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STOCK PLANT AND PROPAGATION PHOTOSYNTHETIC DAILY LIGHT INTEGRAL AND STORAGE INFLUENCE POSTHARVEST PERFORMANCE OF HERBACEOUS CUTTINGS

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STOCK PLANT AND PROPAGATION PHOTOSYNTHETIC DAILY LIGHT INTEGRAL AND STORAGE INFLUENCE POSTHARVEST PERFORMANCE OF HERBACEOUS CUTTINGS

By

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ABSTRACT

STOCK PLANT AND PROPAGATION PHOTOSYNTHETIC DAILY LIGHT INTEGRAL AND STORAGE INFLUENCE POSTHARVEST PERFORMANCE OF HERBACEOUS CUTTINGS

By

Roberto Gerardo Lopez

Herbaceous shoot-tip cuttings of ornamental plants are commonly harvested from stock plants in equatorial countries and then packaged, stored, shipped, and subsequently rooted by greenhouse growers in the United States and Europe. The effects of environmental conditions during this supply chain process on cutting yield, acclimatization, morphology, physiology, rooting, and subsequent growth and development of nonrooted cuttings are unknown on most species. The objectives of this research were: (1) to quantify how photosynthetic daily light integral (DLI) provided to stock plants and during propagation and (2) how post-harvest storage temperature and duration influence the physiology and morphology of six vegetatively propagated herbaceous species. Stock plants of bacopa [*Jamesbrittenia grandiflora* (Galpin) Hilliard], heliotrope (*Heliotropium arborescens* L.), petunia (*Petunia ×hybrida* hort. Vilm.-Andr.), thunbergia (*Thunbergia alata* Bojer ex Sims), and verbena (*Verbena ×hybrida* Groenl. & Ruempl) were grown at 20 °C under DLI treatments ranging from a mean of 4 to 15 $mol \cdot m^{-2} \cdot d^{-1}$. New guinea impatiens (*Impatiens hawkeri* Bull.) 'Harmony Magenta', 'Harmony White', and 'Celebrette Red' were grown at 23 °C under DLI treatments ranging from 6 to 18 $mol \cdot m^{-2} \cdot d^{-1}$. After 12 weeks of treatments, the light saturated maximum photosynthesis, dark respiration, relative chlorophyll content, cutting dry mass, stem caliper, leaf thickness, and cutting yield of bacopa, heliotrope, thunbergia,

and verbena increased as stock plant DLI increased from 4 to 14 $mol \cdot m^{-2} \cdot d^{-1}$. Cuttings were subsequently harvested and stored for 0, 2, 4, or 6 d at 5, 10, or 15 °C followed by 2 d in darkness at 20 °C to simulate shipping. Cuttings were subsequently rooted in a controlled greenhouse environment with overhead mist, a vapor-pressure deficit of 0.3 kPa, and air and media temperatures of ≈25 °C. The percentage of stored bacopa, heliotrope, and verbena cuttings that had initiated roots after 7 or 10 d of propagation was reduced by up to 23% when harvested from stock plants provided with a mean DLI ≥15 $mol \cdot m^{-2} \cdot d^{-1}$. Regardless of stock plant DLI, chlorophyll fluorescence, photosynthesis, and rooting of new guinea impatiens 'Harmony Magenta' were reduced when cuttings were stored >2 d at 5 °C. In a separate experiment, three cultivars of petunia and new guinea impatiens were propagated under DLI treatments ranging from 1.2 to 10.7 $mol \cdot m^{-2} \cdot d^{-1}$. Root dry mass of new guinea impatiens 'Harmony White', 'Harmony Magenta', and 'Celebrette Red' increased linearly and by 867%, 604%, and 580%, respectively, as propagation DLI increased from 1.3 to 6.1 $mol \cdot m^{-2} \cdot d^{-1}$. In petunia 'Tiny Tunia Violet Ice' and 'Supertunia Mini Purple', subsequent time to flower at 20 °C decreased by 3 weeks as the DLI during propagation increased from 1.4 to 10.7 $mol \cdot m^{-2} \cdot d^{-1}$. Collectively, these experiments quantify the importance of controlling shipping and storage temperature and managing the DLI during stock plant production and propagation when producing high-quality herbaceous ornamental cuttings.

DEDICATION

In loving memory of my grandmother, Mrs. Alicia C. Toledo (March 3, 1997) who inspired my curiosity, interest, and passion for plants.

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SECTION I

STOCK PLANT PHOTOSYNTHETIC DAILY LIGHT INTEGRAL: I. INFLUENCES ON INSTANTANEOUS LIGHT RESPONSE, CUTTING QUALITY, AND YIELD

Stock Plant Photosynthetic Daily Light Integral: I. Influences on Instantaneous Light Response, Cutting Quality, and Yield

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Introduction

In 2005, U.S. greenhouse growers imported 868 million non-rooted shoot-tip cuttings of ornamental annuals and herbaceous perennials with a reported wholesale value of US $60 million (USDA, 2006). The three largest exporting countries of cuttings to the United States are Costa Rica, Guatemala, and Mexico, with 299, 287, and 111 million cuttings, respectively (USDA, 2006). Mexico and countries in Central America currently dominate stock plant production for several economic and environmental reasons, including: availability of low-wage labor, proximity to U.S. markets, minimal growing structural investment due to their mild climates, and moderate temperature and light environments (Donnelly and Fisher, 2002).

Knowledge of species-specific cultural and environmental management of stock plants has not always advanced with the rapid transition from seed to cutting production and propagation in the floriculture industry. According to Faust et al. (2005), successful plant production and profitability requires management of the growing environment based on individual species' responses to daily light integral (DLI). Irradiance or DLI can be manipulated for specific crops with supplemental lighting, shading materials, and retractable roofs. Based on the time of the year and location, DLI can range from 5 to 60 $mol \cdot m^{-2} \cdot d^{-1}$ outdoors and 1 to 25 $mol \cdot m^{-2} \cdot d^{-1}$ in a greenhouse (Korczynski et al. 2002). However, DLI, temperature, and photoperiod are not commonly controlled in many equatorial stock plant production facilities. In most instances, DLI is the only environmental factor that can be manipulated in these facilities by adjusting the amount of shade. Light intensity or DLI during herbaceous stock plant production (Kadner, 2005; Moe, 1977; Rapaka et al., 2007a; 2007b) and supplemental lighting (Bertram et al.,

1989; Donnelly and Fisher, 2002) can influence cutting production, quality, stress tolerance, and subsequent performance. For example, as DLI increased from 1.2 to 8.2 $mol \cdot m^{-2} \cdot d^{-1}$, cutting yield, dry mass, and root number of bell flower (*Campanula isophylla* Moretti) stock plants increased by 188%, 33%, 67%, respectively (Moe, 1977). Donnelly and Fisher (2002) reported that the number of viable cuttings harvested increased by as much as 73% when fan flower (*Scaevola aemula* R. Br.) stock plants were provided with supplemental lighting of 2.8 $mol \cdot m^{-2} \cdot d^{-1}$ from high-pressure sodium (HPS) lamps in addition to ambient light (6.2 $mol \cdot m^{-2} \cdot d^{-1}$). In *Portulaca grandiflora* Hook. and *Lantana camara* L., as the pre-harvest stock plant DLI increased, the initial carbohydrate status of cuttings increased and subsequent post-harvest leaf abscission decreased (Rapaka et al., 2007a, 2007b).

The ability of plants to change their morphology, anatomy, and physiology to environmental conditions such as DLI is termed plasticity. The extent of plasticity and acclimation varies among species. Plants exposed to a range of DLIs can also exhibit differences in growth, development, and stress response. Chlorophyll fluorescence (F_v/F_m) quantifies the quantum yield efficiency of photosystem II and has been used to determine photosynthetic potential, stress, and damage to the photosynthetic system. The acclimatization and physiological response to light intensity and DLI can be described with photosynthetic-light response curves and F_v/F_m measurements (Calatayud et al., 2007; Callan and Kennedy, 1995; Funnell et al., 2002; Nemali and van Iersel, 2004; Poulson et al., 2006; Rapaka et al., 2005). For example, estimated quantum yield, dark respiration, and maximum gross photosynthesis per square meter ground area increased by 86%, 162%, and 87% as DLI increased from 5.3 to 19.4 $mol \cdot m^{-2} \cdot d^{-1}$ in wax begonia

(*Begonia semperflorens-cultorum* Hort.) (Nemali and van Iersel, 2004). However, exposure to excessively high light can inhibit F_v/F_m; *Arabidopsis thaliana* (L.) Heynh. 'Columbia' exposed to a 4-h high light treatment (2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$) reduced F_v/F_m by 13% compared to pre-stress values (Poulson et al., 2006). In geranium (*Pelargonium ×hortorum* L.H. Bail.), the photosynthetic efficiency of nonstored cuttings increased as stock plant photosynthetic photon flux (*PPF*) increased from 82 to 359 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Rapaka et al., 2005). To our knowledge, no studies have been published on the morphological, and physiological, responses of herbaceous ornamental stock plants in response to DLI.

We performed experiments to determine how DLI during stock plant production influenced acclimatization, morphology, physiology, stress response, and yield of eight vegetatively propagated annual species. The specific objectives of this study were (1) to quantify the photosynthetic light response (*A/Q*) curves and F_v/F_m of stock plants grown under different mean DLIs (2) to determine species-specific light compensation and saturations points, (3) to determine the effect of DLI on stock plant morphology and cutting quality, and (4) to identify a greenhouse DLI that maximizes stock plant photosynthesis, cutting quality, and yield. We postulate that stock plants grown under higher DLI will have higher photosynthetic rates and produce higher quality cuttings.

Materials and Methods

Stock plant management and culture. Vegetatively propagated stock plants of bacopa 'Breeze Upright White' [*Jamesbrittenia grandiflora* (Galpin) Hilliard], heliotrope 'Baby Blue' (*Heliotropium arborescens* L.), petunia 'Tiny Tunia Violet Ice'(*Petunia*

×*hybrida* hort. Vilm.-Andr.), thunbergia 'Sunny Lemon Star'(*Thunbergia alata* Bojer ex Sims), and verbena 'Aztec Red Velvet'(*Verbena* ×*hybrida* Groenl. & Ruempl) were maintained at 20.9 ± 1.7 °C (mean daily temperature ± standard deviation) under a 12-h photoperiod in greenhouses in East Lansing, MI (43 °N lat.). The photoperiod consisted of a truncated 9-h natural day achieved using blackout cloth from 1700 to 0800 HR and day-extension lighting (≈2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at canopy level) from 1700 to 2000 HR with incandescent lamps.

Three DLI environments were created on 23 Jan. 2006 using no shade or permanent woven shade cloth with an open-weave design that reduced light by ≈30% and 55% (OLS 30 and 50; Ludvig Svensson, Charlotte, N.C.) that surrounded individual benches. In addition, whitewash was applied to the greenhouse glazing to moderate the DLI during experiment repetitions. Line quantum sensors containing 10 photodiodes (Apogee Instruments, Inc., Logan, UT) were placed directly above stock plants in each DLI treatment to measure the *PPF*. From 0800 to 1700 HR, HPS lamps provided a supplemental *PPF* of 10, 35, and 65 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant height when the ambient greenhouse *PPF* was <140 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for the 55%, 30% and 0% shade treatments, respectively. Air temperature was measured on each bench by an aspirated and enclosed thermocouple (TT-E-36; Omega Engineering Inc., Stamford, CT). Temperature and light intensity were measured every 10 s and hourly averages were recorded by a CR-10 datalogger (Campbell Scientific, Logan, UT). To help provide uniform night temperatures of 20 °C, a data logger controlled a 1500-W electric heater, which provided supplemental heat under each bench as needed. The mean DLIs for the three DLI treatments during two-week periods prior to cutting harvests were 7.4, 10.5, and 14.4

$mol \cdot m^{-2} \cdot d^{-1}$ for replication 1 and 4.5, 8.1, and 12.0 $mol \cdot m^{-2} \cdot d^{-1}$ for replication 2. Ethephon (Florel; Rhône-Poulenc Ag Company, Research Triangle Park, N.C.) with a surfactant (Capsil; Aquatrols, Paulsboro, N.J.) was applied every four weeks as a foliar spray at a concentration of 150 or 200 $mg \cdot L^{-1}$ and a volume of ≈ 0.2 $L \cdot m^{-2}$ to abort flower buds.

New guinea impatiens (*Impatiens hawkeri* Bull.) 'Harmony White', 'Harmony Magenta,' and 'Celebrette Red' stock plants were maintained at 24.5 ± 1.1 °C under a 16-h photoperiod that consisted of natural daylengths with day-extension lighting from HPS lamps from 0600 to 2200 HR as previously described. DLI treatments were as described previously and two-week means prior to cutting harvests for replications 1 and 2 were 6.1, 10.9, and 17.3 $mol \cdot m^{-2} \cdot d^{-1}$ for replication 1 and 6.7, 11.4 and 17.2 $mol \cdot m^{-2} \cdot d^{-1}$ for replication 2. Ethephon was applied to new guinea impatiens as described previously but at a concentration of 500 to 750 $mg \cdot L^{-1}$ every two weeks to abort flower buds.

Bacopa, heliotrope, petunia, thunbergia, and verbena stock plants were grown in 15-cm (1.3-L) and new guinea impatiens in 16-cm (2.4-L) round plastic containers filled with a mix containing 70% peat moss, 21% perlite, and 9% vermiculite (Sure-Mix; Michigan Grower Products, Galesburg, MI). Plants were irrigated as necessary with reverse osmosis water supplemented with water-soluble fertilizer to provide the following ($mg \cdot L^{-1}$): 125 N, 12 P, 100 K, 65 Ca, 1.0 Fe and Cu, 0.5 Mn and Zn, 0.3 B, and 0.1 Mo (MSU Special; Greencare Fertilizers, Chicago, IL).

Effect of stock plant DLI on photosynthetic light response. After at least twelve weeks under the DLI treatments, four stock plants were randomly selected from each treatment for shoot or leaf gas exchange measurements. Measurements were performed in the greenhouses between 900 and 1400 HR and were blocked by DLI treatment to

reduce time of day effects. Shoots or leaves were selected at random from sun-exposed shoots in the upper one-third of the stock plant. Photosynthesis (*A*), dark respiration, stomatal conductance, and transpiration measurements were conducted using a portable photosynthesis system (LI-6400, LI-Cor, Lincoln, NE) fitted to a leaf chamber with an LED light source (6400-02B; red at 665 nm and blue at 470 nm) to control the light intensity. The reference CO_2 concentration inside the leaf chamber was 400 $\mu mol \cdot mol^{-1}$ and the flow of air into the chamber was 250 $\mu mol \cdot s^{-1}$. Leaf temperature inside the leaf chamber was maintained at 24 ± 1 °C and 21 ± 1 °C for new guinea impatiens and all other species, respectively, using dual Peltier devices that heated or cooled the air circulating through the chamber. For each DLI treatment, the *A* of leaves was determined at *PPF* levels of 0, 15, 30, 45, 60, 90, 100, 250, 500, 1000, 1250, 1500, 1750, and 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Leaves were allowed to adapt to each *PPF* for three minutes before each data point was recorded. Immediately after measurements were recorded, shoots or leaves inside the 6 cm^2 chamber were excised, placed in plastic packages and stored at 5 °C. Shoot or leaf area was determined by scanning the shoot or leaf through a leaf area meter (LI-3000, Li-Cor, Lincoln, NE) three times and recording the average. Maximum photosynthetic rates expressed in terms of shoot or leaf area were fitted to the following equation

$$A = \frac{\phi Q + A_{max} - \sqrt{(\phi Q + A_{max})^2 - 4\phi Q k A_{max}}}{2k} - R_d$$

using Photosyn Assistant 1.1 (Dundee Scientific, Dundee, England) where Q is light intensity ($\mu mol \cdot m^{-2} \cdot s^{-1}$), ϕ is the apparent quantum efficiency, A_{max} is light saturated maximum photosynthesis, k is the convexity, R_d is dark respiration, and A is shoot or leaf

photosynthesis (Prioul and Chartier, 1977). After determining light response values, light saturation ranges (LSR) for each species and DLI treatment were estimated at 95% of mean A_{max} at a light intensity of 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Landhäusser and Lieffers, 2001). The F_v/F_m of a fully expanded leaf was measured using a portable chlorophyll fluorescence system (Plant Efficiency Analyzer; Hansatech Instruments Ltd., Norfolk, England). Leaves were dark-acclimated for 15 min using the manufacturer's plastic and foam clips before measurements were recorded.

Effect of stock plant DLI on cutting yield and quality. Terminal and internode cuttings of all species were harvested from stock plants under each DLI treatment on 25 Apr. 2006 and 5 June 2006. The number of uniform and harvestable vegetative and reproductive cuttings from individual stock plants were recorded. Terminal cuttings of bacopa, heliotrope, petunia, verbena, and new guinea impatiens each had 4 leaves and a 3-cm stem length; internode thunbergia cuttings had 2 nodes and a 4-cm stem length. Ten cuttings from each DLI treatment were randomly sampled for each of the following measurements. Cutting stem caliper was measured using a digital caliper (Mitutoyo Corp., Aurora, IL). Total chlorophyll (a+b) content was estimated using a SPAD chlorophyll meter (Model 502, Minolta Co., Japan). For each cutting, three SPAD measurements were taken from different positions on the largest fully expanded leaf and the mean was recorded as the value for the cutting. Leaf area of the largest fully expanded basal leaf of a cutting was determined by scanning it through the leaf area meter three times and recording the average. Leaf thickness was measured by examining leaf cross-sections from the center of the largest fully expanded basal leaf under a

microscope at 30x magnification. Cutting dry mass was recorded after drying in an oven at 70 °C for one week.

Data analysis. Data were analyzed using SAS (SAS Institute, Cary, N.C.) mixed model procedure (PROC MIXED) for analysis of variance and regression analysis was performed using Sigma Plot 8.0 (Systat Software, Inc., San Jose, CA).

Results

Bacopa. As the stock plant mean DLI increased from 4.4 to 12.7 $mol \cdot m^{-2} \cdot d^{-1}$, R_d, A_{max}, and ϕ using shoot area increased by 128%, 110% and 69%, respectively (Table 1.1). In addition, the estimated LCP and LSR increased from 41 to 76 and from 1400–1600 to 1650–1850 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively (Fig. 1.1A). The efficiency of photosystem II expressed as F_v/F_m was not influenced by DLI. Net photosynthesis (P_n) was consistently higher for stock plants grown under a high DLI compared to those under a low DLI when the *PPF* exceeded 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Relative leaf chlorophyll content increased from 32 to 42 as stock plant DLI increased from 8.1 to 14.4 $mol \cdot m^{-2} \cdot d^{-1}$ (Fig. 1.2A). Cutting leaf thickness, stem caliper, and dry mass increased linearly by 78%, 49%, and 122%, respectively, and area of the largest fully expanded leaf on a cutting decreased linearly by 45% as stock plant mean DLI increased from 4.5 to 14.4 $mol \cdot m^{-2} \cdot d^{-1}$ (Fig. 1.2B, C, D, and E). The number of cuttings harvested increased at a decreasing rate as DLI increased (Fig. 1.2F).

Petunia. R_d, A_{max}, LSR, and ϕ increased as the mean DLI increased from 4.6 to 9.7 $mol \cdot m^{-2} \cdot d^{-1}$ and then decreased with a further increase in DLI (Table 1.1; Fig. 1.1B). The LCP increased from 34 to 76 $\mu mol \cdot m^{-2} \cdot s^{-1}$, as DLI increased from 4.6 to 13.5 $mol \cdot m^{-}$

$^{2}\cdot d^{-1}$ (Table 1.1). DLI did not have a significant effect on F_v/F_m. P_n was consistently higher at *PPF* >100 $\mu mol\cdot m^{-2}\cdot s^{-1}$ when stock plants were grown under a mean DLI of 8.1 $mol\cdot m^{-2}\cdot d^{-1}$ compared to a mean DLI of 4.6 or 12.1 $mol\cdot m^{-2}\cdot d^{-1}$.

Relative leaf chlorophyll content gradually increased from 24 to 37 as DLI increased from 8.1 to 14.4 $mol\cdot m^{-2}\cdot d^{-1}$ (Fig. 1.2A). Significant linear relationships were observed between DLI and cutting leaf thickness, stem caliper, area of a single leaf, dry mass, and number of viable cuttings harvested (Fig. 1.2B, C, D, E, and F). For example, as mean DLI increased above 4.5 $mol\cdot m^{-2}\cdot d^{-1}$ (providing an additional 3.6 and 7.5 $mol\cdot m^{-2}\cdot d^{-1}$) increased cutting stem caliper by 31 and 43%, dry mass by 67% and 106% and number of cuttings increased by 59 and 136%, respectively.

Verbena. As stock plant mean DLI increased from 4.6 to 14.2 $mol\cdot m^{-2}\cdot d^{-1}$, A_{max}, R_d, and ϕ increased by 1.6, 2.5 and 3.0-fold, respectively (Table 1.1, Fig. 1.1C). The F_v/F_m was positively influenced by DLI and increased from 0.836 to 0.853. There was an increase in the LCP of stock plants with increasing DLI and it ranged between 60 and 95 $\mu mol\cdot m^{-2}\cdot s^{-1}$. Photosynthesis of stock plants saturated at a higher *PPF* when plants were grown at a higher DLI compared to plants grown at a lower DLI.

All parameters of cutting quality increased linearly with DLI except leaf area (Fig. 1.2A, B, C, D, and E). For example, for every 1 $mol\cdot m^{-2}\cdot d^{-1}$ increase in DLI, cutting chlorophyll content, leaf thickness, stem caliper, dry mass, and number of viable cuttings harvested increased by 1.1, 0.02 mm, 0.03 mm, 1.9 mg and 3.2 cuttings, respectively, and area of the largest leaf decreased by 0.20 cm^2.

New guinea impatiens 'Celebrette Red'. As mean DLI increased from 6.6 to 17.0 $mol\cdot m^{-2}\cdot d^{-1}$, A_{max} and R_d increased by 8.1 and 1.0 $\mu mol\ CO_2\cdot m^{-2}\cdot s^{-1}$, respectively (Table

1.1, Fig. 1.1D). There was no consistent effect of DLI on ϕ. As DLI increased from 6.6 to 18.2 mol·m^{-2}·d^{-1}, F_v/F_m decreased from 0.821 to 0.771. The LCP and LSR were relatively low and generally increased from 14 to 27 and 950 to 1350 µmol·m^{-2}·s^{-1}, respectively, as DLI increased from 6.6 to 17.0 mol·m^{-2}·d^{-1}. Above a *PPF* of 250 µmol·m^{-2}·s^{-1}, P_n was consistently higher for stock plants grown under a mean DLI of 18.2 mol·m^{-2}·d^{-1} compared to plants under a lower mean DLI (Fig. 1.1D).

The relationships between cutting chlorophyll content, dry mass, and number of harvested cuttings and DLI were quadratic and increased at a decreasing rate as DLI increased from 6.1 to 17.3 mol·m^{-2}·d^{-1} (Fig. 1.2G, K, and L). As DLI increased from 6 to 17 mol·m^{-2}·d^{-1}, leaf thickness and stem caliper increased linearly by 69% and 26%, respectively, and leaf area decreased linearly by 21% (Fig. 1.2H, I, and J).

New guinea impatiens 'Harmony White'. A_{max}, F_v/F_m, and the LCP were not significantly different when stock plants were grown under a mean DLI ranging from 6.1 to 11.1 mol·m^{-2}·d^{-1} (Table 1.1). R_d was similar when the mean DLI ranged from 10.9 to 17.3 mol·m^{-2}·d^{-1}. However, further increases in DLI significantly increased A_{max}, F_v/F_m, and LCP. The ϕ was not influenced by DLI. The estimated LSR occurred at a *PPF* of 1400 to 1800 µmol·m^{-2}·s^{-1} within the mean DLIs provided (Fig. 1.1E). The P_n of stock plants increased with mean DLI when exposed to a *PPF* of ≥500 µmol·m^{-2}·s^{-1} (Fig. 1.1E).

As DLI increased from 6.1 to 17.3 mol·m^{-2}·d^{-1}, chlorophyll content, stem caliper, dry mass, leaf thickness, and number of harvested cuttings increased by 12 (24%), 0.6 mm (21%), 78 mg (79%), 0.37 mm (100%) and 17 cuttings (57%), respectively (Fig. 1.2G, H, J, K, and L). Additionally, leaf area decreased linearly by 29% from 7.7 to 5.5 cm^2 (Fig. 1.2I).

New guinea impatiens 'Harmony Magenta'. An increase in the mean DLI from 5.9 to 16.7 $mol \cdot m^{-2} \cdot d^{-1}$ increased R_d from 1.3 to 2.3 $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$, ϕ from 0.077 to 0.105 $\mu mol\ CO_2 \cdot \mu mol^{-1}$, and LSR from 200 to 400 to 775 to 995 $\mu mol \cdot m^{-2} \cdot s^{-1}$, while A_{max} nearly doubled (Table 1.1). The mean LCP was 19 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and was not influenced by stock plant DLI (Fig. 1.1F). The F_v/F_m generally decreased from 0.821 to 0.789 as DLI increased. P_n of stock plants grown at a mean DLI of 17.3 $mol \cdot m^{-2} \cdot d^{-1}$ was consistently higher above a *PPF* of 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and saturated at a higher *PPF* compared to plants grown at a lower DLI.

Chlorophyll content, leaf thickness, cutting dry mass, and cutting number increased linearly with increasing DLI (Fig. 1.2G, H, I, K, and L). For example, as DLI increased from 6.1 to 17.3 $mol \cdot m^{-2} \cdot d^{-1}$, the number of harvested cuttings increased by 88% and their dry mass increased by 49%. Cutting stem caliper increased from 2.4 to 2.9 mm as DLI increased from 6.1 to 10.9 $mol \cdot m^{-2} \cdot d^{-1}$ and then remained relatively constant with additional increases in DLI (Fig. 1.2J).

