low-light ‘Lilo’ plants were able to retain (below the pinch) or even accumulate (above the pinch) more chlorophyll than high-light plants, while ‘Amy’ did not exhibit this effect (Fig. 3 A and D).

The lower initial chlorophyll a : chlorophyll b ratio in ‘Lilo’ (0 days, Fig. 3 B and E) may indicate that ‘Lilo’ is also predisposed to better performance in low light because its relative content of light-harvesting complex II is higher than that of ‘Amy’, although it would be necessary to directly measure pigment-protein contents in both cultivars to test this hypothesis.

As judged by chlorophyll fluorescence excitation, low-light acclimatization of light-harvesting function in both ‘Amy’ and ‘Lilo’ appeared sooner in leaves above the pinch than in leaves below the pinch (Fig. 5). While it seemed reasonable to conjecture that this earlier acclimatization above the pinch occurred because these younger leaves were more able to alter their metabolism in low light, further examination revealed that several different patterns all led to the appearance of net acclimatization (Fig. 6). With leaves above the pinch of ‘Amy’, for example, the change with time occurred mostly in high-light (Fig. 6D) rather than in low-light (Fig. 6H) leaves. In this case, net acclimatization (Fig. 5H) resulted because the low-light plants simply maintained their original (day 0) excitation spectrum while the high-light plants changed their excitation spectrum with age.

The information gained in this study may prove useful in poinsettia breeding and evaluation programs. In particular, the
results suggest that cultivars with high total chlorophyll content per leaf area and low chlorophyll a:chlorophyll b ratio may be inclined toward improved interior longevity. Measurements of these leaf pigment levels, which are relatively easy compared to measurements of fluorescence excitation, might prove useful as an early predictor of postproduction performance.

**Literature Cited**