Heliotrope. For every 1 $mol \cdot m^{-2} \cdot d^{-1}$ increase in DLI, A_{max}, and R_d increased by $\approx$0.5 and 0.14 $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$, respectively (Table 1.1; Fig. 1.1G). F_v/F_m increased (from 0.806 to 0.834) with DLI but ϕ remained relatively constant. The mean LCP and LSR was 18.7 and 1425 to 1625 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively, for all DLIs tested. P_n of stock plants grown under the higher DLIs was consistently higher when the *PPF* was $\geq$1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Chlorophyll content, leaf thickness, stem caliper, and dry mass increased linearly by 57%, 67%, 40%, and 62%, respectively, when the mean DLI increased from 4.5 to 14.4 (Fig. 1.2M, N, P, and Q). Area of the largest fully expanded leaf decreased linearly

by 48% as the DLI increased (Fig. 1.2O). The number of viable cuttings harvested increased (from 10 to 29) but at a decreasing rate as DLI increased from 4.5 to 14.4 $mol \cdot m^{-2} \cdot d^{-1}$ (Fig. 1.2R).

Thunbergia. An increase in DLI from 4 to 12 $mol \cdot m^{-2} \cdot d^{-1}$ increased A_{max} and R_d by 5.9 and 1.2 $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$, respectively (Table 1.1, Fig. 1.1G). The ϕ generally decreased as DLI increased from 4.5 to 12.4 $mol \cdot m^{-2} \cdot d^{-1}$. The LCP and LSR increased from 18 to 36 and from 275–375 to 300–500 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively as DLI increased. P_n of stock plants grown under all DLIs decreased or remained constant when the *PPF* exceeded 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Fig. 1.1H).

As DLI increased from 4.5 to 14.4 $mol \cdot m^{-2} \cdot d^{-1}$, chlorophyll content, leaf thickness, stem caliper, dry mass, and cutting number increased linearly from 32 to 40 (25%), 38 to 61 mm (62%), 1.5 to 2.0 mm (33%), 93 to 123 mg (32%) and 11 to 19 cuttings (73%) (Fig. 1.2M, N, P, Q, and R). In addition, the area of the largest leaf decreased linearly from 19 to 15 cm^2 (27%) (Fig. 1.2O).

Discussion

Successful cutting production requires maximizing stock plant photosynthesis to increase biomass accumulation and produce high-quality cuttings that will tolerate environmental stresses during shipping and storage and subsequently root rapidly during propagation (Moe and Andersen, 1988). In this research, stock plant photosynthetic light response parameters, cutting quality, and yield increased as the stock plant DLI increased. For example, as the mean DLI increased from 4 to 14 $mol \cdot m^{-2} \cdot d^{-1}$, A_{max}, R_d, relative chlorophyll content, cutting dry mass, stem caliper, leaf thickness, and cutting

yield increased in bacopa, verbena, heliotrope, and thunbergia. Similar photosynthetic responses to DLI have been reported in wax begonia, calla lily (*Zantedeschia aethiopica* L. Spreng.), and other herbaceous species (Evan and Poorter, 2001; Funnel et al., 2002; Nemali and van Iersel, 2004). For example, R_d increased from 0.3 to 0.4 and A_{max} increased from 6.0 to 10.9 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ for calla lily grown at 28 °C when the DLI increased from 15 to 30 mol $\cdot m^{-2} \cdot d^{-1}$ (Funnel et al., 2002). The rate of photosynthesis per unit leaf area of thorn apple (*Datura stramonium* L.), salvation jane (*Echium plantagineum* L.), tobacco (*Nicotiana tabacum* L.), cape gooseberry (*Physalis peruvianum* L.), plantain (*Plantago major* L. ssp. *pleiosperma*), and radish (*Raphanus sativus* L.) was on average three times higher for plants grown under high light than for those grown under low light (Evan and Poorter, 2001).

With the exception of petunia and thunbergia, all species placed under a saturating intensity of 2000 μmol $\cdot m^{-2} \cdot s^{-1}$ had a higher A_{max} when grown under a high DLI compared to plants grown under a lower DLI. These species apparently have the ability to acclimatize to high DLI, fix more carbon under high *PPF* conditions, and consequently reach a higher A_{max}. The maximum *PPF* that new guinea impatiens stock plants grown under a mean DLI of 6 and 17 mol $\cdot m^{-2} \cdot d^{-1}$ received after 12 weeks of DLI treatments was 300 and 1030 μmol $\cdot m^{-2} \cdot s^{-1}$, respectively. For bacopa, petunia, verbena, heliotrope, and thunbergia stock plants grown under a mean DLI of 4 to 14 mol $\cdot m^{-2} \cdot d^{-1}$, the maximum *PPF* was 520 and 1000 μmol $\cdot m^{-2} \cdot s^{-1}$, respectively (data not presented). Consequently, stock plants grown under a high DLI for twelve weeks acclimated to a high *PPF* by developing smaller and thicker leaves with a higher relative chlorophyll content. As a result, plants responded to a high instantaneous *PPF* by increasing their photosynthetic

capacity and efficiency. In contrast, stock plants grown under lower DLIs acclimated to a low *PPF* by developing larger and thinner leaves with a lower relative chlorophyll content.

Plants that acclimate to high or low DLI also differ in their LCP and LSR (Callan and Kennedy, 1995; Funnel et al., 2002; Nemali and van Iersel, 2004). Except for new guinea impatiens 'Harmony Magenta', there was a significant increase in LCP and an increase in the estimated LSR with DLI in all species studied. Callan and Kennedy (1995) also reported that the LCP of stokes aster [*Stokesia laevis* (Hill) Greene] increased from 25 to 185 $\mu mol \cdot m^{-2} \cdot s^{-1}$ as the *PPF* under which it was grown increased from 120 to 1010 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively. Bacopa, petunia, verbena, heliotrope, and new guinea impatiens 'Harmony White' became light-saturated above $\approx$1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ ($\approx$60% of full sunlight). However, as the mean DLI increased from 4 to 12 $mol \cdot m^{-2} \cdot d^{-1}$, thunbergia became light-saturated between a *PPF* of $\approx$275 and 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ ($\approx$14% to 25% of full sunlight). Similarly, wax begonia and peruvian lily (*Alstroemeria* 'Jacqueline') became light-saturated at a *PPF* of $\approx$530 and 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively ($\approx$23% and 30% of full sunlight, respectively) (Leonardos et al. 1994; Nemali and van Iersel, 2004). Therefore, these three species were unable to utilize a high *PPF*, and in the case of thunbergia, photoinhibition may have occurred at light intensities above 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

Respiration rates varied between 1.3 and 9.8 $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$ among species and tended to increase with DLI. In bacopa and verbena, the two species with the highest A_{max} and ϕ, R_d increased by 128% and 157%, respectively, as mean DLI increased from 4 to 14 $mol \cdot m^{-2} \cdot d^{-1}$. Other studies on ornamental crops have reported an increase in R_d and

LCP with increasing DLI, and it has been suggested that plants grown at a higher DLI photosynthesize more to compensate for the increased respiration rates (Funnel et al. 2002; Moe and Andersen, 1988; Nemali and van Iersel, 2004). In the present study, this tendency was observed in all species except for new guinea impatiens 'Harmony Magenta'. For example, the A_{max}, R_d, and LCP of bacopa grown under a mean DLI of 4.4, 9.1, and 12.7 $mol \cdot m^{-2} \cdot d^{-1}$, was 16.0, 25.5, 33.6 $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$, 4.3, 6.5 and 9.8 $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$ and 41.0, 67.0 and 76.0 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively (Table 1.1; Fig. 1.1A).

Quantum efficiency increased as DLI increased for bacopa (0.117 to 0.198) and verbena (0.034 to 0.105). Nemali and van Iersel (2004) determined that quantum yield of wax begonia was 0.020 $mol \cdot mol^{-1}$ and was not influenced by DLI on a leaf-area basis. In calla lily, quantum yield was 0.029 and 0.016 $mol \cdot mol^{-1}$ for plants grown under a DLI of 15 and 30 $mol \cdot m^{-2} \cdot d^{-1}$, respectively (Funnel et al. 2002). Nevertheless, comparisons in quantum yield and efficiency between different species and studies are difficult to interpret because plants can absorb different fractions of incident light and may be exposed to varying environmental stresses.

F_v/F_m is an indicator of electron transfer efficiency from PSII to PSI and can be used to estimate stress or damage to the photosystem. In new guinea impatiens 'Harmony White', 'Harmony Magenta,' and 'Celebrette Red', F_v/F_m decreased by 2%, 4%, and 6% as DLI increased from 6 to 18 $mol \cdot m^{-2} \cdot d^{-1}$, indicating potential photoinhibition (Table 1.1). These results were supported by our photosynthesis data, which indicated that A_{max} decreased slightly when DLI exceeded 17 $mol \cdot m^{-2} \cdot d^{-1}$ for 'Harmony Magenta' and 'Celebrette Red'. Previous studies have shown that

photoinhibition occurs when plants grown under low light intensities are exposed to higher light intensities (Kitao et al., 2000; Poulsen et al., 2006). In Japanese white birch [*Betula platyphylla* Sukatchev var. *japonica* (Miq.) Hara], F_v/F_m decreased from 0.67 for plants grown under full sunlight to 0.28 for plants grown under 5% of full sunlight after a 2-h exposure to 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Kitao et al., 2000).

Linear and quadratic trends relating cutting quality, morphology, and yield to DLI were observed for all species. As DLI increased from 4 to 14 $mol \cdot m^{-2} \cdot d^{-1}$, cutting yield (biomass accumulation) of petunia, verbena, and thunbergia increased linearly by 195%, 69%, and 69%, respectively. For new guinea impatiens 'Harmony White', and 'Harmony Magenta', as DLI increased from 6 to 17 $mol \cdot m^{-2} \cdot d^{-1}$, cutting yield increased linearly by 57% and 88%, respectively. These results are consistent with Bertram et al. (1989) who reported that cutting yield of begonia (*Begonia* ×*hiemalis* Fotsch) increased by 150% when supplemental lighting increased from 0.1 to 2.9 $mol \cdot m^{-2} \cdot d^{-1}$ in addition to ambient light. Cutting stem caliper and dry mass are measures of cutting quality; cuttings that have thin stems and a low dry mass can root poorly and not have sufficient carbohydrate resources to initiate roots. As the mean DLI increased in all species, cutting stem caliper and dry mass increased linearly with the exception of new guinea impatiens 'Harmony Magenta' and 'Harmony White', respectively. Similar results have been reported in florist's daisy (*Chrysanthemum morifolium* Ramat.), begonia elatior-hybrid, and bell flower; cutting dry mass increased by 94%, 100%, and 33%, respectively, as supplementary irradiance to stock plants increased (Bertram et. al., 1989; Borowski et al., 1981; Moe, 1977). However, Donnelly and Fisher (2002) did not find significant differences in cutting length, stem caliper, biomass, and subsequent rooting of heliotrope,

fan flower, petunia and verbena cuttings harvested from stock plants provided with a mean DLI of 6.2, 7.8, or 9.0 $mol \cdot m^{-2} \cdot d^{-1}$. It is possible that no significant differences in cutting quality were observed because there was only a 2.8 $mol \cdot m^{-2} \cdot d^{-1}$ difference between the ambient and highest supplemental DLI treatment.

Morphological plasticity of all species studied changed with DLI. For example, relative chlorophyll content and stem caliper increased, while basal leaves of cuttings became smaller and thicker with increasing stock plant DLI. Vladimirova et al. (1997) also observed morphological plasticity in belgian evergreen (*Dracaena sanderiana* hort Sander ex Mast.) when exposed to increasing levels of shade; leaf area increased by 19% and internode diameter decreased by 20% as shading increased from 47% to 80%, respectively. The morphological differences reported here are not surprising; sun leaves in high light intensities are generally thicker and smaller in surface area because they develop longer palisade cells to reduce water loss and exposure to the direct sunlight. In contrast, shade leaves generally have more chlorophyll, are thinner and larger to facilitate the capture of more sunlight (Taiz and Zeiger, 2002). In the present study, relative chlorophyll content increased with DLI. Although this is not commonly reported, Kitao et al. (2000) also observed that relative chlorophyll of white birch increased from 22.3 to 37.7 with increasing *PPF*.

Our results collectively indicate that DLI during stock plant production should be closely monitored and controlled to optimize photosynthesis, cutting yield, and quality. The species in this study can thus be categorized according to their physiological and morphological responses to DLI (low, medium or high) similar to the categorization used by Faust et al. (2005). All new guinea impatiens, petunia Tiny Tunia, and thunbergia

could be categorized as medium DLI crops (8 to 12 $mol \cdot m^{-2} \cdot d^{-1}$), i.e., a moderate greenhouse DLI is acceptable for cutting quality and production and photosynthetic efficiency is not compromised. Verbena, bacopa, and heliotrope could be categorized as high DLI crops (10 to 14 $mol \cdot m^{-2} \cdot d^{-1}$) during stock plant production as they exhibited the highest plasticity and were able to adjust their photosynthetic rates according to DLI.

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Table 1.1. Mean dark respiration (R_d), light saturated maximum photosynthesis (A_{max}), apparent quantum efficiency (ϕ), chlorophyll fluorescence (F_v/F_m), light compensation point (LCP), and light saturation range (LSR) in bacopa, petunia, verbena, new guinea impatiens, thunbergia, and heliotrope stock plants grown under various daily light integrals (DLI). Values within columns with the same letter are not significantly by Tukey's honest significant difference test at $P \leq 0.05$.

Mean DLI ($mol \cdot m^{-2} \cdot d^{-1}$)	R_d ($\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$)	A_{max} ($\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$)	ϕ ($\mu mol\ CO_2 \cdot \mu mol^{-1}$)	F_v/F_m	LCP ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	LSR[1] ($\mu mol \cdot m^{-2} \cdot s^{-1}$)
			Bacopa 'Breeze Upright White'			
4.4	4.3 d	16.0 d	0.117 c	0.837 a	40.5 c	1400 – 1600
6.7	5.5 cd	21.4 c	0.141 bc	0.844 a	52.3 bc	1650 – 1850
8.0	5.8 cd	22.8 c	0.124 c	0.849 a	60.9 b	1650 – 1850
9.1	6.5 bc	25.5 b	0.182 ab	0.851 a	67.2 b	1650 – 1850
12.0	8.3 ab	31.5 a	0.187 a	0.855 a	73.7 a	1650 – 1850
12.7	9.8 a	33.6 a	0.198 a	0.854 a	76.2 a	1650 – 1850
Significance						
DLI	***	***	***	NS	***	
			Petunia 'Tiny Tunia Violet Ice'			
4.6	2.7 c	16.8 b	0.089 ab	0.842 a	33.8 c	1275 – 1475
7.2	3.4 bc	17.4 b	0.081 ab	0.844 a	29.3 c	1400 – 1600
8.2	5.8 ab	25.1 a	0.117 a	0.850 a	49.5 bc	1500 – 1700
9.7	7.1 a	25.3 a	0.117 a	0.852 a	58.1 ab	1650 – 1850
12.1	4.1 bc	18.5 b	0.071 bc	0.846 a	57.7 ab	1450 – 1650
13.5	3.0 c	18.2 b	0.062 c	0.844 a	76.0 a	1450 – 1650
Significance						
DLI	***	***	***	NS	***	
			Verbena 'Aztec Red Velvet'			
4.6	2.6 b	16.4 c	0.034 c	0.836 d	60.0 c	1450 – 1650
7.9	3.5 b	16.9 c	0.055 bc	0.842 cd	67.9 bc	1575 – 1775
8.1	3.5 b	18.9 bc	0.065 abc	0.843 bcd	70.5 bc	1600 – 1800
10.4	6.0 a	22.3 ab	0.096 ab	0.846 abc	80.9 ab	1700 – 1800
12.1	5.4 a	24.0 a	0.094 ab	0.851 ab	79.4 ab	1700 – 1800

Table 1.1 continued

14.2	6.7 a	26.7 a	0.105 a	0.853 a	95.4 a	1700 – 1800
Significance						
DLI	***	***	***	***	***	
			Impatiens 'Celebrette Red'			
6.6	1.6 b	9.9 c	0.077 b	0.821 a	14.4 b	950 – 1150
6.7	1.9 b	10.8 c	0.080 ab	0.824 a	13.7 b	1025 – 1225
11.3	1.9 ab	13.1 bc	0.103 a	0.810 a	18.8 b	1025 – 1225
11.7	2.2 ab	13.1 bc	0.077 b	0.809 a	24.8 a	1025 – 1225
17.0	2.6 a	18.0 a	0.099 ab	0.787 b	25.8 a	1150 – 1350
18.2	2.5 a	16.5 ab	0.087 ab	0.771 b	26.9 a	1150 – 1350
Significance						
DLI	**	***	**	***	***	
			Impatiens 'Harmony White'			
6.1	1.6 c	10.7 c	0.086 a	0.832 a	17.1 b	1400 – 1600
6.5	1.9 bc	11.1 c	0.098 a	0.829 ab	19.4 b	1400 – 1600
10.9	2.4 ab	11.7 bc	0.124 a	0.827 abc	23.8 b	1525 – 1725
11.1	2.5 ab	12.4 abc	0.092 a	0.825 abc	32.6 b	1550 – 1750
16.7	2.8 a	14.2 ab	0.114 a	0.820 bc	34.0 a	1600 – 1800
17.3	2.9 a	14.4 a	0.126 a	0.819 c	36.1 a	1600 – 1800
Significance						
DLI	***	***	*	**	***	
			Impatiens 'Harmony Magenta'			
5.9	1.3 b	8.0 c	0.077 ab	0.821 a	17.9 a	200 – 400
6.5	1.6 b	8.4 c	0.086 ab	0.821 a	16.1 a	200 – 400
10.7	1.6 b	10.5 bc	0.079 ab	0.821 a	18.7 a	450 – 650
11.1	1.5 b	11.8 ab	0.072 b	0.816 ab	19.6 a	450 – 650
16.7	2.3 a	14.4 a	0.105 a	0.802 bc	22.4 a	775 – 995
17.1	2.3 a	14.2 a	0.096 ab	0.789 c	19.3 a	850 – 1050
Significance						
DLI	***	***	*	***	NS	

Table 1.1 continued

			Heliotrope 'Baby Blue'			
4.6	1.3 b	11.5 d	0.107 a	0.806 b	15.2 b	1350 – 1550
5.5	1.2 b	12.8 bcd	0.068 a	0.809 bc	15.5 b	1350 – 1550
8.1	1.7 ab	12.0 cd	0.095 a	0.823 abc	18.6 b	1400 – 1600
9.7	2.0 ab	13.7 abc	0.083 a	0.827 ab	18.2 b	1400 – 1600
12.2	2.1 ab	14.7 ab	0.097 a	0.829 a	18.9 b	1525 – 1725
13.2	2.5 a	15.8 a	0.074 a	0.834 a	25.8 a	1525 – 1725
Significance						
DLI	**	***	NS	***	***	
			Thunbergia 'Sunny Lemon Star'			
4.5	1.3 b	11.3 cd	0.010 a	0.766 c	18.3 c	275 – 475
4.8	1.5 b	10.3 c	0.081 abc	0.772 bc	21.8 bc	275 – 475
8.1	1.5 b	13.8 bc	0.070 bc	0.793 ab	20.9 bc	300 – 500
9.0	1.7 b	15.4 ab	0.074 bc	0.793 ab	24.5 b	300 – 500
12.1	2.2 a	16.2 ab	0.095 ab	0.794 ab	31.6 a	300 – 500
12.4	2.5 a	17.2 a	0.064 c	0.797 a	35.8 a	300 – 500
Significance						
DLI	***	***	**	***	***	

NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01 or 0.001, respectively.

[1]Light saturation ranges were calculated at 95% of mean A_{max} at PPF = 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Landhäusser and Lieffers, 2001).

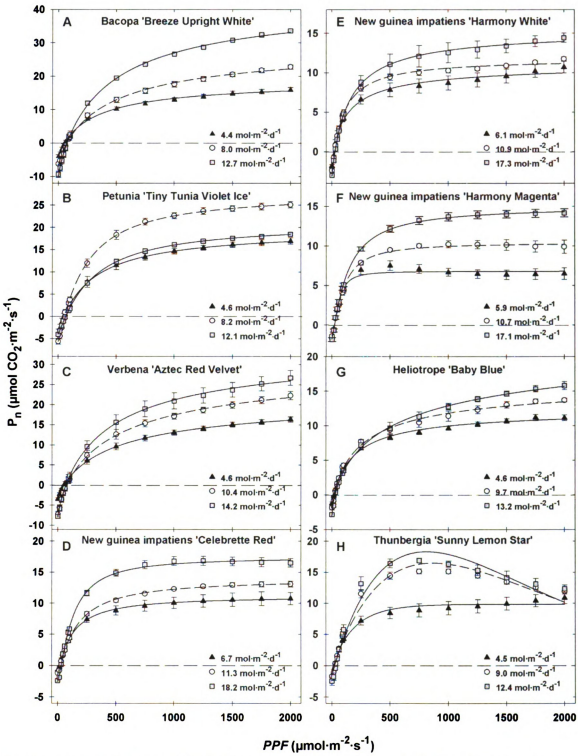

Fig. 1.1. Response of net photosynthesis per square centimeter of shoot or leaf area to photosynthetic photon flux (*PPF*) in bacopa, petunia, verbena, new guinea impatiens, heliotrope, and thunbergia stock plants grown under three daily light integrals. Each symbol represents an average of four plants and error bars represent standard errors of the mean. A nonlinear regression was fitted to the data for each DLI treatment.

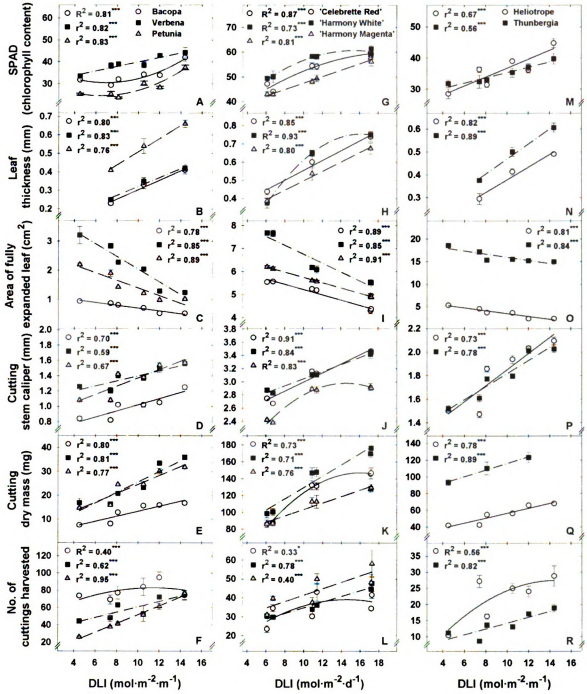

Fig. 1.2. Relationships between mean daily light integral (DLI) and relative chlorophyll content, leaf thickness, area of fully expanded leaf, cutting stem caliper, cutting dry mass, and number of cuttings harvested for eight herbaceous ornamental species including three new guinea impatiens cultivars (G to L). Each symbol represents an average of ten plants and error bars represent standard errors of the mean. Regression lines are presented with corresponding r^2 and R^2. *,***Significant at $P \leq 0.05$, or 0.001, respectively.

SECTION II

STOCK PLANT PHOTOSYNTHETIC DAILY LIGHT INTEGRAL: II. INFLUENCES ON STORAGE LIFE, PHOTOSYNTHETIC EFFICIENCY, AND SUBSEQUENT ROOTING OF HERBACEOUS CUTTINGS

Stock Plant Photosynthetic Daily Light Integral: II. Influences on Storage Life, Photosynthetic Efficiency, and Subsequent Rooting of Herbaceous Cuttings

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Introduction

Most herbaceous ornamental cuttings imported into the U.S. are harvested from stock plants in Mexico and Central America from December to March. Cuttings are shipped via air freight, clear phytosanitary inspections, and then are distributed to rooting stations and greenhouse growers. Nonrooted cutting shipments typically take 2 or 3 days from the time of harvest until receipt by growers (Rapaka et al., 2007a). Therefore, the propagation and production success of U.S. growers has become highly dependent on the quality of harvested cuttings and environmental conditions during their shipping and storage.

Successful short-term post-harvest cold storage of cuttings would allow cutting producers to regulate market supply during surplus production or peak demand to help accommodate propagation schedules (Hentig and Knösel, 1986; Joyce et al., 2000; Rajapakse et al., 1996). However, as shipping and storage duration increase, cuttings of some species can deteriorate from excess respiration, light exclusion, extreme temperatures, moisture loss, pathogen invasion, and ethylene accumulation (Purer, 1988; Rapaka et al. 2007a; Wang, 1987). These abiotic and biotic conditions can influence the aesthetic quality (e.g., necrotic lesions, senescence, desiccation, and chlorophyll degradation) of the cutting and subsequent performance during propagation and finishing. The environmental conditions and duration of storage and shipping of rooted and nonrooted cuttings influence the survival, quality, and rooting of herbaceous and woody ornamental species (Arteca et al., 1996; Conover, 1976; Druege et al., 2000; Garrido et al., 1998; Hentig and Knösel, 1986; Rajapakse et al., 1996; Wang, 1987 and 1994). For example, subsequent root mass and chlorophyll content of geranium (*Pelargonium*

×*hortorum* L.H. Bail.) nonrooted cuttings decreased by 98% and 59%, respectively, as 5-day storage temperature increased from 4 to 25 °C (Arteca et al., 1996).

Several studies have demonstrated that the light intensity provided to stock plants can influence the storage potential, quality, or rooting of cuttings harvested from those stock plants. These studies had species-specific results and primarily focused on storage potential (e.g. survival, quality, photosynthetic efficiency or carbohydrate status) or rhizosphere growth (e.g., rooting percentage, number, and biomass) of cuttings harvested from stock plants grown under different light environments (Bertram et al., 1989; Borowski et al., 1981; Kadner, 2005; Knox and Hamilton, 1982; Leaky and Storeton-West, 1992; Mesén et al., 2001; Rapaka et al., 2005; Rapaka et al., 2007a; Rapaka et al., 2007b). For example, 6% and 44% of variegated swedish ivy (*Plectranthus coleoides* hort. non Benth.) cuttings exhibited shoot damage when harvested from stock plants grown under a DLI of ≈3 and 43 $mol \cdot m^{-2} \cdot d^{-1}$, respectively, after storage at 5 °C for 7 d (Kadner, 2005). In *Portulaca grandiflora* Hook. and *Lantana camara* L., post-harvest leaf abscission of cuttings stored at 20 °C for 2 and 4 d decreased by 77% and 87%, respectively, as the pre-harvest stock plant DLI increased (Rapaka et al., 2007a, 2007b). In florist's daisy (*Chrysanthemum morifolium* Ramat.), the number of roots initiated after 2 weeks increased from 31 to 37 as stock plant DLI increased from 3 to 12 $mol \cdot m^{-2} \cdot d^{-1}$ (Borowski et al., 1981). The number of roots formed in obeche wood (*Triplochiton scleroxylon* K. Schum.) after 8 weeks decreased by 36% as stock plant irradiance increased from 106 to 246 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Leaky and Storton-West, 1992).

In section I, we determined that as stock plant DLI increased, photosynthetic light response parameters, cutting quality, and yield increased. Across all species, leaves

became thicker and individual leaf area was reduced with increasing stock plant DLI. In the present study, we performed experiments to determine how DLI during stock plant production influences the storage potential and subsequent rooting of cuttings exposed to a range of temperatures and durations between harvest and propagation. The specific objectives of this study were 1) to quantify the physiological responses of stored and nonstored cuttings harvested from stock plants grown under different mean DLIs with chlorophyll fluorescence (F_v/F_m), 2) to determine if stock plant DLI and storage temperature and duration influence root initiation and development, and 3) to make recommendations on desirable DLIs for stock plant production and storage temperatures and durations for nonrooted cuttings. Plant species were chosen for study based on recommendations from major floriculture cutting producers. We postulate that cuttings harvested from stock plants grown under higher DLI will tolerate longer storage durations.

Materials and Methods

Stock plant management and culture. Stock plant management and cultural procedures are as described in Section I. An abbreviated description follows, emphasizing the protocols used to create the different DLI environments. Vegetatively propagated stock plants of bacopa 'Breeze Upright White' [*Jamesbrittenia grandiflora* (Galpin) Hilliard], heliotrope 'Baby Blue' (*Heliotropium arborescens* L.), thunbergia 'Sunny Lemon Star' (*Thunbergia alata* Bojer ex Sims), and verbena 'Aztec Red Velvet' (*Verbena* ×*hybrida* Groenl. & Ruempl) were maintained at 20.9 ± 1.7 °C (mean ± standard deviation) under a 12-h photoperiod in glass-glazed greenhouses in East

Lansing, MI (43 °N lat.). The photoperiod consisted of a truncated 9-h natural day achieved using blackout cloth from 1700 to 0800 HR and day-extension lighting (≈2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at canopy level) from 1700 to 2000 HR with incandescent lamps. Three DLI environments were created on 23 Jan. 2006 using no shade cloth or permanent woven shade cloth with an open-weave design that reduced light by ≈30% and 55% (OLS 30 and 50; Ludvig Svensson, Charlotte, NC). Whitewash was applied to the greenhouse glazing to moderate the DLI during experiment repetitions. From 0800 to 1700 HR, high-pressure sodium (HPS) lamps provided a supplemental *PPF* of 10, 35, and 65 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant height when the ambient greenhouse *PPF* was <140 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for the 55%, 30% and 0% shade treatments, respectively. Line quantum sensors containing 10 photodiodes (Apogee Instruments, Inc., Logan, UT) were placed directly above stock plants in each DLI treatment. The *PPF* was measured every 10 s, and hourly averages were recorded by a CR-10 data logger (Campbell Scientific, Logan, UT). The mean DLI for the three light treatments during two-week periods prior to cutting harvests were: 8.2, 11.0, and 15.1 $mol \cdot m^{-2} \cdot d^{-1}$ (replication 1) and 4.5, 8.1, and 12.0 $mol \cdot m^{-2} \cdot d^{-1}$ (replication 2) for bacopa and thunbergia; 6.9, 11.1, and 14.7 $mol \cdot m^{-2} \cdot d^{-1}$ and 4.3, 7.7, and 11.4 $mol \cdot m^{-2} \cdot d^{-1}$ for heliotrope; and 8.4, 11.1, and 15.0 $mol \cdot m^{-2} \cdot d^{-1}$ and 4.5, 8.0, and 11.9 $mol \cdot m^{-2} \cdot d^{-1}$ for verbena.

Stock plants of new guinea impatiens 'Harmony Magenta' (*Impatiens hawkeri* Bull.) were maintained at 24.5 ± 1.1 °C with natural photoperiods and day-extension lighting from HPS lamps from 0600 to 2200 HR as previously described. DLI treatments were as described previously and two-week means prior to cutting harvests for

replications 1 and 2 were 6.4, 11.6, and 18.6 $mol \cdot m^{-2} \cdot d^{-1}$ and 6.9, 11.6 and 17.5 $mol \cdot m^{-2} \cdot d^{-1}$, respectively.

Cutting harvest and storage. Terminal bacopa, verbena, heliotrope, and new guinea impatiens cuttings (4 nodes and a 3-cm stem length) and internode thunbergia cuttings (2 nodes and a 4-cm stem length) were harvested from stock plants beginning at 0800 HR. Cuttings were harvested on 19 Apr. and 5 June 2006 (new guinea impatiens and verbena), 20 Apr. and 6 June 2006 (bacopa and thunbergia), and 1 May and 7 June 2006 (heliotrope). At each harvest, 840 cuttings were collected and separated into two replicated lots, each consisting of 15 cuttings. Cuttings were then placed in sealed polyethylene bags (volume: 0.946 L, thickness: 68.6 μm; Ziploc, S.C. Johnson & Son, Inc., Racine, WI). The sealed bags were placed in cardboard boxes (49 × 34 × 11 cm) and stored in environmental chambers for 0, 2, 4, or 6 d at 5.1 ± 0.8 °C, 9.8 ± 0.3 °C, or 14.9 ± 0.3 °C followed by 2 d at 21.0 ± 0.8 °C to simulate shipping. The nonstored cuttings were directly stuck in a propagation greenhouse.

Propagation environment. Cuttings were stuck in 72-cell (28-mL) plug trays (Landmark Plastic Corp., Akron, OH) in a 50% commercial mix [containing 70% peat moss, 21% perlite, and 9% vermiculite (Sure-Mix; Michigan Grower Products, Galesburg, MI)] and 50% screened coarse perlite (Therm-O-Rock East, Inc., New Eagle, PA) mix and rooted in a glass greenhouse under natural daylengths. Light intensity was modulated with the use of whitewash and shade curtains. A line quantum sensor (Apogee Instruments, Inc.) was positioned in the center of the propagation house and collected and integrated light intensity every 10 s. The actual mean DLI during propagation for replications 1 and 2 was 4.3 and 4.0 $mol \cdot m^{-2} \cdot d^{-1}$, respectively. Air

temperature was measured by an aspirated and enclosed thermocouple and media temperature was measured by a thermocouple placed 2 cm below the media surface. Actual mean air temperatures of the three harvest dates during replications 1 and 2 were 24.5 ± 0.7 and 25.2 ± 1.8 °C and media temperatures were 25.9 ± 2.0 and 25.0 ± 3.3 °C.

Overhead mist containing reverse osmosis water supplemented with water-soluble fertilizer was provided as necessary and delivered ($mg \cdot L^{-1}$): 50 N, 8 P, 42 K, 22 Ca, 1.0 Fe and Cu, 0.5 Mn and Zn, 0.3 B, and 0.1 Mo (MSU Special). Misting was controlled by an environmental computer as a function of time and accumulated *PPF*. Four seconds of misting were provided when the light integral reached 0.20 $mol \cdot m^{-2} \cdot h^{-1}$ or after 60 min, whichever occurred first. A vapor-pressure deficit of 0.3 kPa was maintained by the injection of steam or fine mist (Humidifan Turbo XE, Jaybird Manufacturing; State College, PA).

Data collection and analysis. Cutting visual quality was subjectively evaluated after harvest and storage (day 0), and days 7, 11, and 14 or 16 of propagation using a numerical rating scale from 1 to 5 where 1 = poor (severe injury; yellow or necrotic leaves), 2 = below average (moderate injury or leaf yellowing), 3 = acceptable (minor injury), 4 = good (no injury) and 5 = excellent (similar to fresh cuttings). After harvest and storage and day 11 of propagation, chlorophyll fluorescence (F_v/F_m) of ten cuttings per treatment was measured on the basal portion (upper epidermis) of the most fully expanded leaf using a portable chlorophyll fluorescence system (Plant Efficiency Analyzer; Hansatech Instruments Ltd., Norfolk, England). Leaves were dark-acclimated for 15 min with the manufacturer's plastic and foam clips before measurements were recorded.

The percentage of cuttings that had initiated roots was quantified after 7 d for bacopa, thunbergia, verbena, and new guinea impatiens cuttings that had initiated roots after 7 and 10 d for heliotrope. The percentage of cuttings that had fully rooted into the plug tray cell after 14 d or 16 d for heliotrope was recorded and defined as the marketable plug percentage (MPP). At that time, roots and shoots were separated and root dry weights were recorded after drying in an oven at 70 °C for one week.

Data were pooled for harvests 1 and 2 for all measured characteristics. Data were analyzed using SAS (SAS Institute, Cary, N.C.) mixed model procedure (PROC MIXED) for analysis of variance and pairwise comparisons between treatments were performed using Tukey's honest significant difference test (HSD).

Results

F_v/F_m. The mean DLI provided to bacopa stock plants had no effect on F_v/F_m of cuttings stored at 5 to 15 °C for 0 to 6 d (Fig. 2.1A, B, and C; Table 2.1). However, the F_v/F_m of cuttings decreased as the storage temperature increased from 5 to 15 °C and as storage duration increased from 0 to 6 d. F_v/F_m of heliotrope cuttings stored for 6 d at 5 or 10 °C increased linearly by 123% and 85%, respectively, as stock plant DLI increased from 4.3 to 14.7 $mol \cdot m^{-2} \cdot d^{-1}$ (Fig. 2.1D, E, and F). In contrast, F_v/F_m decreased linearly by 33% after 6 d of storage at 15 °C. With an increase in DLI from 6.7 to 18.0 $mol \cdot m^{-2} \cdot d^{-1}$, F_v/F_m of nonstored new guinea impatiens cuttings decreased from 0.821 to 0.795 (Fig. 2.1G, H, and I). The F_v/F_m of cuttings stored up to 6 d at 10 or 15 °C was similar to nonstored cuttings, but cuttings stored for ≥4 d at 5 °C had a reduced F_v/F_m. Chlorophyll fluorescence of nonstored thunbergia cuttings increased linearly as DLI increased from

4.5 to 15.1 mol·m^{-2}·d^{-1} (Fig. 2.1J, K, and L). Chlorophyll fluorescence of cuttings stored for 2 d at 5 or 15 °C or up to 4 d at 10 °C was similar to control (nonstored) cuttings. The photosynthetic efficiency of nonstored verbena cuttings or those stored for 2 d at any temperature increased linearly with an increase in DLI (Fig. 2.1M, N, and O). However, cuttings stored at 15 °C for 6 d had a reduced F_v/F_m. The mean F_v/F_m of bacopa, new guinea impatiens, thunbergia, and verbena cuttings 11 d after propagation was not influenced by DLI or storage treatments and was 0.836, 0.810, 0.785, and 0.825, respectively (data not presented).

Rooting percentage. At least 80% of nonstored bacopa cuttings or those held at 5 or 10 °C for 2, 4, or 6 d rooted within 7 d when harvested from stock plants under a DLI ≤12 mol·m^{-2}·d^{-1} (Fig. 2.2A, B, and C). However, significantly fewer cuttings stored at 15 °C for 6 d had initiated roots after 7 d of propagation compared to the nonstored control. In general, rooting of heliotrope after 10 d in propagation was inhibited as storage duration increased, regardless of storage temperature (Fig. 2.2D, E, and F). Regardless of storage temperature, no cuttings rooted after 10 d in propagation when stored cuttings were harvested from a mean DLI of 15.1 mol·m^{-2}·d^{-1}. The mean DLI provided to new guinea impatiens stock plants did not influence rooting percentage after 7 d of propagation (Fig. 2.2D, E, and F). Less than 40% of cuttings stored at 5 °C for 6 d had developed roots after 7 d of propagation. In contrast, all new guinea impatiens rooted when stored at 10 or 15 °C for up 6 d. In thunbergia, at least 90% of nonstored cuttings had roots within 7 d, regardless of mean stock plant DLI (Fig. 2.2J, K, and L). Rooting percentage was generally lower than 70% when cuttings were stored at 5 or 10 °C. Rooting percentage of verbena cuttings harvested from stock plants under a DLI of 15

$mol \cdot m^{-2} \cdot d^{-1}$ was generally lower than other DLI treatments (Fig. 2.2M, N, and O). Storage temperature and duration had no consistent effect on rooting when cuttings were harvested from stock plants grown under a mean DLI ≥ 12 $mol \cdot m^{-2} \cdot d^{-1}$.

Visual rating. As bacopa stock plant DLI and storage duration increased, the visual quality rating generally decreased (Fig. 2.3A, B, and C). However, cuttings were generally acceptable (rating ≥ 3) across all treatments. The average quality rating of nonstored heliotrope cuttings was between 3 and 4 (Fig. 2.3D, E, and F). Regardless of stock plant DLI, cuttings were visually unacceptable among all storage temperatures, especially after 6 d of storage. After 4 or 6 d of storage at 5 °C and after 6 d at 10 or 15 °C, new guinea impatiens cuttings were deemed unacceptable (Fig. 2.3G, H, and I). Storage duration had no effect on the visual rating of thunbergia cuttings (Fig. 2.3J, K, and L). Generally, visual ratings of stored cuttings were marginally acceptable or unacceptable (ratings between 2 and 3). Storage temperature did not affect the visual quality of verbena cuttings for the durations tested (Fig. 2.3M, N, and O). The visual quality of cuttings was acceptable even after 6 d of storage, although cuttings were superior when not stored or stored for shorter durations. However, the visual quality rating decreased linearly with stock plant DLI for cuttings stored at 15 °C for 2 to 6 d.

Marketable plug percentage. The MPP provides an indication of the ability of a group of cuttings to root within a desirable period of time (14 or 16 d). In bacopa, the magnitude of the influence of stock plant DLI on MPP was influenced by storage temperature and duration (Fig. 2.4 A, B, and C). Generally, the percentage of nonstored and stored bacopa cuttings that were marketable plugs at 14 d of propagation decreased as the stock plant DLI increased from 4.5 to 15.1 $mol \cdot m^{-2} \cdot d^{-1}$. Cuttings harvested from

stock plants that received a mean DLI of 4.5 $mol \cdot m^{-2} \cdot d^{-1}$ were well rooted when stored up to 6 d at 5 or 10 °C or 4 d at 15 °C. The percentage of nonstored heliotrope cuttings that were marketable plugs after 16 d of propagation decreased linearly from 80 to 0 as stock plant DLI increased from 4.3 to 14.7 $mol \cdot m^{-2} \cdot d^{-1}$ (Fig. 2.4 D, E, and F). Regardless of storage temperature and duration, ≤30% of cuttings were marketable plugs after 16 d of propagation. In nonstored and stored new guinea impatiens, ≤65% of cuttings were marketable plugs after 14 d of propagation (Fig. 2.4 G, H, and I). In addition, no cuttings stored for 6 d at 5 °C were marketable. There was no consistent effect of stock plant DLI on MPP when cuttings were stored. In thunbergia, most nonstored cuttings were fully rooted after 14 d (Fig. 2.4 J, K, and L). After 6 d of storage, ≥50% of cuttings were marketable plugs after 14 d of propagation. Storage temperature and duration had little or no effect on the percentage of verbena cuttings that were fully rooted and marketable (Fig. 2.4 M, N, and O). Generally, the MPP of verbena cuttings decreased with an increase in stock plant DLI in nonstored and stored cuttings.

Root dry mass. Root dry mass of bacopa cuttings stored for ≥4 d at 5 or 10 °C or for 4 d at 15 °C decreased linearly as stock plant DLI increased from 4.5 to 15.1 $mol \cdot m^{-2} \cdot d^{-1}$ (Fig. 2.5A, B, and C). In addition, root mass of cuttings harvested from a low DLI and stored for 6 d decreased with an increase in storage temperature. In heliotrope, as storage duration increased from 0 to 6 d, root dry mass decreased (Fig. 2.5D, E, and F). Root mass of cuttings stored for 6 d at any temperature studied was relatively low. As new guinea impatiens stock plant DLI increased from 6.7 to 18.0 $mol \cdot m^{-2} \cdot d^{-1}$, root dry mass of nonstored cuttings decreased linearly by 25% (Fig. 2.5G, H, and I). In addition, root mass of cuttings stored at 10 or 15 °C was at least as high as nonstored cuttings.

Storage temperature and duration interacted to influence the magnitude of the effects of stock plant DLI on root mass of thunbergia (Fig. 2.5J, K, and L). As DLI increased from 4.5 to 15.1 $mol \cdot m^{-2} \cdot d^{-1}$, the mean root dry mass of stored thunbergia cuttings increased from 26 to 38 mg. Nonstored verbena cuttings had a high root dry mass when harvested from stock plants grown under a DLI ≤ 12 $mol \cdot m^{-2} \cdot d^{-1}$ (Fig. 2.5M, N and O). Cuttings harvested from stock plants grown under a high DLI generally had a low root mass, regardless of storage treatment.

Discussion

In section I, we determined that stock plant photosynthesis, cutting yield, and the production of high-quality cuttings were maximized under high DLI. In this investigation, stock plant photosynthetic DLI influenced the storage life, quality, and subsequent root formation of cuttings. For example, as stock plant DLI increased from 8 to 15 $mol \cdot m^{-2} \cdot d^{-1}$, the percentage of stored and nonstored cuttings that initiated roots after 7 d of propagation decreased from 91 to 77 in bacopa, 80 to 57 in verbena, and 89 to 70 in thunbergia. Similar rooting responses to stock plant DLI have been reported in swedish ivy and geranium (Kadner, 2005; Rapaka et al., 2005). For example, rooting percentage after 12 d of propagation for swedish ivy cuttings harvested from stock plants grown under a DLI of ≈ 3 and 43 $mol \cdot m^{-2} \cdot d^{-1}$ and stored at 1 °C for 7 d decreased from 75 to 29 (Kadner, 2005).

Increased photosynthesis in plants grown under a high DLI may increase initial carbohydrate concentrations in the cuttings. For example, root initiation and development of geranium is dependent upon the availability of transportable sugars from

the stock plant to supply carbohydrates to the region of root regeneration (Rapaka et al., 2005). However, in chrysanthemum, root formation was promoted by a higher sucrose:starch ratio in leaves at harvest (Druege et al., 2000). In addition, Leaky and Storeton-West (1992) suggested that physiological and morphological differences (shoot and internode length, leaf area, stem caliper, and leaf thickness) of plants grown under different irradiances can also have indirect effects on rooting.

DLI during propagation can also influence rooting of cuttings (Section IV) and can impact the photosynthetic efficiency of those cuttings (Rapaka et al., 2005). For example, Rapaka et al. (2005) demonstrated that non-photochemical quenching was reduced when stored and nonstored geranium cuttings were harvested under increasing light intensities of 50 to 380 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and propagated under low light intensities ($\leq 100 \mu mol \cdot m^{-2} \cdot s^{-1}$). Thus, we speculate that long-term stock plant exposure to a high DLI impairs the short-term adjustment to lower light, leading to lower ATP production and reduced rooting. In addition, we postulate that in species that readily root (e.g., bacopa and verbena), rooting is inhibited when cuttings are harvested under a high DLI because the cuttings are not carbohydrate-limited. As carbohydrates are metabolized and become limited, cuttings begin to initiate roots under the lower light conditions of propagation once the photosynthetic apparatus has adjusted.

Thunbergia was the only species in our study in which root mass of stored cuttings increased with increasing stock plant DLI. The increase was most profound when the mean stock plant DLI increased from 4.5 to 8.1 $mol \cdot m^{-2} \cdot d^{-1}$. Similarly, root dry mass of begonia elatior-hybrid (*Begonia* ×*hiemalis* Fotsch), and root number of bell flower (*Campanula isophylla* Moretti) and chrysanthemum, increased by 170%, 67%,

and 19%, respectively, as supplementary irradiance to stock plants increased (Bertram et al., 1989; Borowski et al., 1981; Moe, 1977). However, in begonia elatior-hybrid, increasing stock plant DLI from 4.4 to 6.6 $mol \cdot m^{-2} \cdot d^{-1}$ resulted in a 14% reduction in the number of roots per cutting (Bertram et. al., 1989).

Chlorophyll fluorescence (F_v/F_m) is a non-destructive measurement to rapidly detect if environmental stresses have impaired or damaged the photosynthetic apparatus of plants before visual symptoms of injury occur (Maxwell and Johnson, 2000). In potted rose (*Rosa ×hybrida* L.), chilling sensitivity (variable fluorescence) decreased by >50% after exposure to 0 °C for 1 d (Hakam et al., 2000). In the present study, F_v/F_m was a good indicator of the maximum storage duration at 5 °C before visual tissue injury and reduced rooting occurred in the chilling-sensitive new guinea impatiens. For example, as storage duration increased from 2 to 4 d, F_v/F_m decreased from 0.806 to 0.771. In heliotrope, a 60% decrease in F_v/F_m was observed in cuttings harvested under a low DLI and exposed to 6 d of storage at 5 or 10 °C. Alternatively, if heliotrope cuttings were harvested under a high DLI and stored for 6 d at 15 °C, F_v/F_m decreased by 54%. Under warmer storage conditions, respiration rates of cuttings typically increase, leading to carbohydrate and protein depletion, senescence, chlorophyll degradation, and reduced photosynthesis (Arteca et al., 1996). Swedish ivy cuttings harvested under a DLI of 43 $mol \cdot m^{-2} \cdot d^{-1}$ (high carbohydrates) developed shoot damage after storage at 5 °C, whereas cuttings harvested under a DLI of 3 $mol \cdot m^{-2} \cdot d^{-1}$ (low carbohydrates) were susceptible to damage at 12 °C (Kadner et al., 2005). Consequently, the DLI that leads to maximal photosynthesis of stock plants is not always the best for storage potential and rooting of cuttings.

F_v/F_m, root initiation, and dry mass were higher when bacopa cuttings were stored at 5 and 10 °C. Therefore, we recommend that cuttings can be stored between 5 and 10 °C for up to 6 d plus 2 d of shipping. From our results, we recommend that heliotrope cuttings not be stored before or after shipping to avoid significant decreases in quality, F_v/F_m, and rooting. If cuttings must be stored, quality was least impacted when stored at 10 °C. New guinea impatiens can be stored up to 6 d at temperatures of 10 to 15 °C without significantly impacting quality and rooting. Exposing new guinea impatiens to 5 °C for more than 2 d will cause leaf senescence, and reductions in quality, F_v/F_m, and rooting. Thunbergia can be stored at 5 to 15 °C for up to 4 d without impacting quality.

This and previous research illustrates the importance of closely monitoring and controlling DLI during both stock plant production and propagation of nonrooted cuttings (Section I; Section IV). For example, as stock plant DLI increases, physiological and morphological plasticity, photosynthetic status, cutting quality, and yield generally increase and storage potential and rooting of cuttings can increase or decrease depending on the species. In contrast, as propagation DLI increases, rooting and quality generally increase and subsequent time to flower decreases. Therefore, species-specific DLI management can produce cuttings that can tolerate short-term storage and shipping without significant reductions in visual quality or photosynthetic efficiency, and lead to successful propagation and production of nonrooted ornamental cuttings.

According to the results in Section I and of the present study, stock plant mean DLI of bacopa, heliotrope, thunbergia, and verbena should be maintained between 8 to 12 $mol \cdot m^{-2} \cdot d^{-1}$ to optimize photosynthesis, cutting yield, quality, and have the least impact on storage potential and subsequent rooting. Stock plants of new guinea impatiens

should be maintained between 8 to 14 $mol \cdot m^{-2} \cdot d^{-1}$ to optimize cutting yield and quality, however further increases in DLI may pose challenges for growers in maintaining vegetative stock plants. For example, under high DLI, flower initiation in new guinea impatiens is accelerated and more frequent application of ethephon would be required to maintain vegetative stock plants.

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Table 2.1. Summary of analyses of variance for the effect of stock plant daily light integral (DLI), storage temperature and storage duration on the rooting percentage, visual quality rating, marketable plug percentage, and root dry mass of cuttings of five herbaceous species. Cuttings were stored in darkness for 0, 2, 4, or 6 d at 5, 10, or 15 °C followed by 2 d of simulated shipping at 20 °C. Rooting percentage was quantified after 7 d in the propagation environment for all species except heliotrope, which was evaluated on day 10. Cutting visual quality was subjectively evaluated using a numerical rating scale from 1 to 5 where 1 = poor (severe injury; yellow or necrotic leaves), 2 = below average (moderate injury or leaf yellowing), 3 = acceptable (minor injury), 4 = good (no injury) and 5 = excellent (similar to fresh cuttings). The percentage of cuttings that were marketable (i.e., fully rooted) in the plug cell was determined after 14 d (or 16 d for heliotrope) in the propagation environment.

	F_v/F_m	Rooting (%)	Visual quality	Marketable plug (%)	Root dry mass (mg)
	Bacopa 'Breeze Upright White'				
Source					
DLI	NS[z]	***	***	***	***
Temperature (T)	***	***	*	NS	**
Duration (D)	***	***	***	***	***
DLI × T	NS	**	*	**	***
DLI × D	NS	***	***	***	***
T × D	NS	***	*	***	**
DLI × T × D	NS	***	***	***	***
	Heliotrope 'Baby Blue'				
DLI	**	***	***	***	***
Temperature (T)	***	***	***	NS	***
Duration (D)	***	**	***	**	***
DLI × T	***	*	***	*	***
DLI × D	***	***	**	NS	*
T × D	NS	*	NS	NS	NS
DLI × T × D	***	NS	**	NS	NS
	Impatiens 'Harmony Magenta'				
DLI	**	NS	*	**	*
Temperature (T)	***	***	***	***	***
Duration (D)	***	***	***	***	**
DLI × T	*	NS	*	NS	NS
DLI × D	NS	NS	***	NS	NS
T × D	***	***	***	NS	***
DLI × T × D	NS	*	NS	NS	NS
	Thunbergia 'Sunny Lemon Star'				
DLI	*	***	***	***	**
Temperature (T)	**	*	*	***	NS
Duration (D)	***	NS	NS	**	NS
DLI × T	NS	***	**	***	NS
DLI × D	***	***	*	***	NS
T × D	NS	NS	***	***	***

Table 2.1 continued

DLI × T × D	*	NS	*	***	***
	Verbena 'Aztec Red Velvet'				
DLI	***	***	**	***	***
Temperature (T)	***	***	NS	NS	*
Duration (D)	***	NS	***	*	NS
DLI × T	***	***	NS	***	NS
DLI × D	***	**	***	***	*
T × D	***	***	***	***	NS
DLI × T × D	***	***	***	*	NS

[z] NS, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01 or 0.001, respectively.

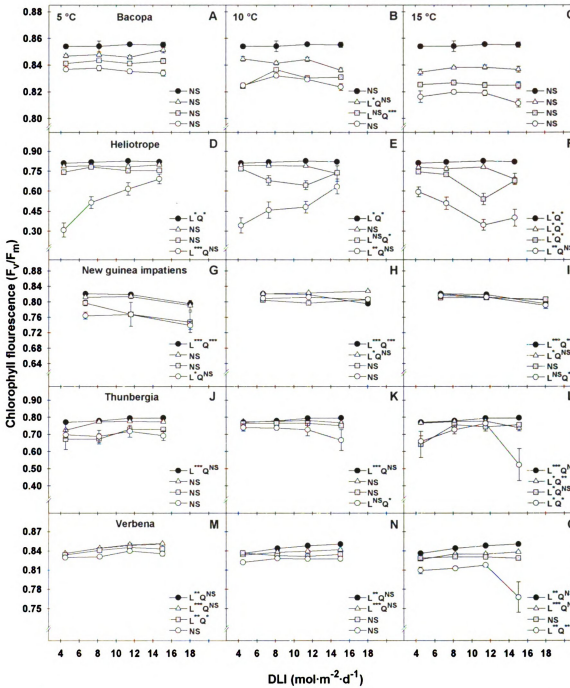

Fig. 2.1. Chlorophyll fluorescence (F_v/F_m) in bacopa, heliotrope, new guinea impatiens, thunbergia, and verbena after storage or harvest for nonstored cuttings. Cuttings were harvested from stock plants grown under three or four daily light integrals (DLI), stored for 0 (shaded circles), 2 (open triangles) , 4 (gray squares) or 6 d (open circles) at 5, 10, or 15 °C followed by 2 d of simulated shipping at 20 °C. Data were pooled for replications 1 and 2, each symbol represents a mean of twenty plants and error bars represent standard errors of the mean. L = linear and Q = quadratic. ^{NS}, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01 or 0.001, respectively.

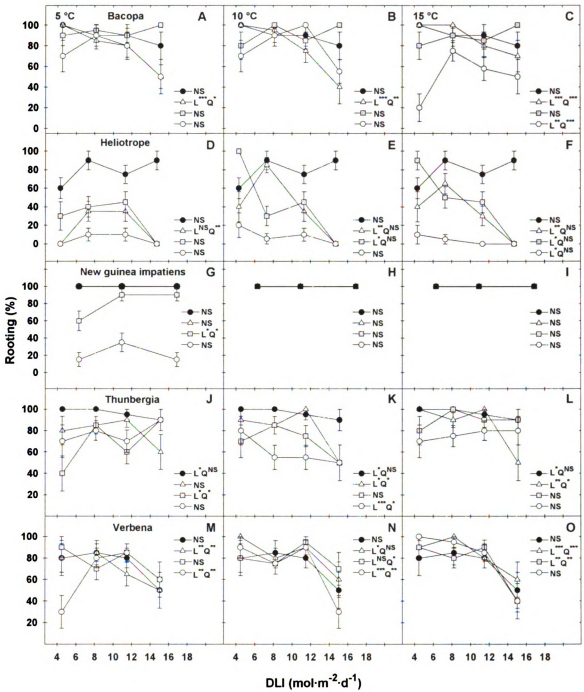

Fig. 2.2. Percentage of bacopa, new guinea impatiens, thunbergia, and verbena cuttings that had initiated roots after 7 d (or 10 d of propagation for heliotrope). Treatments are described in Fig. 2.1. Data were pooled for replications 1 and 2 and analyzed using a binomial distribution with logit transformation. Each symbol represents a mean of twenty plants and error bars represent standard errors of the mean. L = linear and Q = quadratic. ^{NS}, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01 or 0.001, respectively.

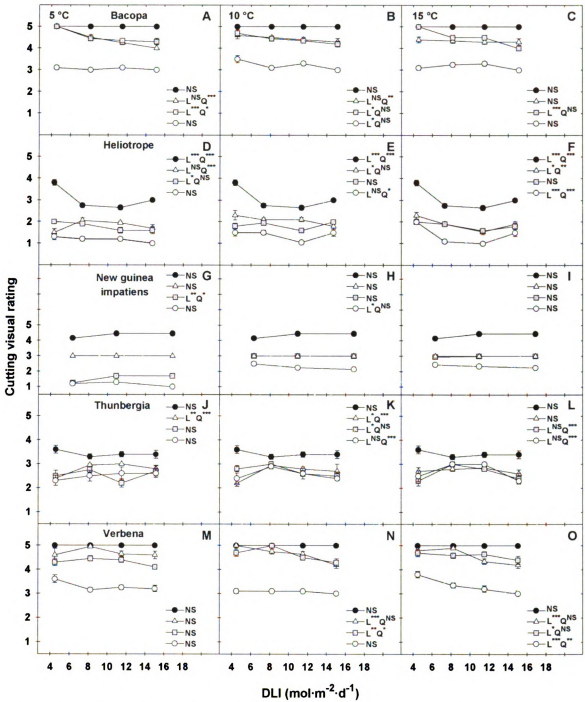

Fig. 2.3. Subjective visual quality rating of bacopa, new guinea impatiens, thunbergia, and verbena cuttings after 14 d (or 16 d for heliotrope of propagation) from 1 to 5 where 1 = poor (severe injury; yellow or necrotic leaves), 2 = below average (moderate injury or leaf yellowing), 3 = acceptable (minor injury), 4 = good (no injury) and 5 = excellent (similar to fresh cuttings). Treatments are described in Fig. 2.1. Data were pooled for replications 1 and 2, each symbol represents a mean of twenty plants, and error bars represent standard errors of the mean. L = linear and Q = quadratic. ^{NS}, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01 or 0.001, respectively.

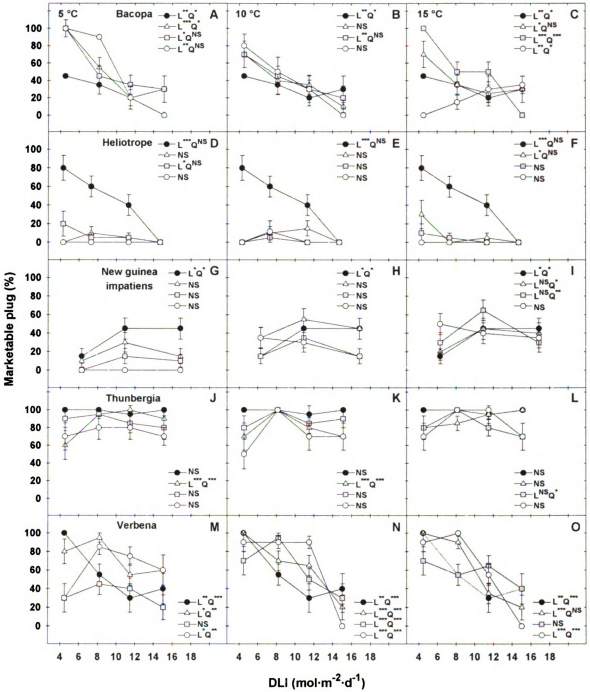

Fig. 2.4. Percentage of bacopa, new guinea impatiens, thunbergia, and verbena cuttings that were marketable or fully rooted in the plug cell after 14 d (or 16 days for heliotrope of propagation). Treatments are described in Fig. 2.1. Data were pooled for replications 1 and 2 and analyzed using a binomial distribution with logit transformation. Each symbol represents an average of twenty plants and error bars represent standard errors of the mean. L = linear and Q = quadratic. ^{NS}, *, **, *** Nonsignificant or significant at $P \leq$ 0.05, 0.01 or 0.001, respectively.

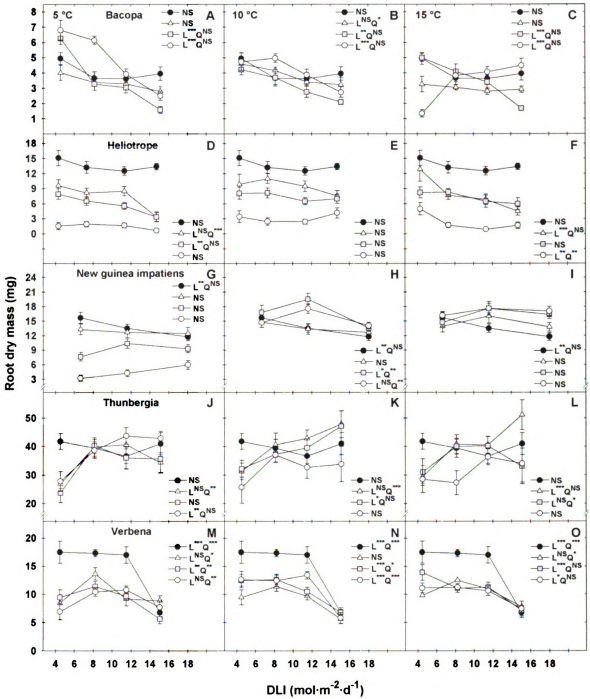

Fig. 2.5. Root dry mass in bacopa, new guinea impatiens, thunbergia and verbena after 14 d (or 16 d in heliotrope of propagation). Treatments are described in Fig. 2.1. Each symbol represents a mean of twenty plants and error bars represent standard errors of the mean. L = linear and Q = quadratic. ^{NS}, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01 or 0.001, respectively.

SECTION III

LOW TEMPERATURE STORAGE INFLUENCES MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF NONROOTED CUTTINGS OF NEW GUINEA IMPATIENS (IMPATIENS HAWKERI)

Low Temperature Storage Influences Morphological and Physiological Characteristics of Nonrooted Cuttings of New Guinea Impatiens (*Impatiens hawkeri*)

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Introduction

The demand for herbaceous shoot-tip cuttings of bedding and potted ornamental crops is continually increasingly in the United States and Europe. A majority of nonrooted vegetative cuttings are produced in Central America, Southern Europe, Northern Africa, and Eastern Asia, then are packaged, shipped, and subsequently rooted by greenhouse growers (Druege et al., 2004). During shipping, cuttings can be exposed to temperature extremes, which can negatively impact their quality and subsequent performance. Postharvest short-term shipping and storage protocols that preserve the quality and subsequent performance of ornamental cuttings are essential to the success of the floriculture industry.

Low temperatures are commonly used for postharvest storage of fruits and vegetables, in vitro propagules, transplants, and rooted cuttings (Heins et al., 1992; Bessembinder et al., 1993; Kubota and Kozai, 1995; Rajapakse et al., 1996). Short-term postharvest storage of cuttings would allow cutting producers to regulate market supply during surplus production or peak demand to help accommodate propagation and production schedules (Hentig and Knösel, 1986; Rajapakse et al., 1996; Joyce et al., 2000). However, cuttings can deteriorate with extended storage from excess respiration, light exclusion, exposure to extreme temperatures, moisture loss, pathogen invasion, and ethylene accumulation (Wang, 1987; Purer, 1988; Rapaka et al. 2007a). These abiotic and biotic factors can influence the aesthetic quality (e.g., necrotic lesions, senescence, desiccation, and chlorophyll degradation) of cuttings and their subsequent performance during production. In addition, the survival and rooting of herbaceous and woody ornamental species can be influenced by environmental conditions during shipping and

storage (Conover, 1976; Hentig and Knösel, 1986; Wang, 1987 and 1994; Garrido et al., 1996; Arteca et al., 1996; Rajapakse et al., 1996; Druege et al., 2000). For example, root mass and chlorophyll content decreased by 98% and 59%, respectively in geranium (*Pelargonium ×hortorum*) cuttings as 5-d storage temperature increased from 4 to 25 °C (Arteca et al., 1996).

In tropical and subtropical ornamental species, low temperature storage can cause chilling injury and tissue necrosis. Impairment of photosynthesis is a major determinant of chilling sensitivity and the development of injury symptoms (Hu et al., 2006). Reductions in photosynthesis and photosystem II (PS II) activity or chlorophyll fluorescence ratio (F_v/F_m) in some cases can be used as indicators of chilling-induced injury and photoinhibition (Maxwell and Johnson, 2000; Allen and Ort, 2001). In potted rose (*Rosa ×hybrida*), chilling sensitivity, determined by the variable fluorescence (F_v), decreased by >50% after exposure to 0 °C for 1 d (Hakam, 2000). In greenhouse ecotypes of tomato (*Lycopersicon esculentum*) exposured to 12/7 °C (day/night) temperatures for 10 d, net photosynthetic rate decreased by as much as 70% from pre-chilling levels (Hu et al., 2006). Further foliar symptoms of chilling injury include brown discoloration, water soaked or wilting tissue, pitting, and lesions (Lange and Cameron, 1998; Joyce et al. 2000; Pompodakis et al., 2005).

New Guinea impatiens is an ornamental plant species usually propagated from shoot-tip cuttings and is used as a bedding and potted flowering plant throughout the world. Larson (1995) estimated that the worldwide demand of New Guinea impatiens cuttings was well over 100 million plants. They are native to the Australian-New Guinea subtropical highlands that have average day and night temperatures of 25 to 30 °C and 18

to 21 °C, respectively (Erwin, 1995). Their base temperature, or the temperature at which the rate of plant development stops, is between 10 and 13 °C (Erwin, 1995). To our knowledge, a science-based protocol has not been developed for short-term storage of New Guinea impatiens.

We performed experiments to quantify how postharvest short-term storage, at a range of temperatures and durations, influences the physiology, quality, growth, and subsequent development of cuttings. The specific objectives of this study were: (1) to quantify the physiological responses of stored and nonstored cuttings with F_v/F_m and gas exchange measurements; (2) to evaluate how storage temperature and duration influence survival, fresh weight loss, root initiation, and visual and marketable quality of nonrooted cuttings; (3) to determine if time to flower, branch development, and plant height are influenced by storage; and (4) to develop recommendations for desirable storage temperatures and durations for New Guinea impatiens cuttings.

Materials and Methods

Plant material and culture. Rooted cuttings of New Guinea impatiens (*Impatiens hawkeri* Bull.) were grown in 16-cm (2.4-L) round containers (Dillen Products, Middlefield, OH, USA) filled with a mix containing 70% peat moss, 21% perlite, and 9% vermiculite (Sure-Mix; Michigan Grower Products, Galesburg, MI, USA) and maintained as stock plants. Plants were irrigated as necessary with reverse osmosis water supplemented with water-soluble fertilizer to provide the following (mg L^{-1}): 125 N, 12 P, 100 K, 65 Ca, 1.0 Fe and Cu, 0.5 Mn and Zn, 0.3 B, and 0.1 Mo (MSU Special; Greencare Fertilizers, Kankakee, IL, USA).

Stock plants were maintained in greenhouses with a 16-h photoperiod at 22.7 ± 1.8 (mean ± standard deviation) and 23.8 ± 1.6 °C and a mean photosynthetic daily light integral (DLI) of 12.5 and 12.0 mol m^{-2} d^{-1} for 'Harmony Magenta' and 'Harmony White', respectively. Light transmission through the greenhouses was reduced by applying whitewash to the glass. The photoperiod was a constant 16 h (6 a.m. to 10 p.m.), consisting of natural daylengths, with day-extension lighting from high-pressure sodium (HPS) lamps that delivered a supplemental photosynthetic photon flux density (PPFD) of 50 µmol m^{-2} s^{-1} at plant height [as measured with a light quantum sensor containing 10 photodiodes (Apogee Instruments, Inc., Logan, UT, USA)]. Ethephon (Florel; Rhône-Poulenc Ag Company, Research Triangle Park, NC, USA) with a surfactant (Capsil; Aquatrols, Paulsboro, NJ, USA) was applied as a foliar spray at a concentration of 500 or 750 mg L^{-1} and a volume of 0.2 L m^{-2} every 2 weeks to abort flower buds. Cuttings were harvested from stock plants at least 7 d after the last ethephon application.

Cutting harvest and storage. Uniform cuttings with four leaves and a 3-cm stem length were harvested from stock plants beginning at 8:00 a.m. on 23 August 2004 and 5 March 2005 ('Harmony Magenta') and 23 July 2004 and 15 November 2005 ('Harmony White'). At each harvest, 600 cuttings were collected and separated into lots of 16 cuttings. In year 1, half of the cuttings were immersed in reverse osmosis water for 2 min (moist cuttings) and the others were kept dry. All cuttings were then placed in 23 × 19 cm high-density polyethylene bags with 12 perforations (≈6 mm in diameter) to allow O_2 and CO_2 exchange (Polyproductos, Guatemala City, Guatemala). The bags were placed in shipping boxes (49 × 34 × 11 cm) and stored in environmental chambers for 0, 1, 2, 3,

4, or 5 d at 0.7 ± 2.2 °C, 5.7 ± 1.8 °C, 10.9 ± 1.2 °C, 15.3 ± 0.7 °C, 21.0 ± 0.5 °C, 26.2 ± 0.7 °C and 30.0 ± 0.6 °C. The nonstored cuttings were directly placed in a propagation house for rooting.

Propagation environment. After storage treatments, cuttings were stuck in 72-cell (28 mL) plug trays (Landmark Plastic Corp., Akron, OH, USA) containing a 50% commercial mix [70% peat moss, 21% perlite, and 9% vermiculite (Sure-Mix; Michigan Grower Products, Galesburg, MI, USA)] and 50% screened coarse perlite (Therm-O-Rock East, Inc., New Eagle, PA) and rooted in a glass greenhouse under natural daylengths. Light intensity was modulated with the use of whitewash and shade curtains (OLS 50; Ludvig Svensson, Charlotte, NC, USA). Air temperature was measured by an aspirated and enclosed thermocouple (TT-E-36; Omega Engineering Inc., Stamford, CT, USA) and media temperature was measured by a thermocouple (TT-E-24; Omega Engineering Inc.) placed 2 cm below the media surface. Actual mean air temperatures were 24.2 ± 1.5 and 25.9 ± 0.8 °C (year 1) and 23.5 ± 2.3 and 23.1 ± 2.2 °C (year 2) and media temperatures were 23.6 ± 1.3 and 24.4 ± 1.4 °C (year 1) and 23.8 ± 2.0 and 23.1 ± 2.2 °C (year 2) for 'Harmony Magenta' and 'Harmony White', respectively.

Overhead mist containing reverse osmosis water and water-soluble fertilizer was provided as necessary and delivered (mg L^{-1}): 50 N, 8 P, 42 K, 22 Ca, 1.0 Fe and Cu, 0.5 Mn and Zn, 0.3 B, and 0.1 Mo (MSU Special). Misting was controlled by an environmental computer as a function of time and accumulated PPFD. A line quantum sensor (Apogee Instruments, Inc.) was positioned in the center of the propagation house and collected and integrated light intensity every 10 s. Four seconds of misting were provided when the accumulated light intensity was 0.20 mol m^{-2} h^{-1} or after 60 min,

whichever occurred first. A vapor-pressure deficit of 0.3 kPa was maintained by the injection of steam or fine mist (Humidifan Turbo XE, Jaybird Manufacturing; State College, PA, USA). The mean propagation DLI during years 1 and 2 were 5.2 and 3.0 mol m^{-2} d^{-1} and 3.8 and 3.5 mol m^{-2} d^{-1} for 'Harmony Magenta' and 'Harmony White', respectively.

Effects of storage temperature and duration on cutting physiology and rooting. Prior to storage, the fresh mass of 5 randomly selected cuttings per treatment was recorded and individually tagged for future reference. Upon removal from storage, the tagged cuttings were re-weighed to determine fresh weight loss percentage. Cutting quality was assessed after harvest and storage (day 0), and days 7, 11, and 16 during rooting using a numerical rating scale from 1 to 5 where 1 = poor (severe injury or wilting/ yellow and/or necrotic), 2 = below average (injury or wilting/leaf necrotic spots), 3 = average (minimally acceptable), 4 = good (acceptable, reduced turgor) and 5 = excellent (above average/ no injury/ turgid).

After harvest and storage, F_v/F_m of ten cuttings per treatment was measured on the basal portion (upper epidermis) of the most fully expanded leaf using a portable chlorophyll fluorescence system (Plant Efficiency Analyzer; Hansatech Instruments Ltd., Norfolk, England). Leaves were dark-acclimated for 15 min within the manufacturer's plastic and foam clips before measurements were recorded. Single-leaf gas exchange measurements of 'Harmony White' were performed when roots had initiated after 11 d of propagation. Net photosynthesis (P_n), dark respiration (R_d), stomatal conductance, and transpiration measurements were performed in the greenhouse between 9:00 a.m. and 1:00 p.m. and were blocked by storage temperature to reduce time of day effects.

Measurements were conducted using a portable photosynthesis system (LI-6400, LI-Cor, Lincoln, NE, USA) fitted to a 6 cm^2 leaf chamber with an LED light source (6400-02B; red at 665 nm and blue at 470 nm) at a PPFD of 0 and 400 μmol m^{-2} s^{-1}. The reference CO_2 concentration inside the leaf chamber was 400 μmol mol^{-1} and the flow of air into the chamber was 250 μmol s^{-1}. Leaf temperature inside the leaf chamber was maintained at 24.8 ± 0.9 °C using dual Peltier devices that heated or cooled the air circulating through the chamber. Immediately after measurements were recorded, leaves inside the chamber were excised, placed in plastic packages and stored at 10 °C. Leaf area was determined by scanning the leaf through a leaf area meter (LI-3000, Li-Cor, Lincoln, NE, USA) three times and the mean was recorded.

The percentage of cuttings that had initiated roots after 11 and 16 d in the propagation greenhouse was recorded. The percentage of cuttings that had survived and fully rooted into the plug tray cell after 16 d was recorded and defined as the marketable plug percentage (MPP). The number of roots and length of the longest root were recorded for each cutting after 16 d in propagation.

Effects of storage and duration on subsequent flowering. Sixteen days after the start of propagation, 10 cuttings from each storage treatment were transplanted into 10-cm square pots containing a peat-based media (Suremix). The plants were grown at 20 °C under a 16-h photoperiod (as described previously). Actual forcing temperatures from transplant until flowering were 22.6 ± 2.5 and 20.0 ± 1.0 °C (year 1) and 24.3 ± 1.6 and 24.1 ± 1.2 °C (year 2) for 'Harmony White' and 'Harmony Magenta', respectively. Mean DLI from transplant to flowering was and 11.0 and 7.0 mol m^{-2} d^{-1} (year 1) and 10.1 and 12.1 mol m^{-2} d^{-1} (year 2) for 'Harmony White' and 'Harmony Magenta', respectively.

Time to flower from the beginning of propagation, number of lateral branches, and plant height (from media surface to the shoot apex) were recorded on the date the first flower opened on each plant.

Data analysis. Cuttings were randomly assigned to each storage treatment. Data were pooled for years 1 and 2 for all measured characteristics. The moisture treatment in year 1 did not significantly influence any of the measured characteristics except visual quality after harvest, and data were therefore pooled. Data were analyzed using SAS (SAS Institute, Cary, NC, USA) mixed model procedure (PROC MIXED) for analysis of variance.

Results and Discussion

Cutting physiology and visual appearance. The maximum potential quantum yield of PS II (dark adapted F_v/F_m) of nonstored cuttings was 0.838 and 0.845 for 'Harmony White' and 'Harmony Magenta', respectively (Fig. 3.1A and B). For both cultivars, F_v/F_m of cuttings stored at temperatures of 10 to 20 °C for up to 5 d remained similar to that of the control cuttings. The F_v/F_m ratio of cuttings stored $\geq$ 1 d significantly decreased after cuttings were removed from storage temperatures <10 °C and >20 °C. For example, as storage duration increased from 1 to 5 d at 0 °C, F_v/F_m of 'Harmony White' decreased by 9% to 65% compared to the nonstored mean (Fig. 3.1A). F_v/F_m data suggests that changes to membranes and photosynthetic systems are occurring at low temperatures (chilling injury) and desiccation is occurring at warmer temperatures. F_v/F_m also been utilized to determine if low temperature injury has occurred in potted and cut rose, and heliotrope (*Heliotropium arborescens*) cuttings (Hakam et al., 2000;

Pompodakis et al., 2005, Section II). For example, F_v/F_m of stored heliotrope decreased by 62% as 5 °C storage duration increased from 0 to 6 d. However, F_v/F_m was not a practical index for assessing low temperature injury of cold-stored roses because there was no correlation between F_v/F_m and vase life (Pompodakis et al., 2005).

As storage temperature and duration increased, the fresh weight loss percentage of cuttings increased linearly in 'Harmony White' and 'Harmony Magenta' (Fig. 3.1C and D). Fresh weight loss of spider flower (*Grevillea*) inflorescences was 16, 26, and 31% after 12 d storage at temperatures of 0, 5, and 10 °C, respectively (Joyce et al., 2000). In geranium cuttings, as storage temperature increased from 4 to 25 °C, fresh leaf weight decreased by ≈60% after 5 d (Arteca et al., 1996). In the present study, a fresh weight loss percentage >20% was characterized by severe wilting in both cultivars. However, turgor of cuttings in all storage treatments was restored within 24 h of being placed in the propagation greenhouse. It is not uncommon for horticultural crops to be exposed to temperature exceeding 30 to 40 °C for short durations during harvest and postharvest distribution (Marangoni et al., 1996). Fresh weight loss of detached fruits and vegetables is often the first result of exposure to high temperature because of the difference in water content between plant tissues and the surrounding air (Marangoni et al., 1996).

Subjective visual quality ratings immediately following storage generally decreased linearly with increasing storage duration and temperature for 'Harmony White' (Fig. 3.1E). However, after 5 d of storage, visual ratings of cuttings stored at ≤5 °C decreased as symptoms of chilling injury became apparent. Initial ratings of cuttings stored at 0 or 5 °C for 1 or 3 d were comparable to or higher than cuttings stored at higher temperatures because symptoms of chilling injury did not appear until 24 h after they

were placed in propagation. Most commercial greenhouse growers visually evaluate shipments of cuttings and those that appear to be in poor visible condition are often refused or monetary claims are filed.

The response of storage temperature on P_n varied with length of storage. No trends relating storage temperature and P_n of 'Harmony White' were apparent after 1 d of storage (Fig. 3.2A). After 3 d of storage, P_n increased linearly from 5.1 to 8.9 μmol CO_2 m^{-2} s^{-1} with storage temperature. After 5 d of storage, P_n increased with temperature up to 20 °C, followed by a decrease at higher temperatures. In addition, P_n of stored cuttings decreased by 28% after only 1 d of storage at 0 °C. Although cuttings stored for 5 d at 0 °C had not rooted during the initial 11 d of propagation, gross photosynthesis (P_n + R_d) of 4.4 μmol CO_2 m^{-2} s^{-1} (Fig. 3.3G; Fig. 3.2A and B). Only cuttings stored at 20 °C maintained a relatively constant P_n of 7.6 to 9.0 μmol CO_2 m^{-2} s^{-1} as storage duration increased from 1 to 5 d. R_d responded similarly to temperature as P_n, however after 1 d of storage, a linear decrease up to 25 °C was observed (Fig. 3.2B). The highest rates of R_d were present in cuttings stored for 3 or 5 d at 0 °C. In general, rooted cuttings with high P_n also had a low R_d. In contrast, P_n of nonrooted geranium cuttings declined before R_d decreased when measured 1 d following 25 °C storage for 0 to 5 d (Arteca et al., 1996).

No trends relating storage temperature and transpiration were apparent after 1 d of storage (Fig. 3.2C). After 3 and 5 d of storage, a linear and quadratic response, respectively, relating transpiration rates to temperature were observed. In poinsettia (*Euphorbia pulcherrima*) cuttings, transpiration increased from 0.2 to 0.3 g h^{-1} after 15 and 20 d in propagation, respectively (Wilkerson et al., 2005). This 50% increase in

transpiration was attributed to the fact that cuttings had formed roots >1 mm in length. From these results, we postulate that the increased ability for gas exchange and transpiration of cuttings exposed to 1 d of storage compared to a longer storage period is a direct result of earlier root initiation and development.

Cutting survival and rooting. After 16 d of propagation, at least 97% of 'Harmony White' and 'Harmony Magenta' cuttings stored for 1 d at temperatures between 0 to 30 °C survived (Fig. 3.3A and B). As storage duration increased to 5 d, survival percentage decreased to 0, 4, and 70 for 'Harmony White' cuttings stored at 0, 5, and 30 °C, and 0 for 'Harmony Magenta' stored at 0 °C. Similarly, sweet basil (*Ocimum basilicum*) is a chilling-sensitive crop and develops darkened lesions and a water-soaked appearance when stored at 5 °C (Lange and Cameron, 1997). Rajapakse et al. (1996) reported that no non-acclimated garden chrysanthemum (*Dendranthema ×grandiflorum*) rooted cuttings survived when stored at –2 to –3 °C for 1 d. However, in some species, chilling sensitivity can be overcome by acclimating cuttings prior to cold storage. For example, sweet basil can survive 5 °C storage if previously chill-hardened for 1 d at 10 °C (Lange and Cameron, 1997). Chill-hardening of New Guinea impatiens cuttings may increase their postharvest tolerance of temperatures between 0 to 5 °C. Therefore, the potential exists for New Guinea impatiens cuttings to be stored with other chilling-insensitive cuttings, but further research is necessary.

The visual quality of both cultivars after 16 d of propagation was not influenced by 1 d of storage (Fig. 3.3C and D). 'Harmony White' cuttings were rated below average (rating of 2) or poor (rating of 1) when stored for at least 3 d at temperatures <15 and >25 °C because of leaf senescence or the presence of necrotic lesions. Results with 'Harmony

Magenta' were similar. Rooted chrysanthemum 'Emily' and 'Naomi' cuttings stored at 0 °C became unmarketable after one week due to leaf necrosis (Rajapakse et al., 1996). Visual quality ratings of cuttings stored at warmer temperatures (15 to 25 °C) improved immediately after storage because they regained turgor after being placed in propagation and did not develop necrotic lesions.

After 16 d of propagation, 65% or 70% of nonstored cuttings were marketable plugs (Fig. 3.3E and F). The percentage of cuttings that became marketable plugs after 16 d of propagation was influenced by storage temperature and duration in both cultivars ($P \leq 0.001$). However, there was no consistent effect of storage temperature on the percentage of cuttings that were marketable plugs in 'Harmony White'. A quadratic increase in marketable plug percentage up to 20 °C after 1 or 3 d of storage was observed in 'Harmony Magenta'.

Root initiation and development was influenced more by long-term exposure to extreme storage temperatures (0 or 30 °C) in 'Harmony White' than in 'Harmony Magenta' (Fig. 3.3G and H). For example, 'Harmony White' and 'Harmony Magenta' cuttings stored at 30 °C for 5 d formed 86% and 7% fewer roots, respectively, than the nonstored control. Arteca et al. (1996) reported similar results in geranium 'Snowmass' cuttings: root weight decreased by 98% when cuttings were stored at 25 °C for 5 d compared to nonstored cuttings. Similarly, the number of roots formed in geranium 'Fire' and 'Katinka' decreased by 34% and 50%, respectively, after 4 d of storage at 21 °C (Mutui et al., 2005). For both cultivars, correlations between F_v/F_m and root number were established (Fig. 3.4A and B). After 5 days of storage, as F_v/F_m decreased, root number after 16 days of propagation decreased.

Under appropriate environmental conditions, New Guinea impatiens cuttings become well-rooted and marketable after 3 to 4 weeks of propagation (Mikkelsen, 1995). In the current study, root evaluations were made after 16 d of propagation to ensure that residual effects of storage on rooting were captured, which may have led to a lower percentage of the nonstored cuttings becoming marketable plugs. Root initiation and development in 'Harmony Magenta' cuttings is generally slower, and is impacted to a lesser extent by environmental parameters such as propagation DLI, than 'Harmony White' (Section IV).

Subsequent flowering. There were no apparent trends on the effects of cutting storage temperature and duration on subsequent time to flower in either 'Harmony White' and 'Harmony Magenta' (Fig. 3.5A and B). In contrast, time to flower and flower diameter were reduced by 13% and 35%, respectively, by low-temperature storage in rooted chrysanthemum 'Emily' and 'Naomi' cuttings (Rajapakse et al., 1996). We believe that time to flower in 'Harmony White' was influenced by the DLI received during forcing. In years 1 and 2, the mean DLIs were 11.0 and 7.0 mol m^{-2} d^{-1} and consequently plants forced in year 1 flowered ≈16 d earlier. According to Whitman et al. (2000), plant temperature of New Guinea impatiens generally increases when grown under a higher DLI and therefore flower earlier.

The number of lateral branches developed at first flowering was influenced by storage duration in 'Harmony White' and by both temperature and duration in 'Harmony Magenta' (Fig. 3.5C and D). However, no consistent trends were apparent. Plant height was influenced by storage temperature and duration, however no apparent trends were observed in either cultivar (Fig. 3.5E and F).

Conclusion

In this study, we have demonstrated that post-storage F_v/F_m of New Guinea impatiens is influenced by storage temperature and duration and can be used as an indicator of postharvest plant quality and rooting success. Although cuttings stored at temperatures ≥15 °C exhibited excessive fresh weight loss, this parameter was not a good indicator of survival and rooting during propagation. In general, cuttings that survived storage treatments flowered at a similar time as nonstored cuttings, and in most instances, branch number was acceptable. Our experiments collectively indicate that New Guinea impatiens 'Harmony White' and 'Harmony Magenta' cuttings can be stored for up to 5 d at temperatures between 10 to 20 °C without severely impacting postharvest photosynthetic recovery, survival, visual quality, and rooting.

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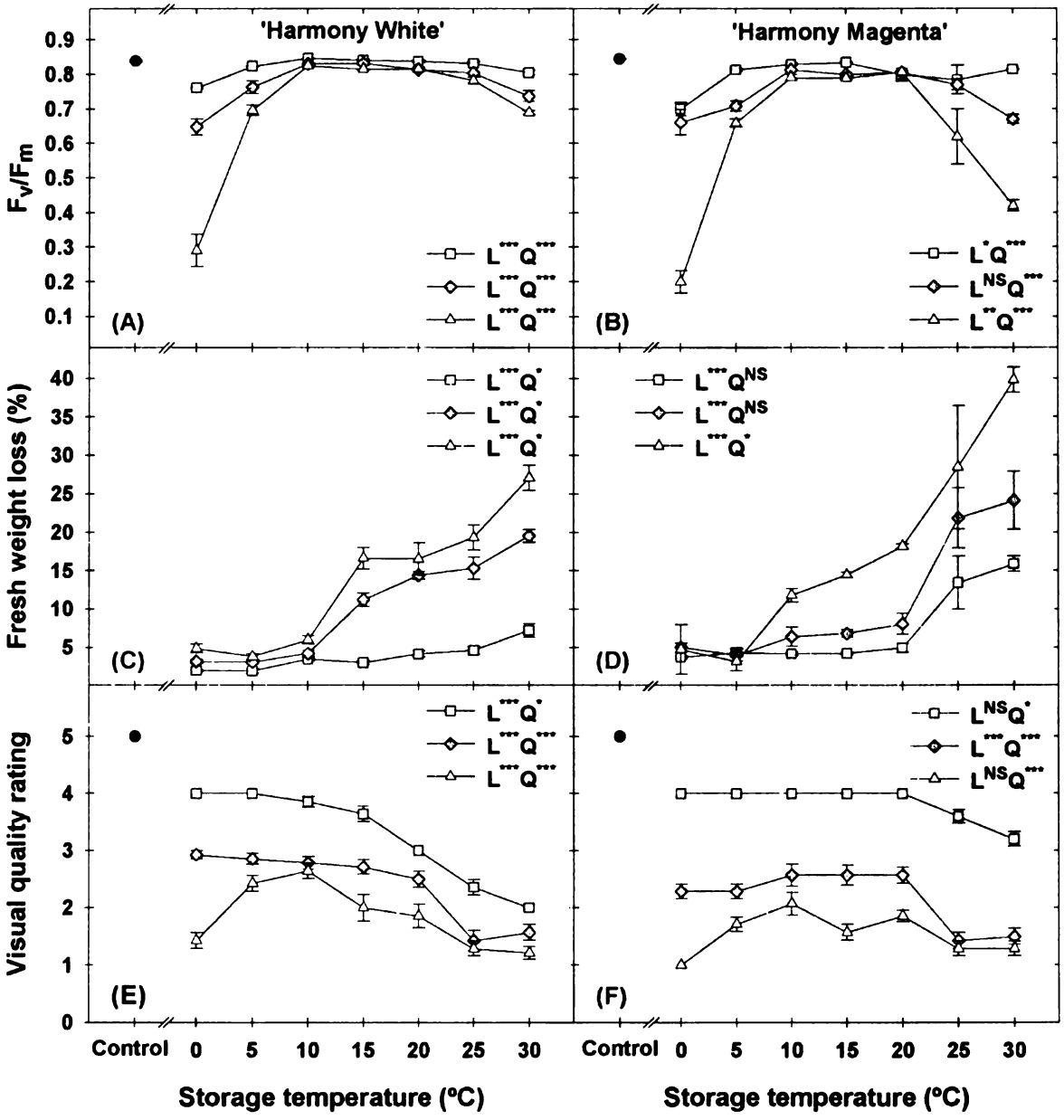

Fig. 3.1. Influence of storage temperature and duration on chlorophyll fluorescence (F_v/F_m), fresh weight loss percentage, and visual quality of nonrooted cuttings of two cultivars of New Guinea impatiens. Cutting quality was subjectively evaluated using a numerical scale from 1 to 5 where 1 = poor (severe injury or wilting/ yellow and/or necrotic), 2 = below average (injury or wilting/leaf necrotic spots), 3 = average (minimally acceptable), 4 = good (acceptable, reduced turgor) and 5 = excellent (above average/ no injury). Cuttings were harvested from stock plants and stored for 0 (shaded circles), 1 (gray squares), 3 (dark gray diamond), or 5 (open triangles) d at 0, 5, 10, 15, 20, 25 or 30 °C and rooted in a propagation greenhouse. Data were pooled for years 1 and 2. Each symbol represents a mean of twenty plants and error bars represent standard errors of the mean. L = linear and Q = quadratic. ^{NS}, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01 or 0.001, respectively.

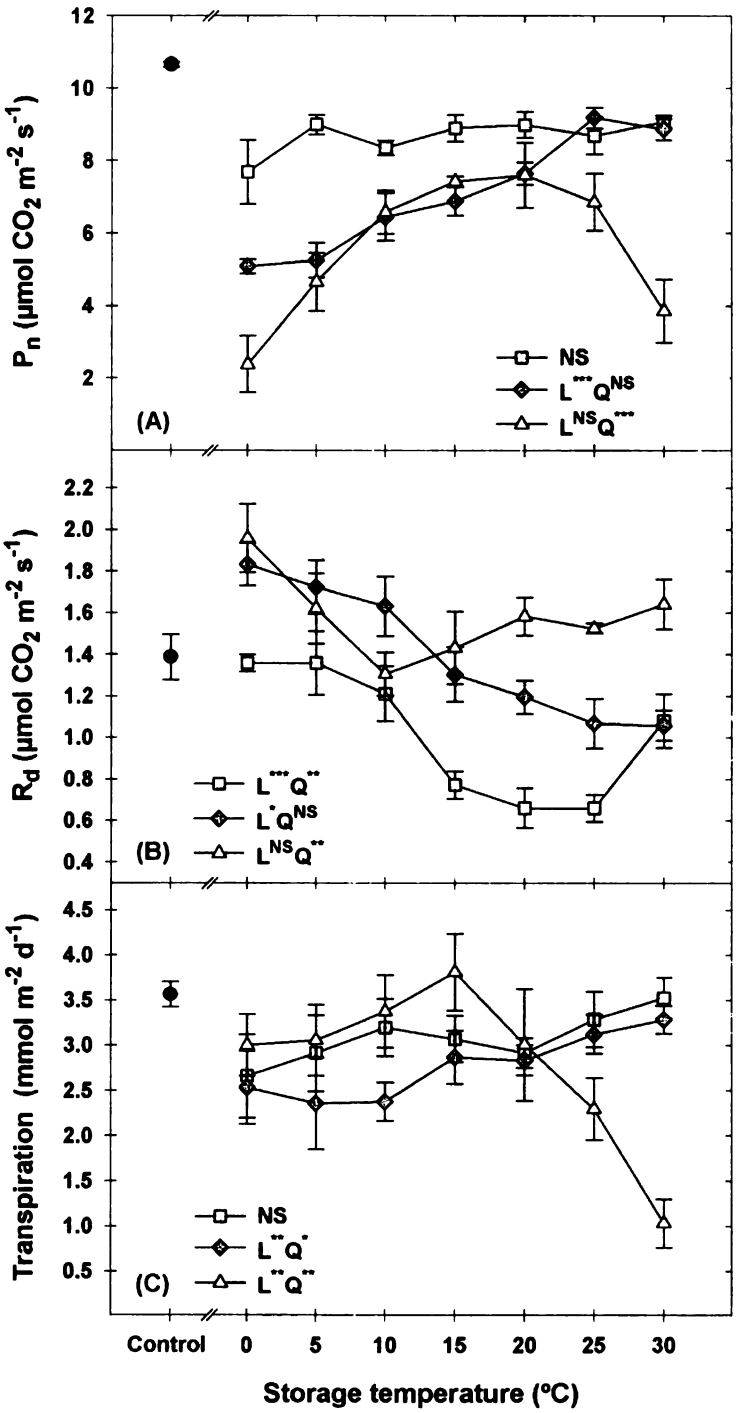

Fig. 3.2. Influence of storage temperature and duration on net photosynthesis (P_n), dark respiration R_d), and transpiration after 11 d of propagation in New Guinea impatiens 'Harmony White'. Cuttings were harvested from stock plants and stored for 0 (shaded circles), 1 (gray squares), 3 (dark gray diamond), or 5 (open triangles) d at 0, 5, 10, 15, 20, 25 or 30 °C and rooted in a propagation greenhouse. Data were pooled for years 1 and 2. Each symbol represents a mean of ten plants and error bars represent standard errors of the mean. L = linear and Q = quadratic. ^{NS}, *, *** Nonsignificant or significant at $P \leq 0.05$, or 0.001, respectively.

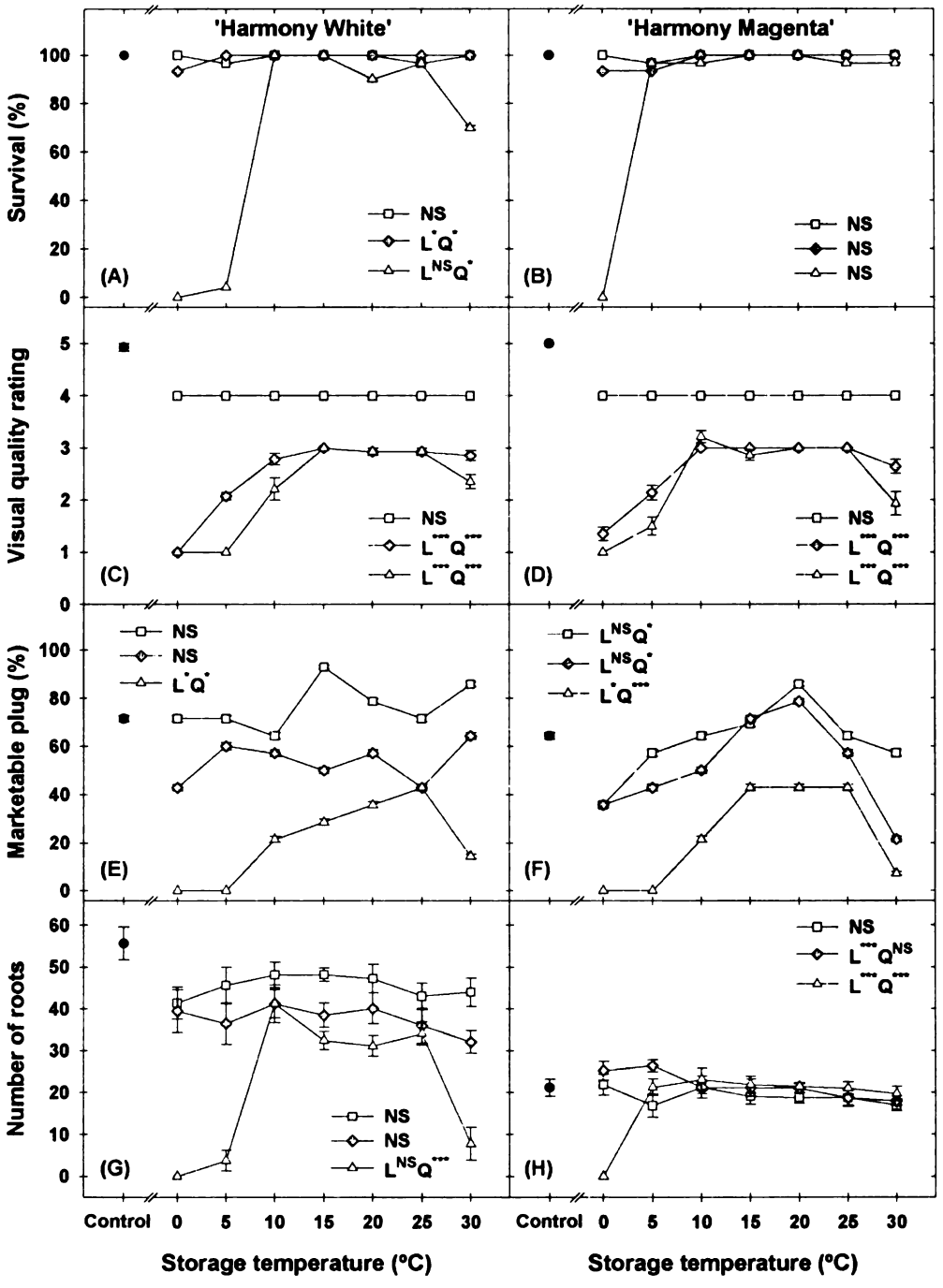

Fig. 3.3. Influence of storage temperature and duration on nonrooted cutting survival, visual quality (described in Fig. 3.1), plug marketability (those that were fully rooted in the plug cell), and the number of roots formed after 16 d of propagation in two cultivars of New Guinea impatiens. Cuttings were harvested from stock plants and stored for 0 (shaded circles), 1 (gray squares), 3 (dark gray diamond), or 5 (open triangles) d at 0, 5, 10, 15, 20, 25 or 30 °C and rooted in a propagation greenhouse. Data were pooled for years 1 and 2. Each symbol represents a mean of twenty plants and error bars represent standard errors of the mean. L = linear and Q = quadratic. ^{NS}, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01 or 0.001, respectively.

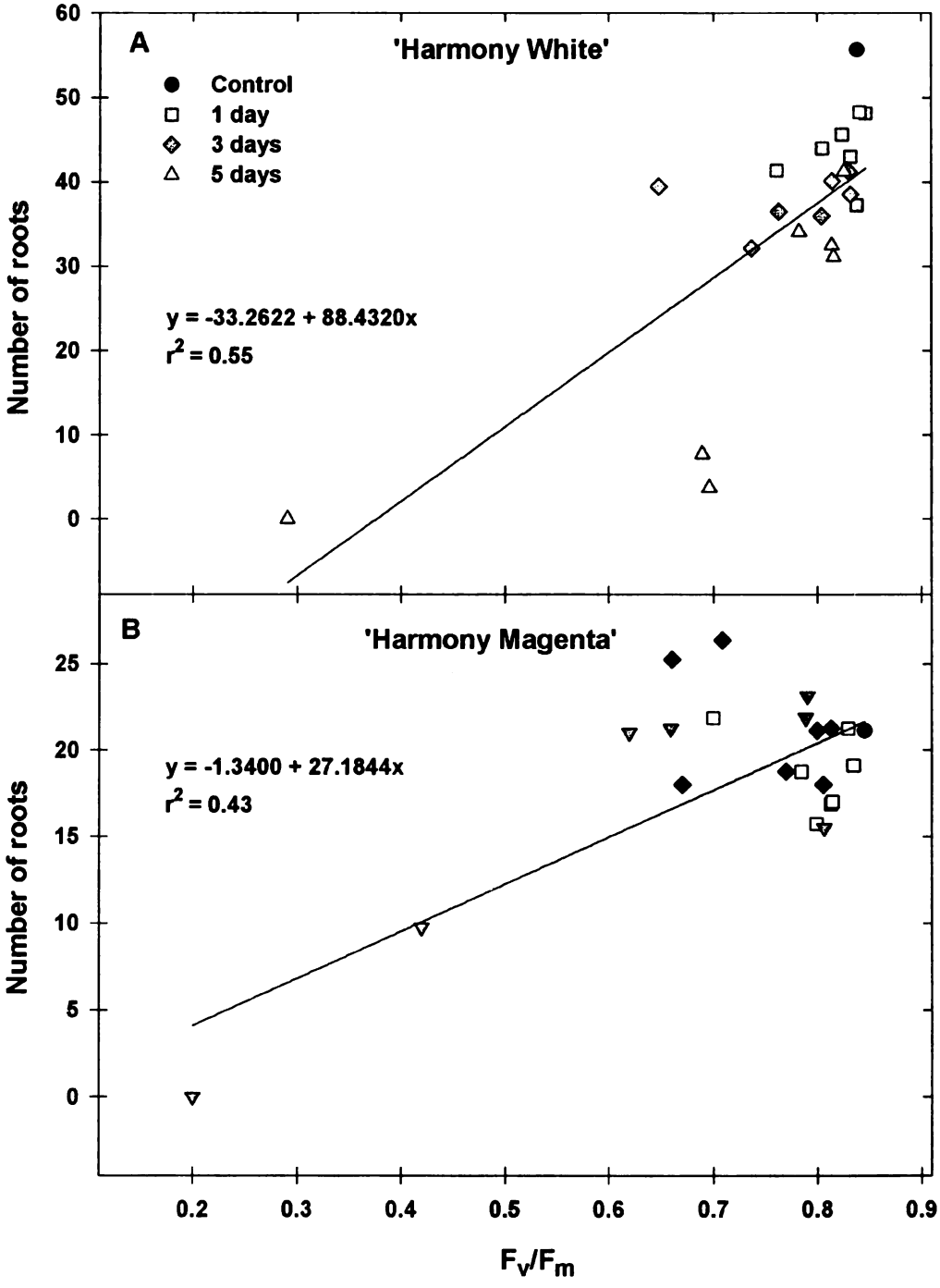

Fig. 3.4. Correlation between chlorophyll fluorescence (F_v/F_m) after harvest (control) or storage and the number of roots formed after 16 d of propagation in two cultivars of New Guinea impatiens. Cuttings were harvested from stock plants and stored for 0, 1, 3, or 5 d at 0, 5, 10, 15, 20, 25 or 30 °C and rooted in a propagation greenhouse. Data were pooled for years 1 and 2. Each symbol represents a mean of twenty plants.

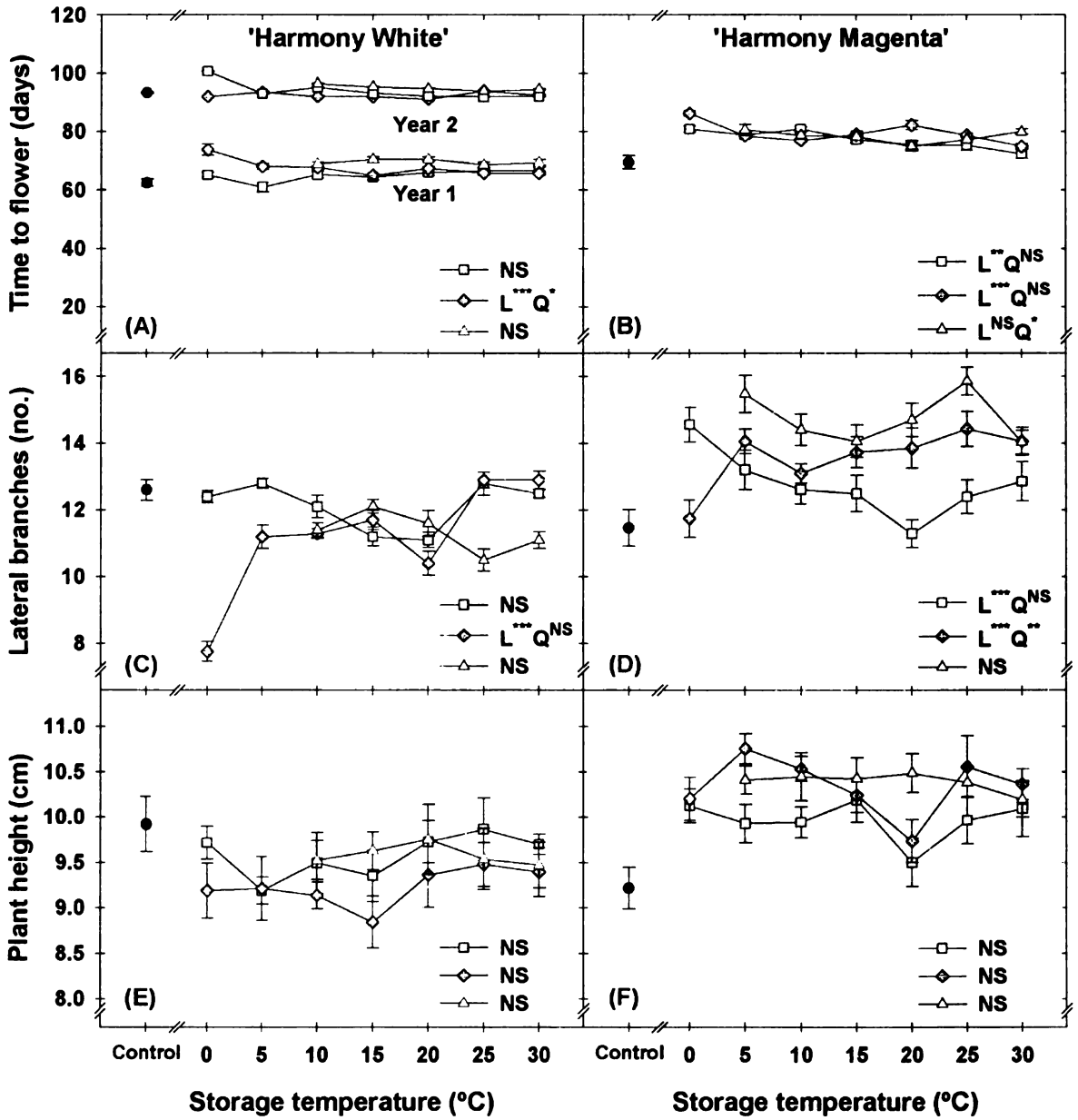

Fig. 3.5. Influence of storage temperature and duration on subsequent time to flower from the beginning of propagation, number of lateral branches, and plant height at first flowering for two cultivars of New Guinea impatiens. Cuttings were harvested from stock plants and stored for 0 (shaded circles), 1 (gray squares), 3 (dark gray diamond), or 5 (open triangles) d at 0, 5, 10, 15, 20, 25 or 30 °C and rooted in a propagation greenhouse. Each symbol represents a mean of thirty plants and error bars represent standard errors of the mean. L = linear and Q = quadratic. ^{NS}, *, **, *** Nonsignificant or significant at $P \leq 0.05$, 0.01 or 0.001, respectively.

SECTION IV

PHOTOSYNTHETIC DAILY LIGHT INTEGRAL DURING PROPAGATION INFLUENCES ROOTING AND GROWTH OF CUTTINGS AND SUBSEQUENT DEVELOPMENT OF NEW GUINEA IMPATIENS AND PETUNIA

Photosynthetic Daily Light Integral during Propagation Influences Rooting and Growth of Cuttings and Subsequent Development of New Guinea Impatiens and Petunia

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Introduction

Annual bedding plants are the largest (51%) segment of the U.S. commercial floriculture industry, with a reported wholesale value of $2.61 billion in 2005 (USDA, 2006a). In the past decade, annual and perennial crops have increasingly been propagated by shoot-tip cuttings; in 2005, U.S. greenhouse growers imported 868 million nonrooted cuttings of annuals and perennials, with a reported wholesale value of US $60 million (USDA, 2006b). Cuttings of bedding plants are typically received from offshore production facilities and propagated in greenhouses from December to March for sales to consumers in spring and early summer. During propagation, the integrated photosynthetic photon flux (daily light integral, or DLI) outdoors can range from 5 to 20 $mol \cdot m^{-2} \cdot d^{-1}$ across northern latitudes of the United States (Korczynski et al., 2002). In greenhouses, light levels can be 50% or less of that outdoors because of structures, glazing, shading, and obstructions (Hanan, 1998). Therefore, the DLI during propagation can be as low as 2 to 5 $mol \cdot m^{-2} \cdot d^{-1}$ and even lower during extended periods of cloudy weather.

Vegetative cuttings require a minimum quantity of photosynthetic light to provide a supply of carbohydrates for callus and adventitious root initiation and development (Haissig, 1986; Veierskov, 1988). Light intensities below this minimum inhibit root growth and development, leading to an extended rooting period and increased probability of rooting failure. Adventitious root formation and growth are influenced by the photosynthetic photo flux (*PPF*) and photosynthesis during propagation of pea (*Pisum sativum* L.) (Davis and Potter, 1981), geranium (*Pelargonium ×hortorum* L.H. Bail.) (Rapaka et al., 2005) and rose (*Rosa ×hybrida* L.) cuttings (Costa and Challa, 2002).

However, rooting of geranium was positively correlated with preharvest leaf sucrose concentrations when propagation *PPF* was low (Druege et al., 2004), such as <100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Rapaka et al., 2005). Conversely, stomatal closure, reduced turgor, and lower osmotic potentials from excessive light can inhibit root formation because of water and temperature stress and photoinhibition (Enfield, 2002; Eliasson and Brunes, 1980; Grange and Loach, 1985).

Numerous studies have been performed to understand the effects of DLI or light intensity on propagation of seedlings, cuttings, or microshoots. These studies have species-specific results and are primarily focused on either shoot growth (e.g., stem elongation and shoot biomass) or rhizosphere growth (e.g., rooting percentage, number, and biomass) and subsequent flowering in herbaceous and woody species. For example, in Japanese maple (*Acer palmatum* Thunb.), the percentage of cuttings that formed adventitious roots under maximum light intensities of 300 and 900 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was 82% and 64%, respectively, after 4 weeks (Behrens, 1988). Similarly, increased light levels during propagation of hibiscus [*Hibiscus rosa-sinensis* L. and *H. schizopetalus* (Masters) Hook. f.] decreased root number, dry weight, and rooting percentage (Kachecheba, 1976). In herbaceous cuttings and seedlings such as celosia (*Celosia argentea* L.), seed impatiens (*Impatiens wallerana* Hook. f.), salvia (*Salvia splendens* Sell ex Roem. & Schult.), marigold (*Tagetes patula* L.) and pansy (*Viola* ×*wittrockiana* Gams.) (Pramuk and Runkle, 2005a), baby's breath (*Gypsophila paniculata* L.) (Islam and Willumsen, 2001), petunia (Cabaleiro and Economou, 1992), and phlox (*Phlox paniculata* L.) (Enfield, 2002), rooting and shoot biomass and quality generally increased with increasing DLI or light intensity. For example, rooting percentage and dry weight of

baby's breath cuttings were greater after 3 weeks in a propagation greenhouse when plants were provided with a DLI of 12 $mol \cdot m^{-2} \cdot d^{-1}$ compared with 7 $mol \cdot m^{-2} \cdot d^{-1}$ (Islam and Willumsen, 2001). In addition, cuttings propagated under a DLI of 12 $mol \cdot m^{-2} \cdot d^{-1}$ subsequently flowered 14 d earlier than cuttings propagated under a DLI of 7 $mol \cdot m^{-2} \cdot d^{-1}$.

Few studies have been published on the effects of DLI during propagation on shoot and root biomass accumulation and the effects on subsequent development of vegetatively propagated bedding crops. Petunia (*Petunia ×hybrida* hort. Vilm.-Andr.) and new guinea impatiens (*Impatiens hawkeri* Bull.) are two of the most valuable annual bedding crops commonly propagated from cuttings, with reported wholesale values in 2005 in the United States of $83 and 80 million, respectively (USDA, 2006a). We performed experiments to quantify the effects of DLI during propagation on rooting, growth, and quality of these species during the rooting stage and to determine whether there were any residual effects of DLI on subsequent growth and development after transplant.

Materials and Methods

Plant material and culture. Petunia and new guinea impatiens stock plants were grown in 15-cm (1.3-L) and 16-cm (2.4-L) round containers (Dillen Products, Middlefield, OH), respectively, filled with a mix containing 70% peat moss, 21% perlite, and 9% vermiculite (Sure-Mix; Michigan Grower Products, Galesburg, MI). Plants were irrigated as necessary with reverse osmosis water supplemented with water-soluble fertilizer to provide the following ($mg \cdot L^{-1}$): 125 N, 12 P, 100 K, 65 Ca, 1.0 Fe and Cu, 0.5 Mn and Zn, 0.3 B, and 0.1 Mo (MSU Special; Greencare Fertilizers, Chicago, IL).

Petunia 'Tiny Tunia Violet Ice', 'Double Wave Spreading Rose', and 'Supertunia Mini Purple' stock plants were grown in glass greenhouses in East Lansing, MI (43°N lat.) at a mean temperature of 20.7 °C under a 12-h photoperiod and a mean DLI of 11.3 $mol \cdot m^{-2} \cdot d^{-1}$. The photoperiod consisted of a truncated 9-h natural day achieved by using blackout cloth from 1700 to 0800 HR, extended with day-extension lighting (≈2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at canopy level) with incandescent lamps from 1700 to 2000 HR. From 0800 to 1700 HR, high-pressure sodium (HPS) lamps provided a supplemental *PPF* of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant height [as measured with a light quantum sensor containing 10 photodiodes (Apogee Instruments, Inc., Logan, UT)] when the ambient greenhouse *PPF* was <140 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Temperatures on each bench were measured by an aspirated and enclosed thermocouple every 10 s, and hourly averages were recorded by a CR-10 data logger (Campbell Scientific, Logan, UT). To help provide uniform night temperatures of 20 °C, the data logger controlled a 1500-W electric heater under each bench, which provided supplemental heat as needed. Ethephon (Florel; Rhône-Poulenc Ag Company, Research Triangle Park, NC) with a surfactant (Capsil; Aquatrols, Paulsboro, NJ) was applied as a foliar spray at a concentration of 150 to 200 $mg \cdot L^{-1}$ and a volume of 2 $L \cdot 10\ m^{-2}$ every 4 weeks to abort flower buds.

New guinea impatiens 'Harmony White', 'Harmony Magenta', and 'Celebrette Red' stock plants were maintained under a 16-h photoperiod at a mean temperature of 23.9 °C and a mean DLI of 9.5 $mol \cdot m^{-2} \cdot d^{-1}$. Light transmission through the greenhouses was reduced by applying whitewash to the glass. The photoperiod was a constant 16 h (0600 to 2200 HR), consisting of natural daylengths with day-extension lighting from HPS lamps that delivered a supplemental *PPF* of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant height.

Ethephon was applied to new guinea impatiens as described previously but at a concentration of 500 or 750 $mg \cdot L^{-1}$ every 2 weeks to abort flower buds.

Harvesting. Approximately 150 uniform 3-cm vegetative petunia terminal stem cuttings were harvested from stock plants on 1 Aug. 2004, 25 Oct. 2004, and 7 Sept. 2005 (Expt. 1) and 16 Aug. and 7 Sept. 2005 (Expt. 2), and ≈150 uniform 4-cm vegetative new guinea impatiens terminal stem cuttings were harvested from stock plants on 22 Aug. 2004, 20 Sept. 2004, and 16 Aug. 2005 (Expt. 1) and 2 Feb. and 16 Aug. 2005 (Expt. 2). Cuttings were harvested beginning at 1000 HR and were stuck in 72-cell (28-mL) plug trays (Landmark Plastic Corp., Akron, OH) in a 50% commercial peat-based mix (Sure-Mix) and 50% screened coarse perlite (Therm-O-Rock East, Inc., New Eagle, PA) mix.

Propagation environment. All cuttings were rooted in a glass greenhouse under a 12-h photoperiod, with an air temperature set point of 24 °C. Air temperature was measured as previously described, and media temperature was measured by 36-gauge type E thermocouples (TT-E-36; Omega Engineering Inc., Stamford, CT) placed 2 cm below the media surface. Actual mean temperatures are provided in Tables 4.1 and 4.2. The 12-h photoperiod consisted of a 9-h truncated natural day (as described previously) extended with light from soft-white fluorescent lamps (BIAX FLE15TBX/L/SPX27; General Electric, Fairfield, CT) (≈3 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at canopy level) from 1700 to 2000 HR. Overhead mist containing reverse-osmosis water supplemented with water-soluble fertilizer was provided as necessary and delivered the following ($mg \cdot L^{-1}$): 50 N, 8 P, 42 K, 22 Ca, 1.0 Fe and Cu, 0.5 Mn and Zn, 0.3 B, and 0.1 Mo (MSU Special). Misting was controlled by an environmental computer as a function of time and accumulated *PPF*. A

line quantum sensor (Apogee Instruments, Inc.) was positioned in the center of the propagation house and collected and integrated light intensity every 10 s. Five seconds of misting were provided when the integrated light intensity reached 0.20 $mol \cdot m^{-2} \cdot h^{-1}$ or after 60 min. A vapor-pressure deficit of 0.3 kPa was maintained by the injection of steam or fine mist (Humidifan Turbo XE; Jaybird Manufacturing, State College, PA).

DLI treatments were created in the propagation environment using no shade cloth or fixed woven shade cloths placed above individual propagation compartments that reduced light by ≈30, 55, or 70% (OLS 30, 50 and 70; Ludvig Svensson, Charlotte, NC). Line quantum sensors (as described previously) were placed directly above cuttings in the four light compartments to measure the *PPF*. Thermocouples and line quantum sensors were connected to a CR10 data logger, and data were recorded every 10 s. Average DLI was calculated for each environment (Table 4.1).

Effects of propagation DLI on rooting (Expt. 1). Ten cuttings per DLI treatment were harvested 8, 12, and 16 d (petunia) or 10, 13, and 16 d (new guinea impatiens) after the start of propagation for each cultivar. The number of roots, length of the longest root, and length of the shoot from the medium level were recorded for each cutting for replications 2 and 3. Roots and shoots were separated and dry weights were recorded after drying in an oven at 70 °C for 1 week. The root-to-shoot dry-weight ratio was also determined.

Effects of propagation DLI on subsequent flowering (Expt. 2). Sixteen days after the start of propagation, 10 petunia and new guinea impatiens cuttings of each cultivar from each DLI treatment were transplanted into 10-cm square pots containing a peat-based media (Suremix). The plants were grown at 20 °C under a 16-h photoperiod (as

described previously), and chlorophyll fluorescence (F_v/F_m) of a fully expanded leaf was measured 24 h after transplant, beginning at 1300 HR, with a portable chlorophyll fluorescence system (Plant Efficiency Analyzer; Hansatech Instruments Ltd., Norfolk, England). Leaves were dark-acclimated for 15 min with the manufacturer's plastic and foam clips before measurements were recorded. Actual finishing temperatures and mean DLIs from transplant until flowering are provided in Table 4.2. Time to flower from the beginning of propagation, number of flower buds, number of lateral branches, and plant height were recorded on the date the first flower opened on each plant, and shoot dry mass was determined as described previously.

Data analysis. A complete randomized design was used that included 10 observations for each DLI treatment. Data were analyzed using SAS (SAS Institute, Cary, NC) mixed model procedure (PROC MIXED) for analysis of variance. Regression analysis was performed using Sigma Plot 8.0 (Systat Software, Inc., San Jose, CA). For new guinea impatiens, the highest DLI treatment was not included in the regression analysis as it was excessive.

Results

Petunia. Root number after 8 and 16 d of propagation increased as DLI increased from 1.2 to 9.5 $mol \cdot m^{-2} \cdot d^{-1}$ in all three cultivars (Fig. 4.1A, F, and K). In petunia 'Tiny Tunia Violet Ice', 'Double Wave Spreading Rose', and 'Supertunia Mini Purple', average root and shoot dry mass after 16 d of propagation increased linearly by 680%, 506%, and 2395% and 106%, 108%, and 147%, respectively, as DLI increased from 1.2 to 8.4 $mol \cdot m^{-2} \cdot d^{-1}$ (Fig. 4.1B, C, G, H, L, and M). After 16 d, shoot length increased from 4.5

to 6.3, 4.7 to 6.3, and 4.7 to 7.3 cm as DLI decreased from 5.9 to 1.2 $mol \cdot m^{-2} \cdot d^{-1}$ in petunia 'Tiny Tunia Violet Ice', 'Double Wave Spreading Rose', and 'Supertunia Mini Purple', respectively (Fig. 4.1D, I, and N). As propagation DLI increased to 8.4 $mol \cdot m^{-2} \cdot d^{-1}$, the root to shoot ratio of cuttings increased from 0.09 to 0.24 in all three cultivars when quantified 16 d after cuttings were stuck (Fig. 4.1E, J, and O).

Subsequent time to flower decreased by 21 and 22 d as the mean DLI during propagation increased from 1.4 to 10.7 $mol \cdot m^{-2} \cdot d^{-1}$ for petunia 'Tiny Tunia Violet Ice' and 'Supertunia Mini Purple', respectively (Fig. 4.2A and G). For petunia 'Tiny Tunia Violet Ice' and 'Supertunia Mini Purple', the relationship between flower bud number or shoot dry weight and DLI at first flowering was linear (decreased by 58% and 63%) and quadratic (decreased by 82% and 58%), respectively, as DLI increased from 1.4 to 10.7 $mol \cdot m^{-2} \cdot d^{-1}$ (Fig. 4.2B, C, H, and I). Time to flower, flower bud number, and shoot dry weight at first flowering of petunia 'Double Wave Spreading Rose' were not influenced by the DLI during propagation (Fig. 4.2D, E, and F). Chlorophyll fluorescence, lateral branch development, and plant height were not significantly influenced by DLI in any petunia cultivar (data not presented).

New guinea impatiens. As the mean DLI increased from 1.3 to 6.1 $mol \cdot m^{-2} \cdot d^{-1}$ during the 16 d of propagation, root number increased linearly by 200%, 108%, and 72% in new guinea impatiens 'Harmony White', 'Harmony Magenta', and 'Celebrette Red' (Fig. 4.3A, F and K). Root dry mass increased linearly from 4.9 to 47.4 mg (867%) in 'Harmony White', 2.7 to 19.0 mg (604%) in 'Harmony Magenta', and 4.9 to 33.3 mg (580%) in 'Celebrette Red' (Fig. 4.3B, G, and L). DLI had little effect on the length of

the longest root after 10 d in propagation, but root length in all cultivars increased linearly as DLI increased when measured after 16 d (Fig. 4.3C, H, and M).

Shoot dry mass when measured after 10 d of propagation increased at a decreasing rate. As DLI increased from 1.3 to 6.1 $mol \cdot m^{-2} \cdot d^{-1}$ shoot dry mass increased linearly by 53%, 32%, and 40% after 16 d of propagation in 'Harmony White', 'Harmony Magenta', and 'Celebrette Red', respectively (Fig. 4.3D, I, and N). After 16 d, the root to shoot ratio of cuttings increased linearly as DLI increased from 1.3 to 6.1 $mol \cdot m^{-2} \cdot d^{-1}$ in all three cultivars (Fig. 4.3E, J, and O).

Subsequent time to flower was hastened by 15, 14, and 19 d in 'Harmony White', 'Harmony Magenta', and 'Celebrette Red', respectively, as the DLI during propagation increased from 1.6 to 10.7 $mol \cdot m^{-2} \cdot d^{-1}$ (Fig. 4.4A, D, and G). Shoot dry mass at flowering decreased from 8.5 to 7.2 g in 'Harmony White', 8.7 to 7.1 g in 'Harmony Magenta', and 11.4 to 8.2 g in 'Celebrette Red' (Fig. 4.4B, E, and H) as DLI during propagation increased. The number of lateral branches at first flowering decreased as DLI increased during propagation in all cultivars (Fig. 4.4C, F, and I). Chlorophyll fluorescence, flower bud number, and plant height were not significantly influenced by DLI in any cultivar (data not presented).

Discussion

Successful and economically viable propagation of vegetative cuttings requires rapid, uniform rooting and the production of high-quality transplants. The transplants must be compact, well branched, large biomass, and fully rooted to ensure survival during shipping and the transition to the finish-production environment (Dole and

Hamrick, 2006). For the petunia and new guinea impatiens cultivars tested, as DLI increased during propagation, root and shoot biomass, the root to shoot ratio, and the quality (shoot height) of rooted cuttings increased. For example, as the mean DLI during 16 d of propagation increased from 1.2 to 8.4 $mol \cdot m^{-2} \cdot d^{-1}$ for petunia and 1.3 to 6.1 $mol \cdot m^{-2} \cdot d^{-1}$ for new guinea impatiens, average root dry mass and shoot dry mass increased linearly in all cultivars. These results are consistent with findings by Pramuk and Runkle (2005a), who reported that an increasing DLI (from 4.1 to 14.2 $mol \cdot m^{-2} \cdot d^{-1}$) during the seedling stage of celosia, seed impatiens, marigold, and pansy increased shoot dry weight per internode by 64%, 47%, 64%, and 68%, respectively. In addition, seedling height decreased with increasing DLI in celosia, seed impatiens, and salvia.

During the early stages of rooting (8 and 10 d for petunia and new guinea impatiens, respectively), the influence of increasing DLI on cutting quality was less profound than at later stages. For example, root and shoot biomass accumulation and length of the longest root increased at a decreasing rate when initially measured and then linearly after 16 d (Figs. 4.1 and 4.3). Studies with woody cuttings have also shown that increased DLI is much more beneficial after root initiation. Grange and Loach (1985) suggested that during the early propagation stages of woody cuttings of *H. syriacus* L., *Viburnum* ×*bodnantense* Stearn, and *Weigela florida* (Bunge) A. DC., the DLI be maintained between 2.5 and 3.3 $mol \cdot m^{-2} \cdot d^{-1}$ to avoid reduced rooting primarily due to loss of water and osmotic potential, turgor pressure, or both. After root initiation, the DLI should be increased because subsequent root growth requires higher irradiances (Grange and Loach, 1985).

Dole and Hamrick (2006) recommend a light intensity of 100 to 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for most vegetatively propagated herbaceous cuttings during the first stage of rooting (propagation to callus formation) which accumulates to a DLI of $\approx$4.3 $mol \cdot m^{-2} \cdot d^{-1}$ under a 12-h photoperiod. During the second stage of rooting (after root initiation), the recommended light intensity is 200 to 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (DLI of $\approx$8.6 $mol \cdot m^{-2} \cdot d^{-1}$ under a 12-h photoperiod). In a previous study, we determined that the photosynthetic light compensation point (LCP) and saturation range of petunia 'Tiny Tunia Violet Ice' stock plants grown under a DLI of 10 to 12 $mol \cdot m^{-2} \cdot d^{-1}$ are 60 and between 1450 and 1850 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively (unpublished data). Cuttings under the lowest and highest propagation DLI treatments of 1.2 and 8.4 $mol \cdot m^{-2} \cdot d^{-1}$ were exposed to a maximum irradiance of 91 and 562 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively, during the 16 d of propagation. Therefore, for most of the 16 d of propagation, cuttings under the lowest DLI were rarely exposed to a light intensity above the LCP, and consequently root biomass accumulation was only 0.10 $mg \cdot d^{-1}$ compared to 1.1 $mg \cdot d^{-1}$ for the cuttings under the highest DLI. Thus, our results indicate that during root initiation of herbaceous cuttings, the DLI should be maintained between 4 and 6 $mol \cdot m^{-2} \cdot d^{-1}$ to avoid a delay in root initiation and excessive shoot elongation and subsequently should be maintained between 6 and 8 $mol \cdot m^{-2} \cdot d^{-1}$ to promote root and shoot biomass accumulation.

With our results, root and shoot biomass of these cuttings can be predicted under a range of propagation DLIs (Figs. 4.1 and 4.2) with air temperatures of 24 to 25 °C. We previously determined that a fully rooted transplant of petunia 'Tiny Tunia Violet Ice' requires >35 roots and a root and shoot dry mass >10 mg and >65 mg, respectively (unpublished data). Therefore, petunia 'Tiny Tunia Violet Ice' cuttings propagated under

a mean DLI of 6 $mol \cdot m^{-2} \cdot d^{-1}$ should be fully rooted within 12 d compared to a cutting rooted under a mean DLI of 3 $mol \cdot m^{-2} \cdot d^{-1}$, which would take ≈22 d with 1.8 $roots \cdot d^{-1}$ and root and shoot biomass accumulation of 0.45 and 3.8 $mg \cdot d^{-1}$, respectively. Similarly, we previously determined that new guinea impatiens 'Harmony White' are fully rooted transplants when they have a root and shoot dry mass >30 mg and >150 mg, respectively (unpublished data). Our data predict that root biomass accumulation will occur at 0.86 and 2.9 $mg \cdot d^{-1}$ when cuttings are propagated under a mean DLI of 2 and 6 $mol \cdot m^{2} \cdot d^{-1}$, requiring 35 and 13 d of propagation, respectively. Greenhouse propagators of these plants could use our models to improve their prediction of propagation time according to the light levels provided to their crops.

To our knowledge, this is the first study that quantifies the effect of DLI during propagation of vegetative cuttings on subsequent plant performance in a common environment. Subsequent time to flower of 'Tiny Tunia Violet Ice' and 'Supertunia Mini Purple' petunia and all three new guinea impatiens cultivars decreased as the DLI under which the cuttings were propagated increased within the range of DLI studied. Similarly, Pramuk and Runkle (2005a) demonstrated that increasing DLI during the seedling stage of annual bedding plants accelerated subsequent time to flower independent of the finish DLI. For petunia 'Tiny Tunia Violet Ice' and 'Supertunia Mini Purple', plant mass (expressed as plant shoot dry biomass) and flower bud number at first flowering decreased as propagation DLI increased (Fig. 4.2). Similarly, plant-quality characteristics (shoot dry biomass and number of lateral branches) decreased for all new guinea impatiens cultivars studied as rooting DLI during propagation increased (Fig. 4.4). Cuttings propagated under the lower DLI treatments took longer to flower; thus, they had

more time to harvest light before flowering, producing more photosynthate for biomass accumulation (e.g., flowers and branches). Similar results have been observed when seedlings or finish plants were grown with either increasing DLI or temperature (Pramuk and Runkle, 2005a, 2005b; Yuan et al., 1998).

We speculate that the 14- to 19-d and 22-d delay in flowering for new guinea impatiens and petunia, respectively, could be the result of flower bud abortion or carbohydrate depletion. The delay in flowering is within ±6 days of the propagation time (16 d), which suggests that flowers are aborting or ceasing to develop when the propagation DLI is low. Alternatively, the carbohydrate status of cuttings under a low DLI is depleted during propagation, and it takes the extra time during finishing for plants to accumulate enough photosynthate to be capable of flowering.

In petunia 'Double Wave Spreading Rose', time to flower was not influenced by propagation DLI but was influenced by finishing DLI (Fig. 4.2D; Table 4.2). In Replication 1, plants flowered in 48 d when the forcing DLI was 13 $mol \cdot m^{-2} \cdot d^{-1}$, whereas in Replication 2, plants flowered in 63 d when the forcing DLI was 7.4 $mol \cdot m^{-2} \cdot d^{-1}$, which suggests that this cultivar has a facultative irradiance response. Plants such as 'Double Wave Spreading Rose' that exhibit a facultative irradiance response typically flower developmentally earlier when provided with higher light (Erwin et al., 2004). In irradiance indifferent plants such as 'Tiny Tunia Violet Ice' and 'Supertunia Mini Purple' flower bud initiation occurs during propagation and further increases in light during finishing do not hasten flowering.

Rapid, uniform, and complete flowering is of primary interest to greenhouse growers so that production time can be minimized. However, our results and those of

similar studies (Pramuk and Runkle, 2005a, 2005b) indicate that a tradeoff exists between rapid flowering and high finish-plant quality. Our model can consequently be used to determine how excessive shading, changing DLI, supplemental lighting, and seasonal propagation will affect subsequent performance (e.g., flowering time and plant quality). For example, petunia 'Tiny Tunia Violet Ice' and new guinea impatiens 'Harmony White' cuttings propagated under an average DLI of 2 and 8 $mol \cdot m^{-2} \cdot d^{-1}$ are predicted to flower in 46 and 33 d and 83 and 69 d, respectively, if subsequently forced at 20 °C.

This information could be used to reduce propagation and production costs and to predict the consequences of propagating vegetative cuttings under excessive shading or during the low-light peak propagation periods of December to February. Our results also show the value of maintaining DLI at 4 to 6 $mol \cdot m^{-2} \cdot d^{-1}$ during first stages of rooting and 6 to 8 $mol \cdot m^{-2} \cdot d^{-1}$ during the second stage to obtain rapid, uniform rooting and high-quality rooted transplants that flower earlier, especially when cuttings are rooted during the darkest periods of the year.

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Table 4.1. Mean media and air temperatures during 16 d of propagation and mean daily light integral (DLI) of three replications of petunia and new guinea impatiens cuttings propagated under four DLI treatments after 8, 12, and 16 d and 10, 13, and 16 d, respectively (Expt. 1).

			DLI ($mol \cdot m^{-2} \cdot d^{-1}$)											
Species and	Temp. (°C)		Shade				Shade				Shade			
replication	Media	Air	70%	55%	30%	None	70%	55%	30%	None	70%	55%	30%	None
Petunia			8 d				12 d				16 d			
1	24.0	24.5	1.8	3.2	6.3	9.5	1.6	2.7	5.4	8.5	1.6	2.8	5.4	8.4
2	24.3	25.4	1.2	1.9	3.4	4.3	1.1	1.7	2.9	3.6	1.2	1.9	3.4	3.9
3	22.4	24.5	3.4	4.7	7.0	8.8	3.2	4.3	6.4	8.1	3.0	4.0	5.9	7.5
New guinea impatiens			10 d				13 d				16 d			
1	24.1	24.7	1.2	2.0	3.7	5.9	1.3	2.1	3.7	5.9	1.3	2.1	3.7	5.9
2	22.5	24.1	2.1	3.4	4.1	6.2	2.0	3.5	4.2	6.4	1.9	3.5	4.2	6.3
3	22.6	24.4	3.8	4.5	6.1	10.4	4.0	4.7	6.3	10.8	4.1	4.6	6.1	10.7

Table 4.2. Mean media and air temperatures, vapor-pressure deficit (VPD), and daily light integral (DLI) during 16 d of propagation under four DLI treatments and average air temperature and DLI during subsequent forcing of two replications of petunia and new guinea impatiens (Expt. 2).

	Propagation							Forcing	
				DLI ($mol \cdot m^{-2} \cdot d^{-1}$)					
	Temperature (°C)		VPD	Shade				Temperature	DLI
Replication	Media	Air	(kPa)	70%	50%	30%	None	(°C)	($mol \cdot m^{-2} \cdot d^{-1}$)
				Petunia					
1	23.5	24.4	0.44	4.1	4.6	6.1	10.7	21.3	13.0
2	22.9	24.1	0.39	1.4	2.0	3.0	4.7	19.9	7.4
				New Guinea impatiens					
1	22.3	25.3	0.38	1.6	2.2	3.4	4.3	20.5	15.7
2	23.5	24.4	0.44	4.1	4.6	6.1	10.7	21.9	13.0

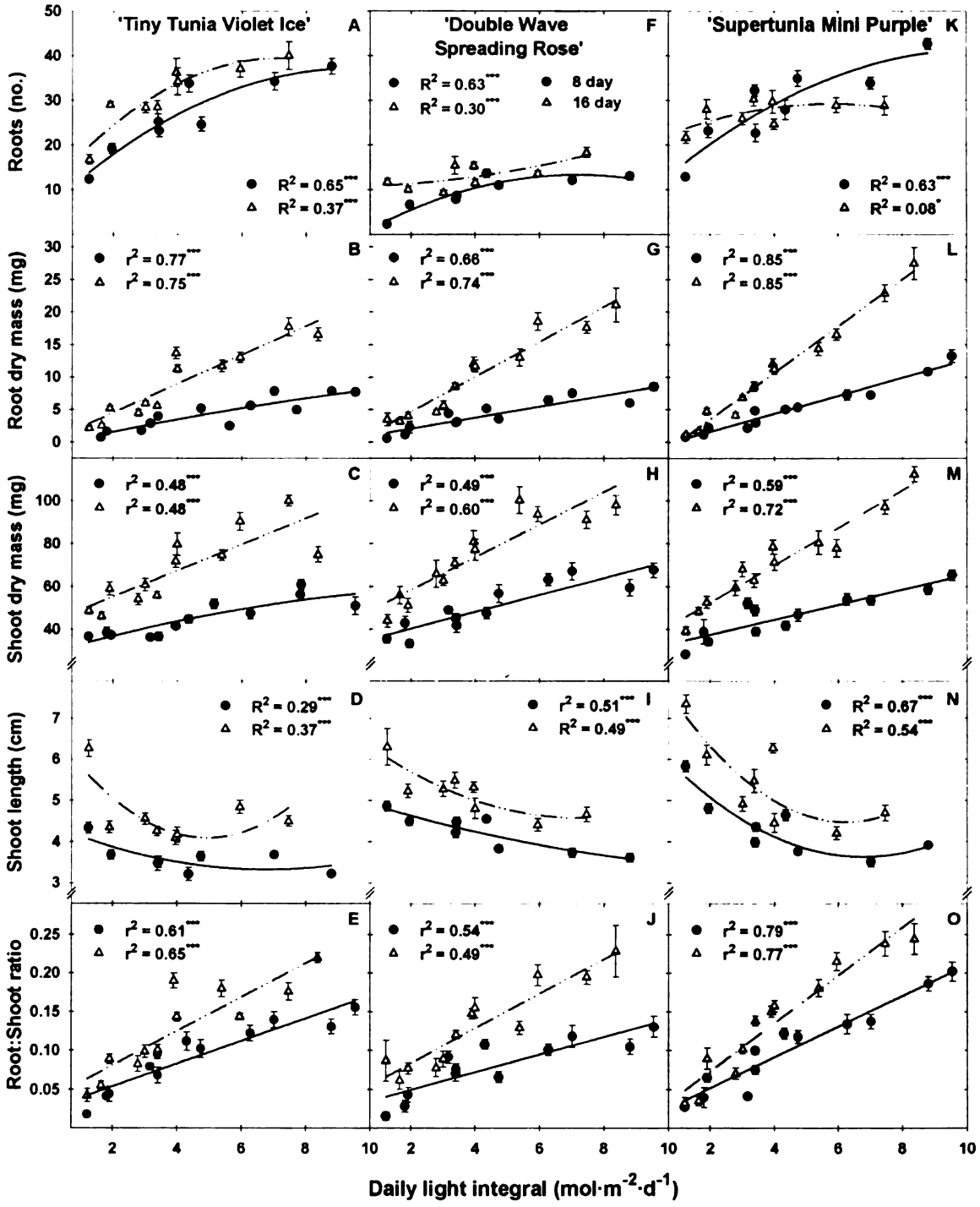


Fig. 4.1. Relationships between mean daily light integral and number of roots formed, root dry mass, shoot dry mass, shoot length, and root to shoot ratio measured after 8 d and 16 d of propagation for petunia 'Tiny Tunia Violet Ice', 'Double Wave Spreading Rose', and 'Supertunia Mini Purple' cuttings. Each symbol represents the mean of ten plants, and error bars represent standard errors of the mean. Regression lines are presented with corresponding r^2 and R^2. Legend in F applies to all figures. *,*** Significant at $P \leq 0.05$ or 0.001, respectively.

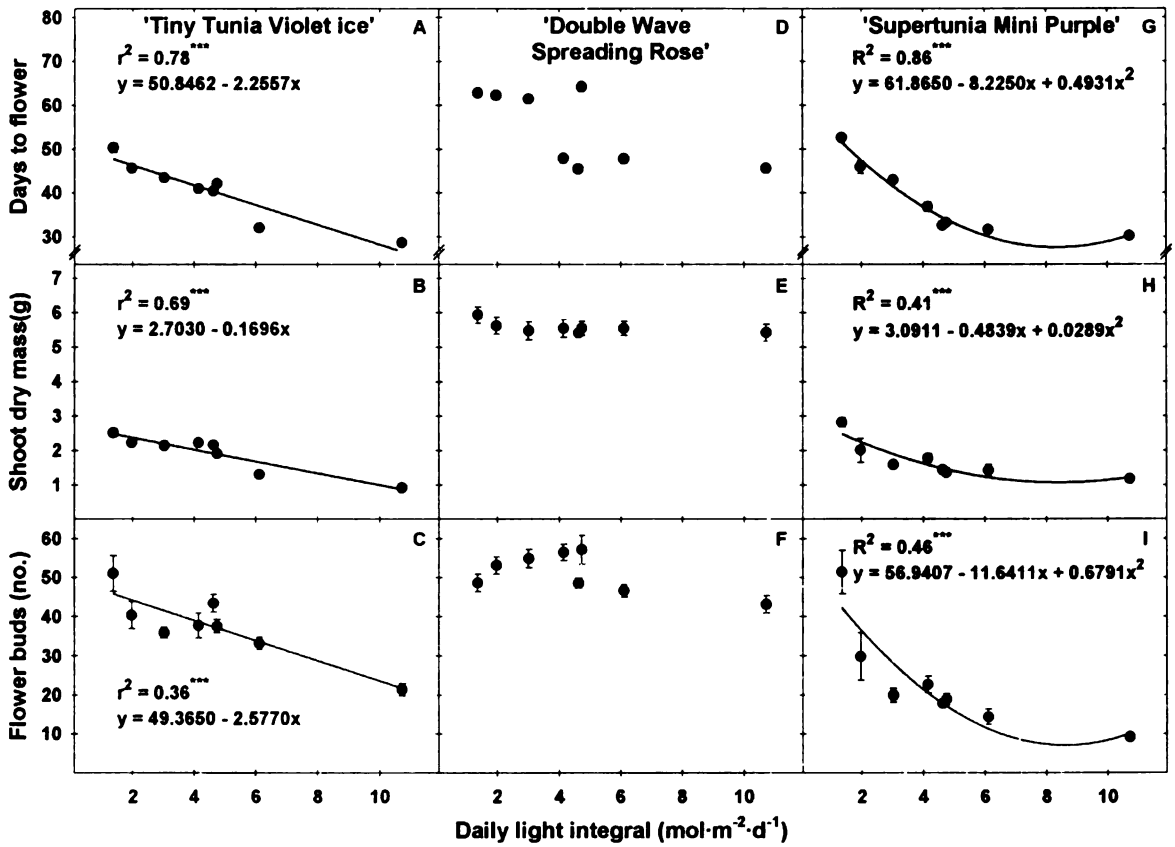


Fig. 4.2. Relationships between mean daily light integral during propagation and days to flower from the beginning of propagation, shoot dry mass, and number of flower buds at first flowering for petunia 'Tiny Tunia Violet Ice', 'Double Wave Spreading Rose', and 'Supertunia Mini Purple' cuttings. Each symbol represents the mean of twelve plants, and error bars represent standard errors of the mean. Equations for regression lines are presented for significant correlations only with corresponding r^2 and R^2. ***Significant at $P \leq 0.001$.

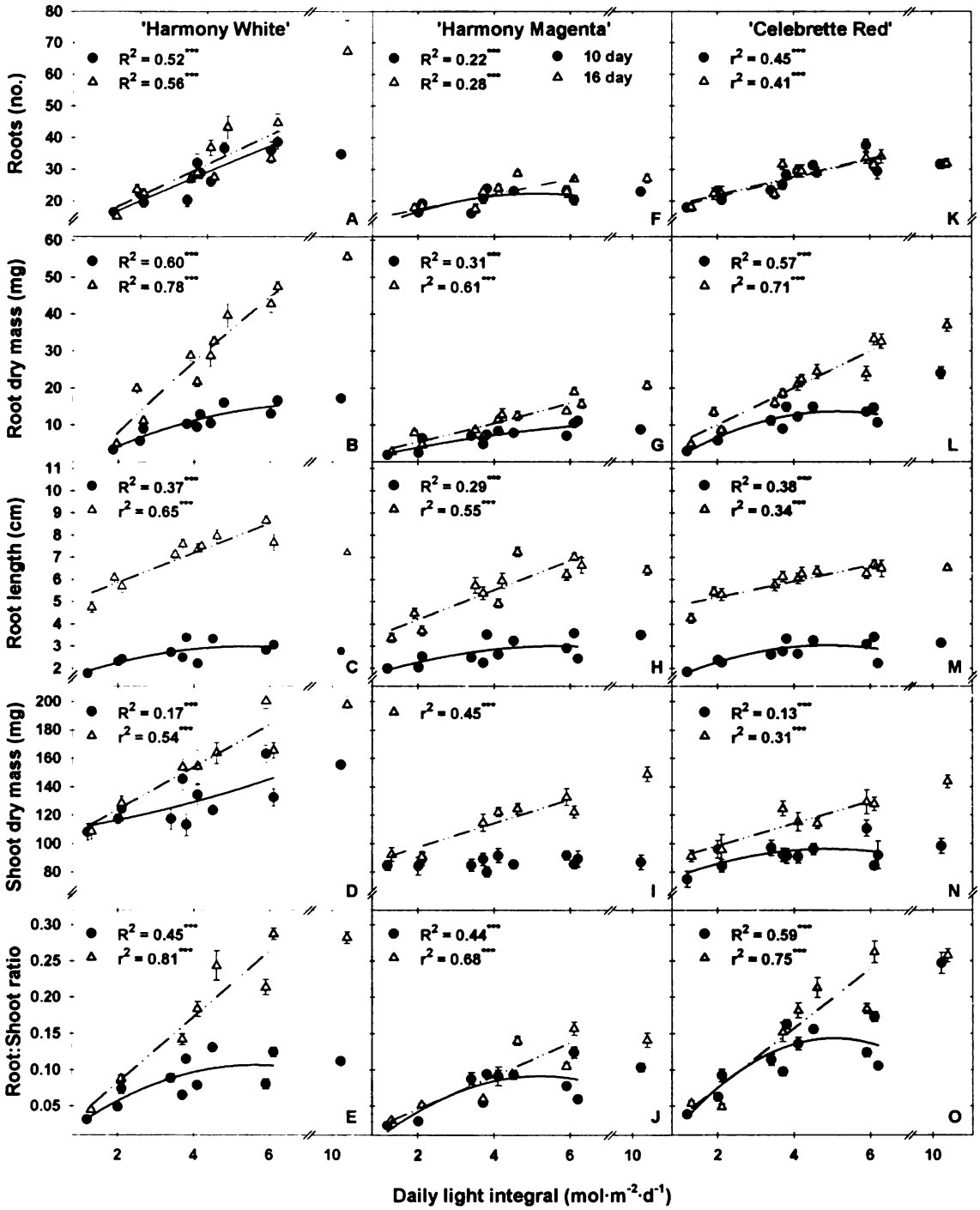


Fig. 4.3. Relationships between mean daily light integral and number of roots formed, root dry mass, length of the longest root, shoot dry mass, and root to shoot ratio measured after 10 d and 16 d of propagation for new guinea impatiens 'Harmony White', 'Harmony Magenta', and 'Celebrette Red' cuttings. Each symbol represents the mean of ten plants, and error bars represent standard errors of the mean. Regression lines are presented for significant correlations only with corresponding r^2 and R^2 are presented. Legend in F applies to all figures. ***Significant at $P \leq 0.001$.

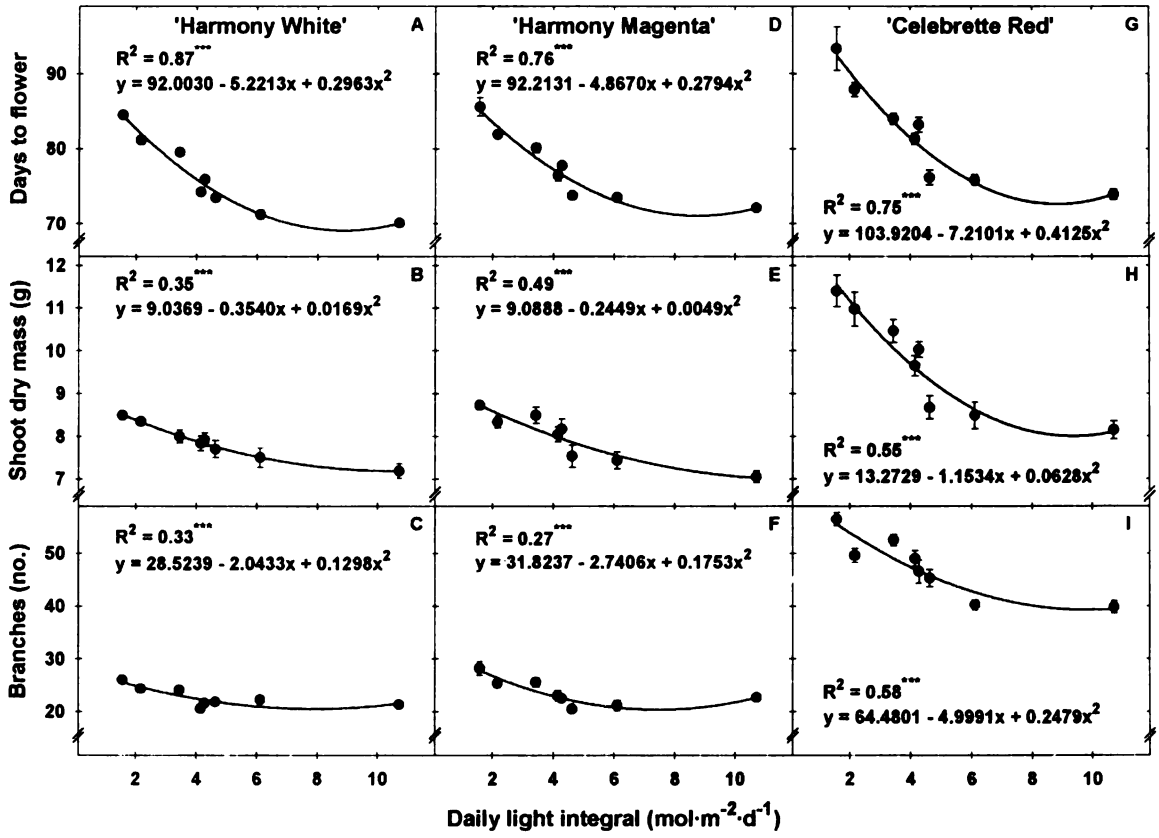


Fig. 4.4. Relationships between mean daily light integral during propagation and days to flower from the beginning of propagation, shoot dry mass, and number of lateral branches at first flowering for new guinea impatiens 'Harmony White', 'Harmony Magenta', and 'Celebrette Red' cuttings. Each symbol represents a mean of twelve plants, and error bars represent standard errors of the mean. Equations for regression lines are presented with corresponding R^2. ***Significant at $P \leq 0.001$.

APPENDIX A

STOCK PLANT AND PROPAGATION PHOTOSYNTHETIC DAILY LIGHT INTEGRAL INFLUENCE PHYSIOLOGY, ROOTING, AND GROWTH OF CUTTINGS AND SUBSEQUENT DEVELOPMENT OF NEW GUINEA IMPATIENS AND PETUNIA

Research Objective

The research objectives of this study were to quantify the physiological responses of new guinea impatiens (*Impatiens hawkeri* Bull.) and petunia (*Petunia ×hybrida* hort. Vilm.-Andr.) cuttings harvested from stock plants grown under different mean photosynthetic daily light integrals (DLI). Cuttings were measured for chlorophyll fluorescence (F_v/F_m), relative chlorophyll content, and gas exchange measurements to quantify their stress tolerance after a simulated shipping treatment. In addition, we determined how stock plant and propagation DLI and, their interaction, influence rooting, cutting growth, and subsequent development of cuttings after transplant.

Materials and Methods

Stock plant management and culture. Vegetatively propagated stock plants of petunia 'Tiny Tunia Violet Ice' were maintained under a 12-h photoperiod in greenhouses in East Lansing, MI (43 °N lat.). The photoperiod consisted of a truncated 9-h natural day achieved using blackout cloth from 0800 to 1700 HR and day-extension lighting (≈2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at canopy level) from 1700 to 2000 HR with incandescent lamps. Three DLI environments were created on 23 Jan. 2006 using no shade or permanent woven shade cloth with an open-weave design that reduced light by ≈30% and 55% (OLS 30 and 50; Ludvig Svensson, Charlotte, N.C.) that surrounded individual benches. In addition, whitewash was applied to the greenhouse glazing to moderate the DLI during experiment repetitions. Ethephon (Florel; Rhône-Poulenc Ag Company, Research Triangle Park, N.C.) with a surfactant (Capsil; Aquatrols, Paulsboro, N.J.) was applied

every four weeks as a foliar spray at a concentration of 150 $mg \cdot L^{-1}$ and a volume of ≈2 $L \cdot 10\ m^{-2}$ to abort flower buds.

New guinea impatiens 'Harmony White' and 'Celebrette Red' stock plants were maintained under a 16-h photoperiod that consisted of natural daylengths with day-extension lighting from HPS lamps from 0600 to 2200 HR as previously described. DLI treatments were as described previously and two-week means prior to cutting harvests are provided in Table 5.1. Ethephon was applied to new guinea impatiens as described previously but at a concentration of 500 to 750 $mg \cdot L^{-1}$ every two weeks to abort flower buds.

Line quantum sensors containing 10 photodiodes (Apogee Instruments, Inc., Logan, UT) were placed directly above stock plants in each DLI treatment to measure the photosynthetic photo flux (*PPF*). From 0800 to 1700 HR, HPS lamps provided a supplemental *PPF* of ≈10, 35, and 65 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant height when the ambient greenhouse *PPF* was <140 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for the 55%, 30% and 0% shade treatments, respectively. Air temperature was measured on each bench by an aspirated and enclosed thermocouple (TT-E-36; Omega Engineering Inc., Stamford, CT). Temperature and light intensity were measured every 10 s and hourly averages were recorded by a CR-10 datalogger (Campbell Scientific, Logan, UT). To help provide uniform night temperatures of 20 °C, a data logger controlled a 1500-W electric heater, which provided supplemental heat under each bench as needed. The mean DLIs and air temperature for the three DLI treatments during two-week periods prior to cutting harvests for replications 1 and 2 are provided in Table 5.1.

Petunia stock plants were grown in 15-cm (1.3-L) and new guinea impatiens in 16-cm (2.4-L) round plastic containers filled with a mix containing 70% peat moss, 21% perlite, and 9% vermiculite (Sure-Mix; Michigan Grower Products, Galesburg, MI). Plants were irrigated as necessary with reverse osmosis water supplemented with water-soluble fertilizer to provide the following ($mg \cdot L^{-1}$): 125 N, 12 P, 100 K, 65 Ca, 1.0 Fe and Cu, 0.5 Mn and Zn, 0.3 B, and 0.1 Mo (MSU Special; Greencare Fertilizers, Chicago, IL).

Cutting harvest and storage. Uniform petunia and new guinea impatiens cuttings (4 leaves and a 3-cm stem length) were harvested from stock plants beginning at 0800 HR. Cuttings were harvested on 25 Apr. and 1 June 2006. At each harvest, 120 cuttings were collected and separated into two replicated lots, each consisting of 20 cuttings. Cuttings were then placed in sealed polyethylene bags (volume: 0.946 L, thickness: 68.6 microns; Ziploc, S.C. Johnson & Son, Inc., Racine, WI). The sealed bags were placed in cardboard boxes (49 × 34 × 11 cm) and stored in environmental chambers for 2 d of shipping simulation at 21.3 ± 0.6 °C.

Propagation environment. Following the simulated shipping treatment, cuttings were stuck in 72-cell (28-mL) plug trays (Landmark Plastic Corp., Akron, OH) in a 50% commercial mix [containing 70% peat moss, 21% perlite, and 9% vermiculite (Sure-Mix; Michigan Grower Products, Galesburg, MI)] and 50% screened coarse perlite (Therm-O-Rock East, Inc., New Eagle, PA) mix and rooted. All cuttings were rooted in a glass greenhouse under a 12-h photoperiod, with an air temperature set point of 25 °C. The 12-h photoperiod consisted of a 9-h truncated natural day (as described previously) extended with light from soft-white fluorescent lamps (BIAX FLE15TBX/L/SPX27; General

Electric, Fairfield, CT) (≈3 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at canopy level) from 1700 to 2000 HR. DLI treatments were created using no shade cloth or fixed woven shade cloths placed above individual propagation compartments that reduced light by ≈30, or 70% (OLS 30, and 70; Ludvig Svensson, Charlotte, NC). Thermocouples and line quantum sensors were connected to a CR10 data logger, and data were recorded every 10 s. Air temperature was measured as previously described and media temperature was measured by thermocouples (TT-E-40; Omega Engineering Inc.) placed 2 cm below the media surface. Actual mean DLI, air and media temperatures during replications 1 and 2 are provided in Table 5.1.

Overhead mist containing reverse osmosis water supplemented with water-soluble fertilizer delivered ($mg \cdot L^{-1}$): 50 N, 8 P, 42 K, 22 Ca, 1.0 Fe and Cu, 0.5 Mn and Zn, 0.3 B, and 0.1 Mo (MSU Special). Misting was controlled by an environmental computer as a function of time and accumulated *PPF*. A line quantum sensor (Apogee Instruments, Inc.) was positioned in the center of the propagation house and collected and integrated light intensity every 10 s. Four seconds of misting were provided when the light integral reached 0.20 $mol \cdot m^{-2} \cdot h^{-1}$ or after 60 min, whichever occurred first. A vapor-pressure deficit of 0.3 kPa was maintained by the injection of steam or fine mist (Humidifan Turbo XE, Jaybird Manufacturing; State College, PA).

Effects of stock plant and propagation DLI on cutting physiology and rooting (Expt. 1). Ten cuttings from each stock plant and propagation DLI treatment combination were randomly sampled for each measurement. Total chlorophyll (a+b) content was estimated using a SPAD chlorophyll meter (Model 502, Minolta Co., Japan). For each cutting, three SPAD measurements were taken from different positions on the largest

fully expanded leaf and the mean was recorded as the value for each cutting on days 7, 11, and 14 from the onset of propagation. After day 7 of propagation, chlorophyll fluorescence (F_v/F_m) was measured on the basal portion (upper epidermis) of the most fully expanded leaf using a portable chlorophyll fluorescence system (Plant Efficiency Analyzer; Hansatech Instruments Ltd., Norfolk, England). Leaves were dark-acclimated for 15 min with the manufacturer's plastic and foam clips before measurements were recorded.

Single-leaf gas exchange measurements were performed 11 and 21 d after the cuttings were stuck in propagation. Net photosynthesis (P_n), stomatal conductance, and transpiration measurements were performed in the greenhouse between 0900 and 1300 HR and were blocked by propagation DLI and cultivar to reduce time of day effects. Measurements were conducted using a portable photosynthesis system (LI-6400, LI-Cor, Lincoln, NE) fitted to a 6 cm^2 leaf chamber with an LED light source (6400-02B; red at 665 nm and blue at 470 nm) at a *PPF* of 1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The reference CO_2 concentration inside the leaf chamber was 400 $\mu mol \cdot mol^{-1}$ and the flow of air into the chamber was 250 $\mu mol \cdot s^{-1}$. Leaf temperature inside the leaf chamber was maintained at 24.5 ± 0.7 °C using dual Peltier devices that heated or cooled the air circulating through the chamber. Immediately after measurements were recorded, leaves inside the chamber were excised, placed in plastic packages and stored at 5 °C. Leaf area was determined by scanning the leaf through a leaf area meter (LI-3000, Li-Cor, Lincoln, NE) three times and the mean was recorded. After 21 d of propagation, roots and shoots were separated and dry weights were recorded after drying in an oven at 70 °C for 1 week.

Effects of stock plant and propagation DLI on subsequent flowering (Expt. 2). Twenty-one days after the start of propagation, 10 petunia and new guinea impatiens cuttings from each DLI treatment combination were transplanted into 10-cm square pots containing a peat-based media (Suremix). The plants were grown at 20 °C under a 16-h photoperiod (as described previously). Actual forcing temperatures and mean DLIs from transplant until flowering are provided in Table 5.1. Time to flower from the beginning of propagation, number of flower buds, number of lateral branches, and plant height were recorded on the date the first flower opened on each plant, and shoot dry mass was determined as described previously.

Data analysis. Data were analyzed using SAS (SAS Institute, Cary, N.C.) mixed model procedure (PROC MIXED) for analysis of variance and pairwise comparisons between treatments were performed using Tukey's honest significant difference test (HSD). Regression analysis was performed using Sigma Plot 8.0 (Systat Software, Inc., San Jose, CA).

Table 5.1. Mean stock plant daily light integral (DLI) and air temperatures for the two-week period prior to cutting harvest. Air and media temperature, vapor-pressure deficit (VPD), and DLI during 21 d of propagation under three DLI treatments. Average air temperature and DLI during subsequent forcing of two replications of petunia and new guinea impatiens

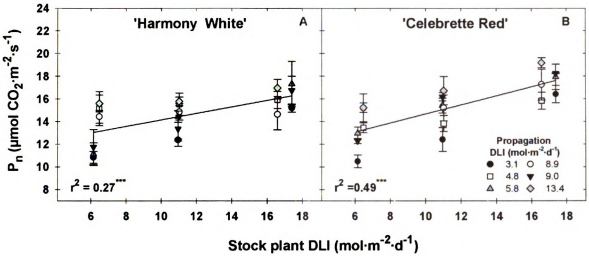
Stock plant			Propagation						Forcing	
						DLI ($mol \cdot m^{-2} \cdot d^{-1}$)				
DLI ($mol \cdot m^{-2} \cdot d^{-1}$)	Temperature (°C)	Rep	Temperature (°C) Media	Air	VPD (kPa)	Shade factor 70%	30%	0%	Temperature (°C)	DLI ($mol \cdot m^{-2} \cdot d^{-1}$)
					Petunia					
7.6	21.5	1	22.4	24.4	0.47	3.1	5.8	8.9	23.5	14.0
10.6									23.3	14.3
14.5									23.5	14.0
4.3	22.0	2	24.4	25.4	0.47	3.4	6.5	11.0	22.5	16.6
7.7									22.6	16.6
11.3									22.5	16.5
					New guinea impatiens					
6.1	24.9	1	22.4	24.4	0.47	3.1	5.8	8.9	23.2	15.3
11.0									23.2	15.3
17.4									23.2	15.3
6.5	23.8	2	24.4	25.4	0.47	3.4	6.5	11.0	23.6	16.6
11.0									23.6	16.6
16.6									23.6	16.6

Table 5.2. Mean chlorophyll fluorescence (F_v/F_m) after 7 d of propagation and relative chlorophyll content (SPAD reading) after 7, 11, and 14 d of propagation for petunia and new guinea impatiens cuttings. Cuttings were harvested from stock plants grown under three different daily light integrals (DLI), received a simulated shipping treatment at 20 °C for 2 d, then were propagated under three different DLIs.

Mean DLI ($mol \cdot m^{-2} \cdot d^{-1}$)			Chlorophyll content (SPAD)		
Stock plant	Propagation	F_v/F_m	7 d	11 d	14 d
		Petunia 'Tiny Tunia Violet Ice'			
4.3	4.8	0.837 fgh	24.9 g	26.1 fg	16.3 f
	8.9	0.844 c–f	27.0 fg	24.5 g	17.8 ef
	13.4	0.842 d–h	25.5 g	27.1 d–g	20.3 d
7.6	3.1	0.852 abc	25.2 g	25.2 fg	22.7 abc
	5.8	0.853 ab	30.8 c–f	29.9 a–f	20.5 cd
	9.0	0.842 b–e	36.0 ab	30.9 a–e	24.3 a
7.7	4.8	0.834 h	27.0 efg	27.0 efg	19.5 de
	8.9	0.846 d–e	29.5 d–g	25.8 fg	20.2 d
	13.4	0.847 a–e	27.4 efg	28.9 b–g	19.5 de
10.6	3.1	0.852 abc	28.7 d–g	34.1 a	21.3 bcd
	5.8	0.848 a–e	30.8 c–f	29.1 b–g	20.5 cd
	9.0	0.843 d–g	35.3 abc	29.8 a–f	22.9 ab
11.3	4.8	0.836 gh	32.3 a–d	32.5 abc	19.4 de
	8.9	0.848 a–e	33.0 a–d	33.6 ab	19.6 de
	13.4	0.843 d–g	30.7 c–f	31.7 a–d	20.1 d
14.5	3.1	0.855 a	32.0 b–e	26.4 efg	17.6 ef
	5.8	0.850 a–d	30.9 c–f	28.0 c–g	19.2 de
	9.0	0.842 e–h	37.0 a	27.2 d–g	20.9 bcd
Significance					
Stock plant DLI		NS	***	***	***
Propagation DLI		***	***	NS	***
Stock DLI × Prop DLI		NS	*	***	***
		Impatiens 'Celebrette Red'			
6.1	3.1	0.841 a–d	50.3 fg	43.6 efg	31.7 bc
	5.8	0.837 a–e	47.9 g	42.8 fg	30.1 c
	9.0	0.821 f	52.6 efg	39.4 g	39.7 a
6.5	4.8	0.828 c–f	53.5 d–g	49.9 bcd	31.4 bc
	8.9	0.828 c–f	52.3 efg	44.5 d–g	32.0 bc
	13.4	0.822 f	53.2 d–g	47.4 c–f	30.9 bc
11.0	3.1	0.828 c–f	53.7 d–g	43.4 efg	30.6 bc
	4.8	0.831 b–f	56.6 c–f	49.6 b–e	31.4 bc
	5.8	0.846 ab	57.6 c–f	47.3 c–f	30.7 bc
11.0	8.9	0.837 a–f	61.5 bcd	52.9 bc	32.6 bc
	9.0	0.826 def	59.2 b–e	49.5 b–e	32.7 bc
	13.4	0.827 c–f	58.7 b–e	50.3 bcd	33.8 bc
16.6	4.8	0.826 ef	66.6 ab	62.3 a	33.9 bc
	8.9	0.837 a–e	70.4 a	66.0 a	33.4 bc

Table 5.2 continued

	13.4	0.834 a–f	62.3 abc	62.9 a	32.6 bc
17.4	3.1	0.847 a	57.6 c–f	54.1 b	29.8 c
	5.8	0.842 abc	56.8 c–f	47.4 c–f	34.6 b
	9.0	0.832 a–f	64.5 abc	52.2 bc	41.2 a
Significance					
Stock plant DLI		***	***	***	***
Propagation DLI		***	***	NS	***
Stock DLI × Prop DLI		***	*	***	***
		Impatiens 'Harmony White'			
6.1	3.1	0.852 ab	54.1 f	43.9 ef	31.8 d–g
	5.8	0.845 a–f	56.7 ef	45.7 def	31.7 d–g
	9.0	0.839 d–h	61.3 a–e	52.7 bc	35.9 bcd
6.5	4.8	0.831 gh	51.7 f	51.1 bcd	36.8 abc
	8.9	0.830 h	57.4 c–f	44.5 ef	28.5 g
	13.4	0.831 gh	57.3 c–f	41.6 fg	31.2 efg
11.0	3.1	0.851 abc	56.9 def	35.1 h	33.2 c–f
	5.8	0.848 a–e	62.1 a–e	40.2 fgh	30.6 fg
	9.0	0.842 b–g	64.1 ab	49.6 cde	32.0 d–g
11.0	4.8	0.834 fgh	62.2 a–e	53.4 bc	38.3 ab
	8.9	0.836 fgh	61.3 a–e	55.4 b	33.4 c–f
	13.4	0.836 e–g	62.9 a–d	49.3 cde	35.5 b–e
16.6	4.8	0.838 e–g	67.2 a	67.0 a	40.4 a
	8.9	0.843 a–g	64.8 ab	64.4 a	36.0 bcd
	13.4	0.840 c–h	66.5 ab	53.9 bc	38.6 ab
17.4	3.1	0.854 a	61.3 b–e	40.1 fgh	34.8 b–f
	5.8	0.851 a–d	63.1 abc	36.1 gh	31.1 fg
	9.0	0.844 a–f	64.6 ab	43.3 f	34.4 b–f
Significance					
Stock plant DLI		***	***	***	***
Propagation DLI		***	***	***	***
Stock DLI × Prop DLI		NS	*	***	*



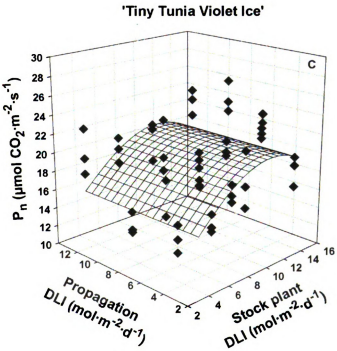


Fig. 5.1. Effects of stock plant and propagation daily light integral (DLI) on mean net photosynthesis (P_n) after 11 d of propagation for new guinea impatiens 'Harmony White' and 'Celebrette Red' and petunia 'Tiny Tunia Violet Ice' cuttings. For impatiens, each symbol represents an average of 3 plants, and error bars represent standard errors of the mean. Regression lines are fitted to data and corresponding r^2 are presented.
***Significant at $P \leq 0.001$.

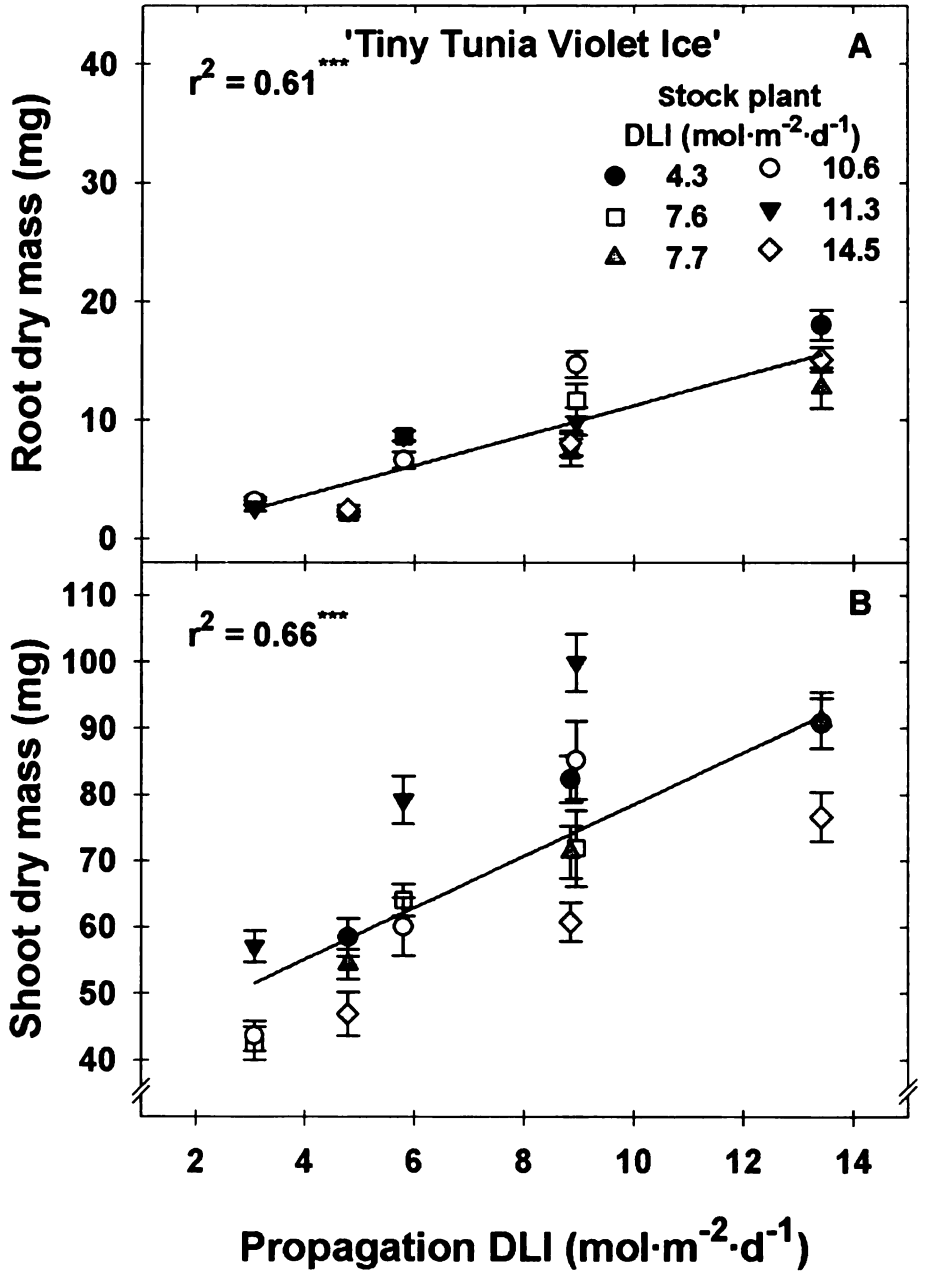


Fig. 5.2. Effects of stock plant and propagation daily light integral (DLI) on mean root and shoot dry mass after 21 d of propagation for petunia 'Tiny Tunia Violet Ice' cuttings. Each symbol represents an average of 10 plants, and error bars represent standard errors of the mean. Regression lines are fitted to data and corresponding r^2 are presented.
***Significant at $P \leq 0.001$.

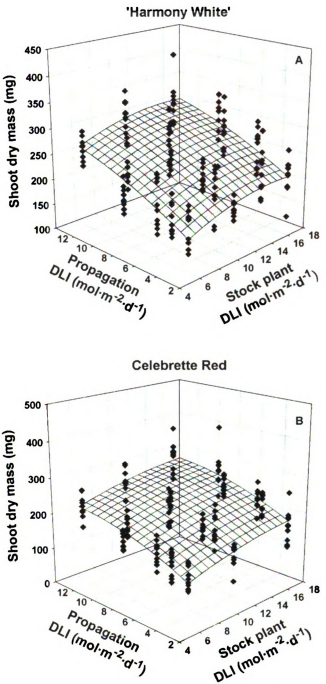


Fig. 5.3. Stock plant and propagation daily light integral (DLI) effects on new guinea impatiens 'Harmony White' and 'Celebrette Red' mean cutting shoot dry mass after 21 d of propagation.

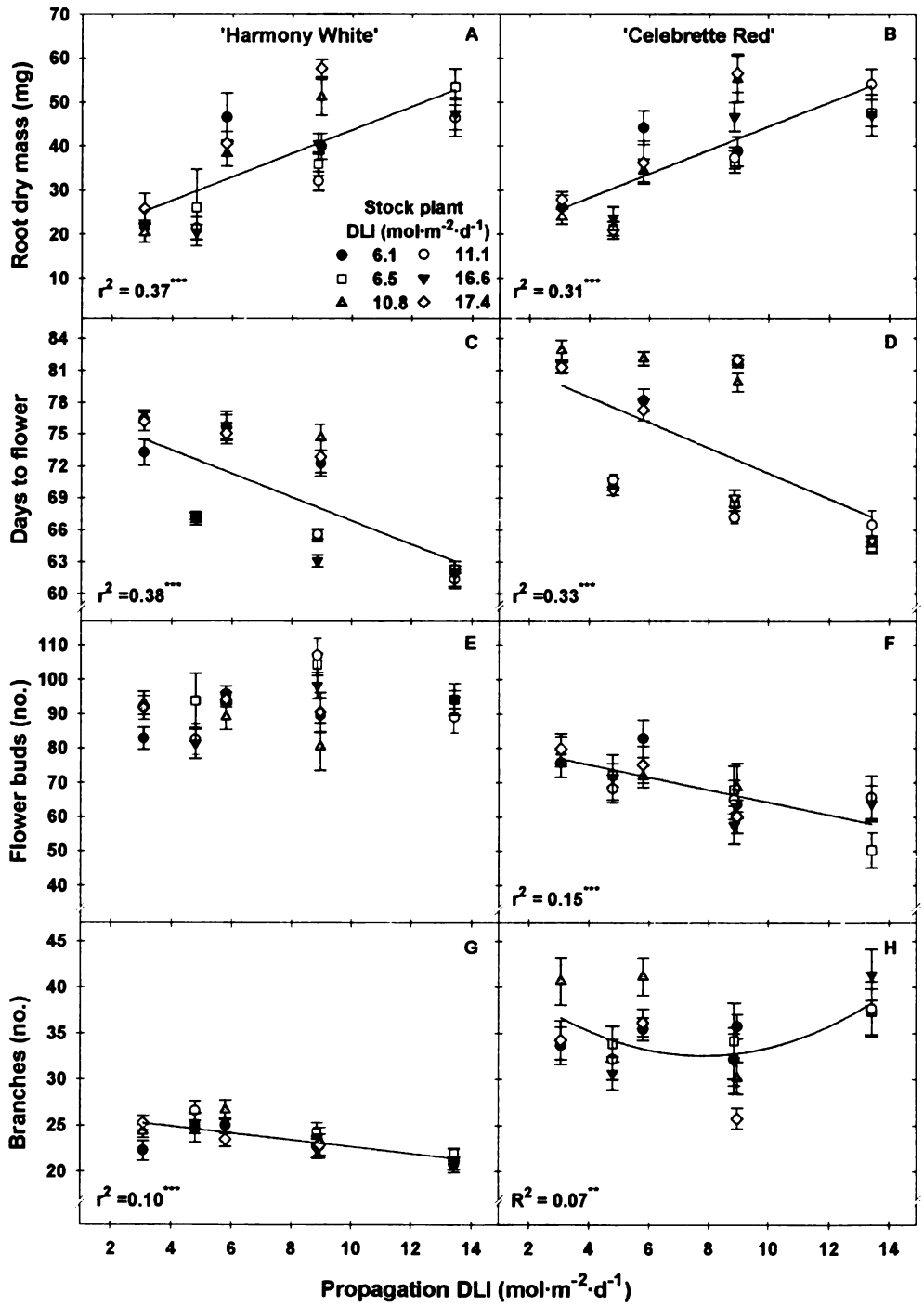


Fig. 5.4. Effects of stock plant and propagation daily light integral (DLI) on mean root dry mass of cuttings after 21 d of propagation and days to flower, flower bud number, and branch number at flowering for new guinea impatiens 'Harmony White' and 'Celebrette Red'. Each symbol represents an average of 10 plants, and error bars represent standard errors of the mean. Regression lines are shown for significant correlations only and corresponding r^2 and R^2 are presented. **, ***Significant at $P \leq 0.01$, or 0.001.

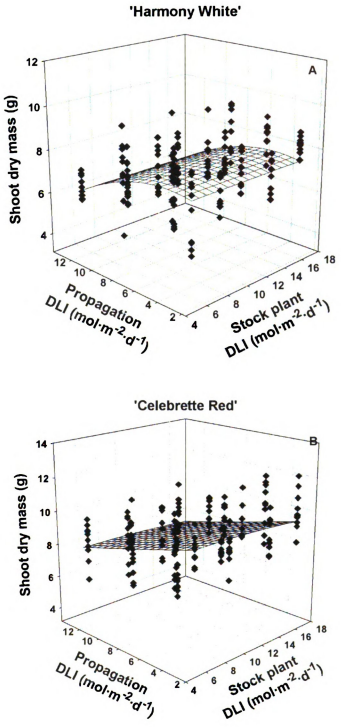


Fig. 5.5. Effects of stock plant and propagation daily light integral (DLI) on mean shoot dry-mass acculmulation at flowering for new guinea impatiens 'Harmony White' and 'Celebrette Red'.

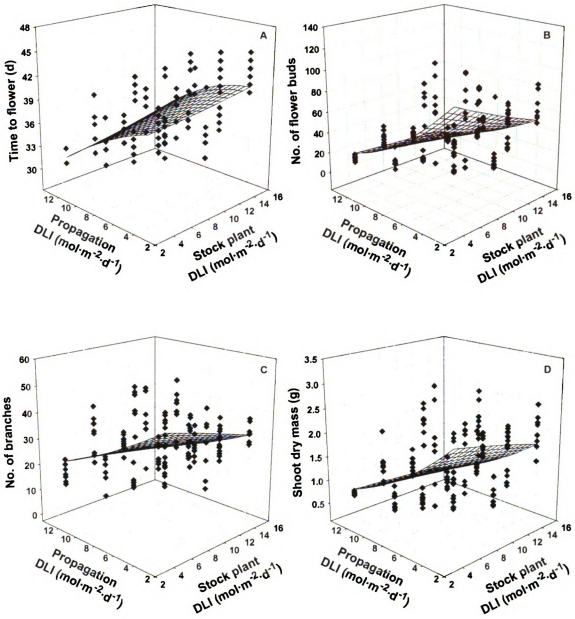


Fig. 5.6. Effects of stock plant and propagation daily light integral (DLI) on mean days to flower, flower bud number, branch number, and shoot dry mass at flowering for petunia 'Tiny Tunia Violet Ice'.